Liver Cancer in the Middle East

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For my daughters: Ophira and Feridey

"Everything should be made as simple as possible, but not simpler."

- Albert Einstein

"Truth does not become more true by virtue of the fact that the entire world agrees with it, nor less so even if the whole world disagrees with it."

"The physician should not treat the disease, but the patient who is suffering from it."

- ben Maimon (Rambam)

"Scientific knowledge is in perpetual evolution; it finds itself changed from one day to the next."

"What we see changes what we know. What we know changes what we see."

- Jean Piaget

"The knowledge of anything, since all things have causes, is not acquired or complete until it is known by its causes."

- Ibn Sina (Avicenna)

"The easiest method of acquiring the habit of scholarship is through acquiring the ability to express oneself clearly in discussing and disputing scholarly problems. This is what clarifies their import and makes them understandable."

- Ibn Khaldun

"We should not be ashamed to acknowledge truth from whatever source it comes to us. One must not be afraid of new ideas, no matter the source."

- Al-Kindi

"A hair divides the false and true; yes, and a single aleph were the clue-could you but find it- to the treasure house, and peradventure to the Master too."

- Omar Khayyam

"Judge a man by his questions, rather than his answers."

- Voltaire

Preface

Primary liver cancer is the fifth most common cause of cancer globally and the second most common cause of death from cancer. The ratio of death to incidence is about 0.9, since most patients who are diagnosed with it, die from it. The global burden of this disease is predominantly borne by less-developed countries and its causes are mainly known. The major cause is chronic infection with hepatitis B (HBV) and the majority of patients are in Asia. Although there are several types of primary liver cancer, approximately 90% are due to hepatocellular carcinoma (HCC), and this book focuses exclusively on this.

The Middle East comprises many countries with a huge range in income per capita, national wealth and its distribution, as well as hygiene and its practices. The causes of HCC and especially the cofactors are also varied. Some countries, such as Egypt and Saudi Arabia, have a very high percent of HCV-based HCC, while others, such as Turkey, Iran, and Kuwait, have a high percentage HBV-based HCC. As in the Western world, obesity and its associated liver diseases is increasingly becoming a cause of morbidity and HCC in the Middle East. Much of the cause of HCC in the Middle East is preventable, as elsewhere, the major approaches being HBV neonatal vaccination and lifestyle changes for obesity prevention and horizontal transmission of hepatitis C (HCV). Unlike other parts of the globe, alcoholism is a lesser cause.

In much of medicine in general and in HCC in particular, the major approaches to decreasing the disease burden and thus mortality depend on prevention (when the causes are known, as for HCC), early diagnosis via surveillance of patients who are known to be at risk (cirrhosis from any cause), and treatment of limited stage tumors (as a result of early diagnosis). Unlike most other tumors, the vast majority of HCC patients actually have two diseases, namely their HCC and an underlying liver disease that was the precursor to the HCC development. Both diseases interact bidirectionally, the liver disease influences HCC incidence and severity, and the HCC growth impairs residual liver function. Thus, consideration of both these co-existent diseases and their severity necessarily informs rational individual patient management decisions.

There is a large body of knowledge about HCC causes, pathophysiological mechanisms, and associated biology and biochemistry. Despite all this, too many patients present for medical care when their disease is at an advanced stage, when surgical therapies (resection, ablation, liver transplantation) with curative intent are no longer feasible. The next series of therapeutic options

consist of the loco-regional therapies, chemo-embolization (TACE), and radio-embolization (TARE), for non-metastatic disease patients. Thereafter come an increasing large choice of systemic therapy options, consisting of recently approved tyrosine kinase inhibitor drugs and immune checkpoint inhibitor drugs, both of which have recently been shown to greatly increase survival in this group of patients.

This book is divided into 4 parts. The first part (chapters "Biological Aspects of HCC", "Changing Etiology and Epidemiology of Human Liver Cancer", "Hepatocarcinogenesis Induced by Environmental Exposures in the Middle East", "Obesity and Hepatocellular Carcinoma: Epidemiology and Mechanisms", "Epidemiology of Hepatitis B Virus in the Middle East", "Hepatocellular Carcinoma in the United Arab Emirates", and "Overview of Clinical HCC and Its Management") considers the causes of HCC and clinical syndromes associated with HCC. The second part gives a descriptive overview of clinical HCC and describes the treatment modalities, with a chapter on treatment selection for individual patients, including settings where choices of therapies are less available (chapters "Cost-Effective Therapies for HCC: Resection and Ablation", "Transarterial Radioembolization in Hepatocellular Carcinoma", "Intra-arterial Chemotherapy and Transarterial Chemoembolization in Hepatocellular Carcinoma", "Radiotherapy for Hepatocellular Carcinoma", "Liver Transplantation in the Middle East", "Individual Patient Assessment and Therapy Decision-Making in a Live Donor-Based Liver Transplant Institute", and "Hepatocellular Cancer in Iran"). The third part gives a series of Middle Eastern country-specific chapters on local clinical HCC experience and practice (chapters "Hepatocellular Carcinoma in Kuwait", "Insights on Hepatocellular Carcinoma in Saudi Arabia", "Hepatitis C-Induced Hepatocellular Carcinoma in the Middle East", "Hepatocellular Carcinoma in the Middle East: An Overview", "Current HCC Clinical and Research in Egypt", "Hepatocellular Carcinoma in Turkey: A Review of Disease Epidemiology and Treatment Outcomes", "Targeting c-Met and AXL Crosstalk for the Treatment of Hepatocellular Carcinoma", "Hepatocellular Carcinoma in Morocco", "Hepatocellular Carcinoma in Lebanon and Its Association with Thalassemia", "An Overview of Hepatocellular Carcinoma (HCC) in Lebanon: A Focus on Hepatitis B- and Thalassemia-Related HCC", "Hepatocellular Carcinoma in Pakistan: An Update", and "Future Directions"). A final part (chapter "The Need for Region-Wide HCC Collaborations" and 27) considers what the next needs are for our subject and proposed useful HCC collaborations across our region.

Malatya, Turkey Brian I. Carr
March 2021

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Part I

Medical Science

Biological Aspects of HCC

Brian I. Carr

1 Etiology: Risk Factors for HCC and Presumptive Causes

The most common risk factors for developing HCC include cirrhosis from any cause, chronic hepatitis B or C viral infection, chronic alcohol consumption, fatty liver disease caused by obesity, and eating foods that have been contaminated by cancer-causing fungal toxins, as depicted in the list of cancer-causing and cancer-preventing substances of Table 1.

2 Biological Characteristics of Human HCC

The prognosis and management of HCC are influenced in most patients by the concurrence of two separate but related and interacting liver diseases, namely, hepatitis or cirrhosis from any cause and HCC. It is likely that each influences the other (i.e., cirrhosis is a precursor to most HCCs and growing HCC can destroy liver parenchyma and thus worsen liver function), and the selection of HCC therapy cannot take place without considering the limitations imposed by the concurrent liver disease. Thus, HCC is "a tale of two diseases."

3 Primary Drug Resistance to Cytotoxic Cancer Chemotherapeutic Agents

For most other cancers that have been studied, after a given number of chemotherapy treatments, the tumors can adapt and become resistant to the cytocidal actions of the cancer chemotherapy. This is called secondary or acquired resistance and is similar to the resistance seen in bacteria after exposure to antibiotics or in insects after exposure to insecticides. HCC is different in that it has primary resistance to a large array of toxins and most chemotherapeutics, without prior exposure to these agents. Work done several decades ago showed that cells that develop in a chronic toxic/carcinogenic milieu acquire a pan-drug resistance phenotype (pleiotropic) as they develop cancers. This is called primary resistance. Thus, trying to overcome this resistance with high doses of chemotherapeutic agents, especially in the presence of chronic liver damage, is often futile at best and dangerous for the liver at worst. Perhaps this is why such a large number of cancer chemotherapy clinical trials failed to produce any meaningful survival advantage for patients with HCC and could usually only be done in selected patients.

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Table 1 Substances of natural origin in the human diet that can cause or prevent cancer

A. *Substances that can cause cancer (carcinogens)*
1. [a]Aflatoxins – fungal contamination of stored rice and grains; ochratoxin A
2. Nitrosamines – fried bacon, cured meats
3. Hydrazines – found in edible mushrooms (false morel)
4. Safrole – found in sassafras plant and black pepper. Oil of sassafras in "natural" sarsaparilla root beer is 75 % safrole
5. Pyrrolizidine alkaloids – found in herbs, herbal teas, and occasionally in honey (e.g., senkirkine [coltsfoot], symphyline [comfrey])
6. Estrogens – from wheat germ, unpolished rice, forage crops
7. Bracken fern carcinogen
8. Methylazoxymethanol or cycasin (cycad plants)
9. Carrageenan – from red seaweeds
10. Tannins – from tea, wine, and plants
11. Ethyl carbamate in some wines, whiskey, and beers
B. *Carcinogens from molds and bacteria in food*
1. Aflatoxins (*Aspergillus flavus*)
2. Sterigmatocystin (*Aspergillus versicolor*)
3. Microcystins – from *Cyanobacteria* in drinking water in China
C. *Tumor antagonists in the diet*
1. Selenium
2. Coffee
3. Antioxidants
4. Phytochemicals, including polyphenols (curcumin from turmeric; resveratrol from red wine)
5. Vitamins A, K, and D. Vitamin A analog (polyprenoic acid, an acyclic retinoid)
6. Flavonols
7. Fish consumption
8. Vitamin K or polyprenoic acid (an acyclic retinoid analog of vitamin A)

[a]Only aflatoxins have strong epidemiologic evidence of association with human HCC. Reproduced with permission from © John Wiley and Sons, 2014; Carr (1985)

4 Vascular Characteristics

There are two different and unrelated vascular characteristics of HCC.

Vascularity: Firstly, it is one of the most vascular of tumors, and HCC has distinctive features on the arterial phase of computed tomography (CT) and magnetic resonance imaging (MRI) scan images. Unlike other organs, approximately a large proportion of the oxygenated blood of the normal liver comes from the portal vein. In contrast, around 80% of oxygenated blood that feeds HCCs comes from arterial outgrowths from hepatic artery branches. This was noted 30 years ago in Japan to offer a potential means for delivering drugs/chemotherapy moderately selectively to the HCC by injecting them into the hepatic artery and thus minimizing the exposure of the underlying diseased liver to drug toxicities. However, the liver is only partially protected, because in cirrhosis there is often hepatic arterial-venous blood shunting and direct intrahepatic arteriovenous connections open up.

PVT: Secondly, HCC has the propensity to invade radicals of the portal vein and grow in its lumen. When the portal vein is occluded by HCC, a characteristic enlargement and vascular enhancement of the portal vein are seen on CT. This is called macrovascular portal venous invasion (PVT). By contrast, microvascular venous invasion is only seen on biopsy or in HCC pathology specimens from liver resection/transplantation. Because the tumor cells are now in a vein, they can/do get carried by the blood stream around the circulation, with the increased possibility of forming distant metastases. Macrovascular invasion very often results in post-liver transplant recurrences and is thus considered a contraindication to transplantation surgery. Microvascular invasion does not seem to carry such a great risk. The reasons are unclear, as the cells are also within the venous lumen. Main branch PVT is considered to be a relative contraindication to trans-arterial chemoembolization (TACE), as HCC cells have blocked the portal vein and the TACE/chemoembolization therapy (transiently) blocks the artery, so the affected liver lobe loses its blood supply and can be severely damaged. Often, if only one of the two major portal vein branches is blocked by the tumor (branch PVT), then TACE therapy can still be safely given to the hepatic artery branches that feed the HCC.

5 HCC Growth Rates

HCCs have been reported to have a wide range of doubling times (growth rates), from 1 month to a year. Without repeated scans over several months, it is difficult to calculate the tumor growth rate of HCC in an individual patient. A newly diagnosed patient could have had a slow-growing 5 cm HCC for 3 years; but another patient with the same size tumor on the first clinic visit might have had only a 2 cm tumor 6 months ago and will thus have an aggressively behaving and rapidly growing HCC. On that first clinic visit, without the knowledge of prior scans, it would have been impossible to know the HCC growth rate. Thus, patients are heterogeneous with respect to their tumor biology, growth rates, and other characteristics. In fact, there is now evidence that HCCs change and evolve as they grow. If true, then a single baseline biopsy might be insufficient for rational patient management decisions, and multiple (liquid) biopsies over time may be a solution.

Size alone may not be so important, as many large HCCs with >8 cm diameter can arise in noncirrhotic liver and are thus quite resectable. Thus, although size is widely seen as a negative prognostic factor, it really depends on the clinical context. A study of platelets (a surrogate for cirrhosis) has shown that very large HCCs grow in a normal platelet environment (low or absent cirrhosis), whereas most patients with smaller and multifocal HCCs have thrombocytopenia. Thus, the cirrhotic and inflammatory context likely influences the ability of the liver to support the growth of an HCC to large size without liver failure due to parenchymal destruction.

Faster-growing tumors are often associated with several "satellite" lesions likely because they invade the surrounding liver. However, there is another mechanism for multifocality, as portal venous invasion by HCC is also a means for tumor spread within the liver (more common than distant metastases). This has significance for resection surgery, where up to 40% of patients have recurrence within 4 years after apparently curative surgery. Such recurrences are observed to be "early" within a few months or "late" after a year or more, which may have different causes. Early recurrence tends to be near the resection site and close to where the removed tumor was located; it is thought to be due to direct tumor extension from microscopic cells that could not have been seen at surgery or on the pre-resection scan. Late recurrences are often in other parts of the liver and may be new primary HCCs. These may occur in cirrhosis because there are millions of proliferating cirrhotic nodules, all being potentially premalignant, and eventually one or more of the nodules develop into new HCCs.

6 The Inflammatory Background

More than 80% of patients with HCC also have disease of the underlying liver that often profoundly affects HCC patient management choices. Most commonly HCC is associated with chronic inflammation (from HBV, HCV, or alcoholism, or their various combinations, or from obesity-associated liver disease), which may lead to cirrhosis, depending on the duration and intensity of the inflammation. Such inflammation may also lead to liver failure, for which only liver transplantation is an effective treatment. Depending on the severity of the underlying liver damage (inflammation/fibrosis/cirrhosis), the ability to perform resection or ablation therapies beyond that needed for a minimal size tumor could be thwarted by the risk of subsequent liver failure after the contemplated surgery. This can also be true for any potentially hepatotoxic medical therapy, such as regional cancer chemotherapy, TKIs, or ICIs. Since many chemotherapeutics also damage the bone marrow where granulocytes and platelets are produced, this combination can produce clinical toxicities. Furthermore, cirrhosis is often associated with bleeding tendencies from failure of the liver to produce sufficient coagulation proteins, in addition to low blood platelet counts thought to be due to splenic destruction of platelets from the back pressure resulting from liver fibrosis. In summary, the fragility of the underlying liver can limit the safety of many therapies other than liver transplantation.

7 HCC Microenvironment

For several decades, it has been thought that tumors arise because one or more growth pathway genes become mutated and are expressed or otherwise activated in a way that leads to excessive stimulation of the growth control pathways of the cell; this is known as the oncogene hypothesis. There is much experimental support for this hypothesis. However, in recent years, it has become clear that the activity of genes is often affected by other factors, either by controls on the gene involved (epigenetic factors), such as methylation, or by not yet well-understood factors in their microenvironment. Thus, both oxygenation and nutrients can affect how a given gene might behave within a cell, including oncogenes. Recent support for this "seed" (gene) and "soil" (cell environment) idea (a hypothesis originally developed for metastases by Stephen Paget (1889): "When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil. … While many researchers have been studying 'the seeds,' the properties of 'the soil' may reveal valuable insights into the metastatic peculiarities of cancer cases." Support for this idea has come from molecular clinical studies in which it has been found that the behavior of HCC can be predicted from knowledge of the pattern of genetic changes (molecular signature) to be found in the nontumorous part of the liver (microenvironment). This environmental influence may have relevance in at least two HCC circumstances:

1. Prediction of the behavior of an individual patient's tumor, such as the likelihood of recurrence after resection
2. The reason for an expected benefit of virus hepatitis therapy (of the "soil") as part of HCC therapy in chronic virus carriers

8 Tumor Microenvironment Systems

Immune and inflammatory mediators: interleukins, chemokines, reactive oxygen molecules, PDL-1

Tumor angiogenesis/vascularization factors: VEGF, PDGF, FGF, and TGF alpha

9 Tumor Microenvironmental Mediators

Cells: hepatic stellate cells, cancer-associated fibroblasts (CAFs), lymphocytes, Kupffer cells, endothelial cells, platelets, tumor-associated macrophages (TAMs), dendritic cells, stem/progenitor cells

10 Noncellular Components

Growth factors (EGF, TGFα, FGF, PDGF, VEGF, HGF, IGF), TGFβ

Proteolytic enzymes: MMPs
Extracellular matrix proteins: laminins, integrins, heparan sulfate proteoglycans
Inflammatory cytokines: IL-6, IL-1, TNFα

11 Platelets and HCC Growth

A significant association has been found in HCC patients, between thrombocytosis and larger tumor volume, high AFP levels, and poor survival. By contrast, thrombocytopenia is also considered as a prognostic factor in HCC. Studies involving clinical parameters of patients with small or large HCCs have shown that along with other factors such as AFP, tumor size is correlated with platelet counts. HCCs associated with thrombocytosis are often found in noncirrhotic liver and tend to be larger-sized tumors. However, HCCs associated with thrombocytopenia are associated with small tumor size, lower blood albumin, and impaired liver function and a fibrotic background. The relationship between platelets and cancer cells is bidirectional, since tumor cells stimulate platelet aggregation, whereas platelets stimulate the growth of tumor cells and promote their metastasis through activation and secretion of several molecules. As tumor cells activate platelets, activated platelets in turn contribute to sev-

eral steps of carcinogenesis. These include the secretions by platelet granules containing (1) growth factors (IGF-1, EGF, VEGF, HGF, transforming growth factor-β (TGF-β), FGF, PDGF, etc.), (2) coagulation factors (prothrombin, fibrinogen, factor V, and factor VIII), (3) pro-angiogenic and anti-angiogenic factors (angiopoietin-1, angiostatin, etc.), (4) MMPs and tissue inhibitor of metalloproteinases (TIMPs) (MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP, MMP-14, TIMP-1, and TIMP-2), (5) pro-inflammatory mediators (C-X-C motif chemokines, such as CXCL4, CXCL7, and CXCL12), and (6) immunologic molecules (C1 inhibitor and IgG) [12,21,70,71]. Recent data identifying the effects of platelet extracts on HCC cell lines have shown that platelets and platelet-derived factors increase cell proliferation, invasion, and migration, whereas they decrease apoptosis and cell AFP levels, through JNK signaling. Secretory platelet granules also trigger angiogenesis by cytokines VEGF, PDGF, TGF-β, IGF-1, and endostatin. Nevertheless, since tumor cells grow without new blood vessels up to 1–2 mm^3, pro-angiogenic factor stimulation is necessary for tumor cells to grow further, which is also provided by platelets. Platelets help the tumor cells to adhere to the blood vessel wall through expressions of P-selectin (CD62P) and αIIbβ3 and enhance both intravasation and extravasation. The "education of platelets by tumor cells" is another recently described and potentially important observation. Studies show that platelets take up pro-angiogenic cytokines, proteins, and RNA which are secreted by tumor cells.

12 Growth Factor Receptors and their Inhibitors (TKIs)

Numerous cellular functions that include tumor cell differentiation, growth, apoptosis, and angiogenesis are mediated via signals from membrane-bound tyrosine kinase receptors. They include EGFR, IGFR, FGFR, Met (HGF receptor), VEGR, IGFR, and PDGFR and they transduce intracellular signals, often via the Ras/MEK/ERK pathway to the nucleus, that often result in transcription factor activation. Two major kinase types are dysregulated in HCC, namely, the tyrosine kinases (TKs) and cyclin-dependent kinases (CDKs), and they are each targets for the treatment of HCC via TKI inhibitors.

Some FDA-approved tyrosine kinase inhibitor (TKI) drugs that inhibit signaling associated with the above growth receptors include sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab. Other drugs, such as bevacizumab, are antibodies that also target growth receptors.

13 Immune Checkpoint Inhibitors (ICIs) and the Liver

The immune checkpoint inhibitors (ICIs) are compounds that target the regulatory signals between T lymphocytes and target cells, as well as other immune cells. T lymphocytes recognize specific antigens on target cells through major histocompatibility complex (MHC) proteins through their T-cell receptors and can induce apoptosis of target cells.

Immune checkpoint proteins, including PD-1/PD- L1 and cytotoxic T lymphocyte antigen 4 (CTLA-4), suppress T-cell inflammatory activity to prohibit overactivation of the immune system and promote self-tolerance. Immune checkpoint inhibitors (ICIs) suppress immune inhibition (suppress the suppressor) induced by PD-1/PD-L1 or CTLA-4 and thereby reactivate T cells to promote their cytotoxicity to tumor cell targets.

ICIs thus prevent the association of programmed cell death protein-1 (PD1) with its ligands, programmed death ligand1 (PD-L1) and 2 (PD-L2), enhancing the T-cell response toward HCCs, and have recently come into clinical use for many tumor types, including HCC. When used in various combinations, recent clinical trials have shown that they greatly enhance HCC responses by shrinkage, with associated increase in patient survival. Examples include nivolumab, a monoclonal antibody against PD-1; pembrolizumab, also a humanized monoclonal antibody against PD-1; atezolizumab, a monoclonal antibody against PD-L1; and ipilimumab, a monoclonal antibody against cytotoxic T lymphocyte

antigen-4 (CTLA-4), a receptor that also functions as an immune checkpoint, to downregulate immune responses. The ICIs appear to have the possibility of enhancing the lifespan of many HCC patients and are perhaps the most exciting development in HCC work in the last 10 years, as of end-2020.

14 Clinically Useful HCC Serum Biomarkers

Alpha-fetoprotein (AFP) is a glycoprotein produced in the embryonic liver and a form of fetal albumin, the synthesis of which is turned off at birth. Hence, an older name is oncofetal antigen, which, like CEA and glypican-3, is resynthesized postnatally in some tumors. It is frequently used and inexpensive and is a simple blood test to perform, but its blood levels are elevated in only 50% of patients with HCC. AFP is not a sensitive enough marker for screening for small, new HCCs but is extremely useful if elevated, when monitoring the HCC response to therapy. The biological function of AFP is still speculative, though there is some evidence for its role in apoptosis. Since it is the fetal form of albumin and albumin has some growth control properties, it may be that AFP has a functional role in the loss of growth control which characterizes the HCC phenotype. Recently, more HCC-specific tests have come into general clinical practice, such as a glycosylated form of AFP (itself, a fetal form of albumin) called AFP-L3.

Des-gamma carboxy prothrombin (DCP) or protein induced by vitamin K absence (PIVKA-2) is an HCC blood biomarker, and US Food and Drug Administration (FDA)-approved kits for measuring it are readily available to clinical labs. Several studies have shown that elevated DCP is commonly elevated in the presence of portal vein thrombosis (PVT). The molecule is really interesting, as it is an immature form of the coagulation protein, prothrombin. The enzyme responsible for catalyzing the immature to the mature form of prothrombin has an absolute requirement for vitamin K. This highlights an important role for loss of vitamin K function in HCC development and suggests that some vitamin K-dependent protein or vitamin K itself might be important in HCC migration, given the association of DCP with PVT. Several attempts have been made to assess the value of high doses of vitamin K in suppressing DCP (it does) and thus suppressing clinical HCC growth. The experimental evidence is good, but the one big randomized clinical trial fell short.

A diagnostic model hepatocellular carcinoma (HCC) has been proposed that incorporates the levels of each of the three biomarkers, AFP, AFP-L3%, and DCP, along with patient sex and age, into the gender, age, AFP-L3%, AFP, and DCP (GALAD) model, but awaits validation for screening.

Glypican-3 is another oncofetal glycoprotein that appears to have prognostic significance as an HCC serum biomarker and is being investigated both for imaging and as a potential target in HCC therapeutics.

15 Clinical Context Is Key

For all HCC parameters, context is key. For a newly presenting HCC patient, we normally do not know at what point the patient is on his/her disease trajectory. Thus, the total clinical context has to be understood to make rational patient management decisions. Tumor size alone is less important, unless we know about the presence of PVT or residual parenchymal liver function. That is why all modern classification systems employ parameters of both tumor aggressiveness (maximum tumor dimension, number of tumor nodules, presence of PVT, and often tumor biomarker levels), as well as liver function parameters. In this approach, two important papers showed that HCC microenvironmental factors may be as important as tumor factors, or more so. Hoshida et al. (2008) showed that gene-expression profiles of tumor tissue failed to yield a significant association with survival. In contrast, profiles of the surrounding nontumoral liver tissue were highly correlated with survival. Utsonomiya et al. (2010) showed that molecular signatures of noncancerous liver tissue can predict the risk for late recurrence of hepatocellular carcinoma.

It has recently been shown that the high rates of recurrence after HCC resection can be significantly reduced by anti-hepatitis viral therapy. Thus, the viral-mediated inflammation must influence the HCC behavior. In summary, there are at least two types of molecular signatures (patterns of genetic changes) and clinical prognostic factors in HCC: those of the tumor and those of the underlying liver.

It has become increasingly clear in recent years that the behavior of a given HCC, and thus the treatment approaches for a patient with HCC, depends on more than just the clinically observed tumor characteristics. This was anticipated in the 1985 staging system of the Japanese hepatologist Kunio Okuda, who brought attention to the need to consider both tumor and liver characteristics in prognosis and therapy. More recently, this approach has been greatly expanded by advances in HCC biology, biochemistry, and molecular understanding. As a result, a fuller understanding of HCC behavior needs to consider genes and gene alterations, tumor stroma (the underlying tissues), tumor neovasculature (the growth of new blood vessels that is necessary to support the increasing mass of the growing tumor), inflammation, supporting liver parenchyma (cells in the liver that support the specialized hepatocytes), and gene/molecular signatures (patterns of genes and their expression through proteins). Although much of this is still in the research realm (at least for the vasculature, inflammation, and molecular signatures), there are rapidly advancing clinical applications. For example, new knowledge of the growth factors that encourage new blood vessel growth has led to the development of several new cancer drugs that target this vasculature, such as bevacizumab or sorafenib. Another example is the use of anti-hepatitis therapy to decrease HCC recurrences after successful tumor resection. The recognition of the importance of the inflammatory microenvironment has had two recent consequences. One is the use of HCC clinical inflammatory markers in patient prognostication. Examples include use of blood levels of C-reactive protein (CRP), Glasgow Index (CRP plus albumin), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR). A second use is the finding that patients being treated with anti-inflammatory agents, such as aspirin for cardio-preventive purposes, seem to have lower incidence of some GI cancers, including HCC. These results point to the possibility of using aspirin or NSAIDs in HCC prevention, possibly as an adjunct to resection. As explained above in the section on HCC growth, absence of cirrhosis likely permits the growth of larger tumors. Counterintuitively, these may be easier to manage with better resultant prognosis, due to absence of the cirrhosis and associated inflammation, rendering hepatic resections safer.

16 Circulating Tumor Cells (Liquid Biopsy)

Precision oncology is becoming increasingly important in the diagnosis and management of patients with various cancers, and liquid biopsy has shown promise as a minimally invasive technique for diagnosis, detection of actionable (therapy) mutations, in the monitoring of tumor evolution and in making rational treatment decisions. Liquid biopsy depends on the observation that many patients with solid tumors shed tumor cells and tumor cell DNA (in addition to a vast amount of nontumor circulating DNA) or its fragments into their circulating blood.

There are several liquid biopsy analytes, including circulating tumor RNA, cell-free micro RNA, exosomes, circulating tumor cells (CTCs), and circulating tumor DNA (ctDNA). This approach permits the use of a minimally invasive means for obtaining clinically useful tumor information without invasive tissue biopsies. Furthermore, since they are based on peripheral blood samples, they can be repeated during the course of a patient's disease at the same time as other routine clinical bloods are drawn for standard tests. However, there is not yet a standardized platform for such testing. Despite this, several blood tests have already been FDA-approved as accompaniments to rational patient selection for several new molecularly targeting therapies, so far in non-HCC tumors. In addition to therapy, some uses of liquid biopsy include the

potential for diagnosis or assessment of postsurgical residual disease and presence of micrometastases. For HCC, measurement of methylation profiles of ctDNA appears to be a promising surveillance tool. Given that AASLD and EASL guidelines do not recommend HCC biopsy for the diagnosis of most patients with a vascular liver mass in a cirrhotic liver, there is a dearth of HCC tissue to examine unless the tumor has been removed by resection or transplantation. Circulating tumor cells offer the potential for repetitive molecular analysis of HCCs over the course of an individual patient's disease.

Further Readings

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Changing Etiology and Epidemiology of Human Liver Cancer

John D. Groopman

1 Introduction

Collectively liver cancer, including hepatocellular carcinoma (HCC) and cholangiocarcinoma, accounts for 8.2% of all reported cancer deaths in men and women, and it is the third/fourth most common cause of cancer mortality worldwide, tied with stomach cancer [1, 2]. Globally, the incidence rates and age of diagnosis of liver cancer vary enormously, and unfortunately the burden of this nearly always fatal disease is much greater in the less economically developed regions of Asia, Central America, and sub-Saharan Africa (Fig. 1) [3]. HCC, perhaps due to a changing pattern of risk factors, is also the most rapidly rising solid tumor in the USA and Central America and is overrepresented in minority communities, including African-Americans, Hispanic/Latino-Americans, and Asian-Americans [4–6]. This increase in the USA may portend a resurgence of this disease in the more economically wealthy countries. Currently, there are more than 840,000 new cases of this nearly always fatal cancer each year and nearly 370,000 deaths annually in the People's Republic of China (PRC) alone [3]. The combined age-standardized rate of mortality from liver cancer for men and women worldwide was 8.5 per 100,000 in 2018 [1]. Further, there are striking sex differences in the age-standardized rate of liver cancer deaths for men and women which was 12.7 and 4.6 per 100,000 people in 2018, respectively. The countries that traditionally are considered as part of the Middle East span two World Health Organization (WHO) regions: Eastern Mediterranean and Europe. Of these countries, Egypt has the second highest age-standardized rate of mortality of liver cancer, 49.0 and 16.7 per 100,000 for men and women, respectively, in the world [1]. Indeed, this mortality rate is only exceeded globally by Mongolia, and the unique circumstances of this liver cancer burden will be discussed in the following sections. While Egypt has the largest population in the Middle East, the next six countries with populations ranging from 20 million to 80 million people (Yemen, Syria, Saudi Arabia, Iraq, and Turkey) all have age-standardized rates of liver cancer deaths for both men and women less than the global average (8.5 per 100,000 people), but as will be discussed later in this chapter, these statistics are likely to change in the next few decades.

For a cancer such as liver cancer that has such a poor prognosis, less than a 15% 5-year survival, the age of diagnosis has a major impact on society [7]. In contrast with most common cancers in the economically developed world where over 90% of cases are diagnosed after the age of 45, in many high-risk regions for liver cancer, onset begins to occur in both men and women by

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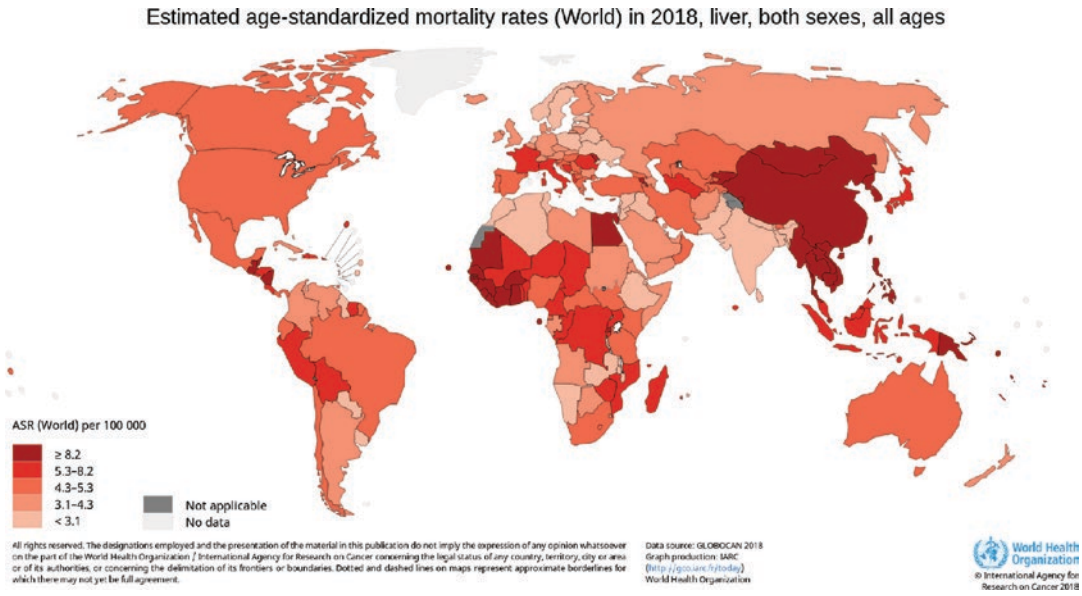

Fig. 1 Age-standardized mortality of liver cancer in men and women worldwide [1, 2]

20 years of age and peaks between 40 and 49 years of age in men and between 50 and 59 years of age in women [8, 9]. This earlier onset of HCC might be attributable to exposures that are both substantial and persistent across the life span and starting early in life. As mentioned above, sex differences in liver cancer incidence have also been well described, and worldwide the number of cases among men was 596,000 and 244,000 among women in 2018 [1, 2, 7]. These human epidemiologic findings are also reflected in experimental animal data for one potent liver carcinogen linked to human HCC, aflatoxin, where male rats have been found to have an earlier onset and higher incidence of cancer compared to female animals [10]. Thus, the consistency of the experimental animal and human data points to the important role that environmental exposures play in sex differences in HCC risk.

This chapter will review the significant data that links exposures to specific environmental toxicants, host factors, and biological agents with the etiology of liver cancer and with a specific commentary for counties in the Middle East. The epidemiologic studies revealing these etiologic factors have been made possible by devising biomarkers reflective of exposure, dose, and risk. The translation of these basic science findings to an understanding of the etiology of HCC has also provided guidance for the development of preventive interventions in high-risk populations. A number of these major investigations will be reviewed, to provide an overview of this very active field of research. Taken together, the etiology of many liver cancers diagnosed today is well understood, and when the underlying genetic diseases of hemochromatosis, alpha-1-antitrypsin deficiency, and copper overload disease are included, probably greater than 90–95% of the risk factors causing today's liver cancers have been identified [11–13]. With the emergence of fatty liver disease as a risk factor for liver cancer, a number of genetic risk factors have been identified including mutations in PNPLA3 that is more common in Hispanic populations [14, 15]. This knowledge base has been actively translated into effective screening tools, and prevention strategies that should continue if implemented effectively mitigate this cancer. Of great concern is the hypothesis that the emergence of new risk factors such as obesity and type 2 diabetes is changing the landscape of liver cancer etiology. With nearly 70% of the US population being

overweight or obese and over 400 million people worldwide being type 2 diabetic, there is an emergent likelihood of dramatically rising hepatic morbidity and mortality [16]. This problem is especially emergent in the countries of the Middle East where a majority of adults are now obese and the rates are rising [17].

1.1 Currently Identified Etiologic Agents for Liver Cancer

Prior prospective cohort studies conducted during several decades across many countries and populations have revealed the critical role that hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, and dietary aflatoxins play, often interacting with each other, in high-risk settings for liver cancer [6, 18]. The identification of these critical risk factors and others such as vinyl chloride and cigarette smoke has often been the result of developing validated biomarkers reflecting these exposure situations. Further, there have been a number of underlying genetic predispositions that have also been linked to increased liver cancer susceptibility.

1.2 The Promethean Liver

The liver has remarkable self-repair mechanisms that can lead to recovery at almost every stage of the progressive etiopathogenesis to cancer including fibrosis and cirrhosis. While Greek mythology poetically captured this biology in the stories of Prometheus, experimental studies have documented this remarkable repair and recovery process [19]. Rodents that undergo 70% partial hepatectomy recover a fully functioning liver within 2–4 weeks following surgery. Some studies from the 1960s demonstrated that rats could be subjected to biweekly partial hepatectomies for a year (20–25 surgeries) and still recover complete liver function [20, 21]. While not as dramatic, humans have also been found to have remarkable hepatic regrowth properties following injury or surgeries. In many respects, it is not surprising that the tissue which is the first line of response to the remarkably diverse number of compounds and nutrients absorbed from the gut into the portal vein would have this type of repair proficiency. A recent review has documented the recovery after several months from fatty liver disease and fibrosis in morbidly obese individuals following weight loss [22]. Thus, quantitative assessments of liver status through the use of biomarkers would be extremely valuable for designing interventions that facilitate this liver repair.

Biomarkers detected from blood samples have become important tools for cancer prevention and control. Many of these biomarkers fall under the rubric of the liquid biopsy. In the case of liver cancer diagnosis, α-fetoprotein (AFP) has been studied as an early diagnostic measurement since elevated levels are detected in patients having liver cancer. Unfortunately, AFP has not proven to be an effective early detection tool in a number of prevention investigations. Other tools such as FibroScan are capable of detecting relatively advanced but not early-stage liver disease [23]. Current liquid biopsy strategies have already been deployed for the early detection of tumor recurrence following initial therapeutic interventions. In a number of studies, mutant tumor suppressor and oncogenes known to have occurred in the primary tumor have been detected as DNA fragments in blood samples. Using the modern tools of nucleic acid detection, these mutant fragments can be readily measured. The analytical challenge of these strategies results from the relatively low number of these DNA fragments emerging in circulation, and this leads to the necessity of collecting large volumes (5–40 ml) of blood in order to have enough fragments of DNA measurement following PCR amplification. Hence, this technology is most applicable to the clinical setting and is not currently practicable for prospective cohort and other environmental exposure studies where much smaller volumes of blood were and are obtained from participants. Given the volumes of blood available in these investigations of healthy individuals, current strategies and technologies are focused on the more abundant proteins and their nucleophilic targets in circulation.

1.3 Hepatitis B Virus

In the case of identifying a role for HBV in liver cancer was the identification of a major biomarker the specific antigen (HBsAg) in blood samples partnered and grounded with cohort investigations [24]. Thus, one of the first breakthroughs in defining a biological agent in the etiology of this disease occurred with a series of studies describing a role for the hepatitis B virus (HBV) in HCC pathogenesis. Historically, a number of investigations found that chronic carriers of HBV, as indicated by sequential hepatitis B surface antigen (HBsAg) positivity at 6-month intervals, were at increased risk of developing HCC [3, 25]. Further, the age of initial infection by this virus was directly related to the prevalence of the chronic carrier state and subsequent accelerated risk for HCC. Approximately 90% of HBV infections acquired in infancy or early childhood become chronic infections, whereas only 10% of infections acquired in adulthood become chronic, and less than 50% of chronic carriers progress to HCC [26–29]. The global burden of HBV infection varies widely, and historically China, Southeast Asia, and sub-Saharan Africa had some of the highest rates of chronic HBV infection in the world, with a population prevalence of over 10% [30]. The public health significance of HBV as a risk factor for HCC is staggering with the consideration that there are still, despite the availability of an effective preventive vaccine, over 400 million viral carriers and between 10% and 25% of these individuals are likely to develop HCC [18, 31, 32]. The biology, serology, mode of transmission, and epidemiology of this viral infection continue to be actively investigated and have been recently reviewed [24, 33, 34]. Collectively, this work directly led to the research that resulted in a vaccine effective against HBV. Indeed, this vaccine has been reported to reduce HCC incidence by up to 70% in a cohort of young vaccinated children in Taiwan that have been followed for up to 30 years [35–40].

Historically, many studies across the globe that explored the relationship between HBV infection and HCC calculated risk estimates ranging from 3 to 30 in case-control studies and from 5 to 148 in cohort studies [41]. For example, an early small hospital-based case-control study from northeast Thailand showed an adjusted odds ratio (OR) of 15.2 for the presence of HBsAg among HCC patients [42]. An adjusted OR of 13.5 was reported from a case-control study in the Gambia [30]. The risk of HCC among HBsAg-positive individuals in Korea from a prospective cohort study of government workers was 24.3 among men and 54.4 among women, adjusted for age, smoking, alcohol use, and diabetes [43]. A similar prospective study from Taiwan found men positive for HBsAg were 223 times more likely to develop HCC than men negative for HBsAg [28]. All of these investigations were grounded and successful because of the measurement of a validated biomarker of high sensitivity and specificity that tracked with the development and risk for HCC. Further, these studies benefited by the high-throughput for the measurement of this biomarker that in turn permitted the recruitment of large numbers of individuals facilitating sufficient power for the study.

The contribution of HBV to the pathogenesis of liver cancer is multifactorial and is complicated by the identification of mutant variants in HBV that modulate the carcinogenesis process [24]. The HBV genome encodes its essential genes with overlapping open-reading frames; therefore, a mutation in the HBV genome can alter the expression of multiple proteins. In many cases of HCC in China and Africa, a double mutation in the HBV genome, an adenine to thymine transversion at nucleotide 1762, and a guanine to adenine transition at nucleotide 1764 ($1762^{T}/1764^{A}$) have been found in tumors [44–46]. This segment of the HBV genome contains an overlapping sequence for the base core promoter and the HBV X gene; therefore, the double mutation in codon 130 and 131 of the HBV X gene reported in human HCC is identical to the 1762 and 1764 nucleotide changes [47]. The increasing occurrence of these mutations has been also associated with the increasing severity of the HBV infection and cirrhosis [45, 46]. This acquired mutation following HBV integration into hepatocytes was originally characterized in

HBV e antigen-negative people [48]. The $1762^{T}/1764^{A}$ double mutation occurs more frequently in people infected with the genotype C strain of HBV, which is the most common genotype found in East Asian patients [49–51]. This double mutation tracks with an increased inflammatory response that becomes stronger as the progression of liver damage transits through chronic hepatitis and into a cirrhosis stage [52]. The underlying mechanism of the effects of HBV e antigen on the biology of inflammation and cirrhosis is still unclear, but there are substantial data that point to modulation of the immune surveillance system and immune tolerance in the presence and absence of this protein [52–54]. The $1762^{T}/1764^{A}$ double mutation has also been demonstrated to affect an increase in the rate of HBV genome synthesis in cellular models [55, 56]. In cellular studies, the $1762^{T}/1764^{A}$ double mutation increased the replication of the viral genome twofold, and in the case of some of rarer triple mutations, an eightfold increase in genome replication was found [54, 56]. A matched case-control investigation of 345 men who died of HCC and 625 controls were nested within a cohort of male hepatitis B surface antigen (HBsAg) carriers from Qidong, China found the HBV $1762^{T}/1764^{A}$ over twice as frequently in cases (81%) as compared with controls. The matched preserving OR of 6.72 (95% CI: 4.66 to 9.68) strongly indicated that cases were significantly more probably than controls to have the mutation. Plasma levels of DNA harboring the HBV mutation were on average 15-fold higher in cases compared with controls ($P < 0.001$). Most strikingly, the level of the mutation in the 20 controls who later developed and died of HCC was on average 274-fold higher than controls who did not develop HCC. Thus, within this cohort of HBsAg carriers at high risk of developing HCC, individuals positive for the HBV $1762^{T}/1764^{A}$ mutation at enrollment were substantially more probably to subsequently develop HCC, with a higher concentration of the mutation in plasma enhancing predisposition for cancer development [57].

Collectively, over 50 years of biomedical research have unequivocally established a role for HBV in the etiology of human liver cancer, and with the availability of an effective vaccine, the impact of this virus can be dramatically reduced. It will take many decades for this to occur because of the need to vaccinate children prior to infection that still occurs very early in life. To those individuals who become infected and therefore not eligible for vaccination, therapeutic drugs are being developed constantly. Hopefully, these therapeutic strategies will become both cost-effective and have minimal adverse effects to accelerate the elimination of HBV as a human carcinogen.

1.4 Aflatoxin

As shown earlier, HCC is among the leading causes of cancer death in most parts of the economically developing world with the unequal distribution of this disease depicted in Fig. 1 [4, 58]. Since the burden of HCC is also coincident with regions where aflatoxin exposure is high, many efforts starting over 50 years ago examined this possible association [59]. These initial studies were hindered by the lack of adequate data on aflatoxin intake, excretion and metabolism in people, the underlying susceptibility factors such as diet and viral exposure, as well as the incomplete statistics on worldwide cancer morbidity and mortality. Despite these deficiencies, early investigations did provide data illustrating that increasing HCC rates corresponded to increasing levels of dietary aflatoxin exposure [60]. The commodities most often found to be contaminated by aflatoxin were common human food staples including peanuts, cottonseed, maize, and rice [61]. The requirements for aflatoxin production are relatively nonspecific since molds can produce these toxins on almost any foodstuff and the final levels in the grain product can vary from micrograms to tens of milligrams [62]. Strikingly in a case of aflatoxin-related deaths in rural villages in Kenya, daily exposures were estimated to be over 50 milligrams [63]. Because contamination of foodstuffs is so heterogeneous, the measurement of human exposure to aflatoxin by sampling foodstuffs or by dietary questionnaires

is extremely imprecise [64]. The development of aflatoxin-specific biomarkers based upon its metabolic activation and subsequent binding to nucleophilic sites in DNA and serum albumin has been validated and used to demonstrate a significant role for this dietary contaminate in HCC across many countries [65].

Many published case-control studies have examined the relation of aflatoxin exposure using various biomarkers and HCC. Compared with cohort studies, case-control studies are both cost- and time-effective. Unfortunately, case-control studies are often initiated long after exposure has occurred, and it cannot be assumed that the exposure has not appreciably changed over time. Additionally, such studies involve assumptions in the selection of controls, including that the disease state does not alter metabolism of aflatoxin. Thus, matching of cases and controls in a specific biomarker study is much more difficult than in a case-control study involving genetic markers [59]. Data obtained from cohort studies have the greatest power to determine a true relationship between an exposure and disease outcome because one starts with a healthy cohort, obtains biomarker samples, and then follows the cohort until significant numbers of cases are obtained. A nested study within the cohort can then be designed to match cases and controls. An advantage of this method is causation can be established (due to the longitudinal nature of cohort studies, there is no temporal ambiguity) and selection bias is minimized. A major disadvantage, however, is the time needed in follow-up (often years) to accrue the cases, especially for chronic diseases such as HCC. This disadvantage can be overcome in part by enrolling large numbers of people (often tens of thousands) to ensure case accrual at a reasonable rate.

Essential to the designation by IARC of the aflatoxins as a Group 1 known human carcinogen were two major cohort studies with aflatoxin biomarkers that have demonstrated the important role of this carcinogen in the etiology of HCC [66]. The first study, comprising more than 18,000 men in Shanghai, examined the interaction of HBV and aflatoxin biomarkers as independent and interactive risk factors for HCC. The nested case-control data revealed a statistically significant increase in the adjusted relative risk (RR) of 3.4 [95% CI: 1.1–10.0] for those HCC cases where urinary aflatoxin biomarkers were detected. For HBsAg-positive people, only the RR was 7 [95% CI: 2.2–22.4], but for individuals with both urinary aflatoxins and positive HBsAg status, the RR was 59 [95% CI: 16.6–212.0] [67, 68]. These results strongly support a causal relationship between the presence of the chemical- and viral-specific biomarkers and the risk of HCC.

A subsequent cohort study in Taiwan has substantially confirmed the results from the Shanghai investigation. Wang et al. [69] examined HCC cases and controls nested within a cohort and found that in HBV-infected people, there was an adjusted odds ratio of 2.8 for detectable compared with non-detectable aflatoxin-albumin adducts and 5.5 for high compared with low levels of aflatoxin metabolites in urine. In a follow-up study, there was a dose-response relationship between urinary aflatoxin metabolite levels and risk of HCC in chronic HBV carriers. Similar to the Shanghai study, the HCC risk associated with AFB_1 exposure was more striking among the HBV carriers with detectable aflatoxin-DNA adducts in urine. The use of aflatoxin biomarkers as efficacy endpoints in primary prevention trials in West Africa has been reported [70]. This study assessed postharvest measures to restrict aflatoxin contamination of groundnut crops. Six hundred people were monitored, and in control villages, mean aflatoxin-albumin concentration increased postharvest (from 5.5 pg/mg [95% CI 4.7–6.1] immediately after harvest to 18.7 pg/mg [17.0–20.6] 5 months later). By contrast, mean aflatoxin-albumin concentration in intervention villages after 5 months of groundnut storage was much the same as that immediately postharvest (7.2 pg/mg [6.2–8.4] vs. 8.0 pg/mg [7.0–9.2]). At 5 months, mean adduct concentration in intervention villages was less than 50% of that in control villages (8.0 pg/mg [7.2–9.2] vs. 18.7 pg/mg [17.0–20.6], $p < 0.0001$). Thus, primary prevention maybe an effective means to reduce HCC burden, especially in areas where single foodstuffs such a groundnuts are major components of the diet.

Recent data utilizing the cancer registry in Qidong, China, has provided some very exciting insights into the role of aflatoxin in liver cancer. Utilizing the availability of serum samples collected over a 30-year period, aflatoxin exposure patterns have been documented. In China, major agricultural reforms in the 1980s led to diminished maize consumption, a major source of aflatoxin contamination. The population-based cancer registry in Qidong, China, has documented a more than 50% reduction in HCC mortality rates occurring across birth cohorts from the 1960s to the 1980s for Qidongese less than 35 years of age although all were born before universal vaccination of newborns. Median levels of the aflatoxin biomarker decreased from 19.3 pg/mg albumin in 1989 to undetectable (<0.5 pg/mg) by 2012. A population attributable benefit of 65% for reduced PLC mortality was estimated from a government-facilitated switch of dietary staple from maize to rice; 83% of this benefit was in those infected with HBV. Food policy reforms in China thus resulted in a dramatic decrease in aflatoxin exposure, which, independent of HBV vaccination, reduced liver cancer risk *[71]*. Now that an extensive HBV vaccine coverage is in place, this augurs even greater risk reductions in the future [72].

Biomarker development in HCC has been further advanced by the molecular biological studies on the TP53 tumor suppressor gene, the most common mutated gene detected in human cancer [73, 74]. Many studies of *p53* mutations in HCC occurring in populations exposed to high levels of dietary aflatoxin have found high frequencies of guanine to thymine transversions, with clustering at codon 249 [75, 76]. In contrast, no mutations at codon 249 were found in *p53* in HCC from Japan and other areas where there was little exposure to aflatoxin [77, 78]. The occurrence of this specific mutation has been mechanistically associated with AFB_1 exposure in experimental models including bacteria [79] and through demonstration that aflatoxin-8,9-epoxide could bind to codon 249 of *p53* in a DNA plasmid in vitro [80]. Mutational analysis of the *p53* gene in human HepG2 cells and hepatocytes exposed to AFB_1 found preferential induction of the transversion of guanine to thymine in the third position of codon 249 [81] [82, 83]. In summary, studies of the prevalence of codon 249 mutations in HCC cases from patients in areas of high or low exposure to aflatoxin suggest that a G-T transition at the third base is associated with aflatoxin exposure, and in vitro and mutagenesis data would seem to support this hypothesis [84].

The remarkable increase in the throughput and cost-effectiveness of DNA sequencing has propelled the analysis of mutations in DNA from tumors at every organ site. When these data are combined with new bioinformatics tools, there has been a revolution in the determination of mutational signatures found in human cancer [85, 86]. Data from a variety of studies have explored these mutational signatures in liver cancer and has revealed a number of insights that are consistent with the etiologic factors that have been identified from epidemiologic studies [87]. Experimental models have also demonstrated a consistency between aflatoxin exposure and a unique set of mutational signatures and liver tumors [88, 89]. With the further development of these technologies, the opportunity for using this mutational signature data for the early detection of liver cancer is an evident opportunity in the near future.

1.5 Hepatitis C Virus

Hepatitis C virus (HCV) is a positive strand RNA virus, and its linkage to liver cancer has been extensively reviewed [90]. People who are chronically infected by HCV represent the high-risk group for its carcinogenic impact. There are upward of 75 million people worldwide who are infected with HCV, and this is primarily due to blood-borne transmission especially among intravenous drug users or in medical situations where insufficient sterilization and handling of needles have occurred. For HCV, the detection in blood of the HCV nucleic acid became an essential biomarker for risk reduction and prevention [91]. From a historic perspective, an HCV-specific test only became available in the early 1990s, and prior to this technology, the attributable etiologic

factor was designated as non-A non-B hepatitis, especially in hepatic cancers diagnosed in Japan [92, 93]. Over the past 25 years, an appreciation for the role of the hepatitis C virus (HCV) has also emerged, and this is undoubtedly contributing to HCC being the most rapidly rising solid tumor in the USA and Japan [94]. Detailed knowledge of the etiology of HCC has spurred many mechanistic studies to understand the pathogenesis of this nearly always-fatal disease [95–97].

As described above, Egypt, the country with the largest population in the Middle East, suffers from the second highest level of liver cancer mortality in the world. Much of this devastating impact is attributable to the extraordinarily high level, in some regions of the country approaching 30% of the population, who have become chronically infected with HCV [98]. This unfortunate circumstance is in large part due to an intervention program that was developed to treat schistosomiasis, an endemic disease in many parts of Egypt. The treatment protocol involved injection of a therapeutic agent for the eradication of the schistosome, and unfortunately many of these injections were done using non-disposable needles and syringes. Since the tests for HCV had yet to be developed during the time of these therapeutic interventions, a large fraction of treated patients unfortunately became infected with the HCV virus. Now decades later, the manifest outcome of chronic HCV infection has led to a dramatic increase in liver cancer incidence and mortality. Hopefully, with the development and certification of new therapeutic drugs that can cure many HCV infections, the impact of this iatrogenic disaster can be blunted in the future.

Similar to the successes that have been attained using high activity antiretroviral therapy toward the HIV virus, basic science research has discovered a number of strategies for impacting HCV infections through the inhibition of its replication process. Over the past decade, this is led to a dramatic increase in the cure of individuals chronically infected with HCV. Many clinical trials and studies spanning different populations have demonstrated the efficacy of these direct-acting antiviral (DAA) agents toward HCV [99]. A challenge to the public health community will be finding and applying the resources necessary for the tens of millions of infected individuals often living in low-resource countries. With these, curative therapies costing thousands of US dollars and HIV implementation model that has been successful in Africa will need to be implemented. Fortunately, the successful development and deployment of some highly effective new drugs that cure HCV infection is a major advance and will hopefully diminish the role of this virus in liver cancer [100, 101].

1.6 Alcohol

Alcohol as a liver carcinogen required no specific biomarker since the prevalence of high alcohol consumption and alcoholic liver disease was easily diagnosed clinically during the early to mid-twentieth century [102, 103]. Alcohol is a recognized human carcinogen and has been causally linked to HCC [102]. Alcoholic cirrhosis and heavy alcohol use have been repeatedly associated with an increase in HCC risk [104]. However, it is unclear if alcohol use in the absence of cirrhosis influences HCC development [105]. Several studies have demonstrated an increased risk of HCC up to fivefold with consumption of more than 80 g of alcohol per day or approximately six to seven drinks per day [104]. The risk of HCC ranges from borderline significant to doubled with chronic alcohol consumption of less than 80 g/day [104]. A synergism between alcohol and HBV and HCV infections has also been described [104, 106, 107]. One issue of importance given the growing association of liver cancer increases with obesity is the role that alcohol, as a basic carbohydrate, plays as a contributor to caloric intake. Further, the impact of alcohol as a carcinogen is likely due to one of its reactive metabolites, acetaldehyde [108].

1.7 Vinyl Chloride

A number of additional environmental exposures have been epidemiologically associated with HCC [66, 109]. Vinyl chloride exposure in occu-

pational settings has been associated with the onset of HCC in workers, and there are the classic studies associating vinyl chloride exposure with angiosarcomas in the liver [110–112]. While vinyl chloride was characterized as a known human carcinogen decades ago, there were still worker exposures that led to this disease being diagnosed today [113]. Studies have reported a multiplicative interaction between vinyl chloride exposure in the workplace and alcohol consumption in the enhancement of HCC [114]. Finally, a synergistic interaction between vinyl chloride workplace exposure and HBV status has been reported in a cohort in Taiwan [115]. Significantly, worker exposure to vinyl chloride has been mitigated through the changing of manufacturing processes and the implementation of new safety protocols in the workplace.

1.8 Cigarette Smoke

Cigarette smoke has been associated and causally linked to many human cancer sites, and there has been a consistent number of studies that have found an association with liver cancer [116]. There are, however, been a number of studies that have not found an association between for tobacco and HCC risk [117]. Nonetheless, meta-analyses of tobacco exposure and cancer risk consistently show a risk for liver cancer and smoking [118, 119]. This has been recently reviewed by IARC [107] and more recently reviewed [120]. Collectively, these findings may reflect that active tobacco users have a compendium of other liver cancer risk factors that underlie these statistically significant results.

1.9 Estrogen-Progestogen

The role of sex steroid hormones in the development of HCC remains obscure; however, in some early studies, an increase risk of HCC was observed among users of the first and second generation of oral contraceptives [41, 121, 122]. Collectively, these hormonal-related increases in HCC were only observed in low-incident countries where exposures to the other major risk factors for this cancer were rare. This epidemiology has been reviewed by IARC in its compendium of monographs on human cancer risk [123].

1.10 Exposures to Radioactive Substances: Plutonium, Thorium-232, and Thorotrast

There have been a number of reports of both occupational and iatrogenic exposures to specific forms of radiation causing liver cancers. This has been observed among workers exposed to alpha-emitters such as plutonium [86]. In these circumstances, the ongoing exposure assessments required for these workers helped to define the dose of radiation, and ongoing health assessments facilitated disease diagnoses. Similar to this exposure and dose situation, the alpha-emitter Thorium-232 that historically was incorporated into an imaging product for the liver was subsequently shown to induce hepatocellular carcinoma in experimental models and in patients [124, 125]. These studies illustrate that high-quality exposure and health assessment can identify these etiologies, and in turn this accelerates preventive interventions.

1.11 Liver Fluke (Opisthorchis viverrini)

There have been a number of liver cancers that are uniquely diagnosed in specific regions of the world. For example, liver fluke has been associated with rare forms of cholangiocarcinoma and other liver cancers. This organism has been determined to be a carcinogen by the IARC evaluations and has been recently reviewed [126–128]. The specific mechanism of action underpinning how liver fluke causes these cancers remains to be elucidated especially since experimental models for this disease process are still needed. There are clear prevention strategies and therapeutic interventions that can be done to eradicate this exposure situation; however, in endemic regions in northeast Thailand, the cultural practices have made these interventions challenging.

2 Changing and Emergent Etiology of HCC

Both experimental models and human investigations have demonstrated a gradation in the increasingly severe pathologies during the etiopathogenesis of liver cancer. The normal functioning liver transits through several distinct pathologies over the course of many years prior to the manifestation of hepatic cancer. These processes include nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), mild fibrosis, and cirrhosis [22, 23, 129]. Each stage of this progressive process can lead to more severe dysfunction such that at the time of the liver cancer diagnosis, upward of 95% of the liver has been functionally destroyed. Even with liver transplantation, patients having advanced liver disease fare poorly. Despite intensive research and development strategies over the last 20 years, a person diagnosed with liver cancer today has less than a 20% 5-year survival. This is among the poorest survival rates for any prominent cancer. It is hoped that the development of early detection strategies could identify people at stages of disease where interventions can be effective.

In addition to the association of HBV, HCV, alcohol, and other toxicants with HCC, in economically developed countries, the dramatic rise in overweight and nonalcoholic fatty liver disease has also been systematically related to increased HCC [22, 130–132]. Of major concern for the future is the role that obesity, diabetes, and general underlying fatty liver disease will play in the development of liver cancer [18, 133, 134]. The problem of obesity is especially of concern for countries through the Middle East since the population for many of these countries is currently young and the increase in obesity and related diseases is increasing dramatically [129, 135]. While the historic risk factors for liver cancer described above are addressed through a spectrum of prevention methods, these new etiologic factors portend an increasing trajectory in the incidence of this disease. Both therapeutic and pre-disease interventions will need to be deployed now to blunt the impact of these risk factors in the decades to come.

The identification of these emergent risk factors and their interactions will likely have to be determined from prospective cohort studies, where each person is effectively their own control. These studies are a powerful resource for the identification and validation of biomarkers having utility in chronic disease outcomes. Many of the success stories in cancer prevention and control emanate from data collected in these prospective cohort investigations. Unfortunately, in the case of chronic disease outcome such as cancer, individuals enrolled in these studies must be followed for decades, and this leads to a slow rate of discovery. Further, a sufficient number of individuals need to be enrolled in the investigations given the relatively low annual incident rates for even the most common cancers. Thus, if today we were to initiate a prospective cohort for liver cancer outcomes, given the current age-adjusted incidence rate of 10 per 100,000, a study encompassing tens of thousands of people followed for many years would be required. Fortunately, there are biologically interlocking prospective cohorts in place now that have samples and outcomes for the discovery and validation of biomarkers. It is highly likely that the emergent risk factors for liver cancer will be modifiable and intervenable.

2.1 Liquid Biopsy Opportunities

The development and validation of biomarkers for early detection of disease or for the identification of high-risk individuals is a major translational effort in cancer research. In the current era, many of the technologies being developed for measuring in blood are DNA fragments with mutations, epigenetic marks in DNA, whole shed cells, micro-RNAs, and proteins; all of these measurements are encompassed by the liquid biopsy umbrella [136]. Indeed, when these marker measurements are combined with medical imaging, occult tumors may be detected and decisions about benign or malignant lesions can be assessed [137]. Since the liver produces a substantial proportion of blood proteins and is highly vascularized, these liquid biopsy methods have been and will continue to be important

epidemiologic and clinical tools. For example, over the past several decades, α-fetoprotein was widely used as a HCC diagnostic marker in high-risk areas because of its ease of use and low cost [138]. Unfortunately, this marker suffers from low specificity due to its occurrence in diseases other than liver cancer. Moreover, no survival advantage is seen in populations when α-fetoprotein is used in large-scale screening [139]. Nonetheless as a proof of principle, these inadequacies have contributed to the need to identify other molecular biomarkers that are possibly more mechanistically associated with HCC development, including hypermethylation of the p16 gene, p15 gene, GSTP1 promoter regions, and codon 249 mutations in the p53 gene [140–143]. Results from investigations of p16, p15, GSTP1 promoter hypermethylation, and p53 mutations indicate that these markers are prevalent in HCC, but there is as of yet limited information on the temporality of these genetic changes prior to clinical diagnosis. Recent studies using combined DNA mutation detection, epigenetic marks, and specific plasma proteins may portend the development of a new liquid biopsy strategy for early detection of liver cancer [144].

Optimism for the liquid biopsy strategy in liver cancer has emerged from several studies demonstrating that DNA isolated from serum and plasma of cancer patients contains the same genetic aberrations as DNA isolated from an individual's tumor [145–147]. The process by which tumor DNA is released into circulating blood is unclear but may result from accelerated necrosis, apoptosis, or other processes [148]. While the detection of specific p53 mutations in liver tumors has provided insight into the etiology of certain liver cancers, the application of these specific mutations to the early detection of cancer offers great promise for prevention [149]. In a seminal report, Kirk et al. [150] reported the detection of codon 249 p53 mutations in the plasma of liver tumor patients from the Gambia; however, the mutational status of the tumors was not known. These authors also reported a small number of cirrhosis patients having this mutation, and the strong relation between cirrhosis and future development of HCC raised the possibility of this mutation being an early detection marker. Jackson et al. [146] used short oligonucleotide mass analysis (SOMA), in lieu of DNA sequencing, for analysis of specific p53 mutations in HCC samples and found concordance between tumor and shed DNA mutations in plasma.

The temporality of the detection of this mutation in plasma before and after the clinical diagnosis of HCC was facilitated by the availability of longitudinally collected plasma samples from a cohort of high-risk individuals in Qidong, PRC, that have been followed since 1992 [151]. The results showed that in samples collected prior to liver cancer diagnosis, a quarter of the plasma samples had detectable levels of the codon 249 mutation. The persistence of this pre-diagnosis marker was borderline statistically significant. The codon 249 mutation in p53 was detected in nearly half of all plasma samples following the diagnosis of HCC. Collectively these data suggest that nearly one-half of the potential patients with this marker can be detected at least 1 year and in one case 5 years prior to diagnosis.

In a pioneering effort using a novel internal standard plasmid, plasma concentrations of p53 codon 249-mutated DNA were quantified by SOMA in 89 hepatocellular carcinoma cases, 42 cirrhotic patients, and 131 non-liver disease control subjects, all from highly aflatoxin-exposed regions of the Gambia [152]. The hepatocellular carcinoma cases had higher median plasma concentrations of the p53 mutation (2800 copies/mL; interquartile range: 500–11,000) compared with either cirrhotic (500 copies/mL; interquartile range: 500–2600) or control subjects (500 copies/mL; interquartile range: 500–2000). Levels of >10,000 copies of p 53 codon 249 mutation/mL plasma were also significantly associated with the diagnosis of HCC (odds ratio, 15; 95% confidence interval, 1.6–140) when compared with cirrhotic patients. Potential applications for the quantification of this alteration of DNA in plasma include estimation of long-term, cumulative aflatoxin exposure and selection of appropriate high-risk individuals for targeted intervention.

3 Summary

All liver cancers emerge from a slowly developing process involving progressive genetic insults and their resulting genomic changes. This process often takes decades to manifest which affords many windows of opportunity for prevention. The advances in modern DNA sequencing technologies have been used on a wide number of human liver cancers with a range of etiological factors that reveal a very complete picture of driver and passenger mutational changes in these tumors [87, 153, 154]. These data will hopefully form a foundation along with other molecular biomarkers for new therapies and early detection and screening methods. Further, as these sequencing methods become extended to characterize microRNAs and proteomic methods help characterize the molecular phenotype of liver cancers, these collective data will help define the preclinical period of tumor development. This will be very valuable for our mechanistic understanding of HCC during the up to 30 years after chronic infection with HBV, HCV, and/or aflatoxin and other exposures prior to clinical diagnosis. These studies may also reveal insights into chronic hepatitis and cirrhosis since 70–75% of all HCC is accompanied by cirrhosis [44, 155].

The molecular epidemiology investigations of aflatoxin, HBV, HCV, and liver cancer probably represent one of the most extensive data sets in the field of environmental carcinogenesis, and this work serves as a template for future studies of the role of other environmental agents in human diseases with chronic, multifactorial etiologies. The development of these biomarkers has been based upon the knowledge of the biochemistry and toxicology of aflatoxins gleaned from both experimental and human studies. These biomarkers have subsequently been utilized in experimental models to provide data on the modulation of these markers under different situations of disease risk. This systematic approach provides encouragement for design and successful implementation of preventive interventions. As the emergence of the new etiologic factors such as obesity and type 2 diabetes becomes more prevalent, particularly in the currently younger populations across the Middle East, the need for early detection and prevention will be critical to blunt this emerging epidemic.

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Hepatocarcinogenesis Induced by Environmental Exposures in the Middle East

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1 Introduction

The most severe complication of a chronic liver disease and the most common form of primary liver cancer is hepatocellular carcinoma (HCC). More than 800,000 people are diagnosed with HCC each year in the world [1]. The general 5-year survival rate is 18%, compared to just 3% 40 years ago. However, if patients were diagnosed at an early stage, the 5-year survival rate is 33%. HCC accounts for approximately 700,000 deaths per year [1–4]. As most HCC cells show the biochemical and morphological features of normal hepatocytes, it is usually predicted that HCC occurs due to the malignant transformation of normal hepatocytes. However, a "stem cell" origin is also suggested by different researchers [5, 6].

HCC has etiologically a very complicated and multifactorial pathology. It has also been associated with exposure to chemical, physical, and biological carcinogens. HCC is also caused by different conditions, including chronic hepatitis and cirrhosis [5, 7]. Like many other solid tumors, several histological and functional changes as well as alterations in different biochemical pathways may lead to the activation of protooncogenes and downregulation of tumor suppressor genes in HCC [8]. Mutations of p53 gene and Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) gene and integration of hepatitis B virus (HBV) DNA into the host genome are the well-known genetic alterations that can cause HCC in humans [9–12].

HCC is more prevalent among men compared to women. In men, 7.5% of all cancers are primary liver cancers, whereas 3.5% of all cancers among women are of liver origin. The gender-specific age-adjusted incident rate (AAIR) ratio ranges from 1.3 to 3.6 worldwide [2, 3, 13, 14]. There were an estimated 383,593 deaths among men and 164,961 deaths among women attributable to HCC for the year 2000, which is quite close to the HCC incidence during the same period [14]. However, the cases and deaths kept increasing through the years. Number of new cases in 2018 was 841,040, corresponding to 4.7% of all cancers. Number of deaths in the same year due to liver cancer was 781,631, which corresponds to 8.2% of the mortality caused by cancer. Age-standardized incidence and age-standardized mortality rate (ASMR) for liver cancer were 13.9 per 100,000 for men, 4.9 per 100,000 for women, and 8.5 per 100,000 individuals in 2018, respectively [15]. The most HCC encountered countries around the world are Mongolia, Laos, Vietnam, Egypt, and Gambia [16].

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2 Epidemiology of HCC in the Middle East

HCC is the third leading cause of cancer-related deaths, and >80% of HCC results in mortality in developing countries. On the other hand, >80% of the new HCC cases are from developing countries. Most of the new cases are suggested to be caused by an underlying viral pathology, particularly HBV or hepatitis C (HCV) [17].

In Middle East countries, liver cancer is a major concern among men, particularly in certain countries like Egypt and Saudi Arabia. Recent reports demonstrate that the incidence of HCC has increased sharply in the last 15–20 years, with a very high incidence in Egypt [18–21].

Egypt has the highest rate of HCC among other Middle East countries (27.37 per 100,000), ranking as the fourth country in the world. In men, the death rate of HCC is 41.94 per 100,000 while in women, it is 14.63. Qatar has the second highest death rate in the Middle East (8.80 per 100,000), ranking as the 45th country. Kuwait, on the other hand, has a death rate of 8.19 per 100,000, ranking as the 49th country [22]. Saudi Arabia is the 100th country around the world, with a rate of 5.5 per 100,000 [17]. HCV is suggested to have a major role in the epidemiology of HCC in Saudi Arabia. A study conducted in Saudi Arabia demonstrated that 23% of chronic liver disease patients carried HBV and 53% of HCV patients had chronic liver disease [23, 24]. In Iran, the prevalence of HCC was suggested to be ~3.1% in total HBV and HCV chronic carriers. The estimated annual incidence of HCC is 1.02 [95% confidence interval (CI): 0.5–1.8] [25]. HCC is a relatively rare cancer in Lebanon. It ranks as the 14th most common cancer among both males and females (with an age-standardized rate of 3.5 and 2.2 per 100.000, respectively) [26]. Results of studies conducted in Lebanon showed that most HCC patients had HBV-related liver cirrhosis, accounting for nearly two-thirds of the subjects followed by HCV and alcohol abuse [27]. In Jordan, HCC was associated with hepatitis D (HDV) as the prevalence of HDV was 23% in chronic liver disease patients and 67% in HCC patients. In contrast, none of HCC patients in Iran were positive for HDV infection [28].

In Turkey, the cancer incidence has been increasing over the years, and HBV infection was found to be the leading cause of HCC, followed by HCV infection and alcoholic liver disease [29]. HCC is the 11th most common type of cancer in males. It annually affects 2.1 per 100,000 males, while in females it is the 15th most common type of cancer, affecting 1.3 per 100,000 subjects. The rough rate of liver carcinoma in the Mediterranean region for males and females was 2.5 per 100,000 and 2.1 per 100,000, respectively [30]. HBV, HCV, and excessive alcohol intake were detected in 56%, 23.2%, and 15.9% of Turkish HCC patients, respectively [29]. However, in general, HCC is less prevalent in the Middle East compared to developed countries. This may be due to low consumption of alcoholic beverages in Middle East, possibly due to religious beliefs [30–32].

These data suggest that HCC is an important global public health problem. In spite of vaccination programs against HBV in many countries, the incidence and mortality from HCC is increasing, particularly in Western countries where the prevalence of HBV is low. Therefore, other than infections like HBV and HBC, environmental risk factors are contributing to the high incidence of HCC in many parts of the world, including the Middle East.

3 What Causes Hepatocarcinogenesis?

HCC is one of the few cancers with clearly defined major risk factors [26, 33–37]:

- Chronic viral hepatitis (HBV and HCV).
- Cirrhosis.
- Nonalcoholic fatty liver disease (NAFLD).
- Advanced hepatic fibrosis.
- Familial tendency to HCC.
- Certain inherited liver diseases (iron overload due to hemochromatosis and copper overload due to Wilson's disease).
- Rare diseases (tyrosinemia, α1-antitrypsin deficiency, porphyria cutanea tarda, glycogen storage diseases).
- Diabetes.

- Obesity.
- Environmental chemicals.

The population attributable risk estimates for liver cancer for each of these risk factors vary among countries. However, chronic infections with HBV and HCV are the most important reasons for HCC development on a global scale, together accounting for over 80% of liver cancer cases worldwide [38].

4 Environmental Hepatocarcinogenesis

Environmental carcinogens are chemical, physical, or biological agents that may lead to carcinogenesis. They may be present in the food, air, or water. The majority of human cancers result from exposure to different environmental carcinogens [39].

The mechanism of action of some of these agents is widely known, but the carcinogenic pathways or mechanisms of some of the agents present in our environment have yet to be fully defined. Toxicologists around the globe believe that current measures to mitigate our exposures are inadequate [39].

International Agency for Research on Cancer (IARC) classifies human carcinogens as [40]:

1. Group 1: carcinogenic to humans.
2. Group 2A: probably carcinogenic to humans.
3. Group 2B: possibly carcinogenic to humans.
4. Group 3: not classifiable as to its carcinogenicity to humans.
5. Group 4: probably not carcinogenic to humans.

An agent is classified by IARC based on scientific evidence derived from human and experimental animal studies and from mechanistic and other relevant data [41, 42].

Environmental hepatocarcinogenesis is an important issue that should be taken seriously by all of the countries, particularly by developing countries like countries of Middle East region. Agents that are known or suspected to cause liver cancer are given in Table 1.

Table 1 Chemical, physical, and biological agents known/suspected to induce HCC in humans in the Middle East and their IARC classifications [45]

Clear evidence for HCC	IARC classification
Hepatitis B virus	Group 1: carcinogenic to humans
Hepatitis C virus	Group 1: carcinogenic to humans
Alcohol consumption	Group 1: carcinogenic to humans
Aflatoxin ingestion	Group 1: carcinogenic to humans
Tobacco smoke	Group 1: carcinogenic to humans
Oral contraceptives	Group 1: carcinogenic to humans
Thorium dioxide, thorium-232	Group 1: carcinogenic to humans
Plutonium	Group 1: carcinogenic to humans
Vinyl chloride	Group 1: carcinogenic to humans
Limited data for HCC	
Anabolic steroids	Group 2A: probably carcinogenic to humans
Arsenic and inorganic arsenic compounds	Group 1: carcinogenic to humans
Betel quid with tobacco	Group 1: carcinogenic to humans
Polychlorinated biphenyls	Group 2A: probably carcinogenic to humans
Trichloroethylene	Group 1: carcinogenic to humans
X- and γ-radiation	Group 1: carcinogenic to humans

HCC hepatocellular carcinoma, *IARC* International Agency for Research on Cancer

5 Environmental Hepatocarcinogenesis in the Middle East

HCC in the Middle is rising due to high exposure to:

- Aflatoxins.
- Alcohol consumption.
- Smoking and exposure to cigarette smoke.
- Heavy metals, particularly arsenic.
- Vinyl chloride.
- Organic solvents.
- Dioxins.

5.1 Aflatoxins

Aflatoxins were first discovered in the 1960s. Although aflatoxins have a low profile in the general population, they are well known by many scientists working in different branches of science. Aflatoxins are secondary metabolites of different *Aspergillus* species (e.g., *Aspergillus flavus*, *Aspergillus parasitius*, *Aspergillus nomius*). They mainly contaminate food and can appear in food chain because of fungal infection of crops. Contamination with different kinds of mycotoxin-producing fungi is both inevitable and unpreventable as these toxins contaminate food during growth, harvest, and/or storage. Several foodstuff (maize, wheat, barley, groundnuts, peanuts, spices, nuts, teas, coffees, milk, beef, and chicken) may contain high levels of different mycotoxins. The most favorable conditions for the production of mycotoxins are high humidity and high temperature [43–48]. The factors affecting aflatoxin production are given in Fig. 1. Human exposure to mycotoxins can be via three different ways: oral, dermal, and inhalation [49, 50]. Concentrations of AFs in final products can vary from <1 μg/kg (1 ppb) to >12.000 μg/kg (12 ppm) [51].

Aflatoxins can be categorized into four major types: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). B forms give blue florescence, while G forms give green florescence in the presence of ultraviolet light. *Aspergillus flavus* produces AFB1 and AFB2, while two other species produce AFG1 and AFG2 [13]. On the other hand, aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) are AFB1 and AFB2 that are metabolites in animal's milk [45, 52–54]. These toxins have both acute and chronic effects, ranging from aflatoxicosis to cancer. Aflatoxins have been shown to be potent carcinogens, mutagens, and teratogens [55–57].

5.1.1 Aflatoxin B1

The International Agency for Research on Cancer classifies AFB1 as "carcinogenic to humans (Group I)." AFB1 is a procarcinogen [45]. In humans, cytochrome P450 enzymes (CYP450s) are responsible for the biotransformation of AFB1 [58, 59]. This particular aflatoxin is first biotransformed to aflatoxin-8,9-exo-epoxide and aflatoxin-8,9-endo-epoxide by CYP1A2 and CYP3A4 [16, 17]. Aflatoxin-8,9-exo-epoxide binds to DNA, and it causes the formation of pre-

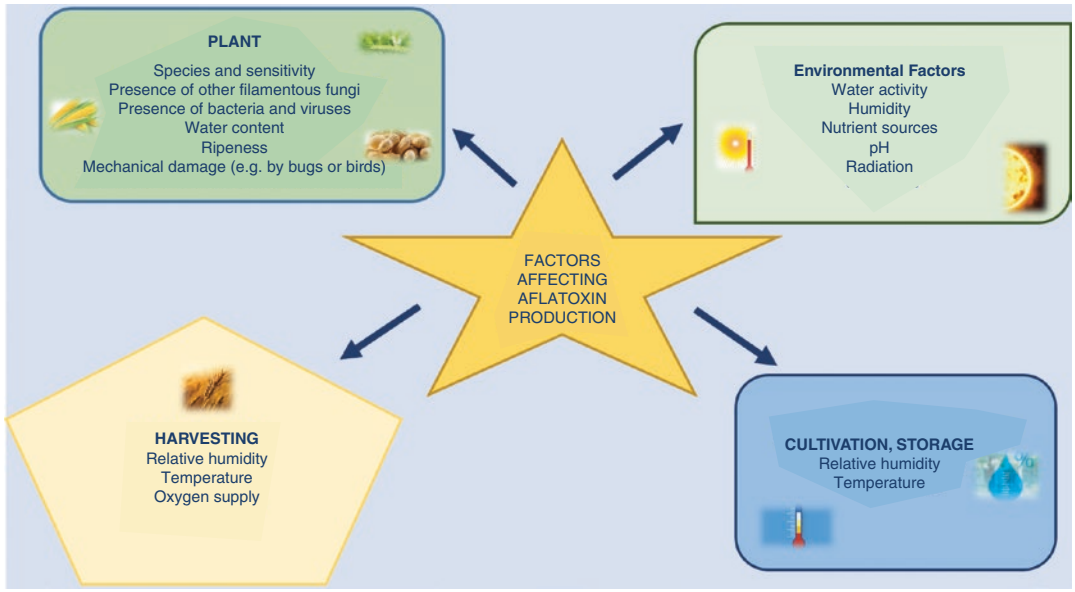

Fig. 1 Factors affecting aflatoxin production

dominant 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy AFB1 adduct. Its pseudo-half-life is short. Later, a stable imidazole ring-opened AFB1-formamidopyrimidine adduct is formed. This adduct can accumulate in the body for several days [60]. Animal experiments showed that formamidopyrimidine adduct could remain detectable for several weeks in rat liver [61, 62]. Individually or collectively, both of these adducts are genotoxic. They can both cause DNA single strand breaks (SSBs) and to a lesser amount of double strand breaks (DSBs). A weak and delayed accumulation of phosphorylated H2A histone family member X (phospho-H2AX) caused by AFB1 metabolites strongly suggests that AFB1-induced DNA damage triggers the activation of ataxia telangiectasia mutated serine/threonine kinase (ATM) [63–65]. The epoxides can also interact with DNA and form a promutagenic and unstable aflatoxin-N^7-guanine adduct [66]. It usually undergoes depurination and is excreted in the urine. However, it can react with serum albumin to form long-lived lysine adducts [67]. Epidemiological studies on populations consuming contaminated diets revealed that both rodents and humans form the same AFB1 metabolites as experimental animals. Subsequent dose-response studies as well as case studies performed on small groups of subjects in India, China, Malaysia, the Gambia, and Kenya evaluated both dietary AF intake and levels of urinary AF biomarkers [68–74]. AF-albumin adducts or AF-lysine adducts were determined in most of the studies [75–78].

The DNA damage caused by AFB1 metabolites is mainly repaired by nucleotide excision repair (NER) in mammalian cells [63, 79]. Other than DNA adducts, they also cause hepatic DNA base damage, particularly 8-hydroxydeoxyguanosine (8-OHdG) lesions in rats [79–81].

The mechanisms underlying the DNA damage checkpoint responses to AFB1 are poorly understood. It was suggested that mammalians are unable to give proper checkpoint responses to genotoxic and mutagenic doses of AFB1. The ATM response to AFB1 exposure is not accompanied by the phosphorylation of three key proteins [Checkpoint kinase 1 (Chk1), Checkpoint kinase 2 (Chk2) or p53], which are involved in the DNA damage checkpoint response [63–65]. The lack of Chk1 phosphorylation indicates that the ataxia telangiectasia and Rad3-related protein (ATR)/Chk1 pathway response is not activated in response to AFB1 metabolites [66]. The lack of Chk1 phosphorylation indicates that the ataxia telangiectasia and Rad3 related protein (ATR)/Chk1 pathway response is not activated in response to AFB1 metabolites [66]. Experiments performed in different cell lines show that although DNA adducts are formed and DNA breaks accumulate, the activation of the tumor suppressor gene p53 fails and cell cycle arrest and apoptotic mechanisms are not activated [63].

Phase I metabolites of AFB1 are detoxified by conjugation with glutathione (GSH), a reaction catalyzed by glutathione S-transferases (GSTs) [48]. The GST activity in humans is lower than rodents, and it suggests that humans are less capable of detoxifying aflatoxin-8,9-epoxides. The biotransformation of AFB1 is given in Fig. 2.

The principal target organ of AFB1 is the liver. It is suggested that AFB1 causes 5–28% of HCCs, particularly in developing countries. This particular aflatoxin leads to hotspot mutation of p53 gene at third base of codon 249. This mutation is caused by G… > T transversion, which in turn causes inactivation of p53 [63, 82, 83]. The detection of p53 mutation is used as a biomarker of both AFB1 exposure and HCC in humans [39, 40]. Literature also shows that AFB1 exposure can lead to both G… > T transversion and A… > T transition in the adjacent codons. In addition, it is also suggested that exposure to AFB1 can alter the activation of p53 in CYP450-expressing human lung cell lines [41]. The different effects of wild-type p53 and mutant p53 are given in Fig. 3.

Other than the alterations in nuclear DNA, AFB1 also attacks mitochondrial DNA, and this effect is suggested to be another underlying factor for the development of HCC, although the impact of mitochondrial gene alterations in the carcinogenic process is not clear yet [84, 85]. Moreover, AFB1 affects oxidative phosphorylation leading to ultrastructural alterations of mito-

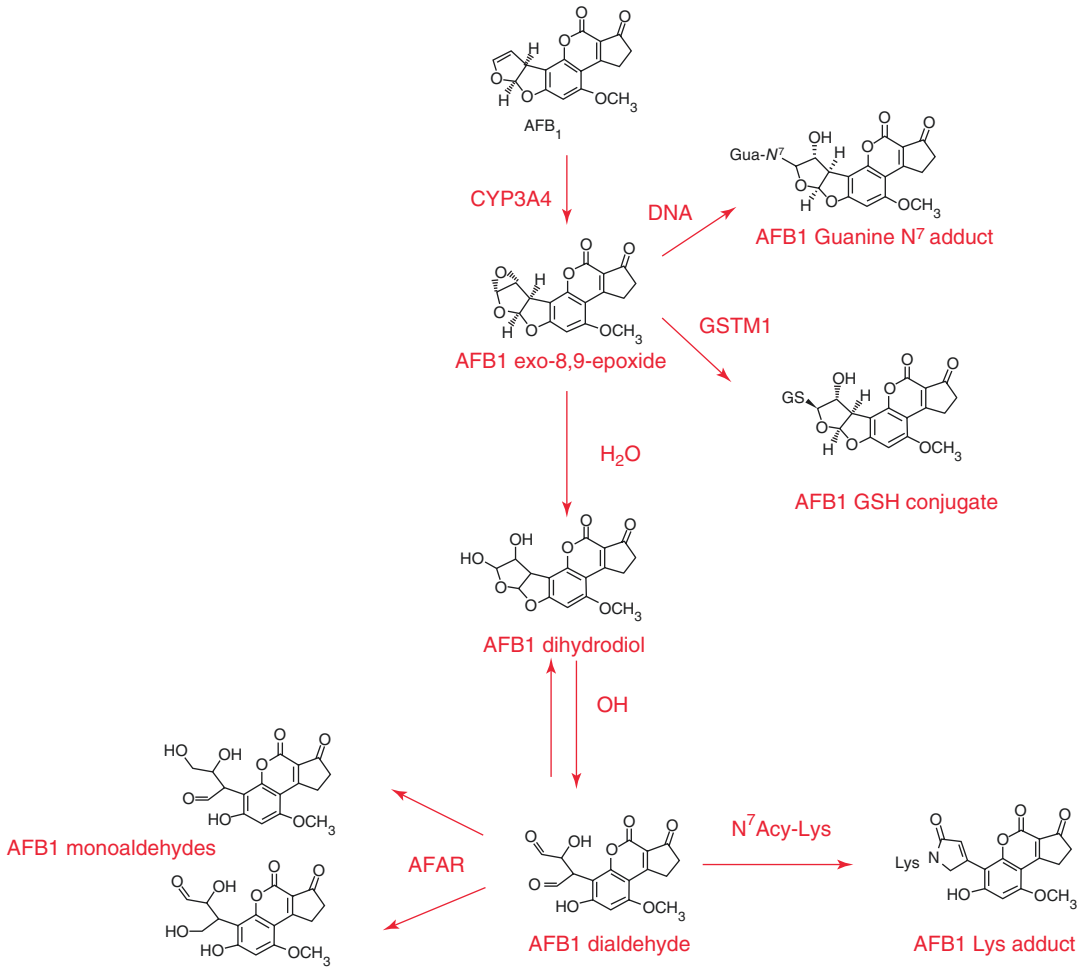

Fig. 2 The biotransformation of AFB1. AFAR aflatoxin-aldehyde reductase, AFB1 aflatoxin B1, CYP3A4 cytochrome P450 3A4, GSTM1 glutathione S-transferase M1, Lys lysine

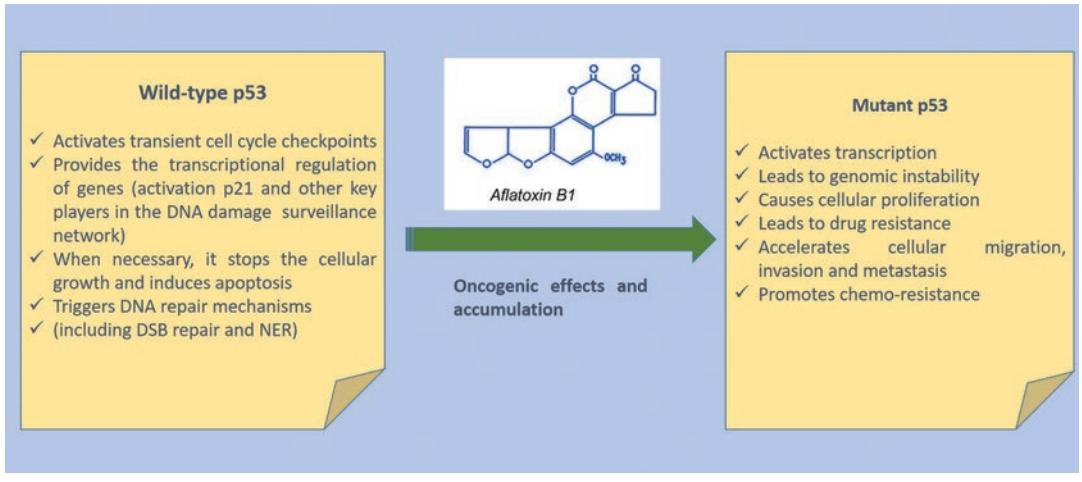

Fig. 3 The different effects of wild-type p53 and mutant p53. DSB double strand break, NER nucleotide excision repair

chondria [43–45]. AFB1 and other aflatoxins can also lead to mitochondria-directed apoptosis [48]. Although humans are prone to the mitochondrial effects of AFB1, rodents are suggested to be more resistant. For example, mouse liver is protected because of the impermeability of mitochondrial membrane to the toxin. In hamsters, it is a more complex process including both a permeability barrier and a possible scavenging system [47].

In humans, other than DNA adducts, antibodies to AFB1 are used as indicators of exposure [86]. Studies also suggest that aflatoxins lead to higher levels of tumor necrosis factor alpha (TNF-α) production and serum lactate dehydrogenase activity [50]. Moreover, AFB1 exposure leads to a Reye-like syndrome, with symptoms like rhinorrhea, vomiting, sore throat, fever, respiratory disturbances like coughing, earache, convulsions, changes in muscle tonus and reflexes, abnormal mitochondrial structure and increases in mitochondrial enzymes (glutamic-pyruvic transaminase and glutamicoxalacetic acid transaminase), structural alterations in the renal cortex, and liver enlargement [87–89].

- AFB1 is also suggested to cause suppression of the cell-mediated immune responses. AFB1 has been shown to induce thymic aplasia, reduce T-lymphocyte function and number, suppress phagocytic activity, and reduce complement activity [90–92].

6 AFB1-Related HCC in Middle East

As indicated above, environmental conditions such as temperature, humidity, and sunlight can favor the survival of mycotoxigenic fungi. The weather conditions in Middle East provide a good survival environment to *Aspergillus flavus* [93]. In the high-incidence countries of Asia and Africa, chronic HBV infection and AFB1 exposure are the major risk factors for HCC. Exceptionally, in Japan and Egypt, the most common risk factor for HCC is HCV infection [94]. However, other than HCV, the contamination rate of food in Egypt is also very high, and therefore, aflatoxin exposure can be suggested as one of the underlying factors of the high prevalence of HCC in this country [93, 94].

Several studies have been performed on the aflatoxin content of different foodstuff in Egypt. Some of these studies observed high amounts of aflatoxin presence while some others did not. Agricultural products were investigated for the aflatoxin content by different researchers. For instance, 90% of hazelnuts (25.0–175.0 ppb), 82% of watermelon and peanut seeds, 35% of soybean (5.0–35.0 ppb), 40% of spices (>0.250 ppb), and 75% of walnuts (15.0–25.0 ppb) were found be positive for aflatoxin contamination [95–97]. A study conducted on 28 samples of dried date palm fruits collected from different shops distributed in Asyut Governorate, Upper Egypt, in 2016 showed that only 1 sample was contaminated with AFB1 (14.4 μg/kg) and AFB2 (2.44 μg/kg) [98]. Another study investigated the levels of AFB1 in corn, wheat, peanut, lupine "termis," white rice, cowpea "lobiya," fava bean, and brown rice. The results showed that AFB1 was present in 64.7%, 53%, 53%, 47%, 47%, 41%, 29.4%, and 29.4% of the samples, respectively [99].

A study on corn samples from Egypt showed that 48.1% of the samples were contaminated with different aflatoxins and the concentration order was AFG1 > AFG2 > AFB1 > AFB2 [100]. A study on maize and rice samples from Egypt showed that total aflatoxin levels detected were 9.75 μg/kg in maize and 5.15 μg/kg in rice [101]. Another study performed on coffee samples from Egypt showed that decaffeinated green coffee beans contained 24.29 μg/kg and roasted coffee beans contained 16.00 μg/kg of total aflatoxin [102]. A study conducted to investigate the natural co-occurrence of multiple toxic fungal and bacterial metabolites in sugarcane grass and juice showed that AFB1 was present in 48% of grass samples and in 58% of juice with a maximum concentration of 30.6 μg/kg and 2.10 μg/kg, respectively. Dietary exposure was assessed using a juice frequency questionnaire of adult inhabitants in Asyut City and revealed that males and females in winter and summer seasons had different levels of exposure to AFB1. The esti-

mated seasonal exposure ranged from 0.20 to 0.40 ng/kg body weight (bw)/day in winter and from 0.38 to 0.90 ng/kg bw/day in summer [103].

Although AFM1 is the major aflatoxin, other aflatoxins can also be present in milk and dairy products. A study conducted on multiple mycotoxins [aflatoxins, ochratoxin A (OTA), and zearalenone] in 61 samples of maize and 17 commercial animal feed samples and of aflatoxin M1 (AFM1) in raw dairy milk samples (n = 20) collected from Asyut City in Upper Egypt showed that AFB1 was detected in both maize (n = 15) and feed (n = 8), with only one maize sample presenting a concentration above the maximum permissible level set by the Egyptian authorities. AFB2 was observed in six maize samples and in one feed sample, with a maximum value of 0.5 μg/kg [104].

Meat and meat products marketed in Egypt were also investigated for their aflatoxin content by different research groups. A study on 215 samples of fresh and processed meat products and 130 samples of spices used in the meat industry collected from different local companies in Cairo, Egypt, showed that processed meat products (beefburger, hotdog, kubeba, sausage, luncheon meat) had higher amounts of aflatoxin-producing fungi when compared with fresh and canned meat. *Aspergillus flavus* (24 isolates) and *Aspergillus parasiticus* (16 isolates) were the predominant aflatoxin-producing fungi isolated from both processed meat products and spices. Out of 150 samples of meat products and 100 samples of spices, AFB1 was detected in 5 samples of beef burger (8 μg/kg), 4 samples of black pepper (35 μg/kg), and 4 samples of white pepper (22 μg/kg). AFB1 (150 μg/kg) and AFB2 (25 μg/kg) were also found in one sample of kubeba. AFB1 and AFG1 were detected in two samples of turmeric and coriander [105]. A study on 50 meat products purchased from different supermarkets in Mansoura City, Egypt, showed that all the meat samples analyzed were contaminated with aflatoxins and OTA with mean values of 1.1 μg/kg. For beef luncheon and for beef burger, the total aflatoxin levels were 3.22 μg/kg and 4.55 μg/kg, respectively. None of the beef luncheon and burger samples exceeded the permissible limits set by the Food and Drug Administration (FDA) for total aflatoxin levels. However, 52% of beef luncheon and 36% beef burger samples had total aflatoxin levels above the Food and Agricultural Organization of the United Nations aflatoxin total (FAO AFT) permissible limit [106].

Other than studies on food, there are also a limited number of studies performing biological samples from Egypt. A pilot case-control study on limited number of sera samples of HCC patients from Egypt showed that aflatoxin-albumin adducts were detected in all of the controls (n = 24, geometric mean 9.0 pg/mg; range: 3.5–25.8 pg/mg) and in 7 HCC-positive individuals while 7 in 22 samples from HCC-positive cases had detectable aflatoxin-albumin adducts (geometric mean 2.6 pg/mg; range: non-detectable–32.8 pg/mg) [107].

It is well known than infants and young children are the most susceptible population to the toxic effects of aflatoxins. A study investigated aflatoxin exposure in Egyptian children (n = 50, 1–2.5 y) by assessing urinary AFM1, AFB1, AFB2, AFG1, and AFG2 levels. Total aflatoxins were present in the urinary samples of 38% of the Egyptian children [108]. A study performed in hepatitis B surface antigen (HBsAg) or anti-HCV seropositive subjects and subjects with fatty liver disease used the univariate logistic regression analysis to determine the odds ratios (ORs) between the diseases and aflatoxin exposure. For HBsAg seropositive subjects, AFB1 OR was 6.2 while for anti-HCV antibodies seropositive subjects, AFB1 OR was 2.5. Multivariate logistic regression analysis for fatty liver showed that only anti-HCV antibody seropositivity had statistically significant OR in comparison with AFB1. The researchers concluded that AFB1 had a definite association with liver diseases [109].

A cross-sectional study conducted on sera from pregnant women showed that aflatoxin-albumin adducts were detected in 34 of 98 (35%) samples (geometric mean of positive samples = 4.9 pg AF-lys/mg albumin [95% CI = 4.1–5.8 pg/mg]). Aflatoxin biomarkers were observed in 41% of the subjects. The frequency and level of these biomarkers in Egyptian women were

modest compared with known high-risk countries [110]. A study performed between January 2005 and January 2006 on 80 cases with HCC (52.88 ± 7.27 y) diagnosed in the Gastroenterology Center, Mansoura University, Egypt, and 20 healthy controls (53.17 ± 6.78 y, 82.5% male) showed that serum AFB1 levels were markedly higher in HCC patients vs. control ($p < 0.0001$). AFB1 levels were significantly higher in males when compared to females, and rural residents had higher AFB1 concentrations vs. urban residents ($p < 0.05$). Moreover, farmers had the highest AFB1 levels vs. other workers ($p < 0.05$, for all). HCV antibody was positive in 70% of the patients. The serum level of AFB1 was markedly higher in HCV-positive patients compared with HCV-negative patients. However, there was difference in the AFB1 levels of HBsAg-positive and HBsAg-negative patients. The serum level of AFB1 was significantly higher in patients with a tumor size >5 cm vs. with tumor size <5 cm. The serum level of AFB1 showed a statistically significant positive correlation with serum alanine transaminase (ALT) and alpha-fetoprotein levels. The researchers concluded that AFB1 exposure might have a crucial role in the occurrence of HCC in the north Nile Delta area and particularly among males, farmers, and rural residents and HCV, cirrhosis, and multifocal hepatoma patients [111].

A study that assessed the hepatic carcinogenicity of AFB1 in wheat handlers in Egypt showed that AFB1-albumin adducts and α-l-fucosidase (AFU) levels were significantly higher among workers employed as bakers compared to mill workers and controls. Mill workers had higher levels of AFB1-albumin adducts vs. controls. In HCC cases, the researchers found a significant correlation between AFU and AFB1-albumin adducts in bakers and between α-fetoprotein and AFB1-albumin adducts. Arginase was negatively correlated with AFB1-albumin adducts in HCC cases, while AFB1-albumin adducts were significantly and markedly correlated with the duration of exposure in bakers [112].

There are also several studies in Iran on the levels of AFB1 in different food. AFB1 and OTA levels were measured in five pistachio cultivars collected from four sites of Iran. The highest mean concentrations of AFB1 and OTA were found in Ahmad Aghaei (4.33 and 2.19 ng/g, respectively) and Akbari (4.08 and 1.943 ng/g, respectively) cultivars from Rafsanjan, Iran. Even in the highest concentrations of AFB1 and OTA in analyzed samples, the levels of these mycotoxins were lower than the corresponding maximum limits set by the European Union (EU) authorities. The researchers suggested that consumption of pistachio cultivated in these regions did not pose any health risk concerning their mycotoxin content [113]. A study on aflatoxigenic fungi and aflatoxin contamination of 40 walnut samples from the Hamedan province, Iran, showed that *Aspergillus* (particularly *Aspergillus flavus*) is the most frequent genus among other fungi. AFG1 and AFB1 levels were 1.7–18.2 ng/g and 0–8.2 ng/g, respectively. A significant difference of aflatoxin contamination was observed between shielded and unshielded walnuts. The AFB1 levels in most of the walnut samples were not above the maximum tolerable limit (MTL) given by EU standard ($p > 0.05$) [114].

In a study conducted on 3356 pistachio nut samples from Iran, AFB1 was detected in 36.7% of the samples (5.9 (±41.7 ng/g), while total aflatoxins were detected in 28.3% of the samples (7.3 ± 53.2 ng/g). AFB1 levels in 11.8% of the samples were above the MTL levels (5 ng/g). Only 7.5% of samples had total aflatoxin levels above the MTL, and the mean total aflatoxin contamination levels were lower than MTL suggested by Iranian regulatory authorities and the proposed draft maximum level of Codex Committee on Food Additives and Contaminants for total aflatoxins (15 ng/g) [115].

The occurrence of total aflatoxin in 35 randomly selected samples of raw walnuts from Iranian supermarkets in Tehran was examined in a study. Total aflatoxin concentrations in the samples ranged from 0 to 112.8 ng/g. Of all samples, 74.3% were contaminated with aflatoxins, and 20.0% of these exceeded the MTL of 15 ng/g set by the Iranian Food Codex [116]. A study conducted on 58 watermelon seeds from Iran

showed that AFB1 was present in 89.2% of the samples with an average level of 8.5 ng/g. The concentrations of AFB1 in 12 samples exceeded the MTL for AFB1 given by Iranian regulatory authorities (5 ng/g), and therefore 18.5% of all the samples was not suitable for human consumption [117]. In another study, 30 bean samples were analyzed for AFB1 contamination. Of all the samples, 16.67% contained AFB1 (>0.2 ng/g) [118]. A study performed in Iran on 65 rice samples revealed that AFB1 was present in 21.5% of the samples and its levels were below than the MTL suggested by Iranian Food Codex [119]. A study on 30 dried apricots and 15 prunes obtained from different parts of Iran showed that 30% of examined apricot samples and 13.33% of the prune samples contained AFB1 levels >0.2 ng/g [120].

A study on 48 commercial baby foods available in the Iranian market showed that 33 out of 48 samples (68.7%) were contaminated with AFB1 (median: 0.11 μg/kg; min-max: 2.602–15.15 μg/kg). The AFB1 levels in 39.6% of the samples were higher than the MTL established by the Iranian Food Codex for baby foods containing milk (0.5 μg/kg). The incidence of AFB1 in rice, wheat, and multigrain infant cereal samples were 90%, 25%, and 100%, respectively, whereas rice-based baby foods contained the highest levels of AFB1 [124]. In another study, the presence of AFB1 was investigated in 150 eggs and 50 chicken livers from the local market of Tabriz, Iran. In 72% of the liver and 58% of the egg samples, AFB1 contamination was observed ranging from 0.30 to 16.36 μg/kg [121].

Fewer studies on food and biological samples from other parts of Middle East are present in the literature. A study performed in Kuwait on milk samples showed that 6% of dairy milk was positive for AFM1, which is the main metabolite of AFB1 in milk [122]. A study from Qatar showed that 8.7% to 33% of the pistachios were contaminated with AFs (>20.0 ppb) [123]. A study performed in Doha, Qatar, on different food samples showed that AFB1 was present in 22% of the baby food ($n = 67$) samples [124]. Another study performed on nuts and spices showed that total aflatoxin levels were 534.15 ng/g and 371.6 ng/g, respectively. The researchers calculated the estimated daily intake (EDI) level using statistical data on average Qatari population. The results indicated Qatari population had high exposure to aflatoxins (with alarming values of margin of exposure) [125].

A study conducted in Saudi Arabia that included 90 bakers, 100 flour milling workers, and 100 controls with no exposure to flour dust found that both serum AFB1-albumin adducts and alkaline phosphatase (ALP) levels were significantly higher in bakers compared to milling workers ($p < 0.0001$, $p = 0.05$). There was significant positive correlation between serum AFB1 and ALT and AST levels in bakers. The researchers suggested that chronic occupational exposure to high concentrations of *Aspergillus* species in occupational environment might cause elevations in serum levels of AFB1 and liver enzymes in workers exposed to flour dust [126]. A prospective study on aflatoxin levels in umbilical cord blood from 201 women living in the United Arab Emirates (UAE) showed that aflatoxins were detected in 110 (54.7%) samples, 27 of which were positive for AFB1, 106 for AFM1, and 31 for AFM2. There was a significant negative correlation ($p < 0.001$) between birth weight and aflatoxin concentrations [127].

Although there are limited number of studies in Arab countries concerning the aflatoxin levels in foodstuff and biological samples, there are several studies performed in Turkey that focused on the levels of different mycotoxins, including aflatoxins in both foods and different biological samples. A study performed on the retail ground samples of 12 different types of seed, pulses, cereal flours, and starches showed the levels of aflatoxins ranged between 0.03 to 3.16 ppb. The percentage of contaminated samples for AFB1, AFB2, AFG1, and AFG2 were 64%, 60%, 72%, and 76%, respectively [128]. A study on 47 samples of corn collected from various street bazaars and market outlets in different regions of Turkey showed that aflatoxins were present in 53% of the samples and total aflatoxin levels ranged between 1.75 and 120.3 μg/kg. Total aflatoxin levels in these samples were above the acceptable limit of 10 μg/kg in Turkey [47].

Dried fruits, vegetables, and nuts are commonly consumed in Turkey. A study examined 284 dried fig samples, collected from fields during drying and from warehouse and processing units in the Aegean region of Turkey in 1986, for aflatoxin contamination. AFB1, AFB2, and AFG1 were detected in 4%, 2%, and 2% of the samples, respectively, particularly in the lower grade of figs taken from the drying stage. The average aflatoxin levels in positive samples were estimated to be 112.3 ng/g for AFB1, 50.6 ng/g for AFB2, and 61.4 ng/g for AFG1 [129]. In another study, 300 samples of hazelnuts and dried figs from Turkey were analyzed for the presence of aflatoxins. Aflatoxins were not detected in shells of the hazelnuts, while six raw hazelnut kernel samples (12%) and five roasted hazelnut kernel samples (8.3%) contained aflatoxins ranging from 0.09 to 11.3 μg/kg and from 0.17 to 11.2 μg/kg, respectively. Sixteen dried fig samples (12.3%) contained aflatoxins ranging from 0.1 to 28.2 μg/kg with a mean value of 3.8 μg/kg. Three hazelnuts and six dried fig samples exceeded the European maximum limits (MLs; 5 μg/kg for hazelnuts and 2 μg/kg for dried figs) [130]. A study on 62 food samples from Istanbul, Turkey, measured the total aflatoxin and AFB1 levels. The total aflatoxin content in dried American cucumber, squash, tomato, okra, and saffron samples was found to be 1.7 μg/kg. AFB1 levels in five dried vegetables (red bell pepper, American cucumber, squash, tomato, and okra), two tea (linden and jasmine flower), and three spice samples (cardamom, galangal, and saffron) were found to be 1 μg/kg. Of the tested samples, 76% exceeded legal limits of total aflatoxins set by the Turkish Food Codex. The highest levels were determined in chestnut (232.9 μg/kg), nutmeg (206.1 μg/kg), and sumac (182.5 μg/kg) [131].

Spices are also highly consumed in Turkey, particularly in east and southeast regions. A study on 33 red pepper samples sold in open and sealed packages on different markets of Turkey showed that AFB1 content in the samples, according to daily consumption data, exceeded the limits set by the European Commission (EC) by almost 150% [132]. A study conducted to determine aflatoxin levels in 138 tarhana powder samples collected from bazaars in Istanbul showed that 32 out of 138 tarhana samples (23.2%) were found to be contaminated with total aflatoxins in the range of 0.7–16.8 μg/kg, whereas 29 samples contained AFB1 ranging from 0.2 to 13.2 μg/kg [133].

A study analyzed 180 red chili peppers (RCP) and berry samples (dried under sunlight and grinded) obtained from two different croplands of Gaziantep and Kahramanmaraş, Turkey, in August, September, and October for total aflatoxin and AFB1 contamination. AFB1 was present in 37 samples, and in these samples, total aflatoxin levels were higher than legal limits. The lowest amounts of total aflatoxin and AFB1 were obtained in August and the highest amounts in October. Statistical analysis showed that there were no significant differences ($p > 0.05$) between aflatoxin content of red chili pepper and berry samples for August, September, and October [134].

A study on 93 organic spices and 37 organic herbs randomly selected from organic markets and organic shops in Turkey showed that AFB1 was detected in 58 organic spice and 32 organic herb samples. Among organic spice samples, the maximum value was detected in cinnamon samples (53 μg/kg). AFB1 was not present in thyme samples. AFB1 levels of 41 organic spice samples were above the EU regulatory limit (5 μg/kg). Among organic herb samples, the highest concentration of AFB1 (52.5 μg/kg) was detected in a rosehip sample. AFB1 levels of 21 organic herb samples were above the regulatory limits of the EU. The researchers suggested that strict measures should be taken to reduce the aflatoxin content of spice and herb samples [135].

A study on unpacked and packed ground red pepper (GRP) and pistachio nut samples obtained from different markets in Turkey from September 2008 to February 2009 showed that 17.1% (14/82) unpacked GRP samples had total aflatoxin levels above the legal limits. Both total aflatoxin and AFB1 were detected in 50.5% (48/95) of unpacked pistachio nuts with the contamination levels ranging from 0.007 to 7.72 ppb [136]. A study on 42 GRP samples that were randomly

collected from retail shops, supermarkets, open bazaars, and apiaries in Şanlıurfa, Turkey, examined the levels of AFB1, AFB2, AFG1, and AFG2. Total aflatoxin levels were <2.5 μg/kg in 16 samples; 13 samples had total aflatoxin levels between 2.5 and 10 μg/kg, while 13 samples had total aflatoxin levels higher than the tolerable limit (10 μg/kg) given by the regulations of Turkish Food Codex and European Commission (EC) [137].

AFB1 levels were determined in 25 cacao hazelnut creams and 15 dried apricot samples randomly collected from traditional retail markets with insufficient chilling facilities in Bursa, Turkey. Mean AFB1 in the cacao hazelnut cream, dried apricot, and cheese were 1076.5 ± 194.4 ng/kg, 1441.3 ± 331.9 ng/kg, and 142.2 ± 18.7 ng/kg, respectively, and all were within the tolerable limits given in the Turkish Food Codex [138].

In a study performed on 63 infant formulas, follow-on formulas, and baby foods that were randomly collected from pharmacies and supermarkets in Ankara, Turkey, AFB1 and AFM1 levels were found in 87% and 36.5% of the samples, respectively. The AFB1 levels ranged between 0.10 and 6.04 ppb [139]. In another study, 3345 commercial Turkish foodstuffs supplied by producers for testing for their own purposes or for export certification were analyzed in Turkey. Foods were categorized as follows: (1) high-sugar products with nuts; (2) nuts and seeds; (3) spices; (4) grain; (5) cocoa products; (6) dried fruit and vegetables; (7) processed cereal products; (8) tea; and (9) baby food and infant formula. Of the 3345 samples, 94% contained AFB1 below the EU limit of 2 μg/kg for nuts, dried fruit, and cereals products. The researchers found that 6% of the 206 contaminated samples were mainly nuts and spices. For pistachios, 24%, 38%, and 42% of a total of 207, 182, and 24 samples tested for years 2007, 2008 and 2009, respectively, were above 2 μg/kg, with 50 samples containing AFB1 at levels ranging from 10 to 477 μg/kg [140]. A study conducted in Turkey showed that 12.28% of the cheese were contaminated with aflatoxins, and hazelnuts and pistachios also had aflatoxin contamination (>4.0 ppb) [141].

Other than foods, there are also studies in Turkey conducted on human milk and sera. In one study, Turkish researchers determined the levels of AFM1 and AFB1 in breast milk samples collected from 75 mothers in Ankara, Turkey. AFM1 levels were between 60.90 and 299.99 ng/L, and AFB1 levels ranged between 94.50 and 4123.80 ng/L [142]. A study performed on serum samples from north and south regions of Turkey in different seasons showed that total aflatoxin concentrations in the Black Sea region was 1.33 ppb (min-max 0.15–3.38 ppb) and 0.90 ppb (min-max 0.18–2.48 ppb) for summer and winter, respectively. In the Mediterranean region, the mean serum concentration of total aflatoxin was determined as 0.55 ppb (range 0.04–1.72 ppb) for summer and 0.45 ppb (range 0.12–1.43 ppb) for winter. The total aflatoxin concentrations in serum samples were statistically higher in summer compared to winter for the two regions. The differences between the regions were statistically significant concerning all samples, with higher total aflatoxin concentrations in the Black Sea region. The researchers suggested that the Turkish citizens living in these two regions were continuously exposed to aflatoxins, particularly in the summer [143]. Another study performed by the same researchers in the Central Anatolia region of Turkey revealed that serum aflatoxin levels ($n = 233$) were 0.98 ± 0.10 and 0.94 ± 0.12 ng/ml and in males 1.35 0.17 and 0.93 ± 0.11 ng/ml in summer and winter, respectively. The serum aflatoxin levels of male subjects were markedly higher than females (~38%). There was no significant seasonal change in AFG1, AFB1, and AFG2 concentrations in the whole population, except AFB2 [144].

A case-control study on chronic HBV-infected patients with or without cirrhosis and liver cancer determined the serum AFB1, AFB2, AFG1, and AFG2 concentrations in Balıkesir, Turkey. The mean AFB1 and total aflatoxin levels in patients without liver cancer and cirrhosis were markedly higher than control group. The mean AFB1 and total aflatoxin levels in patients with chronic hepatitis B and HCC were significantly higher than infected patients with or without cirrhosis. These

results suggested that patients with chronic HBV infection who were exposed to aflatoxins were at increased risk for developing HCC. The authors suggested that the risk of HCC might be prevented by reducing consumption of contaminated foods [145].

7 How to Decrease and Control Aflatoxin Exposure in the Middle East

Several measures have been set to limit aflatoxin contamination in food. Throughout the world, over 100 nations have set MTLs as aflatoxins can lead to mutagenicity and carcinogenicity when populations are exposed to these mycotoxins. Many countries have also put import and export regulations [146–148]. In developed countries, these standards enable public health protection. However, whether these regulations are efficiently applied or not in Middle East is still a debate. Therefore, the measures that should be taken in the Middle East are listed below:

1. Subsistence farms should be put under more regulatory inspection, and their products should be monitored like in developed countries [149, 150].
2. Maize, nuts, and spices are highly consumed in the Middle East countries. These products should be monitored closely. Governments should make spot investigations for the products, especially for those that are not sold without a package [151].
3. Although the Middle East countries produce different foodstuff (particularly maize, dried fruit, dried nuts, and spices) and export these products, they usually find their export markets severely jeopardized by strict aflatoxin standards. Therefore, these countries export foodstuff that are not contaminated by aflatoxins and keep the contaminated domestically. This creates a risk for the development of severe conditions, particularly HCC [152]. FAO (2004) reported that only 60 countries (including 15 African countries) have set MTLs for AFB1 or AFM1 in 2003. The MTLs for AFB1 were 1–20 μg/kg (frequently 2 μg/kg and 5 μg/kg). With regard to AFM1, of the 60 countries, 22 had a limit of 0.5 μg/kg and 34 a limit of 0.05 μg/kg. The differences on AFB1 MTLs suggested by different national and regional food safety regulatory bodies clearly affect the export of the foodstuff. As EU countries have set more strict limits (2 μg/kg for AFB1 and 4 μg/kg for total aflatoxins), countries that have more stringent limits can export more easily, while the shipments of foodstuff from other countries are more subject to rejection along with additional costs [153]. Therefore, Middle East countries should set lower domestic limits for aflatoxins, particularly for AFB1.
4. The Middle East countries should use more sophisticated, affordable, and appropriate methods to determine the aflatoxin content of their foodstuff. In order to achieve this, the governments should supply adequate funding for better and specific laboratories nationwide to determine the aflatoxin content of the foodstuff that will be exported [153].
5. HBV and HCV infections are important risk factors for HCC. Moreover, HDV is also a risk factor for liver cancer, particularly in Jordan. Aflatoxin exposure along with these infections worsens the situation in the Middle East. Therefore, vaccination programs should be more efficient, and serious measures to prevent these diseases should be taken in the Middle East countries. Moreover, society should be informed for the transmission routes of hepatitis infections by education programs, and consciousness should be raised [154–156].

Figure 4 shows to how decrease and control HCC due to aflatoxin contamination in the Middle East.

A non-harmless level for aflatoxin exposure has not been identified yet. Due to climate change and increase in temperatures throughout the world, aflatoxins continue to be a health threat particularly for hot regions, like Middle East. Several regulations were imposed in Middle East countries. However, the prevalence of HCC is

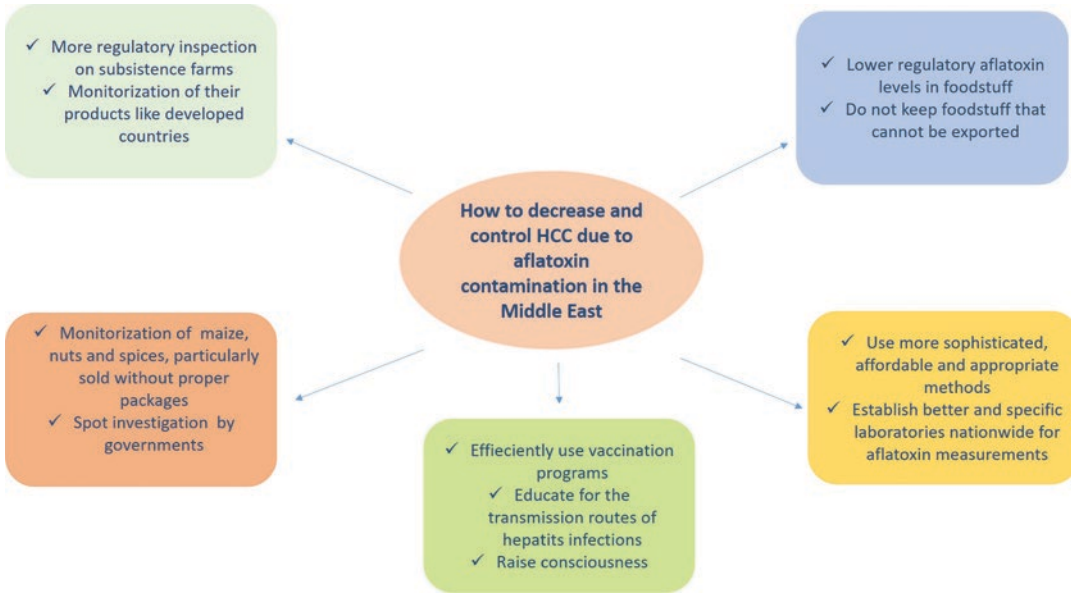

Fig. 4 How to decrease and control HCC due to aflatoxin contamination in the Middle East

still rising in the world and particularly in Middle East countries like Egypt, and there are no easy and inexpensive methods to remove aflatoxins from foodstuff. Studies must continue in order to understand the biochemical and toxicological mechanism/s altered by aflatoxin exposure. A number of approaches have been used to determine aflatoxin exposure in human populations, including analysis of aflatoxin metabolites and aflatoxin-DNA or aflatoxin-protein adducts. These studies must also go on in order to determine a direct association between aflatoxin exposure and HCC. In addition, epidemiological studies must be conducted to measure the aflatoxin levels and aflatoxin adduct levels in HBV and HCV patients. Moreover, serious measures must be taken in the near future, in order to lower the aflatoxin exposure and HBV and HCV infections.

8 Alcohol Consumption

As shown in Table 1, alcohol is classified as a Group 1 carcinogen by IARC since 1988 [157]. Alcohol intake induces the development of HCC among other certain types of cancer. Excessive alcohol consumption may cause fatty liver, acute/chronic hepatitis, and cirrhosis and eventually HCC. Chronic alcohol abuse may lead to three- to tenfold increase in the risk of HCC and may be the underlying factor of 15–30% of HCC cases [158].

9 Biotransformation of Ethanol

Some of the orally ingested alcohol does not enter the systemic circulation. The oxidation of ethanol includes a three-step process [159, 160]:

1. Firstly, ethanol is oxidized in the stomach by alcohol dehydrogenase (ADH) isoforms (i.e., σADH and class I and class III ADH). There is also first-pass metabolism (FPM) that modulates alcohol toxicity. The efficiency of FPM determines the bioavailability of ethanol. When fasted, ethanol passes into the duodenum from the stomach and this passage will minimize FPM. This is suggested to play a role in the higher blood alcohol concentra-

tions seen in the fasted state versus full stomach state. FPM is lower in alcoholics (particularly in females) vs. the whole population. This leads to a high level of blood alcohol concentration in women compared to men after an equal oral dose of ethanol intake. However, the liver is the main organ and therefore the target organ for alcohol, and in the liver most of the biotransformation of ethanol takes place by different isoforms of ADHs in the presence of NAD+. The biotransformation of ethanol by ADHs takes place in the cytosol. In the liver, ethanol is oxidized to acetaldehyde. After this reaction, stable or unstable acetaldehyde adducts can be formed. CYP2E1 also metabolizes a certain amount of ethanol in the microsomes, in the presence of NADPH. This reaction causes an increase in reactive oxygen species (ROS) formation. In alcoholics, CYP2E1 is induced leading to enhanced alcohol metabolism and metabolic tolerance which in turn causes promotion of further alcohol consumption. Moreover, in the peroxisomes, ethanol is metabolized by catalase. While catalase is metabolizing alcohol, it also detoxifies hydrogen peroxide to water.

2. After the oxidation takes place, acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenases (ALDHs) in the mitochondria, and this reaction is irreversible. Again, NAD+ is the cofactor and is reduced to NADH. This leads to an increase in the NADH/NAD+ ratio. Circulating levels of acetaldehyde are low under normal conditions.
3. After acetic acid then leaves the liver, it circulates to peripheral tissues where it is activated to acetyl-CoA, which is the key metabolite produced from all major nutrients (carbohydrates, fats, and excess proteins). Later, the carbon atoms from alcohol are used to produce the same products that are produced from the oxidation of the major nutrients. The products include carbon dioxide, fatty acids, ketone bodies, and cholesterol. Which one of these products to be formed is up to the energy state of the organism. In addition, the nutritional and hormonal conditions are also effective.

The oxidative biotransformation of ethanol is summarized in Fig. 5.

Other than the oxidative biotransformation of ethanol, it can also undergo non-oxidative metabolism. It is minimal compared to oxidation. However, the by-products of non-oxidative metabolism pose pathological and diagnostic importance. The non-oxidative metabolism is suggested to have two pathways at least [160]:

1. One of these pathways includes from the reaction of alcohol with fatty acids, and this reaction causes the formation of molecules called "fatty acid ethyl esters (FAEEs)." FAEEs are weak organic acids, which may have functional roles in humans. FAEEs can be detected in serum and in several other tissues after alcohol ingestion, and they can persist long after the elimination of alcohol. However, the roles of FAEEs in alcohol-induced tissue damage are still being investigated.
2. The other pathway leads to the formation of a phospholipid molecule named as "phosphatidyl ethanol."

The potential mechanisms of alcohol-induced liver carcinogenesis include [161–165]:

1. Ethanol and its metabolite, acetaldehyde, can lead to increased intracellular ROS levels. In addition, in the presence of iron, ethanol causes the formation of hydroxyethyl radical, which in turn causes lipid peroxidation (particularly malondialdehyde and 4-hydroxynonenal formation) and/or protein oxidation. Moreover, ROS can lead to DNA damage.
2. Alcohol leads to the reduction of the levels of certain antioxidants, particularly mitochondrial and cytosolic glutathione.
3. An increase in NADH/NAD+ ratio causes alterations in the rate of metabolic reactions.

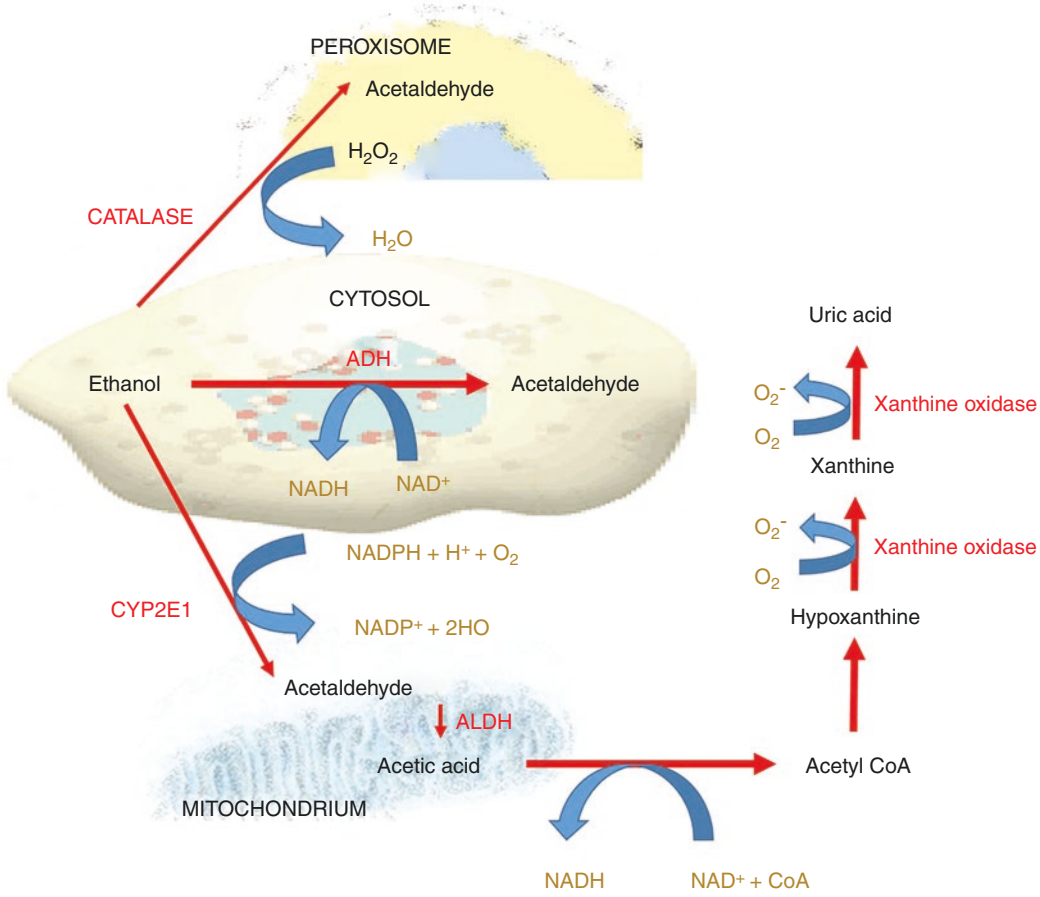

Fig. 5 The oxidative biotransformation of ethanol. ADH alcohol dehydrogenase, ALDH aldehyde dehydrogenase, CYP2E1 cytochrome P450 2E1

4. Ethanol can also lead to decreases in DNA repair, which in turn leads to genotoxicity.
5. Not all amino acids in all proteins are equally likely to interact with acetaldehyde, but the interaction of acetaldehyde with certain amino acids like lysine, cysteine, and some of a group of amino acids called "aromatic amino acids" causes the formation of acetaldehyde-protein adducts. Proteins found in the membranes of erythrocytes, lipoproteins, tubulin, hemoglobin, albumin, and collagen are suggested to be the target of acetaldehyde.
6. Ethanol also causes damage in the mitochondria.
7. Ethanol leads to hypoxia.
8. Ingestion of alcohol causes the activation of Kupffer cells.
9. The interruption of one-carbon metabolism by ethanol causes impaired methylation patterns and altered genetic expression.

The potential mechanisms of alcohol-induced liver carcinogenesis are summarized in Fig. 6.

All these mechanisms one by one or additively may lead to liver injury, enhanced fibrogenesis, and later cirrhosis. The development of liver cirrhosis may result in liver cancer [166–169]. The interaction of with other environmental carcinogens, like cigarette smoke, can accelerate the carcinogenic process. Moreover, as alcohol is a cocarcinogen, it exaggerates liver damage caused by HBV and/or HCV infection and leads to tumor progression [170].

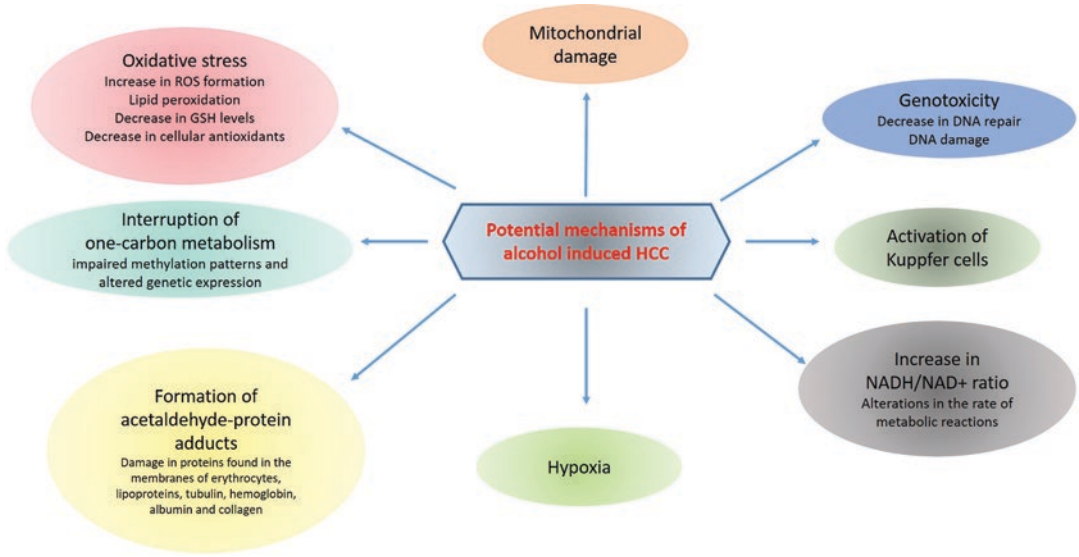

Fig. 6 The potential mechanisms of alcohol-induced liver carcinogenesis

10 Alcohol-Related Hepatocarcinogenesis in the Middle East

Due to religious reasons, alcohol is banned or restricted in most of the Middle East countries, and alcohol consumption is lower than European countries (particularly compared to Russia) and the USA. In the Middle East, Turkey is suggested to have the highest alcohol consumption. In Turkey, the prevalence of heavy episodic drinking was 2.8% in males and 0.2% in females among general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 25.9% in male drinkers and 6.9% in female drinkers. Total alcohol per capita was reported as 33.3 L for males and 11.9 L for females. Age-standardized death rate (ASDR) due to alcohol-related liver cirrhosis was 14.2 per 100,000 in males and 7.5 per 100,000 in females. The prevalence of alcohol use disorders was 8.1% in males and 2.5% in females, while in the same year, WHO European region's prevalence of alcohol use disorders was 8.8% in males and 3.7% in females [171]. In Turkey, beer is highly consumed (63.6%), followed by spirits (27.9%) and wine (8.6%) [172].

According to unofficial reports, the United Arab Emirates has the second most alcohol consumption with an average of 4.3 L per year followed by Sudan with an average of 2.7 L per year and Lebanon with an average of 2.4 L per year. These numbers are below the international average. However, when only drinkers from Middle East are taken into account, the average consumption is significantly higher than the world. A drinker in UAE is estimated to consume 32.8 L of alcohol per year, while in Sudan it is estimated that drinkers consume 24.1 L alcohol, followed by Lebanon with an estimation for drinkers of 23.9 L per year [173]. According to a WHO report published in 2016, the prevalence of heavy episodic drinking was 9.1% in males and 1.1% in females among general population (15+ years) in UAE. In the same report, the prevalence of heavy episodic drinking was 41.7% in male drinkers and 13.9% in female drinkers. Total alcohol per capita in drinkers was reported as 21.9 L for males and 11.9 L for females. ASDR concerning alcohol-related liver cirrhosis was 13.6 per 100.000 in males and 9.5 per 100,000 in females [173]. In UAE, spirits are highly consumed (86.7%), followed by beer (10.3%) and wine (2.9%) [172].

In Lebanon, an average of 1.2 L alcohol consumption in the general population is suggested by a WHO report in 2016. The prevalence of heavy episodic drinking was 2.3% in males and 0.2% in females among general population (15+ years). In the same report, the prevalence of heavy episodic drinking was 24.5% in male drinkers and 6.8% in female drinkers. Total alcohol per capita in drinkers was reported as 29.6 L for males and 10.7 L for females. ASDR concerning alcohol-related liver cirrhosis was 24.1 per 100,000 in males and 13.9 per 100,000 in females [174]. In UAE, spirits are highly consumed (52.4%), followed by wine (29.1%) and beer (18.2%) [172].

In Sudan, an accurate information is not present on the pattern of drinking. The recorded alcohol consumption was given as 1.56 L, while unrecorded alcohol consumption was suggested to be 1 L per year in a report by WHO in 2016. In the same report, alcohol use disorders was suggested to affect 0.54% of the males and 0.06% of the females [175]. In Sudan, other than sprits, wine, and beer, the general population seems to choose different local alcohol drinks (78.5%) followed by alcoholic spirits (13.5%) and beer (18.2%) [172].

In Israel, the prevalence of heavy episodic drinking was 29% in males and 5.7% in females among general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 42.1% in male drinkers and 13.7% in female drinkers. Total alcohol per capita was reported as 9.3 L for males and 3.3 L for females. Age-standardized death rate (ASDR) due to alcohol-related liver cirrhosis was 6.6 per 100,000 in males and 3.3 per 100,000 in females. The prevalence of alcohol use disorders was 9.8% in males and 2.1% in females while in the same year [176]. In Israel, alcoholic spirits a are highly consumed (49.5%), followed by beer (44%) and wine (6.2%) [172].

According to a WHO report in 2016, the prevalence of heavy episodic drinking was 0% in both males and females among general population (15+ years) in Kuwait. In the same report, the prevalence of heavy episodic drinking was 4.8% in male drinkers and 1.1% in female drinkers. Total alcohol per capita was reported as 1.1 L for males and 0.4 L for females. ASDR due to alcohol-related liver cirrhosis was 7.2 per 100,000 in males and 7.8 per 100,000 in females. The prevalence of alcohol use disorders was 0.6% in males and 0.1% in females while in the same year [177]. In Kuwait, beer is highly consumed (58.1%), followed by spirits (30.7%) and wine (10.8%) [172].

The WHO report in 2016 gives the prevalence of heavy episodic drinking as 28.8% in males and 5.3% in females among general population (15+ years) in Qatar. In the same report, the prevalence of heavy episodic drinking was 55.3% in male drinkers and 20.9% in female drinkers. Total alcohol per capita was reported as 4.8 L for males and 1.7 L for females. Age-standardized death rate (ASDR) due to alcohol-related liver cirrhosis was 19.4 per 100,000 in males and 4.9 per 100,000 in females. The prevalence of alcohol use disorders was 0.4% in males and 0.1% in females while in the same year [178]. In Qatar, spirits are highly consumed (84.6%), followed by wine (13.9%) and beer (1.2%) [172].

In Libya, the prevalence of heavy episodic drinking was 0% in males and females among general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 2.5% in male drinkers and 0.6% in female drinkers. Total alcohol per capita was reported as 9.3 L for males and 3.3 L for females. ASDR due to alcohol-related liver cirrhosis was 32.9 per 100,000 in males and 18.4 per 100,000 in females. The prevalence of alcohol use disorders was 0.5% in males and 0.1% in females while in the same year [179]. There is no data on which alcoholic beverage is consumed highly in Libya [172].

The WHO report in 2016 gives the prevalence of heavy episodic drinking a 3.3% in males and 0.3% in females among general population (15+ years) in Oman. In the same report, the prevalence of heavy episodic drinking was 31.3% in male drinkers and 8.9% in female drinkers. Total alcohol per capita was reported as 9.9 L for males and 3.5 L for females. Age-standardized death rate (ASDR) due to alcohol-related liver cirrhosis

was 13.2 per 100,000 in males and 9.5 per 100,000 in females. The prevalence of alcohol use disorders was 0.4% in males and 0.1% in females while in the same year [180]. In Oman, beer is highly consumed (54.6%), followed by spirits (42.2%) and wine (3.3%) [172].

In Egypt, the prevalence of heavy episodic drinking was 1% in males and 0.1% in females among the general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 22.6% in male drinkers and 5.9% in female drinkers. Total alcohol per capita was reported as 17 L for males and 6.2 L for females. ASDR due to alcohol-related liver cirrhosis was 200.4 per 100,000 in males and 121.6 per 100,000 in females. The prevalence of alcohol use disorders was 2.6% in males and 0.2% in females while in the same year [181]. In Egypt, beer is highly consumed (53.8%), followed by spirits (40.3%) and wine (5.4%) [172].

In Jordan, the prevalence of heavy episodic drinking was 1.1% in males and 0.1% in females among the general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 23.9% in male drinkers and 6.3% in female drinkers. Total alcohol per capita was reported as 28.9 L for males and 10.6 L for females. ASDR due to alcohol-related liver cirrhosis was 20.6 per 100,000 in males and 14.6 per 100,000 in females. The prevalence of alcohol use disorders was 0.7% in males and 0.1% in females while in the same year [182]. In Jordan, spirits is highly consumed (75.4%), followed by beer (22.4%) and wine (2.1%) [172].

The WHO report in 2016 gives the prevalence of heavy episodic drinking as 0.3% in males and 0% in females among the general population (15+ years) in Saudi Arabia. In the same report, the prevalence of heavy episodic drinking was 3.6% in male drinkers and 0.8% in female drinkers. Total alcohol per capita was reported as 4.5 L for males and 1.6 L for females. ASDR due to alcohol-related liver cirrhosis was 31.9 per 100,000 in males and 21.8 per 100,000 in females. The prevalence of alcohol use disorders was 0.5% in males and 0.1% in females while in the same year [183]. In Saudi Arabia, spirits is highly consumed (97.9%), followed by wine (1.9%) [172].

In Afghanistan, the prevalence of heavy episodic drinking was 0% in males and females among the general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 2.3% in male drinkers and 0.5% in female drinkers. Total alcohol per capita was reported as 37.9 L for males and 14.3 L for females. ASDR due to alcohol-related liver cirrhosis was 28.2 per 100,000 in males and 19.6 per 100,000 in females. The prevalence of alcohol use disorders was 0.6% in males and 0.1% in females while in the same year [184]. There is no data on which alcoholic beverage is consumed highly in Afghanistan [172].

The WHO report in 2016 gives the prevalence of heavy episodic drinking as 1.1% in males and 0.1% in females among the general population (15+ years) in Iraq. In the same report, the prevalence of heavy episodic drinking was 24.5% in male drinkers and 6.5% in female drinkers. Total alcohol per capita was reported as 14.6 L for males and 5.4 L for females. ASDR due to alcohol-related liver cirrhosis was 9.5 per 100,000 in males and 5.2 per 100,000 in females. The prevalence of alcohol use disorders was 0.5% in males and 0.1% in females while in the same year [185]. In Iraq, beer is highly consumed (76.1%), followed by spirits (22.9%) and wine (1%) [172].

In Bahrain, the prevalence of heavy episodic drinking was 3.8% in males and 0.4% in females among the general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 33% in male drinkers and 9.4% in female drinkers. Total alcohol per capita was reported as 24.5 L for males and 8.7 L for females. ASDR due to alcohol-related liver cirrhosis was 15 per 100,000 in males and 9.1 per 100,000 in females. The prevalence of alcohol use disorders was 2.3% in males and 0.3% in females while in the same year [186]. In Bahrain, spirits are highly consumed (57%), followed by beer (36.6%) and wine (5.3%) [172].

In Syrian Arab Republic, the prevalence of heavy episodic drinking was 0.1% in males and 0% in females among the general population (15+ years) according to WHO report published in 2016. In the same report, the prevalence of heavy episodic drinking was 2.3% in male drinkers and 0.5% in female drinkers. Total alcohol per capita was reported as 17.3 L for males and 6.4 L for females. ASDR due to alcohol-related liver cirrhosis was 17.8 per 100,000 in males and 12.8 per 100,000 in females. The prevalence of alcohol use disorders was 1% in males and 0.1% in females while in the same year [187]. In Syria, spirits is highly consumed (63.5%), followed by wine (27.9%) and beer (8.5%) [172].

11 How to Decrease and Control Alcohol Intake in the Middle East

Although drinking also is forbidden or restricted due to the religious beliefs of most of the countries in the Middle East, alcohol consumption is not very low and can lead to serious health consequences. Therefore, serious measures must be taken in the future:

- People should not keep alcohol at home in order to avoid the consumption.
- Routine traffic checks must be conducted in the countries of Middle East.
- Governments should implement education programs for public and explain the health risks of alcohol consumption. Experts must give detailed information in social media and televisions.
- Cigarette smoking and alcohol consumption together cause an increase in the mortality due to HCC and other cancers. Therefore, citizens should be educated and awareness must be raised in the societies.

12 Smoking

Cigarette smoking is suggested to be one of the main causes of cancer-related deaths worldwide. Although it particularly causes lung cancer, it is also suggested to be related to primary liver cancer. Cigarette smoke includes over 4000 chemical substances, which have hazardous effects. Chronic and heavy smoking causes the development of pathogenesis of liver disease. It induces three major adverse effects on the liver [188]:

- Direct or indirect toxic effects.
- Immunological effects.
- Oncogenic effects.

Over 60 carcinogens have been identified in cigarette smoke, and smoking is suggested to be "carcinogenic to humans" by IARC [189]. Nitrosamines from smoke can cause gene mutations and/or DNA and protein adducts. Nicotine directly promotes cancer progression through the activation of different signaling pathways. Both of these substances facilitate cancer cell growth, angiogenesis, migration, and invasion [190]. On the other hand, several chemicals in tobacco smoke, including 4-aminobiphenyl and polycyclic aromatic hydrocarbons (PAH), are metabolized into reactive carcinogens in the liver [191]. Smoking also increases the production of pro-inflammatory cytokines [interleukin 1 (IL-1), interleukin 6 (IL-6), and TNF-α] that may be further involved in liver cell injury. Because of smoking, it is possible to develop secondary polycythemia. In turn, increased red cell mass and turnover is observed. This phenomenon might be a contributing factor to secondary iron overload disease that promotes oxidative stress of hepatocytes. As smoking-based chemicals have an oncogenic potential, they can increase the risk of HCC in patients with viral hepatitis [188]. The mechanisms of cigarette smoke-induced carcinogenesis are summarized in Fig. 7.

The International Agency for Research on Cancer and the US Surgeon General have stated that studies show a link between tobacco smoking and liver cancer. However, HCC (75% of all liver cancers) and intrahepatic cholangiocarcinoma (ICC, 12%), two dominant types of liver cancer, have been widely studied by the research studies. Furthermore, it is not clear that smoking and alcohol consumption together increase the risk of HCC and ICC. The main causes of HCC

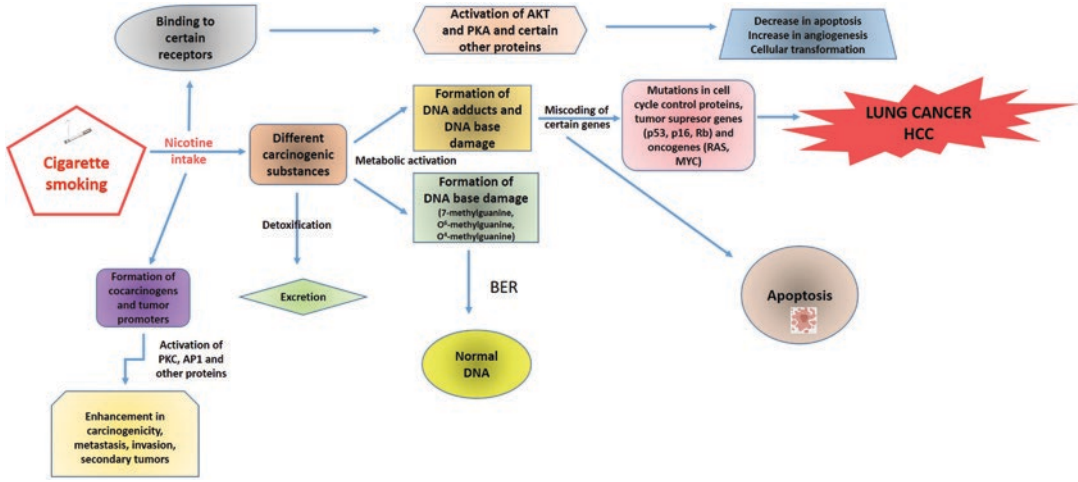

Fig. 7 The mechanisms of cigarette smoke-induced carcinogenesis. AP1 activator protein 1, AKT protein kinase B, BER base excision repair, HCC hepatocellular carcinoma, PKC protein kinase A, PKC protein kinase C

are hepatitis B or C virus, alcohol consumption, obesity, diabetes, and smoking-induced oxidative stress and inflammation [192–194]. ICC has the risk factors such as cirrhosis, HBV/HCV, alcohol consumption, obesity, and diabetes. Nevertheless, the association between smoking and ICC is still unclear. According to the Surgeon General's report, the results are conflicting concerning the dose-response relationships between liver cancer and smoking intensity (number of cigarettes per day and duration) [192, 195].

Reviews of epidemiologic studies and meta-analysis studies on HCC suggested that smoking might be categorized as a risk factor for liver cancer; however, ORs were not very high [192, 195]. A meta-analysis has analyzed 38 cohort and 58 case-control studies in order to evaluate association between liver cancer and cigarette smoking. The increased risk among current smokers was consistent across different regions, study designs, study sample sizes, and publication periods. There was a positive correlation between dose-response and the number of daily consumed cigarettes. Attributable risks for HCC associated with smoking were also determined. The attributable factors HBV and HCV infections were determined as 13% and 21%, respectively, whereas smoking carried an attributable risk of almost 50%. Although smoking conveys only a small increase in relative risk compared to viral infections, its attributable risk was found to be higher [191, 192, 196].

13 Smoking and Liver Cancer in the Middle East

Throughout the globe, China has the most tobacco users (300.8 million), followed by India (205.9 million), Russia (60.2% among men), and Bangladesh (27.9%, overall) [197].

Throughout the Middle East and North Africa, one common denominator, namely, water pipe (also named as shisha, nargile, or rengila) use, is rising among children and young women as water pipe is seen as less harmful, even though one session with the pipe can deliver many times the impact of a single cigarette. Some experts suggest that smoking water pipe is becoming an epidemic and at least 60% of 16–19-year-olds in the Middle East have tried the water pipe [198].

When we have brief look on the smoking status in different countries of the Middle East, Turkey is 1 of 15 countries worldwide with a heavy burden of tobacco-related diseases. WHO's report in 2013 standardized estimate of smoking prevalence and suggested that 25.4% of Turkey's adult population (35.7% of men and 9.9% of women) are daily tobacco smokers [199]. In the UAE, Dubai Health Centers stated

that smoking among 12- to 16-year-olds was between 25% and 30%, considerably higher than the WHO estimate of 13.2% for boys between 13 and 15 years [198]. A cross-sectional study performed in the UAE on 6363 participants suggested that 505 (8.9%) participants had smoked cigarettes, 355 (6.3%) had smoked midwakh, 421 (7.4%) had smoked shisha, and 380 (6.4%) had smoked any other form of tobacco in the previous 30 days. The results showed that 818 (14.0%) adolescents were current smokers (occasional or daily use of at least one form of tobacco). Tobacco use was higher in men compared to women, regardless of age and form of tobacco. Cigarette smoking was popular among men, while shisha was the most smoked tobacco form in women [200].

Golestan Cohort Study has included 165 participants from northeastern Iran: 60 individuals were never users of any tobacco, 35 were using exclusive cigarette, 40 were using exclusive (78% daily) water pipe, and 30 were smoking exclusive smokeless tobacco (nass). Thirty-nine biomarkers of exposure have been evaluated. The results showed that environmental exposure from nontobacco sources also appeared to contribute to the presence of high levels of polycyclic aromatic hydrocarbon (PAH) metabolites, which had adverse effects, including cancer [201].

In another study, which was also a part of Golestan Cohort Study, 50,045 adults (40–75 years) were recruited from northeastern Iran. This study intended to determine the association between different types of tobacco use and earlier deaths. According to the results, 17% of participants reported a history of cigarette smoking, 7.5% chewing tobacco (nass), and 1.1% smoking water pipe. With age, the use of cigarette and different tobacco products showed a decline. The study concluded that regular use of cigarettes, smokeless tobacco, and water pipe was associated with the risk of earlier death, particularly from cancer [202].

In a study from Lebanon, the researchers aimed to estimate the percentage of cancer due to environmental exposures and lifestyle. The results showed that smoking caused most cancer cases, and it caused 1800 new cancer cases by 2018. Percentage attributable risk factor was calculated for liver cancer as 23% and 16% for males and females, respectively [203].

A study from Saudi Arabia explored that the changing trends and patterns of HCC at King Faisal Specialist Hospital and Research Center made a comparative analysis with local, regional, and global trends. Temporal trends indicated a rising incidence of HCC from 2001 to 2014 in Saudi Arabia. It was stated that there were 323 cases in 2001 while 376 cases were diagnosed with HCC in 2015 as per Saudi Cancer Registry. The authors suggested that although there was an improvement in preventive measures, incidence rates of HCC increased in 10 years with a marked regional variation. It was specified that chronic infection with HBV and/or HCV, heavy alcohol consumption, obesity, diabetes, and tobacco smoking were the main factors for this escalation. The researchers concluded that early detection and diagnosis due to expanding healthcare delivery in the country could help to detect this increase [204].

14 How to Decrease and Control Tobacco Use in the Middle East

Smoking causes serious health problems in the Middle East countries. Although men are smoking more than women, the smoking rates among women are rising possibly due to the belief that water pipe smoking is not as harmful as cigarette smoking. Therefore, serious measures must be taken in the future:

- Governments should implement education programs for public and explain the health risks of tobacco use.
- Detailed information in social media and televisions must be given to the public.
- Adolescents must be warned against the serious health effects of smoking.
- Smoking in restaurants and cafes or in public transportation should be forbidden.
- Water pipes also cause serious health consequences as cigarettes. Therefore, awareness should be raised in public on the diseases and pathologies that may arise from water pipe smoking.

15 Rsenic

The metals in the environment are classified in three groups [205]:

- Essential (selenium, copper, and zinc).
- Probably essential (cobalt, nickel, and vanadium).
- Potentially toxic (arsenic, mercury, cadmium, lead, and aluminum).

Heavy metals (e.g., mercury, cadmium, arsenic, chromium, thallium, lead) are found extensively in the environment with a density greater than 5 g/cm^3 [205].

Arsenic is a natural element that is found in rocks and soil, water, and air and in plants and animals. It is one of the most toxic metals present in the natural environment. The major exposure of humans to arsenic is from contamination of drinking water from natural geological sources rather than from mining, smelting, or agricultural sources. Arsenic is present in a variety of industrial products including cosmetics, paints, fungicides, insecticides, pesticides, herbicides, wood preservatives, and cotton desiccants [206, 207].

The World Health Organization (WHO) considered exposure to arsenic (particularly from drinking water) carcinogenic to humans. Chronic arsenic exposure is known to cause various types of cancers including cancers of the skin, bladder, lung, liver, and stomach [208–210]. Long-term exposure to inorganic arsenic through inhalation or drinking water ingestion is causally related to increased risk of cancer in the lung, skin, and bladder. Inorganic arsenic can also increase the risk of kidney, liver, and prostate cancers depending on chronic exposures [211]. The underlying mechanisms of hepatocarcinogenesis caused by arsenic are summarized in Fig. 8.

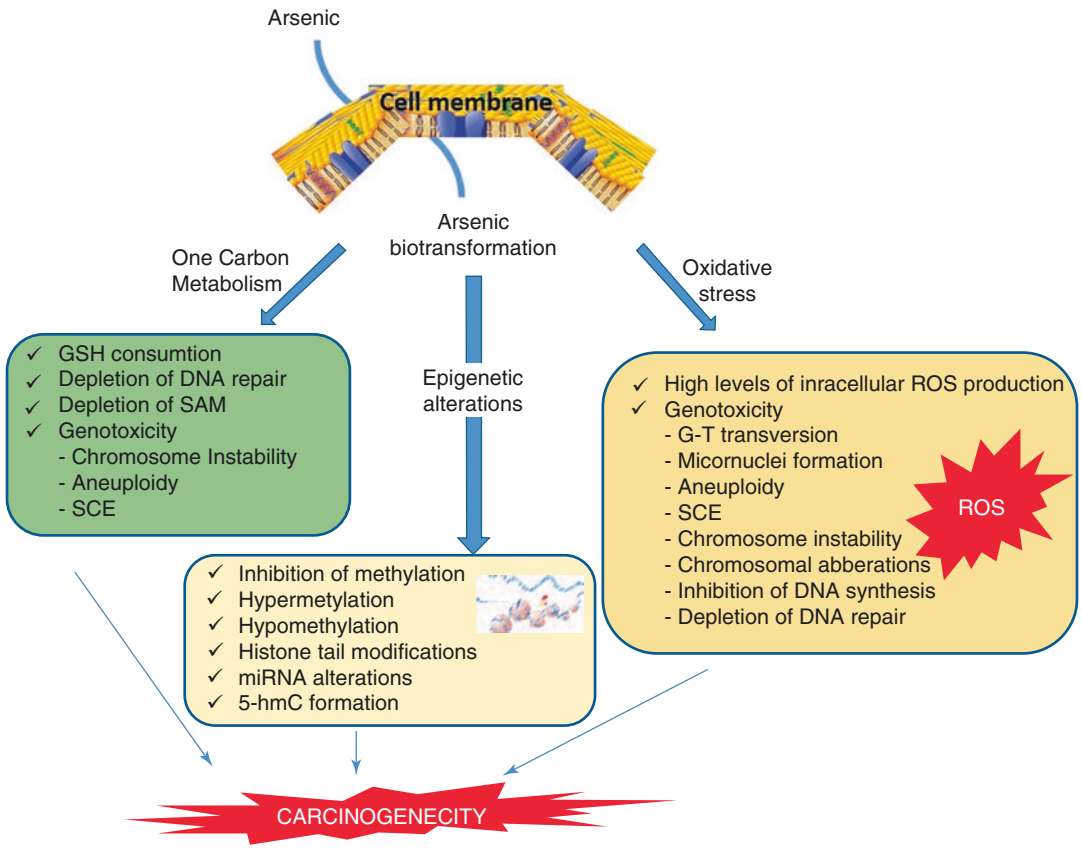

Fig. 8 The underlying mechanisms of hepatocarcinogenesis caused by arsenic. 5-hmC 5-hydroxymethylcytosine, miRNA microRNA, SAM S-adenosyl methionine, SCE sister chromatid exchange

16 Arsenic and Liver Cancer in the Middle East

A study from Simav Plain, Turkey, aimed to compare the deaths related to different arsenic levels in drinking water supplies during 2005–2010. Thus, a two-phase research was designed. Arsenic concentrations of groundwaters were determined in the range of 7.1–833.9 ppb. In the first phase research, public health surveys were conducted on 1003 villagers to determine the distribution of diseases. In the second phase, verbal autopsy surveys and official death records were used to investigate the causes of death. In total, 402 death cases were reported, and cardiovascular system diseases (44%) and cancers (15.2%) were found to be major causes of death. Cancers of the lung (44.3%), prostate (9.8%), colon (9.8%), and stomach (8.2%) were comparably higher in villages with high arsenic levels in drinking water supplies. Furthermore, the majority of cases of liver, bladder, and stomach cancers were also observed in villages with high arsenic levels [208–210].

In a comprehensive review, the researchers investigated the available 221 articles reporting contamination levels of arsenic and mercury in commonly consumed foods in Iran. The results showed that the highest contamination rates of arsenic and mercury were in rice and fish, respectively. It was stated that arsenic and mercury contents in Iranian foods could cause serious health concerns due to the consumption of high quantities of these heavy metals from the main foods [205].

In another meta-analysis on arsenic and lead in rice from many geographical areas in Iran, carcinogenic risk of these heavy metals was estimated for the consumers. The results of 21 carcinogenic risk assessment reports performed between 2008 and October 2017 suggested that the minimum and maximum incremental lifetime cancer risk (ILCR) of arsenic was higher in the 45–54 (4.53×10^{-2}) and 15–24 (5.50×10^{-2}) years' age group consumers while ILCR was higher in 45–54 (2.442×10^{-3}) and 15–24 (2.96×10^{-3}) years' age group consumers for lead. The overall carcinogenesis risk of arsenic (4.864×10^{-2}) was 18.5 times higher than lead (2.623×10^{-3}). All age group consumers were suggested to be at considerable carcinogenesis risk (ILCR>10^{-3}) due to the consumption of arsenic- and lead-contaminated rice [212].

Another study performed in Iran evaluated lead, cadmium, arsenic, and mercury contamination in cosmetic products in both legal and contraband products. The results showed that while lead, mercury, and cadmium contents in the products did not exceed the acceptable limit of the Federal Office of Consumer Protection and Food Safety of Germany (BVL), the arsenic contents of lipsticks, eye shadows, and eyebrow pencils were significantly higher than the BVL standard. The researchers concluded that the contents of arsenic in contraband eye shadows and eyebrow pencils should be taken into serious consideration by the relevant authorities [213].

Studies on the arsenicosis cases in the Middle East and several studies in different developing countries showed that the endemic arsenicosis caused by high levels of arsenic in drinking water is the main reason for blackfoot disease. In a case-control study conducted to examine the clinical characteristics of HCC patients with blackfoot disease of southwestern Taiwan, 65 HCC cases (54 men and 11 women) with blackfoot disease were examined and the clinicopathological features compared with 130 HCC control patients without blackfoot disease. Characteristics such as hepatitis viral infection status, liver function, histological findings, computed tomography scan characteristics, and patient survival were determined. The results showed no differences between HCC patients or their tumors, from study and control areas. However, the researchers observed that artesian well water contains high concentrations of arsenic in southwestern Taiwan and the mortality caused by HCC shows a dose-response increase by concentration of arsenic in the well water [214]. A study from Peru aimed to assess the extent of arsenic contamination of groundwater and surface water. For this purpose, 151 water samples were collected from 12 districts of Peru, and arsenic concentrations were measured in the laboratory using inductively coupled plasma mass spectrometry (ICP-MS). In

86% (96/111) of the groundwater samples, arsenic concentrations were higher than 10 μg/L which is the concentration guideline given by the WHO for drinking water. In 56% (62/111) of the samples, the mean concentration was 54.5 μg/L (range: 0.1–93.1). In the Juliaca and Caracoto districts, in 96% (27/28) of groundwater samples, arsenic was above the WHO guideline. The arsenic concentrations in water samples from the Rímac River running through Lima were exceeding the WHO limit [215].

In a study from Mashhad, Iran, drinking water samples were assessed for probable health risk (noncarcinogenic and carcinogenic risk) for adults and children. Arsenic and other toxic heavy metals (lead, nickel, chromium, and mercury) were determined. The main heavy metal exposure routes for Mashhad residents were suggested to be from drinking water and dermal contact. The results of the study showed that the daily heavy metal intake via water consumption was four to ten times higher than dermal contact. The health risk assessment of heavy metals was evaluated based on daily intake and exposure through dermal absorption and ingestion of drinking water by using hazard quotient (HQ), hazard index (HI), and lifetime cancer risk (CR). The chemical analysis and testing were conducted on 140 water samples. The results of the HQ values of arsenic and heavy metals for combined routes were below the safety level (HQ < 1) for adults, while the HI for children were higher than the safety limit in some stations. Similarly, chromium levels had the highest average contribution of HI of the total heavy metals (55–71.2%) for adult and children population. The average values of total carcinogenic risk (TCR) for the metals by exposure from drinking water for adults and children were 7.38×10^{-5} and 1.33×10^{-4}, respectively. For dermal exposure, the noncarcinogenic and carcinogenic risk level for arsenic and heavy metals were both higher than the American Environmental Protection Agency (US EPA) risk management criterion. On the other hand, the CR total for children and adults was at borderline or higher than the safety level of US EPA risk, and these results suggested that there was a probability of carcinogenic risk for the children and adults due to the intake of the carcinogenic heavy metals by via both ingestion and dermal route [216].

Arsenic distribution in well water has been investigated by a US Agency for International Development (USAID) Middle East Research Cooperation (MERC)-funded project. This study initiated a study that investigates the distribution of arsenic in toenails from arsenic-exposed residents through drinking water. Researchers also have collaborated with Duke Comprehensive Cancer Center and the University of California (UNC) School of Public Health for conducting interdisciplinary research in order to investigate the exposure of arsenic in Union County where the arsenic levels in private wells are very high. It has been specified by the continuous study group that the interface between inorganic isotope geochemistry and traditional epidemiological research could provide a very unique methodology for elucidating the impact of environmental hazards on human health. Preliminary results showed a significant correlation between arsenic in nails and drinking water [217].

A study conducted in Al-Kharj geothermal fields of Saudi Arabia aimed to evaluate the arsenic distribution and associated hydrogeochemical parameters in 27 randomly selected boreholes representing aquifers. Arsenic was detected at all sites, with 92.5% of boreholes yielding concentrations above the WHO permissible limit of 10 μg/L. The maximum concentration was determined as 122 μg/L. Sixty-seven percent of the total composition of the groundwater types were mainly Ca^{+2}-Mg^{+2}-Cl^{-} and Na^{+}-Cl^{-}. The main source of arsenic release as geothermal in nature was evaluated by principal component analysis (PCA). The PCA yielded five components, which accounted for 44.1%, 17.0%, 10.1%, 08.4%, and 06.5% of the total variance. The first component had positive loadings for arsenic and boron along with other hydrogeochemical parameters. The data indicated that the primary sources of arsenic mobilization were derived from regional geothermal systems and weathering of minerals. The rest of the principal components showed reductive dissolution of iron oxyhydroxides as a possible mechanism. Spatial evaluation of the PCA results

indicated that this secondary mechanism of arsenic mobilization might be active and correlated positively with total organic carbon. The aquifers were contaminated to a high degree with organic carbon ranging from 0.57 mg/L to 21.42 mg/L and showed high concentrations ranging from 8.05 mg/L to 248.2 mg/L [218].

In a study from Pakistan, arsenic concentrations were measured in drinking water samples. A total of 4547 drinking water samples were collected from 11 different districts in Punjab province of Pakistan. The variation in arsenic concentrations was very high. Average arsenic concentrations from all districts range from 6 to 12 ng/ml, with an overall average value of 8.5 ± 1.6 ng/ml which was found within the WHO recommended limit of 10 ng/ml [219].

As arsenic can cause several health problems, including cancers of the liver and skin, authorities in the Middle East should highly be concerned about the arsenic levels in Middle East. Although some studies suggest that arsenic concentration in water is not in the recommended limits given by local or global authorities, some suggest the opposite. It is known that in developing countries, well water is highly used in rural areas and arsenic or other heavy metals contaminate the water, which is also used as drinking water in these areas. Therefore, governments should routinely monitor the arsenic content of drinking water and should apply necessary restrictions.

17 Vinyl Chloride

Vinyl chloride monomer (VCM) is a synthetic gas mostly used in the manufacture of polyvinyl chloride (PVC). PVC is a widely used material in plastics. Occupational exposure to VCM is very common, especially in the VCM/PVC production and processing industry [220].

The results of a follow-up study on autoclave workers confirmed that the highest exposures to VCM caused almost tenfold increased risk of HCC compared to workers with low or no exposure [220]. Since the mid-1970s, it is known that occupational exposure to VCM causes the development of angiosarcoma of the liver [221]. VCM is suggested to be a major risk factor for HCC, and in 2007, IARC listed VCM as human carcinogen [222]. The biotransformation and genotoxic effects of VCM are summarized in Fig. 9.

18 Vinyl Chloride and Liver Cancer in the Middle East

There is very limited data on VCM exposure in Middle East although the exposure is suggested to be high, particularly in plastic manufacturing plants. A study performed in Iran evaluated 43 VCM workers who had undergone regular medical evaluation in the last 3 years. No instance of liver dysfunction or angiosarcoma was discovered. The Iranian Standards Institute reviewed the occupational health data and local conditions for VCM provisionally and later set a threshold limit value (TLV)-time weighed average (TWA) of 25 ppm for 8 h in 1976. However, the researchers suggested that such precautionary measures should be revised and improved according to available data as VCM could cause HCC and other liver-related disorders after long periods of workplace exposure [223].

In a study from Kuwait's major power station (Doha West Power Station), spatial distribution of volatile organic compounds (VOCs) was assessed. The researcher collected 24-hour integrated ambient air samples in canisters from ten locations of the stations. According to the results, VCM concentrations were the highest among the other halogenated compounds. It was concluded that halogenated VOC compounds were the dominant group of air contaminants in Kuwait [224].

19 Other Chemicals

Exposure to organic solvents (halogenated, cyclic, aromatic, or aliphatic hydrocarbons, ketones, amines, esters, alcohols, aldehydes, and ethers) is also associated with biliary duct and liver cancers [225]. Persistent organic pollutants, including dioxins, are another group of chemicals

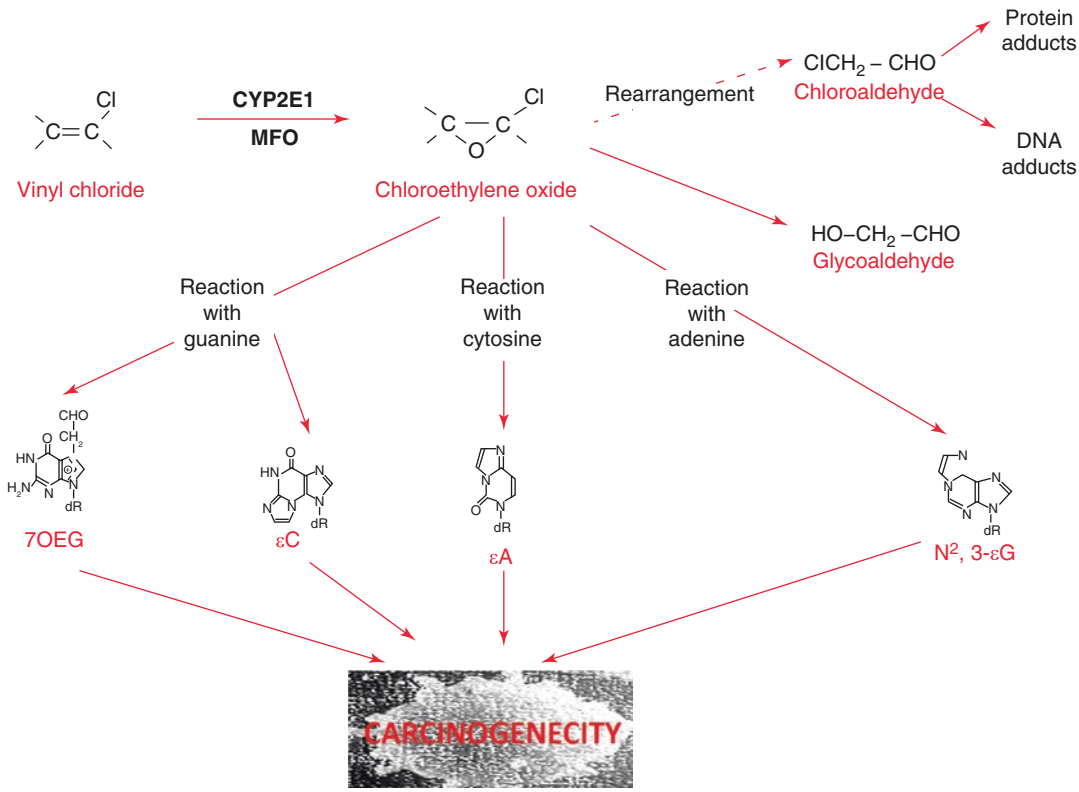

Fig. 9 The biotransformation and genotoxic effects of vinyl chloride. εA 1-N^6-ethenoadenine, εC 3-N^4-ethenocytosine, 7OEG 7-(2-oxyethyl)guanine, N^2, 3-εG N^2,3-ethenoguanine

that are suggested to induce liver pathologies (e.g., fatty liver diseases, cancer) [226].

20 Conclusion

The population attributable risk estimates for liver cancer for each of these risk factors varies among countries in Middle East. Major risk factors of HCC mainly include chronic viral hepatitis, aflatoxin exposure, alcohol consumption, smoking, exposure to inorganic arsenic through drinking water, VCM exposure, and exposure to different organic solvents. On the other hand, oral contraceptives, iron overload, advanced hepatic fibrosis, chlorinated hydrocarbons, radioactive compounds, nitrosamines, oral contraceptives, endogenous hormones, male gender, and genetics are also important risk factors [23, 191].

One or more causative agents can be identified in most HCC cases. Furthermore, HCC can generally be viewed as a complication of cirrhosis, and the incidence is highest when the factors causing liver injury and inflammation (hepatitis) continue to operate. Although chronic HBV infection and chronic HCV infection are the most important risk factors for HCC worldwide, environmental exposures (i.e., high aflatoxin exposure) can lead to HCC. Synergistic and/or additive effects between aflatoxins and viral hepatitis infections may enhance the risk of HCC. Additional studies that show the interactions between aflatoxins and HBV/HCV infections must be performed to predict possible in vivo effects. In addition, it can also be suggested that the possible presence of more than one mycotoxin in different foodstuff might also raise the risk of non-expected effects and possibly HCC in humans.

Aflatoxin and viral hepatitis risk management strategies need to be taken into account as an option to find a solution for HCC in the Middle

East region. Scientific knowledge and improved techniques for harvesting, handling, and storage will reduce or help to eliminate the contamination problem of aflatoxins. Therefore, starting from the farmers, awareness should be raised. Foods and spices should be routinely monitored for the presence of aflatoxins before production and process and prior to consumption. Surveillance on food contaminants including aflatoxins should be conducted by related ministries continuously. Governments must implement necessary measures to ensure that food not complying with the maximum levels of aflatoxins is not marketed in the Middle East and that foods that are not compliable with the country's standards should be eliminated. Governments and professional organizations should develop different education programs on both aflatoxin exposures. Such measures can also decrease the economic loss of the countries due to high aflatoxin content of foods.

Smoking and alcohol are addictions that must be eliminated from the whole world. However, due to physiological and financial problems in Middle East, cigarette use and alcohol consumption are increasing, particularly in the new generation. Due to the cigarette smoking, passive smoking, and ethanol consumption, the incidence of cancers of the lung and liver are rising in the Middle East. Children should be educated starting from primary school for the unwanted health effects and consequences of these addictions. To solve this problem, Middle East countries must implement serious measures. For instance in Turkey, public intoxication and driving under the influence have very high fines. Smoking in cafes and restaurants in inner doors was restricted.

Arsenic contamination in water has been of concern of many governments for decades. Arsenic is one of the important causes of HCC and other types of cancer. However, this problem is still claiming the lives of many in the Middle East as water sources are scarce and serious cleaning procedures are not present in many countries of the region. Therefore, governments must convey financial sources to supply clean and drinkable water to their citizens, and regulatory authorities should investigate the water continuously.

Workplace exposures are always serious underlying causes of different types of cancers. VCM is a known human carcinogen that particularly causes liver tumors and angiosarcomas. Workplace investigations, especially in VCM-producing plants, should be performed frequently and liver parameters should be checked routinely. Middle East countries should perform routine air pollution assessments and particularly check VCM levels in the ambient air.

In conclusion, Middle East countries should implement serious measures for the prevention of HCC-causing factors. The air, water, and food quality should be checked routinely, and citizens should be educated by different governmental and professional programs to avoid consuming food with high aflatoxin content, cigarette use, and alcohol consumption. Moreover, workplaces should be under serious inspection in order to prevent workplace exposures.

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Obesity and Hepatocellular Carcinoma: Epidemiology and Mechanisms

Hikmet Akkiz

Abbreviations

ATG	Autophagy-related protein
BMI	Body mass index
CAFs	Cancer-associated fibroblasts
CD	Choline deficiency
CDAA	Choline-deficient and amino acid defined C
CREB-H	Cyclic-adenosine monophosphate responsive element-binding protein-H
CTGF	Connective tissue growth factor
DCA	Deoxycholic acid
DCs	Dendritic cells
eIF2	Eukaryotic initiation factor 2
FTO gene	Fat mass and obesity-associated gene
FXR	Farnesoid X receptor
GWAS	Genome-wide association studies
HCC	Hepatocellular carcinoma
JAK/STAT	Janus kinase/activator of transcription pathway
LCA	Lithocholic acid
lep	Leptin
lepr	Leptin receptor
MAMPs	Microbial-associated molecular patterns
Mc4r	Melanocortin 4 receptor
MDSCs	Myeloid-derived suppressor cells
NAFLD	Nonalcoholic fatty liver disease
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
NASH	Nonalcoholic steatohepatitis
NCD	Noncommunicable disease
NIDDK	National Institute of Diabetes and Digestive and Kidney Disease
OPN	Osteopontin
PAMPs	Pathogen-associated molecular patterns
PAT	Perilipin,adipophilin,T1p47
PDGF	Platelet-derived growth factor
PNPLA3	Patatin-like phospholipase domain-containing 3
Pomc	Pro-opiomelanocortin
PPAR-gamma	Peroxisome proliferator-activated receptor gamma
RANTES	Regulated upon activation normal T cell expressed and secreted
SNPs	Single-nucleotide polymorphisms
SREBP	Sterol regulatory element-binding protein
T2DM	Type 2 diabetes mellitus
TCPTP	T-cell protein tyrosine phosphatase
TERT	Telomerase reverse transcriptase

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TGFB	Transforming growth factor beta
TLR-4	Toll-like receptor-4
TM6SF2	Transmembrane 6 superfamily member 2
TZD	Thiazolidinedione
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

1 Introduction

Noncommunicable diseases (NCDs) that include cardiovascular diseases, cancer, and diabetes mellitus are responsible for >70% of early deaths worldwide. NCD represents the leading cause of mortality and premature disability [1, 2]. The WHO considers obesity to be excessive fat accumulation that causes many diseases. Obesity is diagnosed at a BMI >30 kg/m^2 [1], and obesity has significant impact on a variety of tissues including the vascular bed [3]. However, the impact of obesity varies widely among subjects even with a similar degree of body mass index (BMI). Genetic and other factors including gender, age, ethnicity, cardiorespiratory fitness, and the body fat distribution may play a role in the development of obesity [1]. Recent studies showed that the metabolic impact of obesity on nonalcoholic fatty liver disease (NAFLD) may also vary among patients with a similar BMI [1, 4, 5]. Obesity is associated with decreased life expectancy. Depending on the severity of obesity and comorbid disorders, obesity may decrease life expectancy by 5–20 years [3, 6]. Obesity is also associated with an increased risk of type 2 diabetes mellitus (T2DM), NAFLD, cardiovascular diseases (such as hypertension and myocardial infarction), osteoarthritis, Alzheimer's disease, and some cancers such as those of the breast, liver, and colon [1, 3, 6]. In addition, obesity may decrease quality of life and promote unemployment, lower productivity, and encourage social disadvantages [1, 3]. For example, osteoarthritis, a common complication of obesity, often leads to disability and early retirement [1].

It is clear that the fundamental cause of obesity is an energy imbalance between the caloric intake and energy expenditure [1, 3, 6]. Obesity develops as a result of low physical activity, sedentary lifestyle, and the overconsumption high-energy foods [1, 3]. Several factors including socioeconomic status, environment, personal behaviors, and genotype-phenotype interactions affect food intake, nutrient turnover, thermogenesis, and lipid utilization of fatty acid [1, 6]. The World Obesity Federation and other organizations including the American and Canadian Medical Associations have identified obesity to be a chronic progressive disease [7]. Reducing the obesity prevalence is among the primary goals of the WHO [7]. The WHO targets a halting of obesity prevalence to 2011 level [1]. The meeting of UN General Assembly on the prevention and control of NCD which has been held on September 2011, highlighted the importance of reducing unhealthy diet and physical inactivity in the development and prevention of NCD [8].

To determine the obesity, different methods have been used including assessment based on anthropometry, bioelectrical impedance analysis, densitometry, and imaging-based methods. Body mass index (BMI) is the most commonly used tool to determine obesity [1, 3, 6]. BMI grossly estimates adiposity and identifies overweight and obesity based on the weight of the individual expressed in kilograms (kg) and divided by the square of the height in meters (m^2) [3]. According to the WHO classification, undernutrition is defined as BMI <18.5 kg/m^2, normal weight as BMI 18.5–24.9 kg/m^2, overweight as BMI 25–29.9 kg/m^2, obesity as BMI >30 kg/m^2, and morbid obesity as BMI >40 kg/m^2 [3]. BMI could be complemented by measuring waist circumference to discriminate between subcutaneous obesity and visceral obesity [3]. It has been shown that low hip fat may protect against diseases; the ratio of waist-to-hip circumferences and the waist-to-height ratio have also been proposed to refine risk assessment. However, BMI is a most commonly used tool to determine the obesity worldwide [3].

Different approaches and treatments at the individual level have been developed over the last

two decades. Dietary education and control, physical activity programs, pharmacotherapy, and bariatric surgery have been recommended to patients. Bariatric surgery has been found to be associated with metabolic improvements including halting or reversing the progression of T2DM independent of weight loss, but adverse effects have also been reported [9]. Currently, 39% of world population is obese or overweight despite efforts to halt the progress of the epidemy [1, 3]. The economic burden of obesity is approximately US 2 trillion. To halt the epidemic, individualized treatment modalities such as precision lifestyle modifications should be complemented with wider population-based approaches and solutions [1, 3]. Currently, researchers started genotype and phenotype analysis in approximately one million obese people in the UK and the USA. In addition, public health strategies have been applied such as taxation to reduce unhealthy fats and added sugar consumption and personalized precision nutrition approaches.

2 Epidemiology of Obesity

During the past 50 years, the prevalence of obesity has increased dramatically worldwide and reached pandemic levels. Researchers from the NCD Risk Factors Collaboration have published a report that explains the causes of the obesity to reach pandemic levels in the past 50 years [1, 10].

The WHO reported that more than 2.1 billion adults were overweight or obese worldwide in 2014. Among them, 1.5 billion were overweight and 640 million were obese [11]. The estimated age-standardized prevalence, of obesity in 2014 was 10.8% among adult men and 14.9% among adult women [1, 10]. These data demonstrated that female gender has been associated with higher risk of obesity. Overweight is more frequent among men [10]. However, according to the Global Burden of Disease Study 2013, the prevalence of obesity and overweight was similar to men and women, to be >36% in both genders [12]. Studies published in the USA have demonstrated that African Americans have a higher prevalence of morbid obesity than other ethnicities [13]. Asian populations have lower BMI than white individuals; however, they have been frequently found to be associated with visceral fat deposition. These features make Asian populations more susceptible to developing type 2 diabetes mellitus at lower BMI levels than white individuals [3, 14].

Between 1980 and 2008, the global age-standardized mean for BMI increased by 0.4 (in men) and 0.5 kg/m^2 (in women) per decade [15]. Between 1975 and 2014, the prevalence of obesity increased from 3.2% to 10.8% in adult men and from 6.4% to 14.9% in adult women [12]. In 2014, 0.64% of men and 1.6% of women had morbid obesity. Between 1975 and 2014, BMI has not changed in adults in North Korea;, however, some countries in sub-Saharan Africa had an obesity prevalence of >30% at the same period [16]. BMI and obesity prevalence dynamics change between countries. Like America and Europe, in many countries, the number of overweight or obese adults has been found to be greater than the number of normal-weight adults [16] (Fig. 1). The rate of BMI increase has been slowing down since 2000 in high-income and some middle-income countries both in children and in adult [1, 16]. It is considered that the adverse consequences of obesity will pose greater threats for public health than hunger or malnutrition [3]. In 2013–2014, the worldwide number of children and adolescents (2–19 years of age) who are obese has been estimated to be 110 million. This number has increased exponentially since 1980 [3]. Furthermore, estimated age-standardized prevalence of obesity in 2014 has been found to be 5% among children [12]. Cross-national analysis of trends in overweight and obesity for both boys and girls (11–15 years of age) in North America and in Europe from 2002 to 2010 has demonstrated a stabilization in overweight prevalence;, however, overall rates of overweight in many countries have been found to be high [3]. Childhood obesity is associated with metabolic complications and chronic disease in adulthood [17].

The prevalence of NAFLD in the general population of North America has been estimated to be approximately 24%. Conversely, the prevalence of NAFLD in South America is 32% [4, 18].

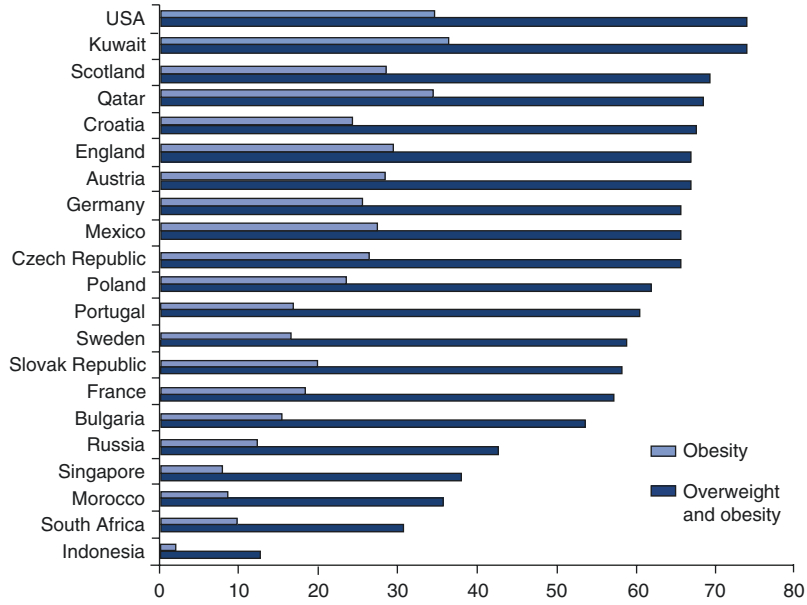

Fig. 1 Countries with the highest adult prevalence rate of overweight and obesity

The prevalence of NAFLD varies among countries depending on obesity. The lowest prevalence of NAFLD has been reported from Peru where obesity prevalence has been found to be only 15% [4, 9]. Other factors such as genetic factors can contribute to the prevalence and outcomes of NAFLD. A study demonstrated that Hispanics of Mexican origin had higher prevalence of NAFLD (33%) than Hispanics of Dominican origin (16%) [9]. The prevalence of NAFLD has been estimated to be 20–30% in the European Union [18]. Approximately 3% of NAFLD patients have been found to be associated with NASH. The prevalence of NAFLD varies among European countries. In Western Europe, the prevalence of NAFLD by ultrasound has been found to be higher (25–30%) than Eastern Europe (20–22%) [4, 8] (Fig. 2). Risk factors for NAFLD (obesity and T2DM) are increasing in Europe. NAFLD prevalence in Asia varies from 15% to 40% and NASH ranges from 2% to 3% [4]. The prevalence of NAFLD in India increased from 28% in 2015 to 31% in 2016. The prevalence of NAFLD in China has been increasing from 3.8% in 1995 to 43.65% in 2015 among Shanghai adults [4]. Lean NAFLD is more common in Asian countries than in western countries. The prevalence of NAFLD in adult population is increasing in the Middle East and in Turkey. A recent meta-analysis suggested that prevalence of NAFLD in the Middle East is 31.79% [18]. Data from Iran suggest that 33.9% of population have NAFLD [18]. A cross-sectional population-based study including 352 individuals from Israel suggests a prevalence of 30% for NAFLD detected by ultrasound. Another cross-sectional study including 483 individuals from Iran demonstrated a 39.3% prevalence of NAFLD. In addition to data from Israel and Iran, NAFLD prevalence is increasing in Turkey. In a recent unpublished epidemiological study conducted in 113,239 healthy individuals in Turkey, the overall prevalence of NAFLD has been found to be 48.3%. The prevalence was higher in those older than 50, in males, and in those with BMI >25 kg/m^2. A rapidly increasing prevalence of NAFLD in Turkey has been found to be associated with the epidemic of obesity and T2DM in this region [4]. There is a significant paucity of data from the rest of the Middle East. However, the rates of obesity and T2DM in the Middle East are quickly increasing. There are few studies on the epidemiology of NAFLD from Africa. A

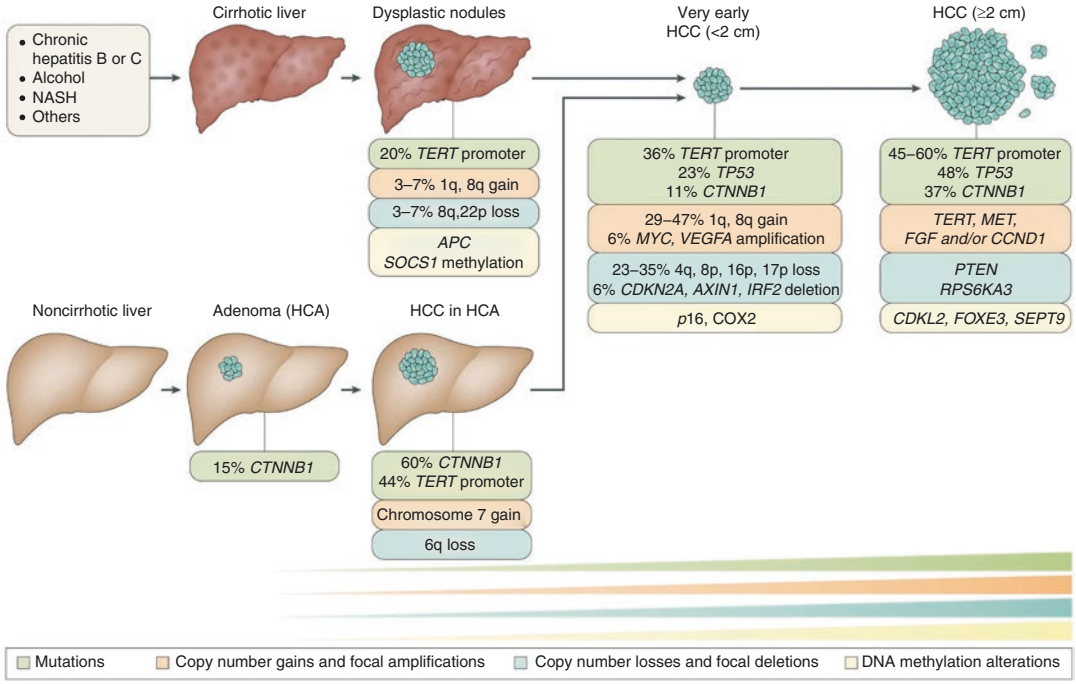

Fig. 2 Hepatocellular carcinoma in cirrhotic and noncirrhotic (adenoma-carcinoma sequence) livers. Most hepatocellular carcinoma develop in cirrhotic liver. Molecular alterations can be detected in dysplastic nodules and early HCC. TERT promoter mutations are the first and most common genetic changes in the hepatocarcinogenesis. TP53 and CTNNB1 mutations develop relatively early in hepatocarcinogenesis. As the process progresses, additional molecular alterations are acquired, including focal DNA amplifications and deletions and DNA methylation of promoter regions. In hepatic adenoma, CTNNB1 mutations are associated with an increased risk of malignant transformation. TERT promoter mutations occur later in hepatic adenoma-related HCC than in cirrhosis

meta-analysis reported the prevalence of NAFLD in Africa to be 13.48% [18].

3 Risk Factors for Obesity

There have been two major drivers of obesity: overeating and sedentary lifestyle. A small daily positive energy balance may provide significant contribution to cumulative weight gain. However, the pathogenesis of obesity is considered to be more complex [3].

Relevant epidemiological approaches such as integrated bioinformatics system analysis, can be used to better understand the causes of obesity, including the intricate interplay between behavioral, environmental, physiological, genetic, social, and economic factors [19]. Body size preference may lead to the development of obesity [1]. Until the beginning of the last century, obesity has been thought to be a symbol of beauty, health, and wealth [1]. During periods of famine, being overweight was a protective factor. In some societies, being obese or overweight provides an advantage to a person for marriage. Obesity has been believed to make people more attractive. In some countries such as the Pacific Islands, a large body size is thought attractive [1]. Among Japanese, obesity develops more rapidly, where social norms favor a small body. The increase in the obesity prevalence started in developed countries in the 1970s, followed by developing countries and more recently by some low-income countries [20]. In Brazil and other developing countries, obesity prevalence typically increases first in people with higher economic status in urban areas, and then the obesity prevalence started increasing in groups of lower socioeconomic status living in rural areas [1, 20].

Since the early 2000s in some developed countries, including France, Norway, Denmark, Sweden, the USA, Japan, and Australia, the prevalence of childhood overweight and obesity has been slowing down [16]. The stable childhood obesity prevalence in developed countries could show that the incidence of new obesity cases continues to be at the same high level or less likely that the duration of obesity has been shortened [1]. However, the great heterogeneity in obesity prevalence between and within countries cannot be explained by only economic situation. Ethnic and other factors may play a role in the obesity prevalence. Obesity prevalence ranges from <5% in countries such as Vietnam, Bangladesh, Laos, and Japan to >50% in Polynesian and Micronesian Islands such as Nauru, Tonga, and Samoa. The significant differences of obesity prevalence between societies indicate the relevance of individual genetic and environmental factors in the development of obesity [16].

As mentioned above, there have been relevant differences between countries in obesity prevalence. There are two key questions regarding obesity: even under same economic situation, why the frequency of obesity varies and how known factors affect certain groups of society differently [1]. Large regional differences in obesity prevalence have been observed in Germany ranging from 29% in cities in northwestern Germany to >29% in Saxony-Anhalt [21]. These regional differences may be related to differences in socioeconomic status, high economic disparity between cities and rural areas, and differences in some measures of sedentary and eating behavior [1, 21]. Regional differences in obesity prevalence have also been reported from the USA. While the lowest rates are in countries of west and northeast, highest prevalence of obesity is observed in the south [22]. In this study, ethnicity, physician density, poverty, unemployment, number of food restaurants per 1000 people and access to supermarket, living in small-town settings, community characteristics such as cultural norm- and values-related diet, physical activity, and ideal weight and body image unique to particular regions or demographic groups have been shown to be associated with obesity outcomes [22]. Economic differences within the society may have a role in the heterogeneity in obesity prevalence and obesity burden [1, 22]. Paradoxically, in the USA, obesity is highly associated with poor black Americans. They eat cheap highly calorific foods at the fast-food chains.

Local environment affects obesity prevalence. Even within a city, significant regional differences in obesity prevalence can occur. In the city of Kiel located in western Germany, obesity has been found to be more prevalent in neighborhoods with increased frequency of overweight and obese parents, overweight siblings, parental smoking, single parenthood, low socioeconomic status, low physical activity in boys, and high media consumption in girls [1, 23]. The local environment may significantly modulate the risk of developing obesity at the individual level. Environment density of fast-food chains, food cultures, transport systems, walkability of the neighborhoods, and active recreation opportunities have been considered to be relevant obesogenic moderators that can have a great influence on obesity in the local and country context [1, 10]. In China, rapid urbanization and increasing number of people using motorized forms of transportation can be some of the main causes of the obesity pandemic [24]. The role of the neighborhood relationships in the development of obesity was investigated in a social experiment [25]. The prevalence of morbid obesity can be reduced by migration of families from the environment with a high obesity prevalence and rich area to a wealthier area. This finding suggest that local microenvironment factors modulate the individual obesity risk [1, 25].

Food marketing including foods or beverages containing high fat and sugar modulates the behavior of children. During advertisements, children consume more energy-dense foods and beverages [26]. Increased food intake as a result of food advertisements may be related to genetic factor in children. Children carrying a high-risk single-nucleotide polymorphism in the fat mass and obesity-associated gene are more sensitive to food marketing than wild-type allele carriers [27]. Among genes that account for BMI variability, obesity-associated gene was the strongest genetic

factor associated with obesity [27]. Several studies have indicated that carriers with obesity-associated gene risk alleles might have decreased satiety responsiveness and excess energy consumption [27, 28]. These data also support a central role of the brain in the modulation of food intake [29]. There have been several potential drivers of obesity pandemic. Unfortunately, we live in obesogenic environments that affect our behavior and lifestyle choices. The obesity prevalence has increased dramatically during the last 50 years worldwide. The pandemic level of obesity is the result of a reduction in home cooking, greater reliance on convenience foods, reduced physical activity, computer-based working, a growing habit of snack consumption, and more persuasive food marketing. In addition, the food industry targets to maximize profits and promotes large portions [30].

The westernization of lifestyle has been considered to be key driver of obesity. That lifestyles lead to obesity more rapidly and to increase in obesity prevalence in populations that do not have time to adapt to these changes. The obesity prevalence is much lower in Pima Indians living in Mexico than in those living in the USA (Arizona). This finding suggests that even in genetically related populations, environmental factors are major determinants in the development of obesity [31]. Another example showing the relevance of environmental factors is, people from Nigeria living in the USA have 20–25% higher mean BMI than the average BMI of Nigerian men and women living in Nigeria [32]. The increase in obesity prevalence has been observed in middle-income countries in which changes in environment and behavior occurred particularly rapidly. For example, obesity prevalence in Jamaica increased more rapidly between 1995 and 2005 than the USA and Nigeria [33]. The relevant difference in obesity prevalence between countries indicates an influence of the local environmental factors on key drivers of obesity pandemic [34]. Changes in the global food system combined with sedentary behaviors are considered to be main factors causing the increase in the obesity prevalence worldwide over the past 50 years [1, 3, 31–33].

Currently, people living in Pacific Islands such as Nauru and the Cook Islands have the highest obesity prevalence in the world [12, 16]. Obesity occurred rapidly in Nauru and Cook Islands in the second half of the past century [12, 16]. Several factors including genetic predisposition, their geographic location, and their lack of capacity to produce sufficient food supplies for own market have been implicated to promote the high susceptibility to emerging obesity in these people [35]. In addition, small, closely networking island societies may be more susceptible to social changes, global markets, and food marketing which may have facilitated the rapid social changes. These changes have been clearly observed in Pacific Islands [36]. Studies conducted on people living in Nauru and Cook Islands have demonstrated that obesity may develop when rapid social changes are introduced to populations with a high degree of interdependence. Obesity prevalence in Cuba declined during the economic crisis that happened in the early 1990s. This finding suggests that obesity is not primarily a product of individual choice and independence [37].

In developed countries, the technical revolution of the past century with mechanization, new modes of transportation, and computerization caused a decrease in energy demands [20]. However, these changes had already started at the beginning of 1900s, whereas the dramatic increase in obesity prevalence emerged from the 1970s onward [1, 38]. Therefore, it was hypothesized that in the most developed countries, energy balance at the population level is characterized by an energy flipping point [20]. In developed countries, this flipping points emerged at the time when the food supply for refined carbohydrates and fats markedly increased between the 1960s and 1970s [39]. In the first half of the past century, decreasing energy expenditure was paralleled by decreasing energy intake despite stable or decreasing energy demands [20]. The availability of cheap and plentiful foods is also reflected by a progressive increase in food waste. However, food supply and food waste data may provide only indirect evidence for the hypothesis that the global system is the main driver of the

obesity pandemic [1]. Environmental and social mechanisms contributing to a continuous decline in energy demands might also contribute to a switch in a populations' energy balance. Changes in body weight of children predicted from increased US food energy supply between the 1970s and 2000s were identical to the measured individual weight gain during that period [1, 38].

Genetic and epigenetic factors may have an important role in the development of obesity. Up to 70% of the interindividual variation in body weight variability may be due to genetic differences between individuals [3]. Genes that determine the susceptibility to obesity can provide information on pathophysiological mechanisms that regulate body weight and fat distribution [3]. Study on monogenic obesity has provided relevant information on biology of obesity in the general population [3]. The molecular mapping of mutations causing monogenic obesity in mice has been one of the first strategies to detect genes that control body weight [3]. The fiindings of these approaches are the finding of the genes-encoding leptin (Lep) and its receptor (Lepr), the melanocortin 4 receptor (Mc4r) and pro -opiomelanocortin (Pomc) affect body weight through in the central nerveous system [40]. Mutations in these genes lead to monogenic obesity. Genome-wide association studies (GWAS) have identified >300 genetic loci for obesity trait. GWAS discovered a common noncoding variant in FTO locus that showed significant association with obesity risk [41]. Studies demonstrated that the FTO locus may regulate the expression of nearby RPGR1P11 or distant IRX3-IRX-5 to influence body weight by regulating appetite, thermogenesis, adipocyte browning, and epigenetic mechanisms related to obesity [42]. Additional GWAS have identified genetic loci associated with adiposity traits, BMI, and waist-to-hip ratio [3, 42].

Epigenetic mechanisms including DNA methylation, histone modification, and noncoding RNAs that modulate gene expression without changing the DNA sequence are sensitive to external factors such as diet and physical activity. Additionally, internal factors such as hormones and genetic factors may affect epigenetic mechanisms [1, 3]. Epigenetic processes are cell, time, and tissue specific. Therefore, it is extremely difficult to study the role of epigenetic mechanisms in some disease. This situation is particularly true for obesity. The role of epigenetics in obesity has been studied mostly in biological models [43]. Sons born to women who were starved during the first half of pregnancy during the Dutch famine have been at a significantly higher risk of developing obesity than comparable subjects. This was later found to be related to epigenetic modifications [44]. Nutritional deprivation of pregnant mothers can have lasting nongenetic effects on body weight of the next generation. Although the methylation of the gene encoding hypoxia-inducible transcription factor 3A (HITF3A) has been considered to be associated with higher BMI, this association was found to be the consequence and not the cause of higher BMI [45]. However, HIF system comprises a key part in energy expenditure and obesity [46]. Two epigenetic studies, involving 10,000 and 7800 individuals, respectively, identified large numbers of DNA methylation loci associated with BMI [47].

4 Adipose Tissue Dysfunction, Insulin Resistance, and Inflammation

Many factors such as adipocyte number, adipocyte size, the overall hormonal microenvironment, and cross talk with other cell types within the adipose tissue bed affect adipocyte function [6]. Fat cells derive from multipotent mesenchymal stem cells that develop into adipoblasts and then to preadipose cells [6]. Maturation is directly related to a complex signal system. Upregulation by peroxisome proliferator**-**activated receptor gamma (PPAR-gamma) is essential. Other transcription factors such as sterol regulatory element-binding protein 1c, CCAAT-enhancer binding proteins, and bone morphogenetic proteins contribute to this process [6]. These transcription factors provide adipocytes significant plasticity and a relevant ability to adapt to overfeeding by means of hypertrophy and hyperplasia. Therefore, adipose tissue might be considered primarily to be

protective tissue that prevents excessive exposure of other organs to fatty acids [6].

The first adaptation in adults to prevent systemic lipotoxicity from the excess calories is hypertrophy. The hypertrophy is followed by a longer-term compensatory mechanism involving fat cell replication (hyperplasia), the predominant mechanism in childhood obesity [48]. Hypertrophic adipocytes develop a gene expression pattern and produce adipokines in foam cells, the fat-loaded activated macrophages that are found in arterial plaques [6, 48, 49]. Protection from excess calories and triglyceride accumulation in the liver, muscle, and pancreatic beta cells requires an extraordinary adaptation by adipocytes that involves activation of several inflammatory pathways [6, 48–50]. The studies demonstrated that the most important pathways in obesity are the NFkB pathway in which free fatty acids (FFAs) activate Toll-like receptor (TLR)-4 in macrophages and adipocytes, the c-Jun N terminal kinase/activator protein 1 pathway, where insulin signaling is inhibited in the presence of tumor necrosis factor (TNF)-alpha, the cyclic-adenosine monophosphate responsive element-binding protein H (CREB-H) pathway, which promotes the secretion of acute-phase proteins such as C-reactive protein and generation of reactive oxygen species, and the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. Excess fatty acids, reactive oxygen species, endoplasmic reticulum stress, and lipid by-products such as diacylglycerol and ceramide activate these pathways, and the pathways are inhibited by adiponectin and thiazolidinediones (TZDs) [51].

Many studies demonstrated that there is a close relationship between hypertrophic insulin resistance and lipotoxicity-induced steatohepatitis. Adipocytes represent only approximately 70% of the total adipose mass [6, 52]. The other components of adipose tissue are endothelial cells, pericytes, fibroblasts, early mesenchymal cells, preadipocytes, and macrophages that play a relevant role in autocrine-paracrine regulation of fat metabolism [6, 52, 53]. Adipocytes and the stromal vascular fraction produce hormones, complement factors, cytokines such as TNF-a, interleukins (Ils), chemokines including monocyte chemoattractant protein 1, enzymes, and peptides known collectively to be adipokines that were previously believed to be secreted only by macrophages [52, 53]. Adipokines include growth factors such as insulin-like growth factor I, transforming growth factor-B, bone morphogenetic proteins, and angiogenic hypoxia-inducible factor 1a and vascular epithelial growth factor [6, 54]. Adipocytes also produce some hormones that regulate glucose and lipid metabolism including angiotensin II, estrogens, glucocorticoids, PPARs, leptin, visfatin, resistin, and retinol-binding protein [6, 54]. Decreased secretion of adiponectin in obesity changes lipid metabolism and insulin sensitivity in the liver. Administration of recombinant adiponectin to adiponectin-deficient obese mice fed high-fat diet dramatically induces hepatomegaly, steatosis, and inflammation. However, ob/ob mice overexpressing adiponectin have been rescued from insulin resistance and diabetes despite a pathological expansion in adipose tissue [54]. In NASH, decreased secretion of adiponectin may promote steatohepatitis and fibrosis. Reducing insulin resistance in the adipose tissue and increasing plasma adiponectin level by TZDs improve lipotoxicity and steatohepatitis in NASH patients [55].

Macrophages have been demonstrated to be key cellular drivers in the onset and progression of NASH. Macrophages are key players of the innate immune system and in the liver comprise Kupffer cells and recruited monocyte-derived macrophages. Liver-resident macrophages play a central role in NASH progression [6, 56].

Understanding the biological and environmental factors that drive the progression to NASH and HCC is fundamentally to the development of robust methods for diagnosis, risk stratification, and therapy. The recent epidemic of chronic liver disease is related to the burden of NAFLD, paralleling the worldwide increase of obesity. It is generally believed that adipose tissue insulin resistance plays a pivotal role in the onset and progression of NAFLD [6, 56]. Briefly, weight gain leads to expansion of adipose tissue and recruitment of macrophages through the secretion of various

chemo- and cytokines [6, 56]. Inflamed and dysfunctional adipose tissue actively releases free FFAs into the bloodstream;, promotes lipotoxicity in the liver, muscle, and pancreas; and contributes to systemic inflammation [6, 56]. In normal liver, resident macrophages or Kupffer cells (KCs) play important roles through cross talk with the different cell types and particularly with hepatocytes. The pro-inflammatory polarization of hepatic macrophages is considered a hallmark of progressive disease in the liver of NASH patients and an attractive therapeutic target [6]. Hepatic lipid accumulation facilitates pro-inflammatory KC polarization possibly as a consequence of FFA excess or signals from surrounding steatotic hepatocytes, such as histidine-rich glycoprotein extracellular vesicles or damaged-associated molecular pattern [6]. More recently, data derived from animal models and in vitro studies suggest that both pro-inflammatory KCs and recruited hepatic macrophages contribute to decreased hepatic insulin sensitivity by inhibiting insulin signaling and activating hepatic glucose production [56].

Macrophages are an important component of adipose tissue. Monocyte chemoattractant protein 1, macrophage inhibitory factor, and necrotic fat cells are powerful stimulants for adipose tissue macrophage recruitment [6, 56]. Activation of adipose tissue macrophages plays a pivotal role in adipocyte dysfunction, adipose tissue insulin resistance, release of excess FFAs into the circulation, and ectopic fat deposition in the liver [6, 57]. There are two types of macrophages: M1 macrophages play a key role in humoral immunity and response to common pathogens that secrete large amounts of pro-inflammatory cytokines including TNF-a, nitric oxide synthase, C-C chemokine receptor 2 and IL-12, M2 macrophages are considered to be tumor associated phynotype that have anti-inflammatory role in the adipose tissue [6, 57]. An increased number of M1 macrophages have been detected in animal fed a high-fat diet and human obesity. Kupffer cells are considered to be adipose tissue macrophages in the liver. Recent studies have identified the dual role of macrophages in the development HCC. In nonalcoholic steatohepatitis mouse model, in which endoplasmic reticulum stress was enhanced in hepatocytes, infiltrating macrophages not only produced TNFa which was associated with enhanced lipogenesis, but more importantly TNF receptor 1 signaling on hepatocytes was promoted and resulted in tumor growth [57]. M1 macrophages differentiate into M2 macrophages in patients with chronic liver disease. Experimental depletion of Kupffer cells prevents high fat-induced and alcohol-induced hepatic steatosis and inflammation in rodents [57]. Adipose tissue macrophage activation develops before Kupffer cell activation. When C57BL/6 mice are fed a high-fat diet, macrophage activation and gene expression of pro-inflammatory cytokines including IL-1B, IL-1R, TNF-a, and TGFB occur quickly in adipose tissue, and animals develop steatohepatitis [57]. M1 macrophage liver infiltration and steatohepatitis develop weeks after adipose tissue dysregulation. Given together, M1/M2 Kupffer cell imbalance is critical to the pathogenesis of NASH and HCC [57].

Adipose tissue consists of adipocytes, macrophages, and other immune cells that have a relevant role in the autocrine-paracrine regulation of adipocytes. In obesity, activation of macrophages promotes the development of dysfunctional, insulin-resistant adipocytes that release excessive amounts of FFAs and cause insulin resistance and lipoapoptosis in the liver, muscle, and pancreatic beta cells. Accumulation of triglyceride-derived toxic lipid metabolites activates inflammatory pathways within hepatocytes and Kupffer cells and other immune cells. Activation of hepatic stellate cells leads to cirrhosis. PPAR-gamma is detected in many cells including adipocytes, hepatocytes, and hepatic stellate cells as well as macrophages and immune cells infiltrating adipose tissue and liver that may be targeted by PPAR-g agonist during TZD treatment in patients with NASH [6].

5 The Molecular Mechanisms of Obesity-Related Hepatocellular Carcinoma

HCC incidence has dramatically increased over the past two decades. The rising incidence of HCC has paralleled an increased prevalence of obesity. Obesity promotes the production of

pro-inflammatory cytokines leading to accumulation of free fatty acids in hepatocytes [58, 59]. This increase has mirrored worsening of the obesity epidemic that affected worldwide. As the rates of obesity continue to rise, NAFLD has become the most common form of chronic liver diseases. One meta-analysis, collecting data from 45 imaging studies, reported an estimated NAFLD prevalence in the global population at 25.24% with a consistent rise in the past decade from 15% in 2005 to 25% in 2010 [58, 60]. NAFLD is strongly associated with metabolic syndrome (obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension). The prevalence of NAFLD is, therefore, dramatically increased in these patient groups. NASH and HCC also increase with a number of metabolic risk factors a patient exhibits [18, 60]. Data highlighting that HCC develops on the NASH background came during the early 1990s. A study from the USA has demonstrated the yearly cumulative incidence of HCC to be 2.6% in patients with NASH-associated cirrhosis compared with 4% in patients with HCV-related cirrhosis [60]. A large US healthcare study found NAFD or NASH to be the most common risk factor for HCC, and NAFLD-related HCC has been recognized to be an emerging indication for liver transplantation [60].

A retrospective study from Korea monitoring 25,947 individuals over an period of 7.5 years reported that NAFLD patients showed a higher HCC incidence than patients without NAFLD (23.1 versus 0.9 per 100, 000 person years). A high NAFLD fibrosis has been associated with the development of HCC [61]. The annual incidence rate of HCC in Japanese patients with NAFLD was similar to that previously reported for the USA, ranging from 0.043% to 0.0627%. In Europe, in countries with high HCV-related HCC incidence, such as Italy or Spain, the prevalence of NAFLD-related HCC has been found to be less noticeable [61]. Conversely, in the UK where the prevalence of viral hepatitis was low and consequently HCC incidence was historically low, a drastic increase in incidence has been noted. NAFLD-associated HCC was documented to be a year-on-year increase [61]. This pattern has also been observed in other studies with the prevalence of NAFLD-related HCC increasing from 2.6% to 19.5% in a French cohort of patients who underwent resection. Some studies reported that survival in patients with NAFLD-related HCC is lower (25.5 months) than that of patients with HCV-related HCC (33.7 months) [61]. Data suggest that precirrhotic NAFLD might confer an increased risk for HCC, independent of cirrhosis NAFLD-associated HCC may exhibit atypical clinical pattern. A large healthcare study suggested that only 46% of patients with NAFLD-/NASH-associated HCC have underlying cirrhosis. Piscaglia et al. have published a prospective multicenter prospective study including patients with NAFLD-related HCC and suggested that NAFLD-associated HCC is more often detected at a later tumor stage and could develop in the absence of cirrhosis but the patients have similar survival rate compared to HCV-related HCC patients [62]. The research group reported that 46.2% of NAFLD-related HCC developed on a noncirrhotic background. Conversely, 97.2% of HCV-related HCC occur in cirrhotic liver. Similar findings have been reported in a German and a Japanese study in which 41.7% and 49% of NAFLD-related HCC patients developed in a noncirrhotic liver, respectively [61].

Several factors underlie the development of HCC. Although currently viral hepatitis is the major risk factor for the development of HCC, they will soon be replaced by obesity and ethanol consumption, both of which cause hepatic steatosis. According to a recent Centers for Disease Control and Prevention report, the incidence of HCC continues to grow at an alarming yearly rate of 2–3%, in part due to the obesity epidemic. There have been considerable efforts to understand how NAFLD and NASH drive HCC development. NASH is unique as it includes chronic hepatitis, necroinflammation, and a metabolic disease. Hepatic lipid accumulation leads to metabolic reprogramming, which is characterized by a combination of cellular metabolic alterations and an accumulation of toxic metabolites that contribute the development of hepatocarcinogenesis. Moreover, NASH underlie a dramatically versatile and dynamic inflammatory microenvi-

ronment. It is not clear how the inflammatory microenvironment, aberrant metabolism, and ongoing liver regeneration contribute to DNA instability and cancer.

NAFLD is a chronic liver disease associated with obesity, insulin resistance, type 2 diabetes mellitus, hypertension, hyperlipidemia, and metabolic syndrome [4]. Race and ethnicity can also be considered a risk for NAFLD [18]. A highest prevalence of NAFLD was observed in Hispanics, followed by non-Hispanic white individuals, and the lowest prevalence was observed in African American [18]. The prevalence of lean NAFLD in the USA was reported to be 7%, whereas the prevalence of lean NAFLD in rural areas of some Asian countries ranges from 25% to 30% [18]. NASH is the histological subtype of NAFLD that leads to liver fibrosis, cirrhosis, liver failure, and HCC. The course of progression can take many years (Fig. 3). Researchers found that 30% of patients with NAFLD and NASH had progressive fibrosis [4, 18]. However, 20% with NASH showed regression over 2.2–13.8 years. These rates of progression and regression can be influenced by a number of genetic and environmental factors [4]. NAFLD is also associated with extrahepatic manifestations that can increase its burden [18]. Gold standard method for NASH diagnosis is histological assessment of liver tissue. The Brunt system was the first histological assessment system proposed to categorize the morphological features of NASH for grading and staging the disease [63]. Perisinusoidal/pericellular fibrosis was recognized as the earliest stage of fibrosis, with subsequent progression to periportal fibrosis, bridging fibrosis, and cirrhosis [63]. The new scoring system, developed and validated by the National Institute of Diabetes and Digestive and Kidney Disease

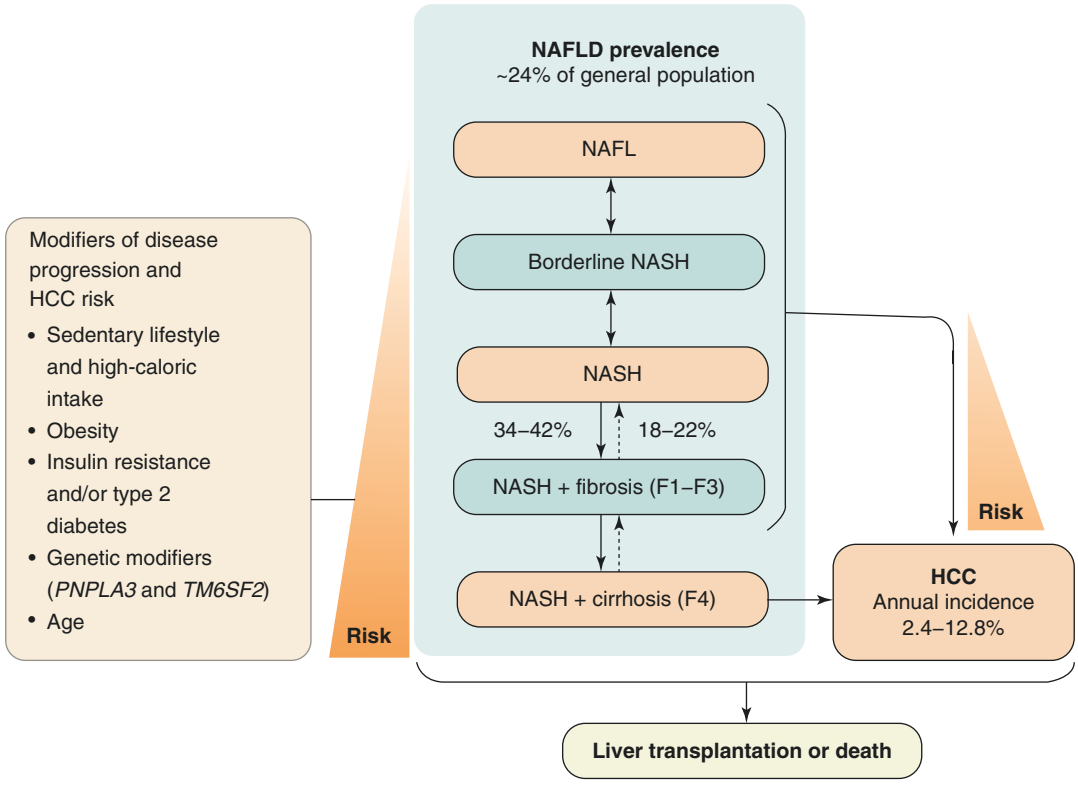

Fig. 3 The pathophysiological states of NAFLD and HCC. Sedentary lifestyle and high ccloric intak emay lead to the development nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and eventually hepatocellular carcinoma (HCC). Annual HCC incidence depending on the disease state (with or without cirrhosis) can vary from 2.4% to 12.8%.

(NIDDK, sponsored NASH CRN Pathology Committee), is currently the most recognized system for scoring NAFLD in clinical trials and experimental studies [63–65].

The studies demonstrated that HCC can develop in the noncirrhotic liver in patients with NAFLD or NASH. In a French cohort study, HCC patients with metabolic syndrome demonstrated mild or no fibrosis in most cases, and HCCs have been found to be well differentiated (28% vs. 64.5%) [66]. In Japanese studies, cirrhosis has not been detected in 38% of 292 NASH-related HCC patients [67]. Prior studies have shown a relationship between obesity and HCC, but have not controlled consistently for well-known risk factors. The pathological process in HCC is hepatocyte death followed by compensatory regeneration and then cell proliferation which may promote tumor growth. A pathophysiologic relationship between obesity, the presence of NASH, and HCC has also been suggested previously. Researchers published a case-control study investigating the impact of early-age obesity on the risk of developing HCC from a large, referral-based cancer center [68]. The researchers used questionnaires to identify common risk factors for the development of HCC and to identify past obesity. Statistical analysis revealed a significant association between early-age obesity and the onset of HCC. This study has investigated obesity as lifelong risk factor for eventual development of HCC, a challenging area with confounding variables. The study has the advantage of a large population and the ability to control for many risk factors for HCC, and the findings are consistent with a growing clinical impression that obesity imparts a substantial risk of HCC. The major limitation regarding the study has been the limited data on the effect of occult cirrhosis on the outcome. This is a challenging issue, as cirrhosis, an established risk factor for HCC, may be present without overt manifestations, probably through silent progression of NASH for which obese patients are at substantial risk [69]. The key question is whether obesity can cause HCC without underlying NASH-related liver injury. Despite this limitation, this study is the first study of this size to control specifically for other known risk factors for HCC. The study is the first study to suggest a temporal relationship between past obesity and the occurrence of future HCC.

Previous studies have implicated the presence of diabetes as a risk factor for development of HCC. Increased insulin resistance has been shown to affect tumor growth in HCC. However, obesity and insulin resistance may not explain whole mechanisms playing a role in hepatocarcinogenesis (Fig. 4). High-sugar diet in mice has

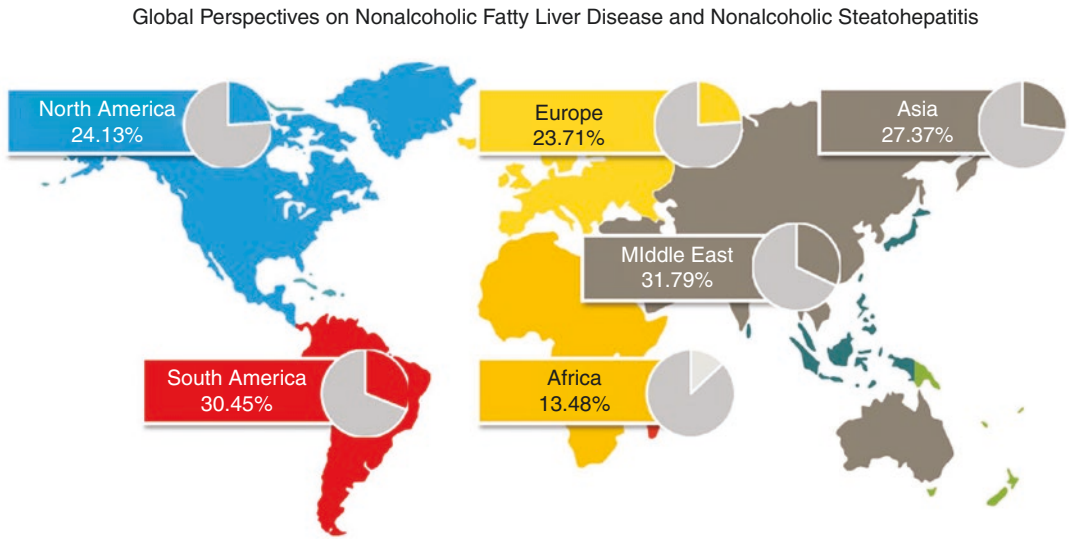

Fig. 4 Global prevalence of nonalcoholic fatty liver disease

been found to be more tumorigenic than high-fat diet. Another study showed that a combination of high-sugar and high-fat diet in mice led to progressive NASH and increased risk for developing HCC. One of the important findings of the study was lower adiponectin levels which have shown an inverse relationship to the development of HCC. Inverse association between adiponectin levels and PNPLA3 gene polymorphism has also been reported. PNPLA3 gene polymorphism has been found to be associated with development of HCC. PNPLA3 gene polymorphisms are considered to inhibit the secretion of very-low-density lipoprotein of hepatocytes [69]. PAT proteins (perilipin, adipophilin, TIP47) are phospholipid membrane-associated proteins on intracellular lipid droplets that regulate insulin-mediated access to stored hydrophobic triglyceride, regulate movement of the droplet within cell, regulate autophagy, and are increased in steatotic hepatocytes. The PAT proteins have also changed expression in different tumor types, such as HCC. Genetic changes in the PAT proteins have been associated with type 2 diabetes and obesity and may inhibit the ability of the lipid droplet to be autophagocytozed [69]. Recently, a study demonstrated that alteration of intracellular lipid droplet metabolism through the GTPase, Rab7, may impact lipid droplet breakdown, a mechanism that has similar implications in the pathogenesis of fatty liver disease as well as hepatocarcinogenesis. These overlapping pathophysiological mechanisms support the argument for increased risk of HCC in patients with obesity and insulin resistance [69].

Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. Grohmannn et al. have shown that the oxidative hepatic microenvironment in obesity activates the STAT-1 and STAT-3 phosphatase T-cell protein tyrosine phosphatase (TCPTP) and increases STAT-1 and STAT-3 signaling [70]. TCPTP deletion in hepatocytes promoted T-cell recruitment and ensuing NASH and fibrosis as well as HCC in obese C57BL/6 mice that normally do not develop NASH or HCC. Attenuating the enhanced STAT-1 signaling prevented T-cell recruitment and NASH and fibrosis but did not prevent HCC. Conversely, correcting STAT-3 signaling prevented HCC without affecting NASH and fibrosis. TCPTP deletion in hepatocytes also markedly accelerated HCC in mice treated with a chemical carcinogen that promotes HCC without NASH and fibrosis [70].

Early mouse studies demonstrated that diet and genetic obesity enhance carcinogen-induced HCC through inflammation-related mechanisms. These studies have not replicated the pathophysiology of NASH-driven HCC, most of which is not associated with carcinogen exposure. Traditionally, NASH-related mouse studies had been based on the use of a hepatotoxic diet including methionine- and choline-deficient (MCD) or choline-deficient and amino acid-defined (CDAA) diets, which cause fat accumulation, hepatocyte death, and inflammation but result in substantial weight loss rather than obesity [71, 72]. Different novel mouse models have been reported to explain the molecular mechanisms that may have pivotal role in hepatocarcinogenesis. Although findings from these models contributed to our understanding of the pathophysiology of NAFLD- and NASH-related HCC, modeling NAFLD and NASH in mice has to be interpreted with care [61]. A recent improvement in modeling NASH is to combine choline deficiency (CD) with a high-fat diet (HFD) leading to weight gain and NASH-like pathology but a rather low rate (25%) of HCC progression [73]. Much more efficient NASH-to-HCC progression (85%) has been achieved by feeding MUP-uPA transgenic mice, which exhibit elevated liver ER stress, with HFD [74].

The liver has vital metabolic secretory and excretory functions in order for whole-body homeostasis. Hepatocytes, the main cells of the liver, are substantially secretory cells responsible for the assembly and secretion of very-low-density lipoprotein (VLDL) and for the synthesis of plasma proteins including albumin, a-1 antitrypsin, apolipoproteins, and coagulation factors. Hepatocytes are responsible for lipogenesis, cholesterol biosynthesis, and glucose and xenobiotic metabolism [5]. Endoplasmic reticulum (ER) in hepatocytes is the main cellular compartment that has critical functions in secretory and transmembrane protein folding, calcium homeostasis,

and lipid biogenesis. ER is involved in NAFLD pathogenesis through the activation of ER stress signaling. However, many factors including hyperlipidemia, inflammation, and viruses can contribute to the dysregulation of hepatic lipid metabolism and liver disease through impairing hepatocyte ER homeostasis. The ER engages unfolded protein response (UPR) pathway to control hepatic protein and lipid homeostasis. UPR decreases the secretory protein load, enhances ER protein folding, and increases clearance capacity by promoting autophagy and ER-associated degradation [5].

Activation of endoplasmic reticulum (ER) stress has been clearly shown to contribute to liver steatosis and NASH [58]. However, the role of ER stress signaling in the development and progression of HCC is much less documented. NAFLD progresses to NASH in response to elevated ER stress. Nakagawa et al. report a novel mechanism in carcinogenesis in which the activation of ER stress signals plays a synergistic role with high diet-induced steatohepatitis to promote the development of HCC [74]. The researchers used the major urinary protein-urokinase plasminogen activator (MUP-uPA) transgenic mouse model to investigate ER stress in hepatocytes. The MUP-uPA transgene induces overexpression of the UPA protein, which accumulates in the hepatocyte ER, leading to ER stress and liver lesions in mice. The researchers observed that the MUP-uPA mice fed with a HFD exhibited greater liver damage, inflammatory infiltration, and increased lipogenesis compared to their control low-fat diet counterparts [3, 74]. In addition, MUP-uPA mice on HFD quickly developed NASH that led to HCC over time. ER stress increases lipogenesis through SREBP activation, oxidative stress, and susceptibility to lipotoxic cell death. The role of ATF6, a major ER stress-activated transcription factor involved in HCC development, should be investigated.

Nakagawa et al. suggested that several mechanisms related ER stress and hypernutrition could work together to develop HCC. These potential mechanisms are HFD-induced SREBP1 activation in MUP-uPA mice, probably enhancing lipogenesis and increasing the degree of hepatic steatosis and, increasing ROS production by ER and steatosis in hepatocytes, Er, and oxidative stress-mediated increase in hepatocyte sensitivity to lipotoxicity and cell death., Pro-inflammatory cytokine expression from activated macrophages which stimulate hepatocyte proliferation and expand HCC progenitors, changes in ER stress activation kinetics and intensities which could lead to hepatocyte transformation [3, 74].

Ma et al. investigated the role of adaptive immunity in progression of NAFLD/NASH to HCC. These researchers used multiple NAFLD/NASH models and human clinical samples, and most of the work has been conducted using Myc-ON transgenic mice in which Myc expression is selectively induced in hepatocytes. When given MCD, these mice exhibited steatohepatitis, fibrosis, and hepatic insulin resistance and developed liver cancer more readily than Myc-ON mice maintained on low-fat diet [75]. A caveat of this approach is that both MCD and CDAA diets cause weight loss and extensive liver damage and may not represent the most accurate NASH model. It was previously shown that $CD4^+$ T cells inhibit initiation of oncogene-driven HCC, through immune surveillance of senescent hepatocytes [75]. Ma et al. showed that release of linoleic acid (LA) by fat-laden hepatocytes and its subsequent uptake by liver resident $CD4^+$ T cells, which oxidize LA in their mitochondria, resulted in endogenous reactive oxygen species (ROS) production and self-inflicted cell death [75]. Incubation of $CD8^+$ and $CD4^+$ T cells with different fatty acid confirmed that $CD4^+$ cells are more susceptible to LA-induced cell deaths than $CD8^+$ cells, but other than greater $CD4^+$ mitochondrial mass, the basis for these differences is not completely clear. The researchers analyzed different $CD4^+$ T-cell subsets and found that total $Foxp3^+$ $CD4^+$ regulatory T-cell (Treg) number had decreased but the frequency $Rorgt^+CD4^+$ T cells had increased, resulting in a remaining $CD4^+$ population producing high amounts of IFNg and IL-17. Together with previous reports of HCC suppression by $CD4^+$ T cells, the new findings suggest that hepatic steatosis drives liver tumorigenesis through suppression of antitumor immunity [71, 72].

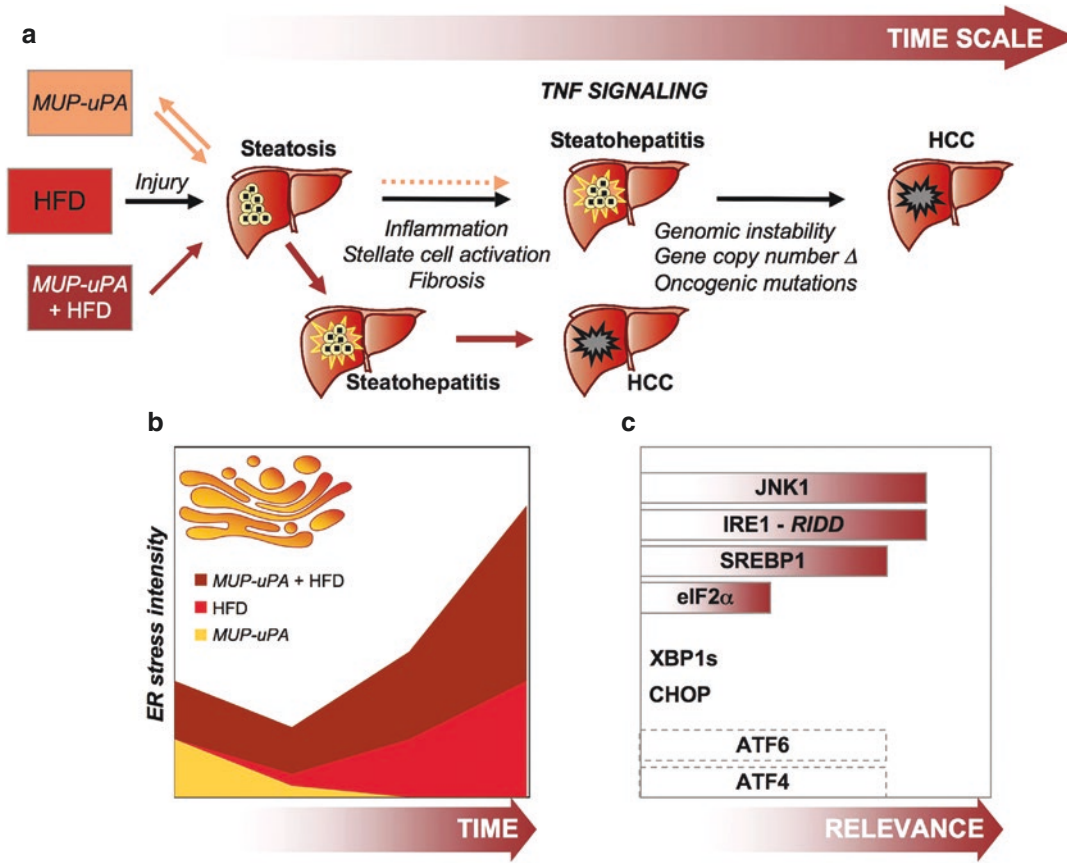

Fig. 5 ER stress in Steatohepatitis-Induced Hepatocarcinogenesis. (**a**) MUP-uPA mice although exhibiting ER stress in the liver, did not develop HCC. On high fat diet (HFD), C57BL/6 mice developed steatosis and HCC. Finally, HFD applied to MUP-uPA mice led to a more penetran HCC phenotype than in wild-type animals. (**b**) Qualitative representation of the intensity of ER stress observed in the models. MUP-uPA mice presented astrong basal Er stress. HFD in MUP-uPA mice led to prolonged and reinforced ER throughout the experimental pipeline, thus correalting ER stress intensity with HCC outcome. (**c**) Qualitative representation of the relevance of ER stress signaling component upon HFD. The IRE arm of the UPR appears to play a significant role in steatosis-induced HCC, because the phosphorilation of IRE1 and the activation of JNK are incerased and 25% of RIDD targets impact lipid methobolism. The activation of SREBP1 also represents an important factor activated upon ER stress taht stimulates lipogenesis. and enhances the steatotic/steatohepatitic phenotype

NAFLD progresses to NASH and HCC in response to elevated ER stress. The onset of simple steatosis requires elevated de novo lipogenesis, and accumulation of hepatocyte-free cholesterol triggers progression to NASH and HCC [76]. It has been shown that caspase-2, which is elevated in NASH, controls the buildup of hepatic-free cholesterol and triglycerides by activating sterol regulatory element-binding proteins (SREBP) in a manner refractory to feedback inhibition. ER stress and TNF induce caspase-2 that controls NASH development. Caspase-2 exerts its pathogenic effect through proteolytic activation of S1P. Caspase-2 ablation or pharmacological inhibition prevents diet-induced steatosis and NASH progression in ER stress mice. Caspase-2 controls adipose tissue expansion and energy expenditure. Caspase-2 also drives SREB cleavage and lipogenic gene expression. The researchers showed that caspase-2 inhibition prevents NASH and HCC in mice [76, 77] (Fig. 5).

NAFLD promotes hepatocellular carcinoma through direct and indirect effects on hepatocytes. The liver is the central organ controlling lipid metabolism and homeostasis. Fatty acids are the most commonly stored and circulating form of energy. Triglycerides, the most common form of fat in foods, are also the most common nontoxic form of fatty acids. The liver synthesizes triglycer-

ides using FFAs and glycerol, which are often obtained from digestion of dietary fat and secrete them into circulation in the form of lipoproteins to provide lipids to other organs. Hepatocyte ballooning caused by accumulation of lipid droplets, is a hallmark of NAFLD. The majority of the accumulated lipids in NAFLD liver are in the form of triglycerides, but triglycerides are generally considered as inert and do not directly cause cell damage or inflammation.

Elevated levels of oxidative DNA damage and abnormal methylation of tumor suppressor genes in the livers of NAFLD patients have been reported. Oxidative DNA damage in the liver of NASH patients has been found to be higher than in patients with other liver diseases. Additionally, more severe oxidative DNA damage has been demonstrated in hepatocytes of patients with NASH and HCC than in those with NASH without HCC.

Oxidative stress is a relevant factor in developing HCC in patients with NASH. Oxidative stress develops as a result of either excessive production of ROS from dysfunctional mitochondria or reduced antioxidant defenses including superoxide dismutase (SOD) and glutathione peroxidase. The contribution of ROS to lipid accumulation and NASH progression in animal models has been described. RS has been suggested to be an independent risk factor for NASH [78]. Impairment of antioxidant cellular mechanisms can lead to an increase in differentiated metabolic pathways related to fatty acid metabolism. This process results in the accumulation of non-metabolized fatty acids and the general disruption of hepatic fatty acid homeostasis that eventually leads to hepatic steatosis and metabolic stress. ROS generation in lipid-rich environment causes lipid peroxidation and release of highly reactive aldehydic derivatives such as 4-HNE and malondialdehyde (MDA) which can cause DNA damage and contribute to malign transformation [79]. Additionally, increased ROS has been detected along as the diseases progress from NASH to HCC. Although it has been suggested that ROS-induced DNA damage leads to HCC, there has not been sufficient evidence on this subject [80]. Excess fatty acid accumulation leads to de novo lipogenesis and b-oxidation that promotes ROS production. The combination of oxidative damage and a proliferative response may promote carcinogenesis. In mouse models, at the early stage of hepatocarcinogenesis, oncogene activation leads to DNA damage and to genomic instability. The Ras-MAPK pathway is dysregulated by multiple mechanisms in HCC [61].

Overexpression of unconventional prefoldin RBP5 interaction has been demonstrated to promote cancer cell survival. In human HCC cell lines or human HCC samples, increased hepatic URI expression causes DNA damage at early stages of hepatocarcinogenesis through the inhibition of enzymes involved in NAD metabolism. It has been shown that HFD-fed mice with hepatic overexpression of URI develop NASH and hepatic DNA damage. In the same study, it was also reported that elevated URI expression in HCC samples correlated with elevated IL-17A expression. Therefore, URI-IL-17A pathway has been suggested to affect NASH-induced HCC. DNA damage in chronic liver disease has been found to be associated with elevated levels of apoptosis and replication which was triggered by caspase 8. Caspase 8 is a relevant protein to mediate efficient proliferation-associated and replication-associated DNA damage in chronic liver damage as found in NASH [81].

Tumor microenvironment plays a key role in the onset and progression of HCC. NASH is a progressive, inflammatory form of NAFLD. HCC may develop in NASH with or without cirrhosis. The HCC tumor microenvironment (TME) is a dynamic ecosystem which includes cancer cells, the cytokines, extracellular matrix, and immune cell subsets.

The immune characteristics of HCC have been established to be a strong suppressor feature. Protumorigenic immune response which mediated through different immunosuppressive cell subsets, cytokines, and signaling plays a key role in the escape of HCC cell from the immune system. In addition, a weak antitumor immunity also contributes to tumor tolerance and progression. Myeloid-derived suppressor cells (MDSC) are increased in pathological conditions and upregulate expression of immune suppressive factors such as arginase and inducible nitric oxide synthase (iNOS). Some tumor-associated cytokines including G-CSF, GM-CSF, and VEGF were shown to induce MDSC infiltration [82]. In addition, local hypoxia plays a key role in in MDSC accumulation in HCC. The MDSCs in fibrotic HCC tissue have been found to be associated with reduced tumor infiltrating lymphocytes.

Tumor-associated macrophages (TAMs) are significant components of the microenvironment in HCC and associated with a poor prognosis of patients with HCC. TAM expression and density can be determined by immunohistochemical. There is strong relationship between TAM density and clinicopathological features and clinical outcomes [83]. Low number of $CD86^+$ TAMs and high number of $CD206^+$ TAMs have been found to be significantly associated with invasive tumor phenotypes and with worse clinical outcomes. TAMs develop from monocytes to functional macrophages and acquire immunosuppressive functions at each stage of its differentiation to maintain tumor microenvironment. Yang et al. demonstrated that tumor cell-derived Wnt ligand stimulates M2 to transduce the polarization of TAMs through Wnt/B-catenin pathway which results in immunosuppression in HCC. Two polarizing phenotypes, M1 and M2, are extremely plastic in response to complex stimuli. M2 phenotype, to be considered as TAM, stimulates tumor onset, progression, and metastasis. HCC-derived cytokines, such as IL-4, IL-13, CSF-1, CCL2, and CXCL12, and connective tissue growth factor (CTGF) promote TAM differentiation from $CCR2^+$ inflammatory monocytes. Osteopontin (OPN) expressed by HCC cells has a positive association with PD-L1 expression in HCC. In addition, it facilitates alternative activation and migration of TAMs through CSF1-CSFR pathway [84]. TAMs can produce angiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGFB). TAMs also promote angiogenesis by expressing matrix metalloproteinase. Cross talk between TAMs and MDSCs leads to decreased production of IL-6 and, IL-12 and, downexpression of MHCII and elevated production of IL-10, a strong inhibitory mediator that impairs downstream $CD8^+$ T-cell and NK-cell cytotoxicity. TAM-derived IL-10 increases intratumoral $Foxp3^+$ Tregs. TAMs in peritumoral stroma of HCC may secrete multiple key pro-inflammatory cytokines such as Il-1B, IL-6, and IL-23 and contribute to the expansion of $CD4^+$ T helper 17 cells (Th17) which suppress antitumor immunity by overexpressing several activation markers such as PD-1, CTLA-4, and GITR [81].

Tumor-associated neutrophils (TANs) affect tumor biological behaviors depending on their polarization, either antitumoral (N1) and protumoral (N2) phenotypes. In some solid tumors, it has been reported that TAN infiltration correlated with tumor progression, which can be considered to be a predictor for monitoring patients receiving anti-PD-1/PD-L1 immunotherapy [81, 84, 85]. TANs mainly suppress antitumor activity through interacting with $CD8^+$ T cells including $CD8^+$ T-cell apoptosis. Loss of hypoxia associated factors, such as HAF, results in inappropriate HIF-1 activation and overproduction of downstream HIF-1 dependent chemokines, RANTES, HIF-1/RANTES upregulation accumulates TANs infiltration, which is associated with NASH related HCC initiation and progression. Cancer-associated fibroblasts (CAFs) have pivotal roles both in HCC cell growth and metastasis. CAFs promote the proliferation, invasion, and metastasis of tumors through secreting various growth factors and cytokines. In 15 HCC patients who underwent hepatic resection, Mano et al. found that BMP4, a regulator of CAF functions, activates hepatic fibroblasts to acquire the ability to secrete cytokines and enhance the invasiveness of cancer cells. CAFs inactivate NK cells and can recruit regulatory dendritic cells (DCs) through IL-6-mediated STAT3 activation and educate them to obtain a tolerogenic phenotype and upregulate Treg production. Briefly, CAFs play an important role in the development and progression of HCC cells [81, 84–86].

6 Modifiers of NAFLD and HCC

Single-nucleotide polymorphisms (SNPs) have been shown to be associated with NAFLD and HCC. SNPs in the genes encoding patatin-like phospholipase domain containing 3 (PNPLA3; rs738409 c444C > G), p.I148M, and transmembrane 6 superfamily member 2 (TM6SF2; rs58542926 c.449 C > T, p.E167K) have been found to be significantly associated with the severity of NASH, cirrhosis, and HCC [61]. The

PNPLA3 polymorphism has been reported to impair mobilization of triglycerides from hepatic lipid droplets. TM6SF2 SNP blocks mobilization of pre-VLDL particles. Several studies have shown that patients carrying the PNPLA3 polymorphism have more than threefold increased risk for HCC. A high prevalence of PNPLA3 gene polymorphism in Hispanics has been proposed to contribute to the high prevalence of NAFLD. PNPLA3 rs738409 GG genotype is more common in Asian patients with NAFLD without metabolic syndrome. Because GG genotype is more common in Asians than Caucasians, this may explain why the two populations have similar prevalence of NAFLD although Asians have a lower metabolic burden [18]. In a retrospective Japanese study, both advanced fibrosis stage (F3 or F4) and PNPLA3 I148M polymorphism have been found to be strong predictors of NAFLD-associated HCC development (HR 24.4 and 6.36, respectively) [87]. European studies including 100 patients with NAFLD and HCC compared with 275 patients with NAFLD who don't have HCC, have shown that PNPLA3 is an independent risk factor for HCC development. Age, gender, BMI, T2DM, and the presence of advanced fibrosis or cirrhosis have been found to be other risk factor for HCC [88]. Whether TM6SF2 rs58542926 polymorphism is independent risk factor for the development of HCC remains largely unknown [89]. Some studies found a weak association between TM6SF2 polymorphism and the risk of HCC. Studies that related patients with alcoholic liver disease demonstrated a relationship between TM6SF2 rs58542926 variant carriage and HCC risk [90]. More recently, MBOAT1 gene rs641738 polymorphism has been shown to be associated with HCC risk in noncirrhotic UK-Italian cohort [91]. Mutations in telomerase reverse transcriptase (TERT) promoter are first and the most common mutations in hepatocarcinogenesis. TERT promoter mutation can be detected in premalignant HCC lesions and in low- or high-grade dysplastic nodules. TERT promoter mutations have been considered to be earliest drivers of hepatocarcinogenesis [92]. TERT promoter mutation is commonly associated with B-catenin mutation in HCC.

Dietary habits, alcohol, physical activity, and socioeconomic factors have been considered to be relevant environmental factors for NAFLD and HCC. In a study including 13 individuals feeding with high-calorie fast food-based meals, elevated serum alanine aminotransferase (ALT) levels and increases in steatosis have been found within 4 weeks [93]. Several studies demonstrate that patients with NAFLD tend to adopt unhealthy eating habits, processed food, and food with high fructose levels and/or high fat content [94]. High intake of carbohydrates such as fructose, is considered to be related to NASH progression [95].

6.1 Intestinal Microbiota and Bile Acid Signaling

Dysbiosis, quantitative and qualitative changes of intestinal microbial composition, has a relevant impact on liver disease. Intestinal dysbiosis and increased intestinal permeability lead to translocation of microorganisms, and the microbial products including cell wall components (endotoxin, B-glucan) and DNA together are referred to as microbial-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) [96]. Immune receptors on liver cells (such as Kupffer cells and hepatic stellate cells) and intestinal lamina propria recognize these patterns which initiate and maintain inflammatory cascades that ultimately lead to liver damage and fibrosis. This damage can progress from cirrhosis to HCC [97]. Several studies suggested the potential role of the gut microbiota in developing of NASH, but the clear relationship between gut microbiota and NASH was not able to indicate yet. Ponziani et al. investigated the association between intestinal microbiota and HCC in 21 patients with NAFLD-related cirrhosis and HCC, 20 patients with NAFLD-related cirrhosis without HCC, and 20 healthy individuals [98].The researchers reported an increased intestine permeability in patients with cirrhosis and HCC compared with cirrhotic patients. The authors have revealed the difference between two groups through increases in lipopolysaccharides and zonulin-1 plasma level compared to healthy

control. Ponziani et al. found that pro-inflammatory mediators such as IL-8, IL-13, chemokine (C-C motif), ligand 3 (CCL3), CCL4, and CCL5 have been significantly increased in the presence of HCC. The researchers also found a positive association between pro-inflammatory mediators and the levels of circulating myeloid-derived suppressor cells (MDSCs). MDSCs are bone morrow-derived cells that have diagnostic and prognostic significance in HCC patients. They found that patients with cirrhosis had a lower gut microbial diversity compared to control, independent of the presence of HCC [98].

A study showed that NASH patients have intestinal dysbiosis. In addition, the bacterial families *Prevotella* and *Enterobacteriaceae* were increased in patients with obesity and NASH when compared with healthy group [61]. In another study, NASH patients showed decreased counts of *Bacteroidetes* and of *Clostridium leptum* when compared to healthy control. The gut microbiota has a role in controlling the composition of bile acids. Bile acids such as cholic acid and chenodeoxycholic acid (CDCA) are important molecules and are synthesized from cholesterol in hepatocytes. Their synthesis is regulated by the farnesoid X receptor (FXR). FXR, a bile acid-activated nuclear receptor, is a regulator of metabolism;, in addition to roles in bile acid homeostasis, it is involved in immune responses, lipid and glucose homeostasis, and insulin signaling [99]. It has been shown that modulation of FXR signaling has beneficial effects on the development of obesity. The gut microbiota has been shown to promote obesity with an increase liver steatosis through systemic FXR signaling.

An association between HCC and altered bile acid metabolism has been found in human and mouse studies. High levels of bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA) in the liver, can induce hepatocyte DNA damage, cell death, and inflammation, promoting hepatocarcinogenesis [100]. A study that used immunohistochemical and mutational analysis in 11 young children with HCC reported a potential link between progressive familial intrahepatic cholestasis and HCC. A mutation in the gene encoding bile acid export pomp, ABCB11, has been found in 10 of 11 patients [101]. Understanding the role of FXR signaling in the context of a metabolic syndrome, NASH and NASH-related HCC will contribute to the management of the diseases.

6.2 Autophagy and HCC

Autophagy is the liposome-dependent catabolic process that regulates the degradation of cytoplasmic proteins and organelles [102]. This process is dependent on autophagy-related (ATG) proteins and can occur in a selective manner by tagging cargos with specific molecular markers such as ubiquitin [103]. In NAFLD, the excess accumulate of triglyceride and FFAs suppresses the onset of autophagy via activation of mammalian target of rapamycin (mTOR) and the suppression of serine/threonine-protein kinase ULK1 activity, leading to increased hepatic oxidative stress [104]. Hepatocytes activate the p62-KEAP1-NRF2 pathway in which phosphorylated p62, an autophagy substrate and adaptor, disrupts the cytoplasmic binding of KEAP1 to NRF2 [105]. The process promotes the expression of pro-survival genes such as glutathione S-transferase and thioredoxin reductase. When the antioxidant capacity of the hepatocytes is exceeded, DNA damage and oxidation develop due to excessive lipid accumulation or impaired autophagy, ultimately resulting in cell death [106].

Previously, KEAP1-NRF2 pathway has been considered as tumor protective in mice. However, the mutations in NFE2L2 gene that encodes NRF2 and KEAP1 are found in 3–6% and 2–8% of HCCs, respectively [107]. In the diethylnitrosamine (DEN) and 2-acetylaminofluorene rat HCC model, Nrf2 or Keap1 mutations occur in 71% of premalignant lesions and in 78.6% of early HCCs. These data have demonstrated the importance of the KEAP1-NRF2 pathway in early-onset events in hepatocarcinogenesis [61]. NRF2 was shown to promote HCC cell proliferation and the expression of biliary or hepatic progenitor marker cytokeratin 19 [76]. Cytokeratin 19 has been found to be associated with metastasis, microvascular invasion, early recurrence, poor overall survival, chemore-

sistance, and cancer stem cell features [76]. p62 plays key role in NRF2 activation and MYC oncogene stabilization which is a major regulator of stemness in cancer, and protects HCC-initiating cells from oxidative stress-induced death [108]. Activation of KEAP1-NRF2 pathway protects cancer cells from harmful effects of ROS and oxidative stress, and disruption of ATG7 and beclin1 helps cells to escape an apoptotic-independent cell death. Therefore, dysregulation of autophagy contributes to the pathogenesis of HCC.

Inflammatory immune response may be associated with ER stress-induced DNA damage and liver cancer in NAFLD patients. Under normal condition, steatosis is not a driver of HCC and needs chronic necroinflammation as a cofactor to drive hepatocarcinogenesis. This effect has been first shown in MUP-uPA mice in which hepatocyte ER stress is induced by plasminogen activator expression and combined with HFD [73]. TNF expressing from inflammatory macrophages, which accumulated in the livers of MUP-uPA mice in response to hepatocyte ER stress, results in NASH and HCC development. Liver samples obtained from NASH patients are characterized by elevated phosphorylation of eukaryotic initiation factor 2 (eIF2) [109].

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Epidemiology of Hepatitis B Virus in the Middle East

Genco Gençdal and Cihan Yurdaydin

Abbreviations

CDC	Centers for Disease Control
CHB	Chronic hepatitis B
HBV	Hepatitis B virus
UN	United Nations
USA	United States of America
WHO	World Health Organization

Hepatitis B (HBV) is one of the most common causes of liver cancer and liver cirrhosis. It is estimated that around 2 billion people are infected with HBV and nearly 300 million people have chronic hepatitis B (CHB). Approximately 850,000 people die each year from complications of HBV infection such as liver cancer and liver failure. According to a 2016 report from the World Health Organization (WHO), 27 million people (10.5% of hepatitis B patients) were aware of their infection, and only 4.5 million (16.7%) of those diagnosed were receiving treatment [1–4]. HBV prevalence varies from country to country depending on geographical location.

Studies on prevalence and incidence of a disease may have important health-related implications for the countries or regions where such studies have been performed as based on those studies health strategies can be developed which at the end may benefit the patients in that country or region. A very good example is the successful campaign against hepatitis C in a low-income country such as Egypt which recently culminated in successfully screening 50 million people for HCV [5]. Once a country with the highest hepatitis C virus (HCV) prevalence in the world (estimated as 10%), this prevalence has greatly decreased, thanks to a very effective collaboration of physicians and epidemiologists with government officials [6, 7] leading to immensely successful negotiations with the biomedical industry with consequent drastic price cuts for Egyptian HCV patients, the aim of which is the eradication of HCV in Egypt [5] which appears now to be a realistic goal. A total of 2.2 million people (4.6%) were found seropositive for HCV, and out of whom close to 70% were further tested and more than one million people or 92% of viremic patients (76% of the tested population) already underwent successful treatment with a sustained virologic response rate of 98% [5].

On the other hand, epidemiological studies in a particular region may have effects far beyond the region. Studies on HBV need to be approached as such. Some high endemic countries for HBV of the recent past have converted themselves to less endemic areas, thanks to the introduction of universal hepatitis B vaccination [8, 9]. China and Taiwan are good examples [8, 10]. Others, such as sub-Saharan African countries, continue

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to be highly endemic. The reason for these local figures to have universal appeal is the increasing immigration of people. The route is mainly from low-income countries to rich countries such as European countries, the USA, Canada, and Australia (Table 1) [11, 12]. In this context, this review deals mostly with a politically unstable and troubled area which contributed to the migration crisis recently in Europe.

The Middle East is mostly made up of developing countries with suboptimal healthcare infrastructure. Given the political instability and the horror of war affecting this area of the world directly or indirectly, reliable data on epidemiology is in general difficult to obtain. In such areas, exploring HBV prevalence in blood donors is attractive because they are based on a large number of individuals and the numbers obtained are reliable. Data obtained from blood donor studies can be considered acceptable indicators of the HBV burden in these countries provided it is understood that these prevalence data may underestimate the real problem because at least in some areas high-risk groups for HBV are rejected from blood donation without pretransfusion blood screening for hepatitis B surface antigen (HBsAg) [13].

In this chapter, we have tried to provide the reader with available data from basically all Middle Eastern countries, and we will provide those data with a country-specific perspective.

Table 1 Estimated number of international migrants according to the UN as of 2017 and migrants as the percentage of the total population in industrialized countries

Major area, region, or country	Number of international migrants (thousands)		Migrants as percentage of total population	
	2000	2017	2000	2017
World	172.604	257.715	2.8	3.4
High-income countries	100.405	164.847	9.6	14.1
Middle-income countries	64.042	81.440	1.4	1.4
Low-income countries	7.733	10.915	1.8	1.6
West Europe				
Sweden	1.004	1.748	11.3	17.6
UK	4.730	8.842	8.0	13.4
Germany	8.893	12.165	11.0	14.8
France	6.279	7.903	10.5	12.2
Spain	1.657	5.947	4.1	12.8
Italy	2.122	5.907	3.7	10.0
Netherlands	1.566	2.057	9.8	12.7
Switzerland	1.571	2.506	21.9	29.6
North America				
USA	34.814	49.777	12.3	15.3
Canada	5.512	7.861	17.9	21.5
Oceania				
Australia	4.386	7.036	23.0	28.8
Asia				
China	508	1.000	0.0	0.1

Adapted from United Nations, International Migration Report 2017 [12]

1 Turkey

In 1998, the national vaccination program was launched in Turkey. With this program, risk groups and infants started to be vaccinated. Turkey before the vaccination program was considered a country with higher intermediate endemicity (5–8%) for HBV [8]. The Turkish Ministry of Health declared the incidence of hepatitis B infection to be 8.26 by 2002 and 4.2 by 2010 per 100,000 people [14]. According to the study of 22 blood centers, which included the results of 6.24 million donors between 1989 and 2004, the prevalence of HBsAg among blood donors peaked in 1991 (5.2%) and then gradually and consistently declined to 2.1% in 2004 [15]. In a population-based, cross-sectional, national study using random sampling, conducted between 2009 and 2010, HBsAg positivity was found to be 4.0% [16]. This prevalence may have decreased further in recent years. However, the effect of international migration is not considered in this scenario. Migrants accounted for only 2% of the Turkish population in the year 2000. The Syrian war led to a surge in international immigration with close to five million refugees now located in Turkey. Migrants' share in the population has therefore increased drastically and as of 2017 accounts for 6% of the Turkish population [12]. In this context, an important study needs mentioning. In this study from 2017, Kose et al. reported among Syrian refugee children aged

0–18 an alarming 4.2% HBsAg positivity rate [15]. The good news is that in the very same year, the Turkish Ministry of Health, with support from UNICEF, the WHO, and local nongovernmental organizations, conducted a mass countrywide vaccination campaign, at which HBV vaccine was included, to more than 400,000 refugees and migrant children [17, 18]. Turkey has been providing to each Syrian refugee the same health insurance opportunities as Turkish citizens. The drugs used in the treatment of chronic HBV in Turkey are fully covered by public health insurance. HBV genotype in Turkey is exclusively genotype D [19].

2 Saudi Arabia

Despite the national neonatal vaccination program that started in 1989, HBV continues to be a major health problem in Saudi Arabia. Recent studies conducted in Saudi Arabia show that the HBV prevalence is approximately 1.3–3.5%. HBV genotype is mainly D. Due to the impact of the vaccination program, HBV prevalence in the younger population (<30 years of age) is lower than in the older population. It is estimated that the prevalence of HBsAg in the Saudi population over the age of 40 is between 3% and 6% [8, 20, 21].

Some studies on high-risk populations such as intravenous drug users and HIV-positive patients have shown a much higher rate of HBV infection [22, 23]. In a new study, it was reported that the number of CHB patients over 60 years old in Saudi Arabia in 2015 was higher than in 2010 and 2012. More importantly, the rate of patients with comorbidities such as cirrhosis and HCC in the CHB population in 2015 was higher than in 2010 and 2012. In Saudi Arabia, although the overall prevalence of HBV may have decreased significantly, CHB complications will increase significantly in the next 20 years due to the aging of individuals infected before the vaccination program [24]. According to a United Nations (UN) report, it is estimated that the proportion of the immigrant population in Saudi Arabia represents 37% of the total population as of 2017 (Table 2). These immigrants are mainly workers from Asian or non-Asian countries. Data on this immigrant population is scarce. According to one study, only 43% had been vaccinated against HBV [25]. The antiviral agents commonly used as first-line treatment of CHB in Saudi Arabia are tenofovir disoproxil fumarate and entecavir [26].

Table 2 Estimated number of international migrants according to the UN as of 2017 and migrants as the percentage of the total population in countries of the Middle East

	Number of international migrants (thousands)		Migrants as percentage of total population	
Country	2000	2017	2000	2017
Turkey	1.281	4.882	2.0	6.0
Saudi Arabia	5.263	12.185	25.3	37.0
Yemen	143	384	0.8	1.4
Iraq	211	367	0.9	1.0
Iran	2.804	2.699	4.2	3.3
Jordan	1.928	3.234	37.8	33.3
Israel	1.851	1.962	30.8	23.6
Lebanon	693	1.939	21.4	31.9
UAE	2.447	8.313	77.6	88.4
Kuwait	1.128	3.123	55.0	75.5
Oman	624	2.073	27.5	44.7
Bahrain	239	723	36.0	48.4
Qatar	360	1.721	60.7	65.2

Adapted from United Nations, International Migration Report 2017 [12]
UAE United Arab Emirates

3 Yemen

Yemen is a hyperendemic country for HBV. The prevalence of HBV in Yemen is reported to be 12–18.5%, and most studies to determine prevalence have been conducted in tertiary healthcare centers and include hospitalized patients with acute or chronic hepatitis or blood donors. As these populations are known to be highly selected, such studies may not reflect the true prevalence in the population [27–32]. The mode of transmission of HBV in Yemen has not been adequately clarified but is likely to be horizontal. History of blood transfusion, advanced age, male gender, and healthcare personnel are stated as risk factors. In Yemen, vaccination against HBV was ini-

tiated in 1999, and its coverage reportedly increased to 87% in 2008 [33–37]. Recent political unrest may have had a negative impact.

4 Iraq

HBV infection in Iraq has declined in the past few decades. Hepatitis B among blood donors was reported as 3.6% in 1973, 4.1% in 1984, and less than 1% in the 1990s. It has been suggested that this decrease is the result of prevention and control programs adopted by the state, such as safe blood transfusion and safe injections, in addition to the initiation of the vaccination program [38]. In Baghdad, the prevalence of HBsAg in blood donors was 0.6%, higher in men (0.8%) than in women (0.5%). It has been reported to be 0.7% among all blood donors in Babylon province. In various studies, the prevalence of HBsAg in Najaf province was reported as 0.6% and in Karbala as 3.5%. HBV prevalence in the latter region has steadily increased since 2011, due to high migration to Karbala during attacks of the self-declared Islamic state of Iraq from some northern cities of Iraq [38, 39].

5 Syria

Hepatitis B vaccine was added to the national vaccination program in 1993 in Syria. Genotype D is common. Home births are still common in the country, and there are no estimates of children who received the first hepatitis B vaccine within 24 hours of birth. In early 2002, HBsAg positivity was reported to be 5.3% and 10.8% in drug users and sex workers, respectively, in Syria. In 2004, in a study involving 3168 people, the rate of HBsAg positivity was found to be 5.6%. In the same study, it was reported that there is a distinct regional difference, with hepatitis B seroprevalence being 10.5% and 10.6% in the north of the country (Aleppo) and in the east of the country (Hasshat), respectively. In a retrospective study involving 11,015 Syrian pregnant women with a mean age of 25 ± 6:02 between January 2012 and January 2018, 1.1% HBsAg seropositivity was reported [40, 41]. It has to be added that the war in Syria with its consequences has likely had its toll also on the health infrastructure with less compliance to routine childhood vaccinations as pointed out already in the study of Syrian refugee children in Turkey [15]. It would be not surprising to see the same trend in Syrian children who did not leave the country.

6 Lebanon

The current status of hepatitis B infection in Lebanon is not well known due to the lack of sufficient published studies on the subject. When the studies conducted since 1972 are examined, it is seen that most of these studies were carried out by the virology and biochemistry departments of some hospitals. These studies are related to certain areas and specific groups of individuals and cannot be generalized to the whole population. According to data in these studies, the prevalence of hepatitis B has been reported from less than 2% to more than 3% in Lebanon [42–46]. Genotype D is common. HBV prevalence in the general population is estimated to have declined following the inclusion of the neonatal hepatitis B vaccination program in the Lebanese vaccination schedule in 1998. According to 2007 WHO data, the frequency of hepatitis B was estimated between 1.6 and 2.2%. In a study involving 6 Lebanese provinces and 31,147 people from January 2011 to December 2012, 542 (1.74%) were identified with HBV. HBV exposure was reported to be higher in South and Nabatiyeh (1.9%) compared to those from Beirut (0.73%). These results have been attributed to inadequate sanitation, low socioeconomic status, extended family size, and high migration rates from high endemic areas such as African countries. In 2016, HbsAg positivity was found to be 1.6% in a study involving 3769 hemodialysis patients. [47–49]. Lebanon is another country hosting a large community of immigrants (32% of the general population). The HBsAg prevalence in this group is expected to be higher than in the native population, but data in this group is lacking.

7 Israel

The HBV vaccination program in Israel has been carried out since 1992. In various studies conducted in Israel, 1.5–7% Hbsag positivity has been reported in the adult population. It has been reported that the HBV incidence has decreased to 0.5/100,000 in 2015. The HBV prevalence is estimated to be 0.96% recently. A recent large study showed that 868,714 people (22.6%) were exposed to HBV, while 15,258 people were HBsAg positive (1.75%). In this study, the prevalence of HBV in the Arab population was higher than in the Jewish population (2.98% and 0.76%, respectively). Genotype D is common [50–54].

8 Iran

In Iran, the national vaccination program against HBV infection started in 1993. According to the reports of the WHO in 2001 and the Centers for Disease Control and Prevention (CDC) in 2005, prevalence of CHB infection among the people of Iran was between 2% and 7%. This wide range of prevalence was mainly due to variance in prevalence rates among different geographical regions in Iran as a consequence of different socioeconomic level in addition to traditions and cultures. HBV infection prevalence in the general population of Iran has been reported as 2.9% (95% CI: 2.5–3.4%) before 2010 and as 1.3% (95% CI: 0.9–1%) after 2010. The HBV vaccine has effectively reduced the incidence of new cases in the country. In Iran, according to a recent meta-analysis, the prevalence of hepatitis B was reported to be 2%. The prevalence of hepatitis B in blood donors in Iran has been reported to be approximately 0.58% [8, 55–59].

9 Qatar

HBsAg screening in the State of Qatar started in 1983. There are very few published studies that address the prevalence of hepatitis markers in blood donors or in the general population. In a retrospective cohort study covering the years 2002–2006, it was reported that the HBV prevalence in Qatar was 4.7%. Another study in blood donors reported a HBsAg positivity of 0.9%. Qatar is home to a significant number of expatriates that make up more than 80% of its residents, most of which come from countries suffering from poor health systems in Asia and Africa. In a study published between 2010 and 2014 on the epidemiology of hepatitis B in Qatar, most cases were reported in non-Qatarians (89.4%) over 5 years [60–62].

10 Jordan

The prevalence of HBV before vaccination in Jordan has been reported as 9.9%. In 1995, the HBV vaccine was integrated into the childhood vaccination program. There has been a fourfold decrease in HBV prevalence over the last 30 years, indicating the successful implementation of this program. In the Polaris study, the estimated prevalence of HBV in Jordan in 2016 was reported to be 2.4%. In two separate studies, HBsAg positivity in hemodialysis patients was detected as 4% and 5.6% in 2003 and 2006, respectively [63–67]. These studies do not take into account the effect of migration which is an important problem of this country. According to the UN, Jordan is hosting some three million refugees and hence is home to the second largest refugee population. Overall, one-third of the population are immigrants.

11 UAE

To date, no prevalence estimates have been made for the general population of the United Arab Emirates. A previous report showed that the prevalence of HBsAg among young adults admitted to police college in Abu Dhabi was 0.3%. In addition, in a study of 2000 pregnant women aged 15–45, the HBV prevalence was found to be 1.5%. In a recent study, HBsAg positivity was detected in 2.15% of patients of a dental hospital [68–70]. The vast majority (88%) of people living in the UAE are foreigners who came for work.

12 Kuwait

Studies estimate the prevalence of HBV carriers to be 1% in the general population. However, this prevalence is higher (3.5%) among blood donors born abroad [8, 71]. It is important to add that Kuwait is one of the countries with a very large foreign-born population – 75% of the general population consist of non-Kuwaitis [12].

13 Oman

Before the introduction of vaccination in Oman, the prevalence of CHB virus infection was estimated at 2–7%. In a 2005 study, HBsAg seroprevalence was 2.3% among students born before the national hepatitis B vaccination program and 0.7% among students born after introduction of the vaccination program. A study evaluating the effect of vaccination efficiency and scope revealed that 15 years after the initiation of HBV vaccination in newborns, the prevalence of CHB in children fell from 2.3% to 0.5% [72, 73]. As in many countries in this area, nearly half of the population (45%) is foreign born.

14 Bahrain

The prevalence of HBV infection in Bahrain from 2000 to 2010 was 0.58%. However, the prevalence of infection has been reported to be significantly higher in patients who have undergone dental procedures and surgical operations and among citizens born abroad [73, 74]. HBV genotype has been reported as genotype D in 61% to be followed by genotype A in 10% [75]. In Bahrain, similar to Oman, 48% are foreign born.

15 Conclusions

Epidemiology of HBV is a dynamic process everywhere, even more so in the Middle East for several reasons; the richer countries in the area such as the UAE, Qatar, Oman, Kuwait and Bahrain, Israel, and Saudi Arabia contain a large cohort of immigrants (Table 2). In the UAE, Qatar and Kuwait natives represent a minority in their own country, and in countries such as Bahrain and Oman, there is an even split between natives and foreigners. In these areas, the impact of foreign born on HBsAg prevalence in the country is obvious. This needs to be addressed in the respective countries, and health strategies including screening, vaccination, and treatment have to be implemented. Poorer countries such as Iraq, Syria, Lebanon, Jordan, and Yemen have to deal with direct and indirect consequences of political instability such as civil war and sudden surge of refugees. Effective preventive strategies such as infant vaccination may be interrupted under these circumstances. Data provided in this text based on scant available studies should be approached with caution as they may not hold true in the long run. The concern here is that the downward trend in HBV prevalence reported here (Table 3) also for the Middle East may change. For the rich countries of the West facing a continuous wave of immigrants, adaptation of health strategies accordingly is warranted. However, no country has so far adopted a policy of screening of immigrants or refugees for hepatitis viruses despite the recommendation of the Centers for Disease Control and Prevention in the

Table 3 Estimated HBsAg prevalence rates in countries of the Middle East and the year universal vaccination had started in those countries

	General population (%)	Year of the start of universal vaccination against HBV
Turkey	2–3	1998
Saudi Arabia	1.5–3	1989
Yemen	>8	1999
Iraq	1	1993
Syria	3–4	1993
Iran	1–2	1993
Jordan	2–3	1995
Israel	1–2	1992
Lebanon	1	1998
UAE	2	1993
Kuwait	1–2	1990
Oman	1	1990
Bahrain	<1	1993
Qatar	1–2	1989

USA that all immigrants originating from countries with an HBV seroprevalence greater than 2% should be screened for CHB infection and vaccinated if found to be susceptible [76]. Further, several studies suggest that screening individuals coming from a country with a HBV prevalence of 1% is cost-effective [77, 78].

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Hepatocellular Carcinoma in the United Arab Emirates

M. Jawad Hashim, S. Sadaf Rizvi, and Gulfaraz Khan

1 Demographics of UAE

The United Arab Emirates, often referred simply to as The Emirates, is a federation of seven states, namely, Abu Dhabi, Dubai, Sharjah, Ajman, Fujairah, Ras Al Khaimah, and Umm Al Quwain. Over the last 20 years, UAE has witnessed a population boom, increasing by over three times [1]. The current UAE population is estimated to be 9.8 million, and approximately 70% of whom live in Dubai and Abu Dhabi. Expatriates account for more than 80% of the total population [2]. Due to the large proportion of single expatriate workforce, mostly from the Indian subcontinent, UAE has a very unusual gender and age distribution; males constitute over 70% of the population, and more than 65% are in the 25–54-year age range [1]. This demographic group has elevated rates of HCC risk factors such as obesity, chronic viral hepatitis, and alcohol intake.

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2 Epidemiology of Liver Cancer in the UAE

Cancer is the third leading cause of death in the UAE, after cardiovascular diseases and traffic injuries [3]. Like many other countries around the world, the incidence of cancer is growing in the UAE. Recognizing this health problem, the UAE government has included cancer as one of the key performance indicators in its National Health Agenda to assess the nation's healthcare goals [4]. The UAE National Cancer Registry (NCR) reported a cumulative total of 3818 new cancer cases from January 1 to December 31, 2014 [3]. More recent estimates indicate that this number has risen to 4707 new cases, with 56% occurring in females [5]. The top 5 most frequent cancers in UAE were breast, colorectal, thyroid, prostate, and leukemia [5].

In terms of hepatocellular carcinoma (HCC), the UAE has a small but rising burden of this malignancy (Fig. 1a, b). This trend may reflect the increasing rates of obesity, alcohol use, and population aging. Within the Middle East North Africa (MENA) region, UAE has moved up in ranks from 17th highest incidence rate three decades ago to the 13th position recently [6]. The gradually increasing number of cases of hepatocellular carcinoma will place additional demands on the healthcare system, especially for clinical oncology services. The UAE NCR recorded 68 cases in 2014 [3]. Although the rise is concern-

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ing, the overall burden is low at about 220 new cases in 2019.

Age-standardized rates (which adjust for changes in underlying population) show a stabilization of the number of cases (Fig. 1c, d). This implies that the apparent rise in the total number of cases is partly due to changes in population structure such as aging. The annual incidence of 2.37 cases per 100,000 is lower than the global average (6.9 cases per 100,000) and markedly lower than Egypt (14 cases), Japan (36.5 cases), and South Korea (38 new cases annually per 100,000 population). Moreover, the incidence in UAE is also lower than neighboring countries such as Saudi Arabia and Qatar. Overall, the burden of HCC is relatively low in the UAE.

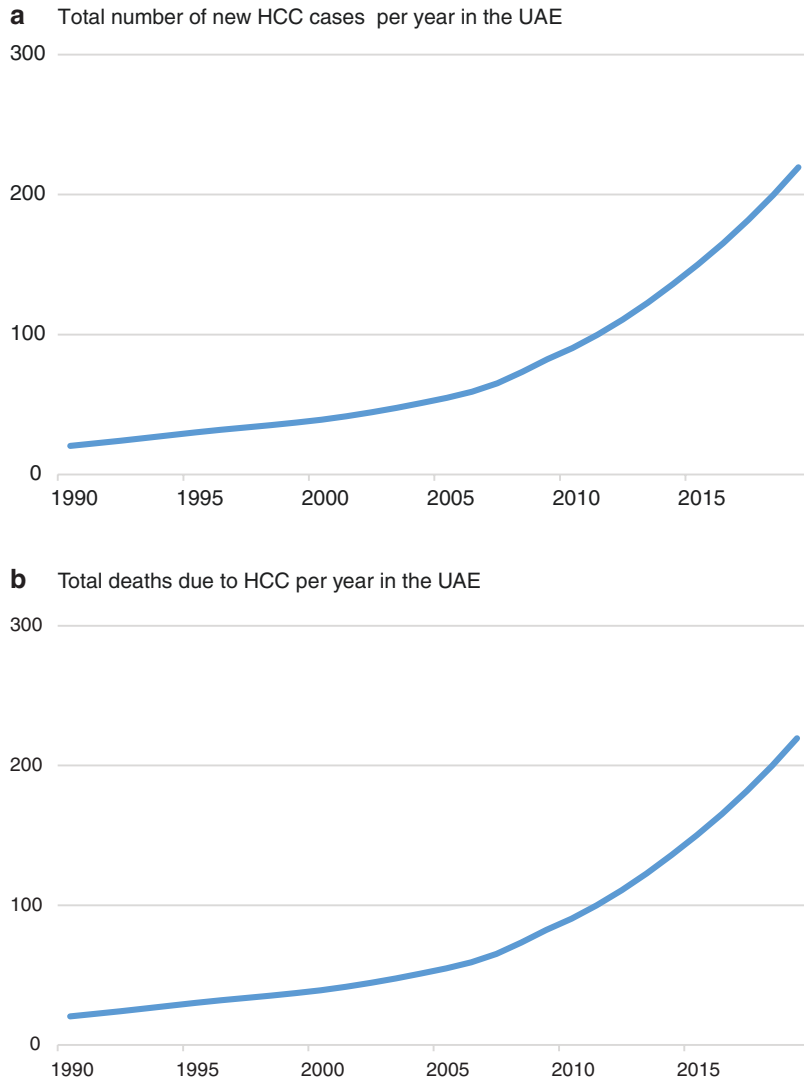

Fig. 1 Burden of liver cancer in the United Arab Emirates, 1990 to 2019, based on Global Burden of Disease 2019 data [6]. (**a**) Total number of new HCC cases per year in the UAE. (**b**) Total deaths due to HCC per year in the UAE. (**c**) Age-adjusted incidence rate (new cases per 100,000 standardized population). (**d**) Age-adjusted mortality rate (deaths per 100,000 standardized population)

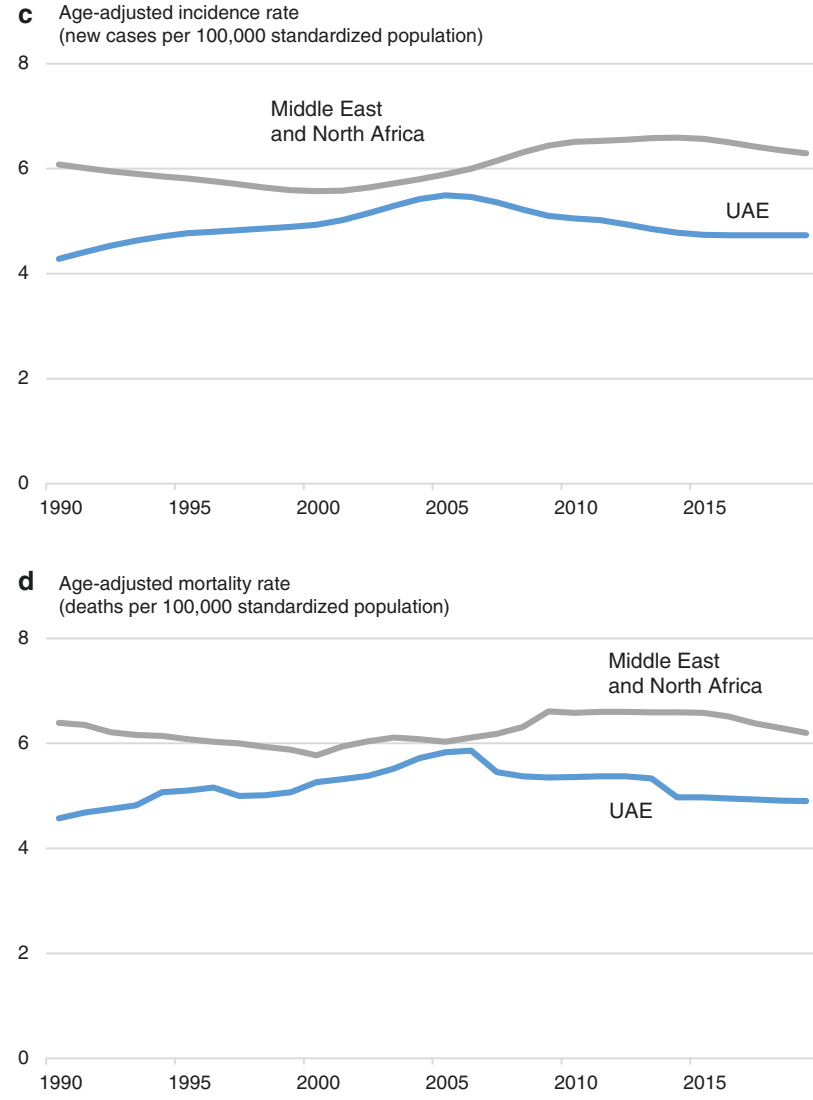

Fig. 1 (continued)

3 Risk Factors for Hepatocellular Carcinoma in the UAE

The main risk factors for HCC in the UAE have been chronic infections with hepatitis B and C viruses, accounting for 74% of all HCC cases (Fig. 2). Although both HBV and HCV target the liver, the two viruses are very different. HBV is a small, enveloped, partially double-stranded DNA (3.2 kb) virus belonging to the *Hepadnaviridae* family. HCV on the other hand is an enveloped single-stranded RNA (9.6 kb) virus belonging to the *Flaviviridae* family. Both viruses are primarily transmitted via exposure to infected blood or body fluids through sexual contact and sharing contaminated needles. In terms of seroprevalence, certain regions appear to have higher burden compared to others. For example, HBV is particularly prevalent in Africa and Western Pacific regions, while high prevalence of HCV has been noted in the European and Eastern Mediterranean regions

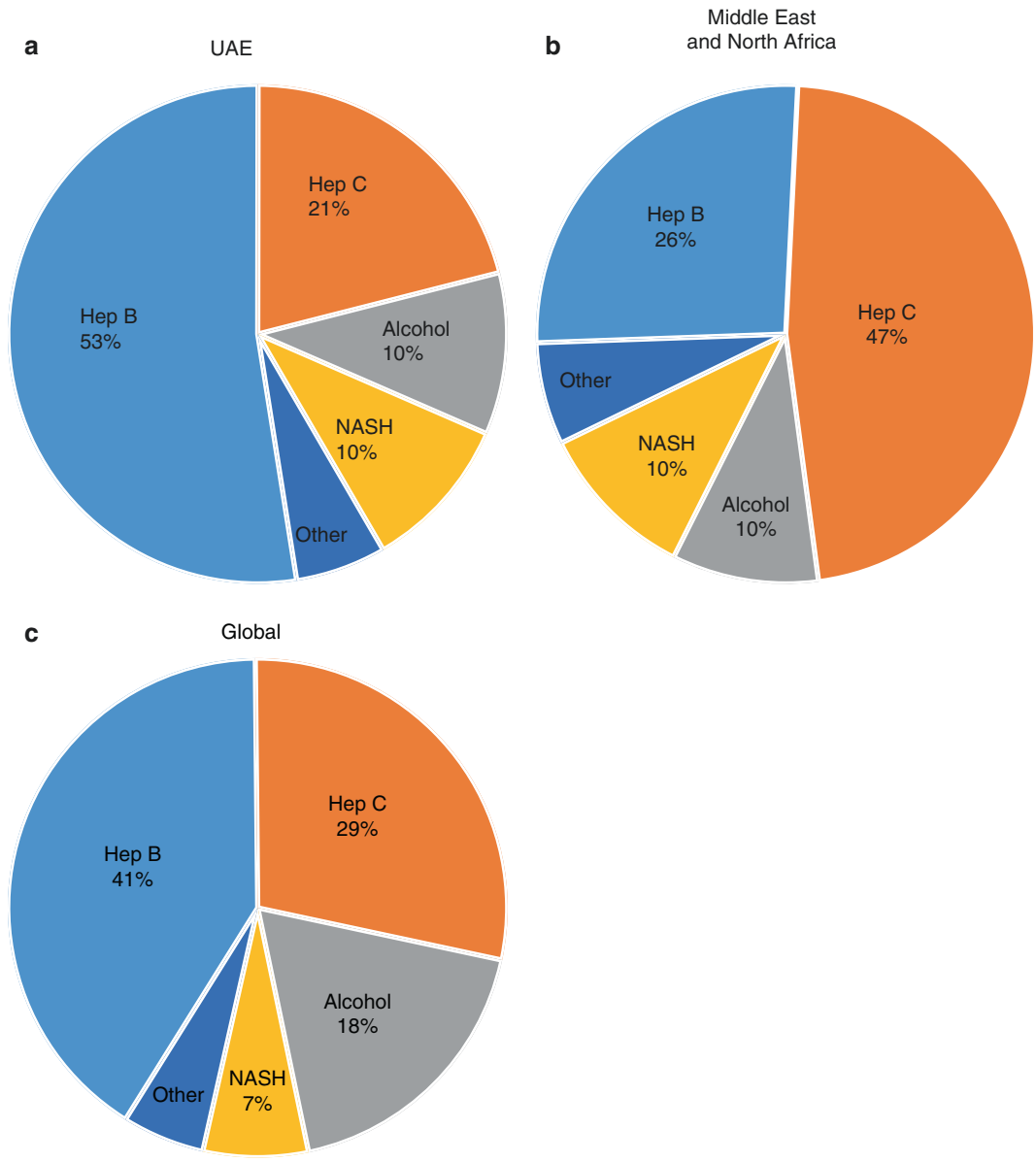

Fig. 2 Relative contribution of different causes of liver cancer incidence in (**a**) the UAE, (**b**) Middle East North Africa region, and (**c**) global [6]

[7]. It is expected that the burden of viral-associated HCC will decrease in the future, as vaccination and antiviral treatments become standard practice. However, this reduction may well be overshadowed by the alarming increase in other HCC-associated risk factors, notably diabetes and obesity. Within the last 50 years, both of these risk factors have more than tripled [8, 9]. This has been particularly evident in the Gulf region. Although both diabetes and obesity are associated with cancer, the causal association is particularly strong for HCC. Indeed, it is estimated that these two risk factors independently contribute to approximately 25% of all HCC cases [10].

Rates of liver cancer attributable to hepatitis B virus in the UAE are lower than most western

countries such as Spain, Portugal, the UK, and the USA [6]. Vaccination at birth for hepatitis B is close to universal coverage due to a legislated mandate and federal funding. There is 98% coverage for the third dose of hepatitis B vaccine among children in the UAE [11]. The effect of universal vaccination is likely to further decrease the role of this risk factor for HCC within the UAE. All expatriate residents in the UAE are periodically screened for hepatitis B (along with other infectious diseases such as HIV) for their visa renewal. Among citizens, the rates are decreasing. For instance, about 1.5% of local pregnant women are positive for hepatitis B (HBsAg) [12].

Hepatitis C (anti-HCV) prevalence is also fairly low in the UAE at 0.1%, comparable to developed nations [13]. The rates are reported to be higher among resident expatriate workers from neighboring countries [14]. HCC attributable to chronic hepatitis C is considerably lower in the UAE compared to western countries such as France, Germany, the UK, and the USA [6]. For example, the incidence of liver cancer due to hepatitis C in the UAE is 0.5 cases annually per 100,000, while the corresponding rate is 6.2 cases in Spain. Screening for hepatitis C in the UAE meets international standards in clinical laboratories and for blood transfusion. As most healthcare workers are internationally trained and experienced, awareness of blood-borne transmission and infection control measures is well established and strictly implemented, thus minimizing the transmission of the virus by these routes. Similarly, transmission of HCV via injecting drugs does not appear to be a dominant contributor to HCV seroprevalence in this region [15].

Nonalcoholic fatty liver disease, which includes nonalcoholic steatohepatitis (NASH), is rising rapidly as a risk factor in the UAE [16]. The current prevalence of NASH (4.1% of adult population) is expected to more than double over the next decade [17]. This is likely due to rapid changes in social lifestyle from a nomadic existence and labor-intensive fishing to affluent world-class amenities. Sedentariness and a calorie-rich diet have led to rising rates of obesity and diabetes in both citizens and expatriates [18].

Alcohol is an important risk factor for HCC. In some countries, notably in the Eastern European region, alcohol is estimated to contribute to more than 50% of all HCC cases. Recent estimates for MENA region show that alcohol-associated HCC cases are on the rise in some of the countries, namely, Qatar, Turkey, and Egypt [19]. Unfortunately, no published data is available on the contribution of alcohol in the development of HCC in the UAE. Similarly, aflatoxin is also a well-known causative risk factor for HCC [20, 21]. Foods with high levels of aflatoxin have been reported from a number of countries [20]. However, once again little or no reliable data is available on the impact of aflatoxin on HCC in the UAE. A study published over 20 years ago reported that some foods in the UAE may contain aflatoxin [22], which could lead to exposure of neonates from maternal ingestion of aflatoxin-containing food [23].

4 Clinical Features of Liver Cancer in the UAE

Age of presentation in the UAE starts in the 40s and peaks in the 80- to 90-year-old age group (Fig. 3). Males are more commonly affected than females. In the UAE, the male to female ratio for HCC incidence is 2.7:1. This is higher than the global average of 2.3:1. At older ages (80+ years), the male to female ratio tends to be closer to 2:1. Furthermore, males have a markedly higher risk of dying from HCC than females [14].

High-income Middle Eastern countries, such as the UAE, have a greater proportion of hepatitis B- than hepatitis C-associated HCC. These countries have generally lower rates of liver cancer compared to low-income neighboring nations. There is a paucity of clinical research data on liver cancer from the Middle East region. Thus, it is difficult to compare HCC stage at diagnosis, treatment, and survival rates in the UAE with other countries. Regionally, HCC tends to be an aggressive malignancy that is often diagnosed at a late stage with associated poor survival [24]. Even with liver transplantation, recurrence rates can be 10% within 2 years [25].

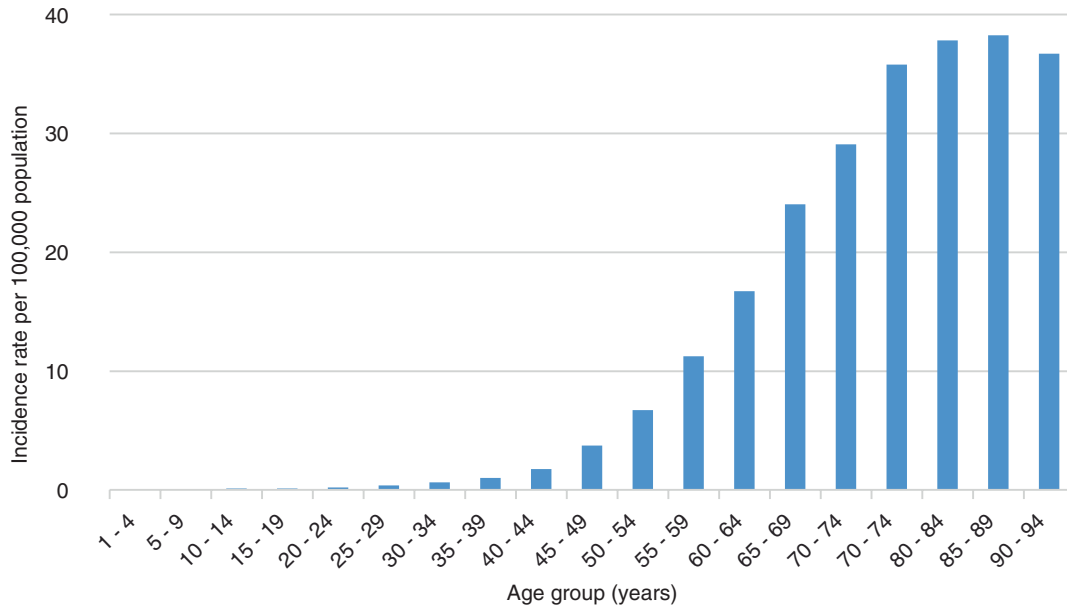

Fig. 3 Age distribution of new cases of liver cancer in the UAE in 2019 [6]

5 Health Issues Facing UAE and Its Healthcare System

It is pertinent to view HCC in the context of diseases prevalent in the UAE and the challenges faced by its healthcare system. UAE is a rapidly developing country which is transitioning from reliance on petrochemicals to a more diverse, knowledge-based economy. Dubai and Abu Dhabi have become world centers for trade, finance, travel, and tourism. With rising affluence and lifestyle change, there is increasing consumption of highly processed foods and hepatotoxic substances such as alcohol, leading to metabolic steatohepatitis.

The leading causes of death in the UAE are ischemic heart disease, road injuries, stroke, chronic kidney disease, and diabetes. Malignancies are ranked lower, and among neoplasms, liver cancer ranks at 12th place [6]. This is much lower than global trends, where liver cancer consistently ranks amongst the top 5 leading causes of cancer death [26, 27]. According to the UAE official cancer registry, the top 5 leading causes of cancer death in the country are breast, lung, colorectal, leukemia, and stomach [3].

Cancer care in UAE, like all other aspects of the healthcare sector, has witnessed enormous transformation over the last couple of decades. Developments have occurred throughout the entire spectrum of the specialty, including diagnosis, screening, early detection, prevention, palliative care, and management. Major cancer care centers are located in urban regions such as Abu Dhabi, Dubai, and Sharjah. These tertiary care hospitals provide advanced world-class clinical services including endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance imaging (MRI), trans-arterial chemoembolization (TACE), and minimally invasive and robotic hepatobiliary surgery. Systemic chemotherapy is available for advanced HCC. In 2018, the first liver transplantation in the UAE was performed at Cleveland Clinic Abu Dhabi [28]. Posttransplantation care and expertise is available for recipients travelling from abroad. Palliative care and visiting home nursing are increasing in capacity with growing demand as the population ages in the UAE.

6 Policy Recommendations

Key public health messages for liver cancer prevention may be potentially useful. Avoiding alcohol consumption, limiting processed foods that are rich in fat and simple sugars, increasing physical activity, vaccinating against hepatitis B, and avoiding reuse of medical sharps should be emphasized. Due to the diversity of people living in the UAE (more than 200 nationalities), culturally appropriate, multilingual approaches to health promotion are essential. Risk factors such as chronic hepatitis and alcoholism are higher among expatriate workers in the UAE [29].

More aggressive case finding for chronic hepatitis C may be useful toward reducing HCC. With the availability of effective oral antiviral regimens for hepatitis C, universal access to this lifesaving treatment should be pursued.

Among healthcare workers, refresher training, systems processes, and strict enforcement of infection control measures may be useful. Recognition and accreditation may provide additional incentives. Exposure to hepatitis C virus occurs in high-risk groups, often linked to medical care. In particular, disinfection protocols are critical for medical instruments used in intravenous access, vascular procedures, endoscopy, surgery, and dentistry. Immunity against hepatitis B among healthcare workers should be assessed as booster series may be needed [29]. Immunity from childhood hepatitis B vaccination wanes to 50% among UAE medical students [30].

Finally, it is worth mentioning that the UAE government heavily subsidizes healthcare for both its citizens and expatriates. Many patients with HCC in the UAE receive advanced cancer care, including chemotherapy and oncologic surgery, free of cost.

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Part II

Clinical Section

Overview of Clinical HCC and Its Management

Brian I. Carr

1 Clinical Risk Factors

Risk factors for developing HCC in patients with cirrhosis include older age, male gender, and severity of compensated cirrhosis, independent of etiology or cause of the cirrhosis (most commonly from hepatitis B virus [HBV], hepatitis C virus [HCV], or alcoholism). Mixed infection with HBV and HCV, HCV and HIV, or HBV plus alcohol greatly increases the HCC risk as well.

Common clinical diseases associated with an increased risk for developing HCC:

- Cirrhosis from any cause.
- HBV or HCV chronic infection.
- Alcohol chronic consumption.
- NASH/NAFLD (nonalcoholic steatohepatitis, typically from obesity).
- Aflatoxin B_1- or other mycotoxin-contaminated foods.

Less common diseases associated with an increased risk for developing HCC:

- Primary biliary cirrhosis.
- Hemochromatosis (increased iron).
- α_1 Antitrypsin deficiency,
- Glycogen storage diseases (rare metabolic diseases).
- Citrullinemia (rare metabolic disease).
- Porphyria cutanea tarda (rare metabolic disease).
- Hereditary tyrosinemia (rare metabolic disease).
- Tyrosinemia type I (rare metabolic disease).
- Wilson's disease (increased copper).
- Autoimmune hepatitis.
- Alagille syndrome of infants.

Patients with any of the diseases that predispose them to HCC can be exposed to a variety of additional factors that increase their risk for HCC, including diet, alcohol, and possibly obesity. In the Middle East, both HBV and HCV feature as prominent causes, with HBV high in Turkish HCC patients and HCV high in Egyptian HCC patients.

The leading cause of HCC in Asia and sub-Saharan Africa is hepatitis B infection. Patients have a higher risk of developing HCC if they have hepatitis B infection and are alcoholics or have hepatitis B infection and are exposed to fungal toxins.

The leading cause of HCC in Japan, Western Europe, and the USA is cirrhosis that is caused by hepatitis C. People can get hepatitis C through contaminated blood transfusions, syringes, and needles or drug abuse. It is thought that the rate of HCC development is approximately 2% yearly for chronic HBV cirrhotic patients and 3–8% per year for chronic HCV cirrhotic patients.

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Increasingly, a sedentary lifestyle and accompanying obesity, which has developed into epidemic proportions, is coming to be regarded as a major source of disease and mortality from its associated diseases, including HCC. A chapter has been devoted to this increasingly important topic (H. Akkiz).

Cirrhosis can typically take 10–15 years to develop after hepatitis viral infection is established, and HCC typically develops after an additional 10 years or more of cirrhosis. Cirrhosis is thus a premalignant disease. Many patients may die of liver failure from their cirrhosis without developing HCC. Conversely, many patients with cirrhosis can receive curative liver transplants without developing HCC. In general, HCC incidence appears to track portal pressure (one measure of this is reflected in thrombocytopenia, a surrogate marker). Cirrhosis occurs in about 10–15% of alcoholics, of whom about 15–20% develop HCC at a rate of 3–4% per annum. Alcohol is not a direct carcinogen, but HCC likely develops as a consequence of alcohol-induced oxidative stress (reactive oxygen species), which then affects downstream cellular lipids, proteins, DNA, and cell signaling pathways. Reactive oxygen species are also thought to be important in iron and copper accumulation disorders as well as in nonalcoholic steatohepatitis (NASH), resulting from fatty liver disease.

The current obesity epidemic is associated with nonalcoholic steatohepatitis (NASH), which requires liver biopsy for diagnosis and may also lead to a symptomless form of cirrhosis. NASH is distinct from the usually harmless fatty liver by being associated with liver inflammation. Nonalcoholic fatty liver disease (NAFLD) may or may not be associated with NASH. NAFLD is associated with metabolic syndrome and diabetes mellitus type 2, which in turn can be associated with HCC.

2 Epidemiology

The major cause of HCC in Asia (where HCC is globally most prevalent) and sub-Saharan Africa is chronic HBV. There are over 300 million HBV carriers worldwide who may develop HCC with or without the development of the intermediate step of cirrhosis, unlike HCV in Western countries, where cirrhosis is a necessary intermediate step. The conversion rate for chronic HBV carriers to HCC is thought to be approximately 2–3% per annum. HBV is a DNA-binding virus and may directly influence gene function. The incidence of HCC is lower in alcoholic cirrhosis, NASH, and hereditary hemochromatosis. In China, Southeast Asia, and sub-Saharan Africa, aflatoxin B_1 is the most potent naturally occurring liver chemical carcinogen known (a group 1 carcinogen) and is a fungal product that contaminates stored rice, peanuts, ground nuts, and maize and is an important cause of HCC. The carcinogen is produced by the carcinogenic fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Concomitant HBV plus alcohol and HBV plus aflatoxin B_1 exposure are thought to substantially increase the HCC risk; less is known of aflatoxin B_1 combinations with HCV.

The major risk factor for developing HCC in Japan, Western Europe, and the USA is HCV-mediated cirrhosis, mainly from transfusion with contaminated blood or use of contaminated syringes or needles through medical or recreational drug use. The mechanism of HCV-mediated carcinogenesis is complex and it does not bind to DNA like HBV. It is thought that the risk for HCC development in HCV-based cirrhosis is approximately 3–5% per year. HCV seems to relate to HCC mainly via the development of cirrhosis, and the risk seems proportional to the severity and duration of the HCV-induced hepatic inflammation and fibrosis that are part of the resulting cirrhosis. Studies involving outbreaks of HCV from contaminated blood transfusions have indicated that it takes decades to develop HCC. However, now that donors and their blood in blood banks are screened for HCV, it is thought that HCV infections will sharply decrease over the next 30 years. Evidence of this trend is already clearly available in Japan.

Globally, HCC is now the fifth most common type of cancer and the third most common cause of death from cancer. The reason for this discrep-

ancy is due to the fact that a high proportion of patients die from this disease (overall ratio of mortality to incidence is about 0.9). The number of new HCC cases varies from country to country. Asian and sub-Saharan African countries have more new cases of HCC than Western countries. This difference is probably due to how each population is exposed to different risk factors. Examples of these risk factors include having hepatitis or eating food that is contaminated by fungal toxins. Generally, men have a higher rate of HCC than women. This may be due to increased tobacco smoking and alcohol consumption in males, which are both risk factors for developing HCC.

The cause of the gender discrepancy in HCC arising from cirrhosis (>80% of HCC cases) is unclear for viral causes. However, for chemical causes, such as aflatoxin B_1 contamination of foods, animal studies have shown that male rodent livers are better able to metabolize the carcinogen to its DNA-reactive and thus carcinogenic form.

There are about 750,000 new global cases annually; it is the fifth most common cancer in males and the seventh most common in females. There is a male predominance in incidence, varying from 9:1 male/female to 2:1 male/female cases, depending on the country, except in low-cirrhosis Western countries where the ratio approaches 1:1.

Most HCC cases worldwide occur in developing countries. The world's highest incidence rates are found in Eastern Asia, followed by Southeast Asia and then Central Africa. Southern Europe has moderately high rates, as do Central America and Polynesia. Low rates occur in Western Europe, the USA, and South America, with the lowest being in Northern Europe, Australia/New Zealand, and South-Central Asia. The large global variation is thought to be due to differences in exposure to causative factors, such as hepatitis virus or carcinogen contamination of foodstuffs, but not to ethnicity. Supporting this, studies of migrant populations, such as Japanese or Jews living in various locales, show changes in HCC incidence in the same ethnic group, but living in different locations.

3 Prevention: Primary, Secondary, and Tertiary

Many risk factors for developing HCC can be prevented. There are three types of prevention: primary, secondary, and tertiary prevention.

- Primary prevention consists of reducing the chance of developing HCC.

  Examples of primary prevention include destroying contaminated food, vaccinating neonates against hepatitis B, and screening blood at blood banks for hepatitis C. Currently, the most important is screening pregnant women for hepatitis B and vaccinating newborns against hepatitis B.

  Destroying aflatoxin B_1-contaminated, spoiled foodstuffs is simple in theory but can result in a major financial burden to farmers in impoverished regions in rural China or Africa where it is most common. Prevention of the *Aspergillus* mold from growing in the first place, by storing grains, such as peanuts in refrigerated silos, is likely the most effective preventive measure in these areas but requires capital outlay for refrigeration in these farming communities.

  The near-universal neonatal vaccination against HBV is already showing dramatic decreases in both HBV and the resulting HCC in children and adolescents in those areas with a high incidence of HBV. This approach is likely to cause a huge decrease in Asian HCC in the coming decades.

  The elimination of HCV-contaminated blood in blood banks in Europe and Asia is expected to contribute to a major decrease in HCV infection, although recreational drug abuse remains a problem.
- Secondary prevention means that the patient has risk factors and attempts to prevent HCC from developing. Examples of secondary prevention include treating patients who have chronic hepatitis B or C infections with antiviral therapy and counseling to reduce alcohol consumption.

  Once HBV infection has taken place, viral treatment strategies are needed and

have become increasingly effective in recent years in decreasing the blood-viral load (sustained virological response). It is expected that this will interfere with the development of cirrhosis and minimize the development of HCC. Although the data are preliminary, evidence has been published from meta-analyses of the effectiveness of HBV therapy. The treatment of chronic HCV infection, with resulting undetectable HCV blood levels, has recently been shown to greatly diminish HCC incidence rates. For both patients with chronic HBV or chronic HCV, a treatment-induced sustained virological response has been found in several studies to reduce the HCC incidence rate by >50%. It remains to be determined if this will be true of patients with HCV who also have cirrhosis.

Since alcohol consumption is a lifestyle choice and a contributor to HCC development, it would seem that alcohol counseling might be effective in either alcohol consumers or for alcohol consumers who are also HBV or HCV carriers, but the effects of an intervention are likely to be greater when undertaken at younger age or at earlier phases of the hepatitis. Cigarette smoking is a significant HCC cofactor, and the same principles apply here as for chronic alcohol overconsumption.

- Tertiary prevention involves the new and effective therapies that can suppress hepatitis in patients who have had their HCC surgically treated, but in whom the etiological factors are still active (HBV, HCV, alcohol). Tertiary prevention became available in recent years and has resulted in major decreases in HCC recurrence rates in areas where HBV therapies have been introduced on a wide scale. This is a major recent public health advance. It is unclear that HCV therapies can decrease HCC recurrences, as HBV therapies seem to do.

4 Surveillance

Screening for HCC is possible because there are several known risk factors and causes, and thus a large percent of patients who are at risk for HCC development can be identified. The earlier a cancer is detected by screening in general, the sooner and more effectively it can be treated. Furthermore, evidence is beginning to show that patients with HCC who are treated earlier, as a result of surveillance, have longer survival rates, at least for HBV-based HCC patients.

Screening for HCC usually involves an abdominal ultrasound scan. This can determine if there is a tumor, its size estimated, and possibly its growth after repeated scans. Abdominal ultrasound scans should be performed approximately every 6 months for patients at risk for developing HCC, such as those with cirrhosis from any cause or subsets of chronic HBV carriers. Screening can also include the cheap serum biomarker test for alpha-fetoprotein (AFP). Unfortunately, it is not elevated in over 50% of small HCCs – just when a serum biomarker screening test is most needed. Other serum biomarkers have also not yet been shown to be cost-effective in screening.

5 Diagnosis

An HCC diagnosis can be made with dynamic contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) or by contrast-enhanced abdominal ultrasound (US). If the lesion demonstrates specific imaging characteristics of vascularity with washout, a diagnosis of HCC can be made radiographically, obviating the need for a biopsy.

6 Diagnostic Approach

Lesions <1 cm are too small to be definitively diagnosed by further imaging or biopsy. They

should be monitored at short intervals of 3–6 months for about 2 years. If the lesion disappears or remains 1 cm, the patient may return to routine surveillance at 6-month intervals. If the lesion grows beyond 1 cm, or if a new ≥1 cm lesion develops, or if the AFP level is rising, a CT or MRI is obtained.

Lesions ≥1 cm are evaluated by contrast-enhanced CT or MRI. Biopsy for histologic confirmation is not typically regarded as necessary if the lesion fulfills typical imaging criteria for HCC.

Typical HCC imaging features are defined as arterial phase hyperenhancement of the suspected nodule on scan, with washout in the portal venous, delayed, or hepatobiliary phases.

A resection or ablation procedure will anyway provide biopsy confirmation at the time of the procedure. When an initial imaging modality cannot provide a diagnosis with confidence, a different imaging modality seems reasonable as a follow-up.

Although the need for biopsy has become less compelling, based on recent guidelines, as practicing oncologists, we prefer core biopsy proof of HCC. Furthermore, as molecular testing for prognostic and therapeutic subset identification becomes more accepted and useful, biopsy will be needed, just as it is for management of other solid tumors. Biopsy proof is certainly obtained for suspicious vascular liver masses without evidence of cirrhosis. Given the importance of the HCC-surrounding liver for prognostic molecular signatures (chapter "Biological Aspects of HCC"), we are also including biopsy of the non-HCC liver in our biobank collection for future studies.

All patients have a scan of the chest and abdomen, to identify the extent of the HCC and the HCC aggressiveness characteristics of maximum tumor dimension, number of tumor foci, and presence of PVT. Blood tests include hepatitis B and C markers, HCC serum biomarker, AFP and DCP levels, complete blood count and differential count and PT, liver function tests of total bilirubin, ALT, AST, ALKP, GGT, albumin, creatinine, as well as CRP and ESR levels. Calculation is made of BMI, NLR, PLR, and Glasgow Index.

7 Staging Systems for HCC

Staging is important both for prognosis and therapy selection. Several systems are in use, but their common agenda is to take into account both liver disease severity and tumor aggressiveness factors of maximum tumor size, multifocality, and PVT.

The Barcelona Clinic Liver Cancer (BCLC) staging system includes factors for tumor stage, degree of liver function, and performance status of the patient and has been endorsed by EASL and AASLD. However, it includes fairly limited treatment recommendations. Korean, Japanese, and Chinese systems have been proposed, which may in part reflect there different HCC populations.

For all our patients, minimum workup includes a high-quality CT scan or MRI of the chest and abdomen with contrast liver images, together with tests for hepatitis B and C, complete blood count, and liver function tests, to include GGT (especially helpful in that 50% of patients with low AFP levels), as well as serum AFP and DCP levels. BMI is also assessed and we routinely add inflammation markers CRP (together with albumin, for the Glasgow Index) and ESR and compute NLR and PLR for prognosis evaluation.

Following diagnosis, a multidisciplinary setting is usually considered appropriate for discussing the disease extent and treatment options. Especially in clinical trial settings, a psychological evaluation is used for quality of life assessment, as well as for liver transplant donors and recipients.

A. Child-Pugh (CP) score for cirrhosis grade							
Factor	1 point		2 points		3 points		
Total bilirubin (μmolL)	<35		35–50		>50		
Serum albumin (g/L)	>35		28–35		<28		
PT INR	<1.7		1.71–2.30		>2.30		
Ascites	None		Mild		Moderate/severe		
Encephalopathy	None		Mild		Severe		
Scores:	**Class A**		**Class B**		**Class C**		
	5–6 points		7–9 points		10–15 points		
	100%; 1-year survival		80%: 1-year survival		45%: 1-year survival		
B. Some HCC staging systems							
(1) CLIP classification							
Variables	0 points		1 point		2 points		
(i) Tumor number	Single		Multiple		–		
Hepatic replacement by tumor	<50%		<50%		>50%		
(ii) Child-Pugh score	A		B		C		
(iii) a Fetoprotein (ng/nL)	<400		≥400		–		
(iv) Portal vein thrombosis (PVT)	No		Yes		–		
(2) Okuda classification							
Tumor extent[a]	Ascites			Albumin (g/L)		Bilirubin (mg/dL)	
≥50%	<50	+	–	≤3	<3	≥3	<3
(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)

CLIP stages (sum of points): CLIP 0, 0 points; CLIP 1, one point; Clip 2, two points; CLIP 3, three points
Okuda stages: stage 1, all negative; stage 2, 1 or 2 (+); stage 3, 3 or 4 (+)
[a]Extent of liver occupied by tumor; CLIP Cancer of the Liver Italian Program

(3) BCLC staging	
Stage 0: very early	CP A; single nodule <2 cm; PS 0–1
Stage A: early	CP A-B; 1 nodule or 2–3 nodules <3 cm; PS 0–1
Stage B: intermediate	CP A-B; multinodular; PS 0–1
Stage C: advanced	CP A-B; PVT or N1 or M1; PS 0–2
Stage D: terminal	CP C; any T, N, or M; PS >2

CP Child-Pugh score, PS ECOG performance status, N1 lymph node involvement, T tumor size, M1 metastasis

Predictions of the BCLC classification:

(a) Early-stage HCC patients (stage 0 and A) may benefit from potentially curative treatments (liver transplant, resection, radiofrequency ablation).
(b) Intermediate-stage (stage B) or advanced-stage (stage C) patients may benefit from TACE, RFA, or systemic TKI or ICI therapies.
(c) End-stage disease (stage D) patients are offered supportive care and palliation.

8 HCC Clinical Biomarkers in Circulating Blood

Alpha-fetoprotein (AFP) is a glycoprotein produced in the embryonic liver and a form of fetal albumin, the synthesis of which is turned off at birth. Hence, an older name is oncofetal antigen, which, like CEA and glypican-3, is resynthesized in some tumors in adult life. It is frequently used and inexpensive and is a simple blood test to perform but is elevated in only 50% of patients with HCC. AFP is not a sensitive marker for screening

for small HCCs but is extremely useful if elevated, when it can be used to follow the response of an individual patient to therapy or to see if therapy fails. It is also beneficial when used after surgery, resection, or ablation for tracking the possibility of recurrence, again, only in those patients in whom it was initially elevated at baseline. However, its use as a screening tool in surveillance is limited, when the search for small HCCs is the goal.

Recently, more HCC-specific tests have come into general clinical practice, such as a glycosylated form of AFP (itself, a fetal form of albumin) called AFP-L3.

Des-gamma carboxy prothrombin (DCP) or protein induced by vitamin K absence (PIVKA-2) is an HCC-secreted biomarker, and US Food and Drug Administration (FDA)-approved kits for measuring both AFP-L3 and DCP are readily available to clinical labs. Several studies have shown that elevated DCP is common in the presence of portal vein thrombosis (PVT). The molecule is really interesting, as it is an immature form of the coagulation protein, prothrombin. The enzyme responsible for catalyzing the immature to the mature form of prothrombin has an absolute requirement for vitamin K.

A diagnostic model has been proposed that incorporates the levels of each of the three biomarkers, AFP, AFP-L3%, and DCP, along with patient sex and age, into the Gender, Age, AFP-L3%, AFP, and DCP (GALAD) model, but awaits validation for screening.

Glypican-3 is another oncofetal glycoprotein that appears to have prognostic significance as an HCC serum biomarker and is being investigated both for use in imaging and as a potential target in HCC therapeutics.

None of these markers alone or together have yet been shown to be cost-effective for surveillance though.

9 Therapies for HCC

The aims of therapy for HCC, as with other solid tumors of patients, is to firstly improve quality of life and secondly to improve lifespan or survival time. In all of oncology, the patient's medical personnel must strike a balance between the objective of increasing survival as compared to untreated patients on the one hand and, on the other hand, to consider the availability, side effects (toxicities), risks, and costs of the intended approaches. In this section, a general overview of best treatments for the appropriate stage of the disease will be considered, but in chapter "Transarterial Radioembolization in Hepatocellular Carcinoma", consideration will also be given to reasonable therapies to offer to HCC patients in less well-developed countries.

Overall, the complexities of patient decision-making, involving as they do, treatment of the underlying liver disease and how it affects both medical and surgical treatment choices, are best done in a multimodality setting. This normally requires the following specialities to be represented in the discussion: diagnostic radiology, interventional radiology, liver surgery, medical oncology, hepatology, and usually, pathology and psychosocial services. We have found that this provides an optimum environment for therapy decision-making and therapy sequencing, as well as identifying the personal needs of the individual patient to be able to cope with the needed treatments that are being suggested. Quite often, there is more than one reasonable approach to an individual's HCC and associated liver pathologies and comorbidities.

10 Therapies with Curative Intent: Injection, Ablation, Resection, and Liver Transplantation

Survival after the diagnosis of almost all solid tumors is typically best if the total identified tumor mass can be completely removed. This is most readily achieved for small HCCs (usually $\leq$3 cm diameter in a minimally cirrhotic or non-cirrhotic liver). The tumor size constraints are typically due to the functional reserve of the underlying diseased liver, as well as the proximity of the tumor to major hepatic blood and bile vessels.

Percutaneous injection of ethanol (PEI) or *acetic acid* is a time-honored, cheap, and easily performed procedure, requiring only a syringe and needle and an ultrasound machine to guide the needle placement, as well as the cheap agent to be injected. It has generally been supplanted by the other ablation techniques. However, it may be very useful for a resource-poor region. Typically, two to three small satellite lesions can also be treated this way, with repeat treatments every few months, as dictated by tumor growth or recurrence found on radiological follow-up. Patient tolerance is typically very good.

PEI has generally been superseded by *radiofrequency ablation* (RFA) or *microwave ablation* (MWA) procedures, which can be performed nonoperatively (percutaneously) or at open surgery. Excellent tumor necrosis can be obtained for small tumors and with minimal patient toxicities. Several studies have shown that even better tumor control can be achieved by combining RFA with TACE. Other forms of local ablation, such as hypothermia or hyperthermia, have fallen into disuse.

For all but minimal tumors, *hepatic resection*, involving various amounts of resected liver, depending on the size of the tumor and extent of the underlying liver disease, has been the standard of care for decades, where applicable. Multiple satellites can be removed at the same time, and often resection can be combined with RFA to distant liver nodules. Increasingly, hepatic resection is being done laparoscopically, by experienced surgeons. Major hepatic resections can be used only for patients with excellent liver function, Child-Pugh (CP) class A without portal hypertension or elevated bilirubin. The reason is that such resection depends on liver regeneration postsurgery, which is compromised by chronic liver disease. Limited resection can be used in the presence of CP A or B7 cirrhosis and sometimes even in the presence of tumor invasion of a bile duct or portal vein, though not the main trunk. Up to 50% 5-year recurrence rates are reported in large numbers of centers, with very low operative mortality (see chapter of Cost effective therapies B. Isik).

Liver transplantation differs from all the other HCC treatments in that it can cure two diseases simultaneously, namely, both the HCC and the underlying liver disease, usually cirrhosis. Using the well-established Milan criteria of a single HCC nodule not >5 cm or up to three nodules, not >3 cm, approximately 75% survival rates are typical, which is similar to transplantation survival rates for noncancerous liver diseases. Recently, several centers have shown similar survival rates for slightly larger HCCs. Regardless of size, the results are much poorer when macroscopic PVT is present that is observed radiologically. Many patients also have microscopic PVT that is usually found on pathological examination of the explanted liver. Survival rates are not as great for these patients but still much superior to the survival of patients with macroscopic PVT. More recently it has become apparent that patients with high pretransplant serum AFP levels also have poorer survival and this has now become part of the selection process for patients awaiting liver transplantation. Exact AFP cutoff levels have not yet been agreed upon, although the range of 500–1000 ng of AFP/mL seems to offer useful cutoff guidance. Even with the most restrictive criteria for liver transplantation, between 10–15% of patients have HCC recurrence within 5 years, often in the lung and to a lesser extent in the new liver. Due to the persistent loss of patients in the variable and sometimes long months of waiting for a cadaveric donor, some centers now offer live donor liver transplantation (LDLT), which does not involve several months of waiting and therefore no loss of patients on the transplant list. As centers evaluate extending the Milan criteria to more advanced tumors, which then require downstaging for tumor control, even LDLT transplant recipients often require several months of waiting while their HCCs are brought under control (downstaged).

11 Management of HCC Patients with Tumors Not Suitable for PEI, Ablation, Resection, or Liver Transplantation

This group constitutes the majority of diagnosed HCC patients. Even the practitioners of transplantation, resection and ablation (PEI, RFA, MWA) usually do not think of these therapies as

curative, although some use the phrase "curative intent." The thinking is that if all visible tumors which have been seen on CAT scan are removed, then the patient (or a subset of patients) might be cured. Although theoretically possible, this is often unlikely. Considerations of tumor biology, explained earlier in this chapter, indicate the reasons. HCC typically arises on a liver that is chronically diseased from HBV or HCV or alcoholism or dietary aflatoxin consumption or, as recently recognized, from obesity/metabolic syndrome. Any of these etiologies lead in most patients to a degree of cirrhosis, which is regarded as a key intermediate step between cause and HCC development. This process usually takes upward of 10 years, although the degree of cirrhosis is clearly linked to the probability of subsequent HCC development. Thus, in a cirrhotic liver, there may be millions of cirrhotic nodules, and many of them can be premalignant and eventually develop into HCC. Thus, it is only a matter of time before a patient with cirrhosis develops either liver failure, or HCC, or both. Therefore, after resection or ablation, there are multiple other residual cirrhotic liver nodules that are in the process of developing into HCC. This likely explains the high levels of recurrences postsurgical resection. This, however, does not explain recurrences post liver transplantation. For that, it is necessary to learn about how tumors of other organs spread, such as breast cancer to the liver, lung, and bone. The concept of circulating tumor cell micrometastasis was developed to explain this, which has received recent experimental support from evidence in patients with a variety of tumors, including HCC. In these patients, circulating tumor cells (CTC) in the blood have been found, as well as circulating tumor DNA (ctDNA) fragments. Consistent with this is the observation that a proportion of HCC patients during the 5 years after liver transplantation for HCC develop lung metastases. Their origins are thought to be from circulating tumor cells in the bloodstream.

As with any aspect of HCC therapy, considerations of both tumor properties (maximum tumor diameter, number of tumor nodules, portal vein invasion and thrombosis [PVT], and serum levels of tumor markers, especially AFP) need to be taken into account, as each has prognostic implications. However, unlike most other tumors, patients with HCC usually have a second potentially life-threatening disease, namely, hepatitis or cirrhosis, and the extent of the resulting liver damage has a major impact on the choice of which therapy might be safe. The indications and contraindications for liver transplantation, resection, or ablation are given in the chapters of S. Yilmaz and B. Ishik.

12 Trans-arterial Chemoembolization or TACE for HCC Confined to the Liver

The majority of HCCs are unsuitable for the above therapies at the time of diagnosis, because of the presence of portal hypertension, poor liver function, tumor multifocality, portal vein tumor thrombosis (PVT), extrahepatic tumor spread, old age, or comorbidities, and thus they are BCLC stages B, C, or D.

TACE has been the most commonly used non-surgical treatment modality for these patients, if they are nonmetastatic, and has been the standard of care for intermediate stage HCC patients, BCLC B. The principle upon which it is based is that while the portal vein is the predominant supplier of oxygenated blood to the liver, the hepatic artery supplies over 80% of the oxygenated blood to the HCC (but not to other tumor types in the liver). Any therapeutic agent that is injected into the hepatic arterial branch feeding the HCC will thus be relatively selective in targeting the HCC and will to a considerable extent spare the underlying liver parenchyma. Thus, through a needle placed percutaneously into the inguinal artery in the groin, a catheter is threaded into the arterial system and then into the liver (chapter by R. Kutlu) and to the hepatic arterial branch that feeds the tumor to be treated. High concentrations of cytotoxic cancer chemotherapy drug(s) can in this way be injected into the artery feeding the tumor. Usually, embolizing particles are also injected. This embolization has two purposes. By slowing down the hepatic arterial blood flow, it has been shown that increased amounts of cancer

chemotherapeutic agent are thereby taken up by the tumor. Additionally, some measure of tumor necrosis is achieved by injection of the embolizing particles. In addition to TACE, trans-arterial embolization, which uses only embolic materials, and hepatic arterial infusion chemotherapy (HAIC), which uses only antitumor chemotherapeutic agents, has each been used as treatment modalities. The chemotherapy drugs most commonly used are doxorubicin, cisplatin, or a combination of doxorubicin and cisplatin plus mitomycin C. However, there is little clinical trial evidence to support the choice of one of another nor the optimal dose of any of these drugs. This author prefers cisplatin over doxorubicin, due to its safety in cirrhosis, in which a high proportion of patients have portal hypertension-associated baseline thrombocytopenia. In recent years, doxorubicin-based drug-eluting beads have been tried, but they do not seem to be superior to bland embolization. The procedure can be repeated on a schedule of every 6–12 weeks, or on demand, when there is evidence of residual vascular tumor, increasing tumor mass or new nodules, or rising serum AFP levels. The main toxicities are liver-related or transient abdominal pain from the embolization (post-embolization syndrome). A range of 30–70% response rates has been reported. However, modern evidence on survival advantage is largely lacking. The special case of presence of PVT deserves mention, as it is present in 30–40% of HCC patients. It was considered as a relative contraindication to TACE in the past, since TACE can damage the hepatic artery and PVT occludes the portal vein. However, in experienced hands, this still seems to be a moderately safe procedure, even in the presence of branch but not main stem PVT (see chapter of Trans-arterial chemotherapy and chemo-embolization T. Balli).

13 Trans-arterial Radio-Embolization or TARE for HCC Confined to the Liver

TARE has gained increased traction in recent years, due to its relative safety and effectiveness in patients who have PVT and its lesser toxicity profile compared to TACE. It consists of the intrahepatic arterial injection of glass or resin microparticles (Therasphere or SirSpheres) that have radioactive ^{90}Ytrium as part of their structure (so it cannot be separated from the particle). It is a pure beta emitter, with a 64-hour half-life (background radiation levels are reached by 10 days; it decays to stable ^{90}zirconium)) and a maximum path length of 1 cm (so the medical staff or patient family cannot get irradiated from it after injection into the patient). It seems to give higher tumor response rates compared to systemic sorafenib, but not to enhanced survival. Nor are there comparative head to head studies of the two commercial products. Likewise, there are few phase III randomizations of TACE versus TARE results. However, some recent comparisons of TACE versus TARE show similar survival results and costs, on a 1-year perspective. Post TARE toxicity is mainly fatigue and post-embolization pain is generally absent. However, post-TARE liver toxicity, including increased levels of serum bilirubin, can occur in about 25% of patients, often non-transiently. In some centers, TARE has become a standard for locoregional therapy in place of TACE (see chapter of Transarterial radio-embolization R. Kutlu). Therasphere was approved by FDA in April 2021 for HCC therapy.

14 PVT Therapy

The presence of PVT is a major therapeutic challenge, especially since many patients have poor liver function. Several radiation approaches have been taken. TARE has been shown to be safe and can be effective in this setting, using either Therasphere or SirSpheres. However, external beam radiotherapy (EBRT) is looking increasingly attractive, either with intensity-modulated radiotherapy (IMRT) or stereotactic body radiotherapy (SBRT). Furthermore, TACE combined with EBRT has been shown to result in superior survival in patients with PVT. Recently, PVT therapy with SBRT and downstaging of the primary tumor followed by liver transplantation have resulted in prolonged post liver transplant survivals.

15 Treatment of Metastatic HCC or Patients Who Have Progressed on TACE or TARE: Systemic Therapies

First line This subject is rapidly changing, with new recommendations every 12 months or so at this moment. The discussion below and recommendations must therefore be considered tentative and subject to change. The main nonsurgical treatments for advanced and nonmetastatic HCC confined to the liver are TACE and TARE. For metastatic HCC or for patients who have already received and failed to respond to TACE or TARE or who have main branch PVT, systemic therapies are the mainstay of treatment. At the time of writing, three therapies are considered as a first-line therapy after TACE or TARE or in TACE-/TARE-ineligible patients and who have Child-Pugh class A cirrhosis as well as ECOG performance status 0–1. They include the current first choice in first line, which is combination of atezolizumab (Tecentriq) plus bevacizumab (Avastin), which was approved in 2020, and then, for patients who are intolerant to the component drugs of this combination, either sorafenib (Nexavar, approved in 2007) or lenvatinib (Lenvima, approved in 2018). For patients who have failed/progressed on combination atezolizumab plus bevacizumab, there are several TKI choices for second-line therapy. They include lenvatinib, sorafenib, regorafenib, or cabozantinib or ramucirumab for patients with serum AFP levels ≥400 ng/mL. For those patients who received lenvatinib or sorafenib as first-line therapy and then progressed, reasonable choices for the second line of therapy include atezolizumab plus bevacizumab, cabozantinib, regorafenib, or ramucirumab for those patients with serum AFP levels ≥400 ng/mL.

Multiple combinations of either immune checkpoint inhibitors (ICI) or ICIs plus TKIs are in progress and thus may supplant sorafenib or lenvatinib in the first line post TACE/TARE. Furthermore, as the response rates of these combinations continue to improve and approach the responses seen with either TACE or TARE therapy, these two locoregional therapies might even be supplanted in the future by the newer systemic therapies, using ICI or ICI/TKI combinations, which seem to have both higher responses and survival than sorafenib in comparison trials and higher survival than has been reported for TACE or TARE, without direct comparison trials. Current trials that appear promising include the combination of cabozantinib plus atezolizumab, as well as the combination of two ICIs, nivolumab and ipilimumab, durvalumab plus tremelimumab, as well as combination pembrolizumab plus lenvatinib.

There is a decades-long history of systemic chemotherapy for HCC, almost entirely without survival advantage, when tested in prospective, randomized clinical trials. This approach has therefore been mainly abandoned, especially since the approval of oral sorafenib, a tyrosine kinase inhibitor (TKI). That was the first FDA-approved drug that was not a cytotoxic agent but was developed as an inhibitor of the Raf-1 protein and thus of its downstream signaling mediators, MEK and ERK. It also has multiple other targets, including vascular endothelial growth factor receptor-2 (VEGFR-2), platelet-derived growth factor receptor (PDGFR), and c-kit. It was FDA-approved based on the phase III SHARP trial (published in 2008), in which median overall survival for sorafenib was a statistically significant 10.7 months versus 7.9 months for placebo. A confirmatory phase III trial in Asian patients also showed significant median overall survival advantage for sorafenib of 6.5 months versus 4.2 months in the placebo group. Thus, in Asian patients, survival in the sorafenib arm of the trial was worse than in the western placebo arm of the SHARP trial. Several other findings were of note in the SHARP results. Partial responses were only 2%, while 70% of both sorafenib and placebo patients had stable disease. Furthermore, responses were higher in patients who had HCV as compared with those who had HBV. There was a dissociation between a significantly improved survival (if only10 weeks) for sorafenib and extremely low response rates (compared to TACE and TARE). Perhaps this means that for TKIs, which do not function

primarily by killing HCC cells, unlike cytotoxic chemotherapy, but rather by modulating their growth, stable disease may be an important end point in its own right. If so, this requires a major shift in our thinking about end points for medical therapies for HCC. Furthermore, the sorafenib toxicities were considerable, with a high percentage of patients having clinically meaningful tiredness and lethargy, as well as hand-foot syndrome, skin rash, hypertension, hoarseness, anorexia, weight loss, constipation, and alopecia. In addition, the SHARP trial did not demonstrate an improvement in quality of life for sorafenib versus placebo.

The REFLECT trial was a phase III noninferiority study in previously untreated metastatic or unresectable HCC patients, comparing lenvatinib to sorafenib. The median overall survival was 13.6 months for lenvatinib and 12.3 months for sorafenib. Based upon these results and its toxicity profile, lenvatinib was preferred by many to sorafenib in the first line.

However, everything has changed again with the superior responses and survival results of the IMBrave150 phase III trial, in which previously untreated HCC patients were randomized to receive the combination of atezolizumab (Tecentriq, an ICI) plus bevacizumab (Avastin, a monoclonal antibody targeting angiogenesis) versus sorafenib (Nexavar). Objective response rates were 27.3% of patients in the combination arm versus 11.9% for the sorafenib arm. In addition, 5.5% of patients in the combination had complete responses (disappearance of HCC) compared to none for sorafenib. The overall survival was 19.2 months for the atezolizumab plus bevacizumab combination arm versus 13.4 months for the sorafenib arm. Furthermore, the quality of life was also improved in the combination arm, with many patients continuing to work. The FDA has just given a breakthrough therapy designation for the combination of lenvatinib (Lenvima, a TKI) plus pembrolizumab (Keytruda, an ICI), which gave an ORR of 46% and OS of 22 months. A phase III study is ongoing. Multiple other TKI plus ICI combination trials are underway.

16 Therapies for Patients Who Failed Sorafenib

Second line regorafenib, cabozantinib, ramucirumab, nivolumab, pembrolizumab, nivolumab plus ipilimumab.

Until 2020, there were two approved first-line (or post TACE/TARE) therapies, namely, sorafenib and lenvatinib, and six second-line FDA-approved therapies for sorafenib intolerance or failure. They were regorafenib (TKI), cabozantinib (TKI), ramucirumab (TKI), nivolumab (ICI), pembrolizumab (ICI), and nivolumab plus ipilimumab (ICIs) (Checkmate 040 study). However, at the time of this writing (March 2021), combination atezolizumab plus bevacizumab has been approved in the first line and is so superior to the other agents that current standard of practice is to use it first in the first line. This has changed thinking about best choice in the second line – another first-line TKI or one of the second-line therapies? This so-called sequencing algorithm is currently being evaluated, based upon multiple factors including individual patient tolerance to TKIs, AFP level (ramucirumab was approved for patients with elevated AFP; REACH-2 trial).

Currently, multiple clinical trials are in progress, combining PD-1 and CTLA-4 inhibitors with various ICIs and/or TKIs, as well as combinations of ICIs with TACE or TARE.

17 Combination Systemic or Regional Therapies Combined with Surgery: Bridge to Transplant, Downstaging for Transplant, and Adjuvant (Posttransplant or Resection)

Bridge to transplant The variable long waiting lists in some liver transplant centers mean that some patients who qualify for a cadaveric liver transplant (within Milan criteria) can nevertheless drop out of the transplant wait list if their

HCC grows sufficiently during the months of waiting. Considerable effort is currently expended on keeping the HCCs of these patients from growing and thus preventing a disqualification of the patient from receiving a cadaveric liver transplant. Both locoregional therapies and systemic therapies appear to be suitable in this "bridge to transplant" context.

Downstaging Many patients have HCCs that are larger than permitted by the Milan criteria for cadaveric liver transplantation but who have good liver function and thus the possibility of therapy-mediated tumor shrinkage or downstaging, so that their tumors then might decrease to within transplant criteria. Due to a higher response rate, TACE or TARE are currently used in this situation, but as the newer ICI-containing combinations induce responses above 30% of patients, they may in the future supplant locoregional therapies in this setting. Furthermore, as various centers expand the size of tumors that they are willing to transplant, the combination of neoadjuvant therapy plus transplantation may increase the posttransplant survival of these patients, by bringing the tumors under pretransplant control.

Adjuvant therapies postsurgery After potentially curative hepatic resection, approximately 50% or more of patients develop recurrences in the residual liver within 5 years of resection. This is unsurprising from knowledge of the biology, since the whole of a cirrhotic liver is potentially capable of generating new HCCs in the post-resection parenchyma or capable of being invaded by HCC cells via microscopic portal vein invasion from the resected primary HCC. This is an important unmet need in HCC therapy. Multiple trials using chemotherapy have failed to decrease recurrences, as did the sorafenib in the STORM trial. Nevertheless, at least two studies showed a post-resection survival benefit for hepatic arterial delivery of ^{131}I-Lipiodol (gamma radiation emission) therapy.

PVT There have also been multiple publications on the treatment of PVT with various forms of radiation (external beam and SBRT, among others) in the neoadjuvant setting, prior to resection or live donor liver transplantation (LDLT) for HCC, or as a means of shrinking a tumor sufficiently away from a main vessel to enable safe surgery (see chapter of Radiotherapy for HCC A. Shamseddine).

18 Hepatitis Therapy and HCC

Neonatal vaccination against HBV is a great modern public health triumph in gradually eliminating HBV-based HCC: primary HCC prevention. There is no equivalent HCV vaccine. HCC incidence in untreated HCV patients is directly related to the stage of fibrosis and cirrhosis. Direct-acting antiviral agents (DAAs) can cause a >90% decrease in HCV viral load and a decrease, but not an elimination, in HCC incidence rates, especially in patients without cirrhosis. This is an important example of secondary HCC prevention. There are several reports of a more aggressive HCC phenotype (increased AFP and PVT) in those DAA-treated patients who do develop HCC. Furthermore, the timing of DAA treatment in patients who already have HCC is controversial and should likely be deferred till after HCC treatment. Furthermore, the effects of DAA therapy on time to recurrence post HCC treatment and the aggressiveness of recurrences are currently unclear.

There has been a two decades-long decline in HBV-based cirrhosis and HCC incidence in HBV patients who were treated with nucleos(t)ide analogs. However, it requires prolonged treatments and does not result in a cure, due to the persistence of covalently closed circular HBV DNA. In contrast to HCV patients with HCC, patients with HBV-based HCC are advised to be treated with nucleoside analogs before their HCC treatments, to prevent further liver injury and reduce the risk of HCC recurrence after HCC treatments (tertiary prevention). In addition, nucleos(t)ide analog treatments seem to increase survival even in nonsurgically treatable HCC patients.

Conclusions HCC patients typically have two diseases, the tumor and the underlying liver, which likely have bidirectional influences.

Multiple factors impact the therapeutic approach in an individual patient, including the extent of the liver damage and the site and aggressiveness characteristics (maximum tumor size, number of tumor nodules, presence and extent of PVT). The most reasonable practical approach is the multidisciplinary tumor board that best includes interacting surgeons, oncologists, diagnostic radiologists, interventional radiologists, radiation oncologists, hepatologists, nursing, palliative care team, and psychosocial support colleagues, as well as representatives from the tumor registry and clinical research.

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Cost-Effective Therapies for HCC: Resection and Ablation

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Abbreviations

AASLD	American Association for the Study of Liver Diseases
AFP	Alpha-fetoprotein
ALPPS	Associating liver partition and portal vein ligation for staged hepatectomy
BCLC	Barcelona Clinic Liver Cancer
CT	Computed tomography
EASL	European Association for the Study of the Liver
ECOG	Eastern Cooperative Oncology Group
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
ICG	Indocyanine green clearance
MELD	Model for end-stage liver disease
MVI	Microvascular invasion
MWA	Microwave ablation
PEI	Percutaneous ethanol injection
RFA	Radiofrequency ablation
TACE	Transarterial chemoembolization
TNM	Tumor node metastasis
US	Ultrasound

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1 Liver Resection for Hepatocellular Carcinoma

Therapeutic approaches in the treatment of hepatocellular carcinoma (HCC) involve tumor stage, liver function, tumor biology, and patient comorbidities. Decision of treatment option also depends on the capabilities of the center, familiarity of the physicians to the liver diseases, and budget of the social security system.

Surgical resection can be performed safely in patients with non-cirrhotic livers. These patients account for 5–10% of HCC in Western countries [1]. This incidence is 30–50% in Eastern Asia and majority of Africa where HBV is endemic [2, 3]. Major hepatic resection in these patients is associated with mortality and morbidity rates less than 4% and 33%, respectively, and 5-year overall survival of 50% [4–7]. Even re-resection of the recurrences in selected patients offers 5-year survival rate of 80% [8]. On the other hand, in one-third of patients with nonalcoholic fatty liver disease in whom HCC developed, there will be no sign of cirrhosis, and postoperative complication rates are high in these patients with impaired liver regeneration [9–11]. Perhaps a biopsy to evaluate the liver parenchyma in the preoperative period may be suitable when imaging studies suggest possible steatosis.

Resection is also suitable in patients without clinically significant portal hypertension and in Child A class patients. Resectability decision of a

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tumor needs precise evaluation. Recently perioperative mortality after resection in cirrhotic patients is reported to be under 5% [12–14]. In fact, it is not possible to standardize a resectability definition. Localization of the tumor and multinodularity are important anatomical points. AASLD guideline summarizes resectable HCC from variable references as those (i) minor hepatectomy with one to three unilobar lesions, with an upper size limit of 5 cm for single lesions and 3 cm for more than one lesion (some trials accept two lesions up to 4 cm), (ii) without radiographic evidence of extrahepatic disease or macrovascular invasion, and (iii) occurring in the setting of minimal or no portal hypertension and in the absence of synthetic dysfunction (Barcelona Clinic Liver Cancer stage 0 or A) [15]. On the other hand, Roayaie et al. suggested that selection criteria for resection may be expanded with favorable outcomes [16]. EASL guidelines included patients with portal hypertension for minor hepatectomy if MELD score is <9 [17]. They also emphasized on liver decompensation with a 9% risk of mortality. Solitary tumor is associated with favorable prognosis where size is not a significant factor [18]. Major hepatectomy which is defined as resection of three or more Couinaud segments is achievable with no evidence of portal hypertension and with a MELD score <9 [19, 20]. However, the most important aspect is leaving an adequate hepatic remnant after resection which depends on the functional reservoir of the liver. Mostly the postoperative course after resection in patients with resectable tumors and without portal hypertension is uneventful, and 5-year survival rate is nearly 70% [1]. In patients with elevated bilirubin and portal hypertension with or without multifocal disease, 5-year survival rate is <30% whatever their Child-Pugh score is [21, 22]. Postoperative decompensation characterized by persistent jaundice and/or ascites beyond 3 months is seen in 10–12% of patients, and this condition is associated with mortality in the first year [19, 23–25]. The determinative factor for the dimension of tumor which can be resected is the functional capacity of the future remnant. As far as this capacity is enough, large resections, greater than three segments, can be performed safely. An indocyanine green clearance (ICG) test can estimate liver function. An ICG retention rate of 14% at 15 minutes is acceptable for major hepatectomy where 22% is enough for minor hepatectomy [18, 26]. Also more recently developed LiMAx® (Humedics, Berlin, Germany) test is a useful diagnostic test to predict postoperative failure risk [27].

The future liver remnant volume in patients without cirrhosis should be 25–30%, in patients after chemotherapy should be 30%, and in patients with evidence of cirrhosis should be 40% [28–31]. Although currently portal vein embolization is the first-line choice for small liver remnant, in 20–30% of the cases, resection cannot be performed due to insufficient hypertrophy, disease progression, and complications [32, 33]. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) was introduced by Schnitzbauer et al. for future liver remnant hypertrophy in patients with advanced colorectal liver metastasis [34, 35]. Regenerative effect of ALPPS seems due to alterations in portal flow and systemic response to parenchymal transection inducing hepatocyte proliferation and remodeling [36]. How much regenerative response can be achieved in a fibrotic liver tissue may be questionable, but an animal model revealed an attenuated but present ALPPS-derived regeneration [37]. Although there are concerns about the feasibility of the procedure, in a review by Zhang et al., it was concluded that ALPPS is a safe and feasible approach to treat selected patients with unresectable HCC [38]. However patients with a model of end-stage liver disease (MELD) score of more than 10 revealed increase in mortality [39]. Careful selection of the patients to avoid liver failure, small for size syndrome, and perioperative 31% mortality rate are essential while patient is evaluated for ALPPS [29, 40, 41]. To induce the hypertrophy of future remnant liver, portal vein embolization has also been used [42]. Liver resection for HCC following portal vein embolization is a safe procedure with morbidity rates of 19–55% and mortality rates of 0–12% [32, 43–51]. The 5-year overall survival ranged from 44% to 72% in patients who underwent surgical resection for HCC after portal vein embolization. If portal vein embolization is combined with transarterial chemoembolization, the hypertrophy of

the future remnant liver is greater because the arterial flow is also occluded [52].

Curative resection attempts for intermediate and advanced HCCs are controversial. Koh et al. reported a systematic review of 74 articles for intermediate and advanced Barcelona Clinic Liver Cancer (BCLC) stage HCC [53]. Current guidelines don't propose surgical resection for these stages. However, Koh et al. revealed in this review that the median 5-year overall survival after resection for BCLC B is 38.9 (range: 10.0–57.0%) which is better than other treatment choices. The same study also demonstrated that median 5-year overall survival after liver resection for BCLC C cases is 20% which is poor. Surgical resection also seems as a good choice in this report for multifocal HCC with the median 5-year overall survival of 54.0% (range: 29.9–75.5%). In a review by Kim et al., the median 5-year overall survival rate was 65% after resection for HCC within "Milan criteria" including multifocal HCC [54]. Kokudo et al. evaluated the survival benefit of resection in HCC patients with portal vein tumor thrombosis [55]. They reported that as long as the thrombus is limited to the first-order branch, resection is associated with longer survival than nonoperative approach. The survival benefit was not statistically significant in patients with thrombus invading the main trunk or contralateral branch.

Another group of patients are the ones with extrahepatic spread. Because the presence of extrahepatic disease is considered as a contraindication for surgical therapy, most of the patients treated for metastatic disease are previously resected or transplanted ones. Encouraging results have been obtained from metachronous extrahepatic metastasectomy. Extrahepatic metastasectomy after initial treatment which is transplantation or resection is associated with 20-month increase in median overall survival when compared to treatment of these patients with sorafenib alone [56]. Berger et al. reported 1-, 2-, and 5-year overall survival rates for extrahepatic metastasectomy as 77.4, 53.1, and 25.1%, respectively. Patients who underwent lung resections had better median overall survival compared to metastasectomy performed for other sites. In this same report, it was shown that patients with ≤ 2 metastases benefit from metastasectomy [56]. In a recent study, Yoh et al. reported their repeat surgery results for both intra- and extrahepatic recurrences [57]. They concluded that surgery for recurrent HCC may yield long-term survival for both intra- and extrahepatic recurrences in selected patients.

There is no consensus on the type of the resection to be performed. Anatomical or non-anatomical resections may be preferred. Makuuchi who introduced anatomical liver resections suggests this procedure to minimize the volume of noncancerous but cirrhotic liver to be resected with optimal outcomes [58]. Intrahepatic metastases occur from tumor cells carried via portal venous branches. Anatomical resection defined by Makuuchi et al. is resecting one or more segments by mapping the corresponding portal branch of the tumor [59]. Nonanatomical or limited resection is removal of the tumor with adequate tumor-free margins (Fig. 1). In a

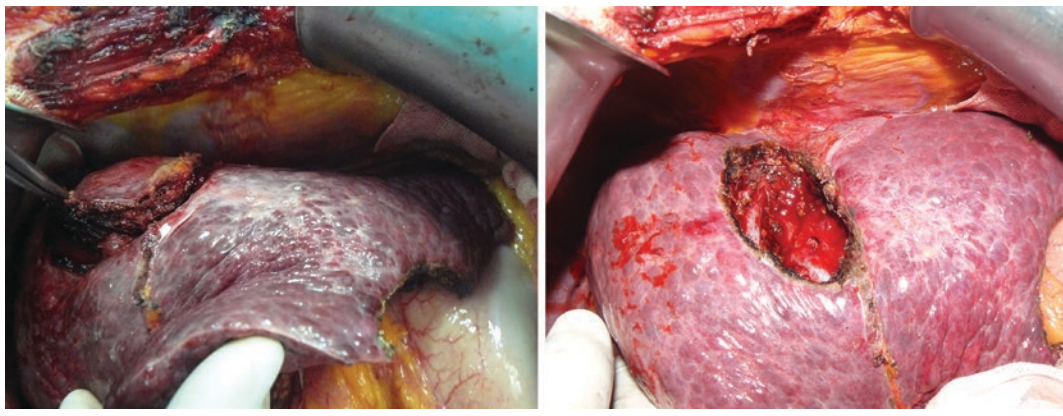

Fig. 1 Nonanatomic resection of two HCC nodules in the cirrhotic liver

meta-analysis by Jiao et al., overall survival benefit at 3 and 5 years was superior and statistically significant in anatomical resection group [60]. Again statistically significant disease-free survival benefit from anatomical resection was gained at 1, 3, and 5 years. On the other hand, this same study suggests a subgroup of patients with poor liver reserve function who can benefit from nonanatomical resection. Tumor located at the liver margin, tumor diameter >5 cm, or multiple tumors in different hepatic segments should be removed nonanatomically but with adequate surgical margins. This suggestion is made for preservation of more remnant liver volume.

The recurrence rate after resection is about 70% at 5 years. The prognostic factors influencing this outcome are tumor differentiation, micro- and macrovascular invasion, and satellite nodules [18, 61]. Liver resection should be the treatment for resectable HCC with sufficient liver function in the regions where transplantation is not possible. The 5-year overall survival rates of 81.7%, 77.2%, 44%, and 28.2% for TNM stages I, II, IIIA, and IVA patients after resection, respectively, are reported by Fan et al. [18]. There is also limited data in the literature about 10-year survival after resection for HCC. Recently Linn et al. reported their results for actual 10-year survivors and 10-year recurrence-free survivors after liver resection [62]. Their actual 10-year overall survival rate was 31.5%, and the actual 10-year recurrence-free survival was 18.6%. They revealed that only age and absence of cirrhosis were the most important predictors of 10-year survival. They also showed their results with patients with unfavorable prognostic factors. They had patients who survived beyond 10 years with AFP >1000 ng/mL, ruptured HCC, and margins of <1 mm.

Liver resection is still an effective treatment option for HCC both in cirrhotic and noncirrhotic livers. Although liver transplantation is the definitive treatment for HCC and the underlying disease, the procedure needs a big teamwork and equipped hospital. Also the cost of the procedure is not standard with many variables from comorbidities of the patient to posttransplant follow-ups and complications. The guidelines have disparities about the resection of HCC for both single and multiple lesions. Careful patient selection for resection offers comparable overall survivals with transplantation for HCC patients.

2 Local Ablative Treatments: Percutaneous Ethanol Injection, Radiofrequency Ablation, and Microwave Ablation

HCC is the most common primary liver cancer. When all liver cancers are considered, the 5-year survival rate is below 20% [63]. Traditional treatment methods for HCC are surgical resection and liver transplantation. While the rates of local recurrence are reduced with surgical resection, liver transplantation may completely remove the tumor and also cure the underlying liver disease. For these reasons, the results of these treatments are better. However, a recent study showed that only less than 10% of HCC patients are eligible for surgical resection [16]. Patients who are not surgical candidates may benefit from locoregional treatments.

2.1 Percutaneous Ethanol Injection

Percutaneous ethanol injection (PEI) is one of the first described ablation therapy for hepatocellular carcinoma (HCC) [64]. It is inexpensive and well-tolerated with few complications. PEI is performed with the guidance of ultrasound (US). This allows to perform precise targeting of the lesion, to administer sufficient amount of ethanol and real-time manipulation of the needle, and to control the ethanol distribution within the tissue.

HCC is a hypervascular tumor that mostly develops in the cirrhotic liver. Its hypervascularity helps homogeneous distribution of ethanol, and a relatively stiff cirrhotic liver keeps ethanol within the softer tumor. Ethanol causes cell dehydration and coagulation necrosis and also induces endothelial cell necrosis which triggers thrombosis of tumor vessels.

All the features mentioned above make PEI an effective treatment option for HCC, especially in early stages. Best results for PEI can be achieved in single HCC lesions less than 3 cm in diameter.

PEI is used less frequently because of the widespread use of RFA and its better results. However, it can be used safely in patients who cannot reach other treatment options.

2.2 Radiofrequency Ablation

Radiofrequency ablation (RFA) is a highly effective, safe, and most commonly used thermal ablation therapy to date. It destroys tumor tissue with the heat generated by radiofrequency effect. This effect, which is achieved by using high-frequency (460–500 kHz) alternating current, is created with the help of a special needle inserted into the tumor tissue under US or CT guidance. When the tissue temperature reaches 60–100 °C, protein denaturation, sudden cell death, and coagulative necrosis of the tumor develop. In order to reduce local recurrence, 5–10 mm liver tissue around the tumor should be ablated as a safety margin to destroy possible adjacent micrometastases with the tumor tissue as well. This helps to reduce local recurrences.

Tumor size should be <4 cm to achieve complete cure, but best results are obtained in BCLC stage 0 HCCs. EASL guidelines recommend RFA therapy as the first-line treatment for very early-stage HCC (single tumor <2 cm) rather than surgical resection [65]. RFA can be used for single tumor <5 m or up to three tumors <3 cm without extrahepatic metastasis. The main purpose of using RFA in tumors larger than 5 cm is to reduce tumor size before chemotherapy or to relieve pain [66].

RFA treatment also has some limitations like charring around the electrodes which limits heat distribution and heat-sink effect if tumor is adjacent to vascular structures (>3 mm). Bile duct damage, liver failure, vascular damage, and liver abscess are the most common major complications of RFA [67, 68].

2.3 Microwave Ablation

Microwave ablation (MWA) uses needlelike probes that broadcast microwaves. These microwave-emitting needle probes are placed in the tumor tissue percutaneously, laparoscopically, or with open surgical technique. These microwaves (900–2400 MHZ) cause oscillation of water molecules in the soft tissue, and this vibration and molecular friction produce high amount of heat which causes coagulation necrosis and ablation of tumor. MWA provides higher intra-tumoral temperatures compared to RFA. While RFA causes conductive heating, MWA provides active heating and is less prone to heat-sink effect. Since multiple probes can be activated at the same time, the treatment of large or multiple tumors can be performed more rapidly, and no grounding is required since no electric current is used. Also, MWA is less painful than RFA.

HCC differs from other types of cancer in that the two severe diseases, cirrhosis and cancer, coexist. Therefore, its staging should be supported by other parameters that show the severity of the liver disease which have direct impact on mortality in these patients. BCLC staging system, which is most widely used, has been validated by different clinical studies and helps to determine treatment options according to stage of the cancer.

Basically, the main treatment options for HCC are liver transplantation and surgical resection. Best overall and disease-free survival rates are achieved with these treatments. However, the majority of HCC patients are not suitable for these treatments. While most patients are diagnosed in the advanced stages, some of the patients which are diagnosed in the early stages cannot be treated with these options for various reasons (comorbidities, portal hypertension, insufficient hepatic function, inability to tolerate general anesthesia, tumor location, etc.).

Patients with preserved liver function, excellent performance (ECOG 0), and tumor size <2 cm and without vascular invasion are classified as BCLC stage 0 and single tumor or two to

three nodules ≤3 cm who are Child-Pugh score A or B are BCLC stage A. According to the BCLC classification, ablation therapies can be used for curative purposes for these groups of patients who are not candidate for surgery. RFA, which has less complication rate and is more cost-effective compared to surgical resection, provides good results as surgical resection especially in patients with stage 0. In a recent meta-analysis by Majumdar A et al., management of patients with very early- and early-stage HCC is analyzed. Although there was no significant difference in all-cause mortality between surgery and RFA, cancer-related mortality was lower in surgery group. Serious adverse events were higher in surgery group as expected. In patients not suitable for surgery, those treated with PEI had higher mortality rates at maximum follow-up than those treated with RFA [69]. Another network meta-analysis by Gui-Qi Zhu et al. evaluated 14 randomized controlled trials. Compared to surgical resection, PEI was associated with a significantly increased proportion of dead patients with small HCC, whereas RFA showed no significant effect on proportion of dead of these patients. Surgical resection was superior for overall and recurrence-free survival compared to ablative therapies but with more adverse events [70].

In a recent population-based study from Taiwan, Yun-Jau Chang et al. analyzed 4496 patients treated with either RFA or PEI. Patients treated with RFA had better overall survival, disease-free survival, and local recurrence-free survival at 3, 5, and 9 years than patients treated with PEI. Median overall survival and recurrence-free survival were 72.1 and 45.2 months in the RFA group, while 61.5 and 41.9 months in the PEI group, respectively [71].

In a study conducted in China, HCC patients with solitary tumor (2.1–5.0 cm) were divided into two groups. While resection was applied to one group, RFA and PEI combination (RFA was performed 3–5 min after PEI in the same session) was applied to the other group, and these patients were analyzed in terms of overall survival and disease-free survival. PEI-RFA combination therapy was found to be superior to resection in terms of overall survival, disease-free survival, complication rates, length of hospital stay, and cost [72].

RFA can also be used in combination with transarterial chemoembolization (TACE). A meta-analysis of seven randomized controlled trials showed that RFA plus TACE significantly improved the survival rates of patients with HCC at 1 and 3 years compared to RFA alone. When subgroup analysis was performed according to tumor size, it was seen that this difference was more pronounced in HCCs larger than 3 cm for survival rates at 1, 3, and 5 years, and there was no significant difference between the two groups in tumors smaller than 3 cm [73]. This combination can be applied in two ways. When RFA is applied first, thermal damage is inflicted on the tissue in sublethal doses, and this increases the effect of chemotherapeutic agents by reducing the cellular resistance. When TACE is applied first, as the arterial flow of the parenchyma decreases, the heat-sink effect is reduced and RFA provides a more effective ablation in a larger area [74].

One of the important factors that make ablation treatments challenging is tumor localization. Especially, tumors close to the liver capsule, large vascular structures, intraabdominal organs, and diaphragm make application difficult which often leads to unsatisfactory ablation. To prevent this, different methods such as RFA application with laparoscopic or open surgical technique instead of percutaneous technique or creating artificial ascites have been tried. Zachary Makovich et al. analyzed HCC patients with tumors adjacent to large vascular structures or the diaphragm treated with MWA. When compared to the control group, it was found that the rates of local recurrence in these patients were statistically higher. Although the procedure-related complication rates were detected as 20.9% in the risky group and 10.9% in the control group, it was stated that those were not statistically significant. Median survival rates were also similar for both groups [75].

Microvascular invasion (MVI) is one of the prognostic factors for HCC but can only be detected by biopsy. Sunyoung Lee et al. investi-

gated the effect of MVI on early recurrence after surgery or RFA treatment in patients with HCC smaller than 3 cm. Using laboratory and radiological data, they developed an MVI scoring formula and validated it. Early recurrence rates were found to be significantly higher in patients with high MVI risk scores and those treated with RFA. However, early recurrence was observed less frequently in high-risk patients treated with surgery [76].

One of the most important factors affecting overall survival in HCC patients is recurrence. Tumor recurrence may occur early (<2 years) or late (>2 years). Early recurrence is generally related to the biological features of the tumor. Late recurrence is considered to occur due to de novo carcinogenesis and likely emerging in another focus rather than the primary tumor. After RFA, early recurrence is usually encountered after inadequate ablation of the primary tumor or surrounding satellite foci. Studies about late recurrence after RFA are lacking. In a recent study by Yi Yang et al., the risk factors of late recurrence after RFA were investigated. Male gender, multiple tumors, and cirrhosis were found to be independent risk factors [77].

Ablation treatments are also an important option in patients with recurrence HCC. These treatments are good alternatives, especially when recurrence occurs in patients with reduced liver volume after surgical resection. Although there are studies in the literature that report that repetitive surgical resections give better results in these patients, there are also studies stating that there is no difference in comparisons with RFA. In a recent meta-analysis conducted by Junjie Liu et al., it was observed that there was no significant difference between RFA and recurrent surgical resection in patients with recurrent tumors within Milan criteria when 1-, 3-, and 5-year survival rates were compared. Surgical resection provides survival advantage when tumor size exceeds 5 cm [78].

There are certain treatment options for HCC that have been defined and standardized according to the stage of the disease. However, not every center or country has the chance to reach the most ideal treatment. This may be due to economic reasons or lack of qualified personnel. Ablation treatments provide overall survival rates close to surgical resection, with lower cost and less complication rates, especially in early-stage HCC. The severity of the underlying disease and the characteristics of the tumor are the most important factors affecting the success rate. These techniques can be used safely in selected patients.

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Transarterial Radioembolization in Hepatocellular Carcinoma

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1 Introduction

Hepatocellular carcinoma (HCC) is one of the deadliest cancers. It is the most common primary liver cancer, sixth most commonly diagnosed, and fourth cause of cancer-related mortality worldwide and represents about 75–85% of primary cancers [1, 2].

There are mainly three modes of treatment: (1) surgical treatments, e.g., resection and transplantation; (2) interventional oncologic liver-directed therapies (ablation, bland embolization, chemoembolization, radioembolization, etc.); and (3) systemic chemotherapy which is indicated for advanced stages [3]. Interventional oncology (IO) has a spectrum of treatment options for the treatment of HCC. In addition to the widely used and well-established IO procedures like radiofrequency ablation and transarterial chemoembolization (TACE), transarterial radioembolization (TARE) with Yttrium-90 (^{90}Y) is becoming an indispensable part of HCC management [4]. Despite these options, the prognosis is poor especially for advanced-stage patients that only one-third of them might benefit from curative therapies, in addition to the fact that underlying liver diseases predispose to new tumor formation [5].

Although majority of the patients are in intermediate or advanced stages at the time of presentation, therapeutic options are limited, but radioembolization with ^{90}Y, which is a form of localized brachytherapy, has an important role in all stages of HCC with curative intent to palliation [3, 6–8].

2 Radioembolization

The Liver has a dual blood supply, and about 95% of the tumoral blood supply is provided by hepatic artery which makes it possible to embolize the tumor and deliver higher concentration of chemotherapeutics or radiotherapeutics selectively to the liver tumors by avoiding systemic effects [5]. Due to the hypervascularity of hepatic tumors, radioembolization, which could be regarded as a form of brachytherapy, allows localized radiotherapy to liver tumors limiting the dose to the normal parenchyma (Fig. 1) [9, 10]. The isotope ^{90}Y, loaded to glass or resin microspheres, is the most commonly used isotope in TARE [11]. Downstaging the tumor for resection and transplantation; bridging to transplantation, palliation, and maximizing the survival; and intention to treat are the main goals of TARE [12].

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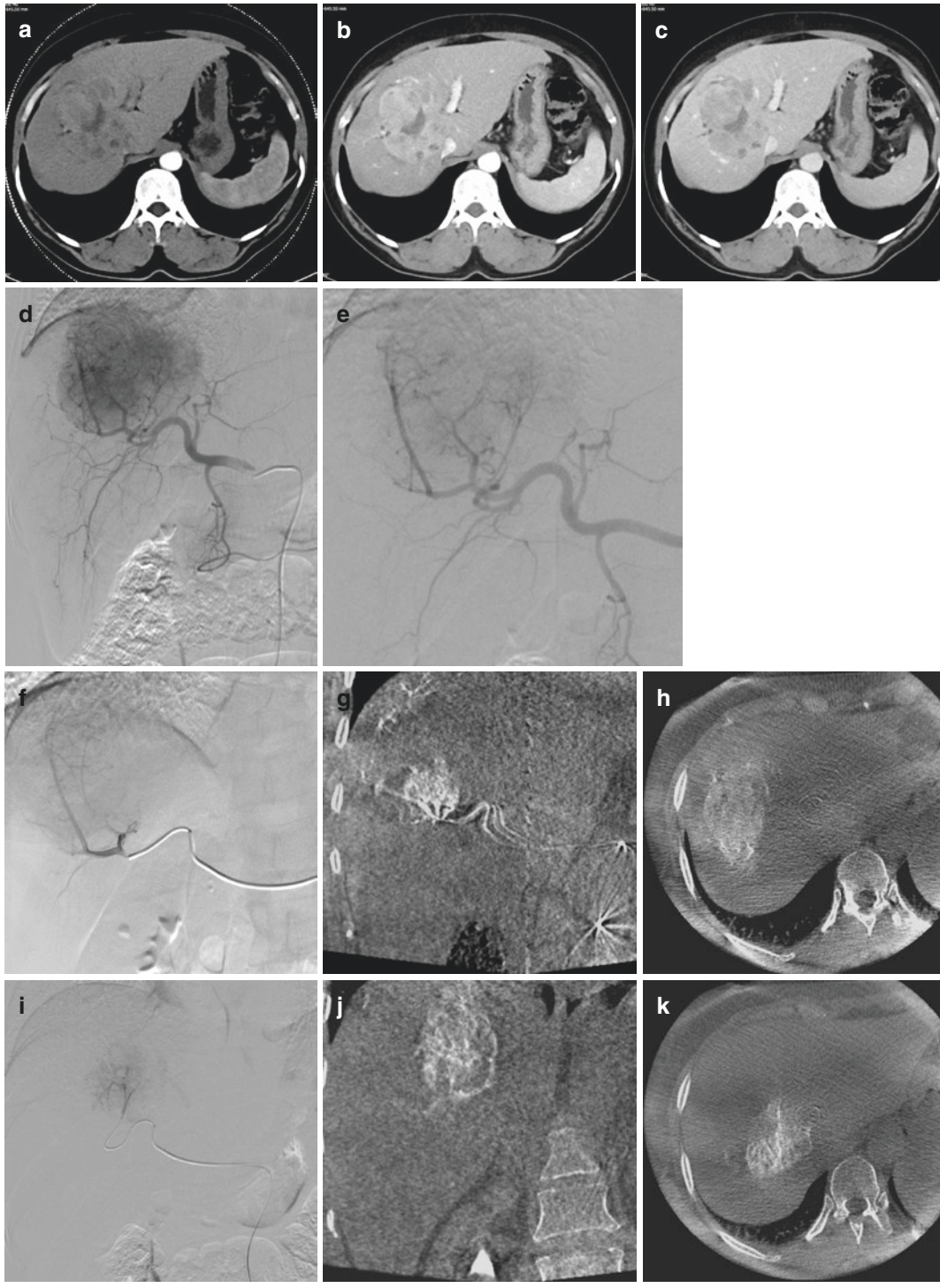


Fig. 1 Axial arterial (**a**), portal (**b**), and venous (**c**) phase CT images show 7 × 4 cm HCC lesion in segment 8. Proper hepatic artery DSA images (**d**, **e**) show a hypervascular tumor that has a dual supply from right anterior and posterior sectorial hepatic artery branches. Selective injection DSA images (**f**, **i**) and corresponding coronal (**g**, **j**) and axial (**h**, **k**) cone-beam CT images demonstrate dual supply of the tumor. PET CT image (**l**) after administration of two vials of ^{90}Y embedded glass microspheres separately from each feeding artery shows total coverage of the lesion. Corresponding control axial CT images in all phases obtained 3 months after TARE show total necrosis of tumor

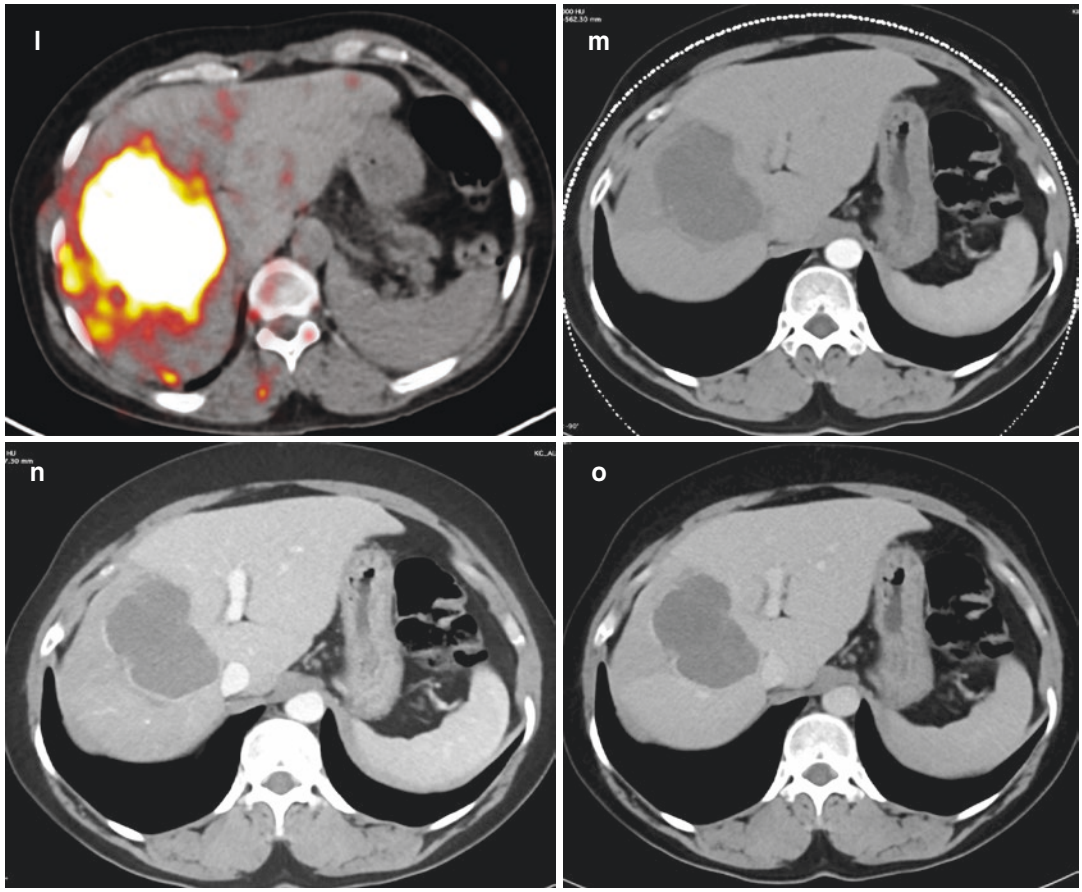


Fig. 1 (continued)

Hepatic arterial variations, flow dynamics, parenchymal reserve, tumoral arterial supply, device-related properties, and activity principles are among the main limiting factors from the point of optimal use of TARE [4]. Ionizing radiation causes unrepairable DNA breaks through prolonged exposure that in turn leads to cellular decompensation and apoptosis. The continuous brachytherapy exposure will also damage the cells in different phases of mitosis. TARE also may decrease the intratumoral pressure that helps to improve reoxygenation [4]. TARE has many advantages over other intra-arterial locoregional liver-directed therapies. It is usually an outpatient procedure and could be performed in cases with portal vein thrombosis or compromised portal vein blood flow, and postembolization symptoms are usually minimal. When compared to TACE, TARE has improved time to progression though no significant difference in mortality [10, 13].

3 Radionuclides and Microspheres for TARE

The type of microspheres can be grouped based on the embedded radioactive isotope (^{90}Y or ^{166}Ho) or microsphere material (resin, glass, or poly-l-lactic acid). These microspheres all have different production processes, physical characteristics, and methods of use. The most important characteristics of the different microsphere types are summarized in Table 1. The comparative properties of the four radionuclides in use in microsphere labeling are given in Table 2.

Table 1 ^{90}Y and ^{166}Ho loaded microsphere characteristics

Isotope	Yttrium-90 (^{90}Y)		Holmium-166(^{166}Ho)
Half-life	64.1 h		26.8 h
Decay product	Zirconium-90 (^{90}Zn)		Erbium-166 (^{166}Er)
Radiation emission	β (max 2.28 MeV)		β (max 1.74 and 1.85 MeV) γ (max 81 and 1.38 keV)
Energy per activity	49.67 J/GBq		15.87 J/GBq
Tissue penetration	2.5 mm mean, 11 mm max		2.5 mm mean, 8.4 mm max
Imaging	PET (internal-pair production) SPECT (bremsstrahlung)		SPECT (γ-imaging) MRI
Material	Glass (ceramic)	Resin	PLLA
Product name	TheraSphere®	SIR-Sphere®	QuiremSphere®
Size	20–30 μm	32.5 ± 5 μm	20–50 μm
Density	3.3 g/cc	1.6 g/cc	1.4 g/cc
Spheres per vial	$1.2 – 8 \times 10^6$	$40 – 80 \times 10^6$	33×10^6
Specific activity per sphere	2500 Bq	40–70 Bq	450 Bq
Max activity per dose	20 GBq	3 GBq	15 GBq
Number of microspheres (for 3 GBq activity)	1.2–8 million	40 million	8–12.5 million
Surrogate particle/scout dose	^{99m}Tc-MAA	^{99m}Tc-MAA	^{99m}Tc-MAA/^{166}Ho microspheres
Dosimetry method recommended by manufacturer	MIRD based	BSA method	MIRD based
Handling for dispensing	Not required	Required	Not required
Splitting one vial for two or more patients	Not possible	Possible	Not possible
Specific gravity	High	Low	Low
Embolic effect	Low	Moderate	Moderate

Table 2 Radionuclides used for TARE

Radionuclide	Half-life (hours)	Form and probability of decay	Average/maximum beta emission (MeV)	Average/max range in tissue (mm)	Type of imaging
Y-90	64.2	β^+ positron	0.94/2.20	2.5/12	Bremsstrahlung Planar/SPECT PET
Re-188	17	β γ (155 keV)	0.76/2.12	3.8/11	Planar/SPECT
Ho-166	26.8	β γ (81 keV)	0.66/1.85	2.2/10.2	Planar/SPECT MRG
I-131	482.4 h (8.04 day)	β γ(364 keV)	0.192/0.61	0.8/3	Planar/SPECT MRG

3.1 ^{90}Y Microspheres

Radioactive ^{90}Y can be produced by neutron irradiation of stable Yttrium-89 (^{89}Y) or by chemical separation from the parent isotope Strontium-90 (^{90}Sr), a fusion product of uranium. ^{90}Y is a suitable radionuclide to treat cancer with an appropriate safety profile. It is a nearly pure (99.99%) β-emitter with a half-life of 64.1 hours and decays to stable Zirconium-90 (^{90}Zn). Maximum beta particle (β–) energy of 2.28 MeV results in an energy release of 49.67 J/GBq and penetration range in water or soft tissue of 2.5 mm (mean) and 11 mm maximum [10]. Imaging of the radiation emission from ^{90}Y is a challenge due to the absence of γ-radiation emission. SPECT images can only be acquired by the detection of bremsstrahlung, secondary γ-radiation produced by slowing of the beta particles in tissue, a modality with very limited spatial resolution. Actually, ^{90}Y has a minor branch to the first excited state of ^{90}Zn at 1.76 MeV (0+ −0+ transition). As a result, once in every 32 million (31.86×106) decays, an electron-positron (β– / β+) pair is created. This

process is called internal-pair production and enables positron-emission detection with PET at high ^{90}Y activities [14].

3.1.1 Glass Microspheres

Glass ^{90}Y-microspheres (Therasphere®, Boston Scientific, Marlborough, MA, USA) are produced by incorporating ^{89}Y oxide into the glass matrix of the microsphere and subsequent activation by neutron bombardment in a nuclear reactor facility [15]. Glass ^{90}Y-microspheres have a relatively high density and a high specific activity per sphere (2500 Bq/sphere) compared with the other microsphere types. Therefore, to administer the same treatment activity, less glass microspheres need to be injected than resin microspheres. As a consequence, the embolic effect is much smaller during injection, so the entire treatment dose can be injected at once with a lower risk of stasis and particle reflux. The microembolic effect of glass microspheres is reported as a significant decrease in tumor enhancement in the cone-beam CT delayed phase images [16]. Main advantages of glass microspheres are no physical manipulation required and due to relatively low number of microspheres given, embolic effects are limited. Therefore, glass microspheres can be also used in patients with portal vein thrombosis; oxygenation is maintained in tumors, and objective responses induced by the irradiation are theoretically improved; and in nearly all cases, the target tissues receive more than 95% of the planned absorbed dose without reaching flow stasis. The specific gravity of glass microspheres is high compared to resin microspheres and may theoretically limit microsphere distribution. The difference in specific gravity is not reported to have a proven effect on clinical outcome [4, 10]. Specific activity is the approximate activity of each microsphere (glass 2500 Bq, resin 75 Bq per microsphere) and is an important factor for dose administration [4]. Moreover, to improve the uniformity of dose distribution within a lesion, the same activity can be injected choosing among different numbers of spheres (i.e., different initial activity) administered after different decay intervals. With crossfire effect, multiple microspheres create lethal radiation exposure [10]. The low number of spheres may result in inadequate tumor coverage for very large tumors, although the number of spheres can be tailored to the needs by selecting a higher activity vial and by using it later after some degree of decay. With extended shelf-life method, it is possible to allow an increased number of glass microspheres (decayed to the second week of their allowable shelf-life) to be administered for the same planned absorbed dose, therefore allowing better tumoral distribution of the microspheres without causing additional adverse radiation-related events [17]. So, increased embolic load and lowered activity per microsphere theoretically resulted in better tumor coverage and, hence, improved response rates [18].

3.1.2 Resin Microspheres

The production process of resin ^{90}Y-microspheres (SIR-Spheres®, Sirtex Medical Limited, North Sydney, Australia) is different; in this type of microsphere, ^{90}Y cations in solution are chemically incorporated onto the bland microsphere surface by binding to the carboxylic group of the acrylic polymer matrix [19, 20]. Resin microspheres have a much lower density than glass microspheres, which could potentially result in a more distal distribution in the tumor vasculature [21]. Furthermore, the relatively low specific activity requires injection of a higher number of microspheres, approximately 20–80 million. Since this involves a greater embolic effect, stasis of blood flow may occur during administration. Therefore, resin ^{90}Y-microspheres must be administered carefully by hand injection in smaller aliquots, with intervening angiography to reevaluate pace of flow and degree of stasis. Glass and resin microspheres may be used in different or similar tumor types and disease extents, but it remains controversial how the differences in distribution patterns impact treatment efficacy.

The activity vial can be tailored for the patient in the nuclear medicine radiopharmacy if needed. Resin microsphere injection system allows direct monitoring of the treatment because the infusion is performed with alternating injections of sterile

water and contrast medium. In theory, the lower specific gravity is in favor of a better suspension. Main limitation of resin microsphere is the 24 hours of shelf life of the device which is restricting clinical flexibility and patient scheduling. The need for human technical manipulation may result in methodological errors. Resin microspheres are more embolic which might lead to whole-dose delivery failure and transient hypoxia, limiting the effect of radiation.

The main difference between glass spheres and resin spheres is shown in Table 1. Major difference is the activity per sphere; in a glass sphere, activity is about 2500 Bq per sphere with respect to 50 Bq in resin sphere. Glass spheres offer vials between 3 and 20 GBq while resin spheres offer standard 3 GBq vials. Finally, for the same chosen activity, the higher number of resin spheres could provide more uniform dose distribution, with a higher biological effect (toxicity and efficacy). The influence of gravity of glass microsphere can quoted but never demonstrated on biodistribution [4, 22–26].

3.2 ^{166}Ho (Holmium-166) Microspheres

The isotope ^{166}Ho emits both high-energy β-radiation and low-energy γ-radiation. It has a shorter half-life than ^{90}Y (26.8 h) and decays with a relatively high dose rate to the stable element Erbium-166 (^{166}Er). ^{166}Ho emits β-radiation at two energy levels, maximum 1.74 MeV (48.7%) and 1.85 MeV (50%), with a maximum soft-tissue range of 8.4 mm. The resulting energy release is much lower (15.87 J/GBq) than with ^{90}Y; therefore, a larger administered treatment activity is required to achieve the same radiation-absorbed dose in liver tissue [27]. The biodistribution of ^{166}Ho microspheres can be visualized on SPECT, using the low-energy γ-radiation (81 keV, 6.2%; 1.38 keV, 0.93%), and with magnetic resonance imaging, utilizing the paramagnetic properties of ^{166}Ho [28]. Holmium microspheres that come with a special management system, unique dosing, and imaging possibilities have become available as well. Additionally, a scout dose of ^{166}Ho microspheres can be used instead of 99mTc-macroaggragated albumin during the preparatory angiography procedure. Thus far, two prospective phase I and phase II clinical studies have been performed on ^{166}Ho radioembolization in a population of liver metastases from mixed origins. These studies showed that a mean whole-liver dose of 60 Gy is safe and induces tumor response [29, 30].

3.3 ^{188}Re-Lipiodol (Rhenium-188)

As a generator product, ^{188}Re has good availability. Unlike the ^{90}Y produced in the reactor, it can be obtained from the generator, providing a great advantage for ^{188}Re, and permits preparation of the ^{188}Re radiopharmaceutical “on demand” in any hospital radiopharmacy housing the generator. Since it is possible to obtain enough ^{188}Re from a generator for about 6 months, the production cost is lower when compared to ^{90}Y [31]. The physical characteristic is useful for clinical use: a short physical half-life of 16.9 h, high maximal beta energy of 2.1 MeV, and soft-tissue range around 10 mm maximum, similar to ^{90}Y. The gamma-emission of 155 keV (15% abundance) allowed pre- and post-therapeutic scans for biodistribution studies and dosimetry [32]. Nowicki et al. investigated the feasibility of ^{188}Re treatment in patients with primary and metastatic liver tumors, and the median overall survival was 7.1 months; calculated progression-free survival is 5.1 months [33]. Although there are studies similar to this in the literature [34–36], ^{188}Re microsphere has not yet found widespread use as ^{90}Y microsphere.

3.4 ^{131}I-Lipiodol (Iodine-131 Lipiodol)

^{131}I is a beta emitting radionuclide with a physical half-life of 8.04 days. The maximum and mean beta particle energies are 0.61 MeV and 0.192 MeV, respectively. Additionally, ^{131}I emits a principal gamma photon of 364 keV (81% abundance) [37]. The beta radiation of ^{131}I is

responsible for its therapeutic effects, while gamma radiation makes the distribution of the radiopharmaceutical visible.

Lipiodol is an ester of fatty acids derived from poppy seed oil which is used to diagnose and treat HCC. It was initially used as a radiological contrast medium and was found to have higher uptake in HCC, relative to normal liver tissue [38]. This compound contains an iodine^{127} moiety, which can be exchanged for iodine^{131} (I^{131}), to create a compound that delivers targeted, internal, beta, and gamma radiation. Early studies showed that I^{131} lipiodol could induce tumor necrosis and significantly prolong survival in inoperable patients [39]. Treatment with I^{131} lipiodol has been used since the 1990s as palliation for HCC, as it is well-tolerated with few complications or side effects [40, 41].

According to biodistribution data, more than 75% of the ^{131}I-lipiodol stays following the arterial administration in the liver, and the remainder reaches the lungs. ^{131}I-Lipiodol treatment was at least found effective as chemoembolization and is tolerated much better in the treatment of HCC with portal thrombosis and also as an adjuvant to surgery after the resection of HCCs. In the cases that severe liver dysfunction represents theoretic contraindication for radioembolization as well as for TACE, ^{131}I-Lipiodol is an alternative therapy option especially in tumors smaller than 6 cm [42].

Although ^{131}I-lipiodol therapy provides an economically viable alternative, long half-life (t1/2 = 8.04 days), low β-energy [Eβmax = 0.61 MeV (89.3%), 0.33 MeV (7.3%), 0.25 MeV (2.1%)], need for the isolation of patient post-therapy, and high nonspecific lung uptake, which drastically limits the administered dose, make it a less preferred clinical choice [32].

4 Dose and Activity

Dose and activity are two components related to the topic of dosimetry in microsphere therapy. Dose refers to the amount of energy of radiation that is taken up by the tissue within the body and is measured in gray (Gy). Activity refers to the amount of ionizing radiation and is measured in either curie (Ci) or becquerel (Bq). In microsphere treatment, the term dose (Gy) is used for desired radiation to be delivered to the tumor tissue in the liver, and the term activity (GBq) is used for radiation that is delivered to the target organ (i.e. liver) [43].

TARE naturally targets most tumors as a function of increased vascular density. Since the radiation source is attached to each microsphere, the radiation effects depend on the pattern of their accumulation within the tumor vasculature. This concept requires the distinction between the applied radioactivity and the final tissue exposure when planning a treatment dose as: The dose is the biological effect of radiation measured in gray (Gy) and depends on four factors [4]:

1. Activity: Radioactive decay per unit of time is usually expressed as decrease per second or becquerel (Bq). Most TARE activities are implemented in the range of billions of decays per second or gigabecquerel (GBq).
2. Volume: the amount of tissue in which activity is located.
3. Distribution: Variations in vascular compartments that affect the geographic accumulation of microspheres result in nonuniform irradiation patterns.
4. Radiation susceptibility: radiosensitivity and repair abilities of both tumor and normal parenchyma.

Therefore, activity (GBq) is only one factor in determining the dose (Gy), and the biological effects of TARE should not be overly simplified by assuming uniform distribution of activity within a target volume [44–46].

4.1 Determining Treatment Activity

After the patient is found suitable for treatment in the ^{90}Y microsphere treatment, the stage of determining the appropriate treatment activity is started. There are different methods that differ according to the type of radiomicro-

sphere used for the treatment dose. To date, different methods for the calculation of the amount of radioactivity to be administered have been applied, namely, empirical and dosimetric ones.

There are two commonly used methods to estimate the amount of activity delivered by ^{90}Y microspheres to patients using resin microspheres: a BSA model and a (two-compartment) partition model. An activity detection method generally used for glass microspheres and medical internal radiation dose (MIRD) is referred to as single partition model. Much confusion has arisen as the terms partition model (refers to a two-compartment partition in the use of resin microspheres) and MIRD partition (refers to a single-compartment model used with glass microspheres) have both been abbreviated to the partition model. It is important to notice that the partition model and MIRD partition represent distinct and different methods with significant differences in calculated activity [47, 48].

4.1.1 Empirical Methods

Empirical methods have been tested for resin spheres and are based on a broad estimate of tumor involvement (T) in the liver [tumor volume/(tumor + liver volumes)]. The first empirical method proposed for SIR-Spheres® is based only on T: The larger the tumor burden, the higher the recommended activity in increments of 0.5 GBq per 25% tumor burden.

Empiric Method Calculation

- Tumor <25% of the total mass of the liver by CT scan = use 2 GBq whole-liver delivery.
- Tumor >25% but <50% of liver mass by CT scan = use 2.5 GBq whole-liver delivery.
- Tumor >50% of liver mass by CT scan = 3 GBq for whole-liver delivery.

BSA

The second empirical method proposed for SIR-Spheres® incorporates body surface area (BSA, measured in square meters). Therefore, the activity to be administered is:

$$A\left(\text{GBq}\right) = \left(\text{BSA} - 0{,}2\right) + \text{Tumor volume / Total liver volume.}$$

$$\text{BSA}\left(\text{m}^2\right) = 0.20247 \times \text{Height}\left(\text{m}\right)^{0.725} \times \text{Weight}\left(\text{kg}\right)^{0.425}.$$

Empirical methods are in use with reported objective responses and low incidence of toxicity. Nevertheless, this approach may intrinsically expose patients to the risk of unnecessary toxicity or tumor underdosage. It must be noted that these methods do not take into account the degree of tumor uptake. Therefore, dosimetric methods should be generally recommended.

The BSA method, to date, has been the most prospectively studied model due to its implementation in several randomized clinical trials [49]. It is also the most frequent method used in dosing resin microspheres. The BSA method generates a hypothetical volume of liver based on the body surface area with dose modulations for tumor burden, large lung shunt percentage, and poor liver function. The main benefit to the BSA method is a generally well-tolerated toxicity profile. The BSA method is otherwise limited by its lack of anatomic accuracy, disregard of preferential distribution, inability to calculate segmental administrations, and inflexibility to angiosomal demands. As such, some contemporary practices have abandoned the BSA method due to its aforementioned limitations.

The BSA model assumes a relationship between the physical size of the patient and ability to tolerate increasing dosage. The concept that larger patients (not necessarily with larger livers) are more tolerant to increased dosages of ^{90}Y has been shown in the literature [50].

The BSA model was also found to have a lower risk of liver toxicity than the empiric model in the aforementioned cohort of 680 patients treated with resin microspheres, where 21 of 28 cases of radiation-induced liver disease (RILD) occurred from a single center using the empirical model [51].

4.1.2 Partition Method

The purpose of the partition method is to give the maximum dose to the tumor, while the lowest possible dose is given to the liver parenchyma excluding the tumor. This method is based on MIRD theoretical foundations and takes into account tumor and nontumor liver tissue separately. The partition model equation uses patient-specific tumor and liver volumes, along with predetermined T:N from pretreatment 99mTc-MAA SPECT/CT and/or cone-beam CT (CBCT). Thus, this method represents the most tailored treatment planning algorithm, allowing for accurate estimation of absorbed dose to the tumor, nontarget liver tissue, and lungs. Prior research has shown that treatment planning with the partition model based on 99mTcMAA SPECT/CT can improve clinical outcomes [52].

Partition Model (Two-Compartment): With this method, SIRT activity calculations are based on the two-compartment model, which allows the amount of radiation delivered to the tumor to be more accurately optimized with an optimum dose of >120 Gy. A higher degree of compatibility is required to arrive at the derivation of equations for activity (and dose) for the lung, normal liver, and tumor. As a result, although this method is theoretically more robust, it has not been widely adopted.

A higher degree of complexity is required to arrive at the derivation of the equations relating to the activity (and dose) to the lung, normal liver, and tumor. As a result, this method, although theoretically more sound, has not been widely adopted [10, 53].

Clinical studies have shown that the background liver parenchyma in cirrhotic patients can tolerate up to 70 Gy of radiation without evidence of radiation-induced hepatitis. Based on this information, the two-compartment partition model was able to optimize the amount of transmitted activity to ensure that tumors receive the minimum amount of radiation needed to cause cellular destruction while protecting the background liver from exposure to excess radiation to minimize the risk of inducing radioembolization-induced liver disease (REILD) [10].

TARE treatment with glass microspheres (Thera-Sphere) uses a simplified single-compartment MIRD model based on the size of the entire liver regardless of the amount of tumor burden with the following formula:

$$\text{Activity GBq} = \left(D \times m\right) / 50.$$

D is the dose administered in grays, and m is the mass in kilograms. Using this formula, it can be said that a dose of 50 Gy will be administered to 1 kg of tissue if 1 GBq of ^{90}Y is given. The dose given to the treated mass also depends on the percent residual activity (R) in the vial after treatment and the LSF, which is calculated beforehand. These factors are accounted for in the following formula:

$$D = A \times 50 \times \left(1 - \text{LSF}\right) \times \left(1 - R\right) / m.$$

The MIRD method is a common model adopted for glass microsphere administration. It requires volumetric calculation of the targeted hepatic tissue and incorrectly assumes a uniform distribution of activity within the volume. Like BSA, the MIRD method does not differentiate the amount of radiation distributed into the tumor and liver parenchyma. While there is abundant safety data to support MIRD utilization with glass microspheres, the specific activity range of this product can vary by orders of magnitude by demand, and the authorized user should be aware of this potential [54].

A practical method based on the hepatopulmonary shunt ratio and liver lobe and/or segment volume and based on a simple internal dosimetric approach is widely used in routine practice to determine therapeutic activity in ^{90}Y glass microspheres. With the software developed to facilitate the calculation in determining the treatment dose, the treatment dose can be calculated practically by using the liver lobe volume where the patient will be treated and the hepatopulmonary shunt ratio obtained from the hepatic artery perfusion scintigraphy data. This software provides the capability to visualize prospective dose distribution and assess the absorbed dose delivered to the target lobe or the tumor and normal tissue. By

allowing for pre- and posttreatment dosimetry, this software can help determine the effectiveness of a patient's ^{90}Y SIRT with confidence. It can be used to interactively tailor the absorbed dose per perfused volume by adjusting the injected activity. The software tools can be customized to a patient's specific tumor presentation and anatomy (personalized dosimetry) (TheraSphere ^{90}Y glass microspheres user's manual).

5 Posttreatment Bremsstrahlung and PET/CT Imaging

Imaging to assess microsphere distribution to the planned liver parenchyma to be sure that there is no nontarget distribution of them after TARE is necessary. Unintended activity leaks could lead to the development of severe complications [55–57].

Post-procedure imaging with bremsstrahlung SPECT and PET/CT can measure defining treatment response and nontarget site embolization. Since ^{90}Y is pure beta emitter, after being administered to the patient, the X-rays created by the Bremsstrahlung effect can be viewed under gamma camera. It is recommended that imaging be done within the first 24 hours after treatment. The ^{90}Y bremsstrahlung SPECT/CT is difficult to measure due to a photopic, collimator detector scattering and lack of septal penetration caused by high-energy bremsstrahlung photons. To quantify, it requires additional compensation that is not readily available in most commercial systems [58–60]. However, the image is still useful in qualitative comparison between delivered and planned deliveries and in controlling extrahepatic uptake [61].

^{90}Y PET/CT is another option for post-therapy imaging. Lhommel et al. [62] showed that imaging ^{90}Y microspheres with PET/CT was feasible, even with the low positron yield of 32 ppm per decay [63]. Studies have shown that time-of-flight (TOF) information helps with quantifying the noisy ^{90}Y PET images [64, 65]. Thus, ^{90}Y post-therapy imaging is capable of providing the delivered activity distributions with a spatial resolution of a few mm. These can then be converted to absorbed doses, preferably using the voxel-level methods.

Finally, 90Y PET/CT is the most promising modality to replace bremsstrahlung SPECT/CT due to its superior qualitative and quantitative capability. It will play an important role in reorganizing the safety and efficiency profile of radioembolization.

5.1 Anatomy

Variant anatomy is common and reported to be seen in about 40% of population [12]. In order to avoid or at least minimize nontarget embolization of nontumor bearing liver tissue and extrahepatic organs during the TARE, proper knowledge of relevant anatomy is essential [10]. Therefore, a thorough understanding of normal liver anatomy and in particular arterial anatomy together with variations is essential for a successful radioembolization procedure. These variations could be determined before angiography by a properly performed CT examination. A special attention is required for replaced and accessory arteries. They could be the part of dual supply of a tumoral lesion.

In addition to hepatic arterial variations, there are also variations inside the tumor that alter the effectiveness of intra-arterial therapies. Contrast enhancement patterns and Tc-MAA deposition should be evaluated in order to predict intralesional radiation watershed areas that could be managed by increasing either the number of particles or activity [4].

Hepatic tumors are hypervascular and receive supply primarily from hepatic arteries and could also receive parasitic arterial supply from adjacent segments and neighboring organs. Inferior phrenic (Fig. 2), internal mammary (Fig. 3), intercostal, omental, cystic, and adrenal arteries should be evaluated as a potential route of blood supply to the tumors especially in the ones located near the surface of the liver or after intra-arterial therapies [66]. Bare area of the liver is also a frequent site of parasitic supply from

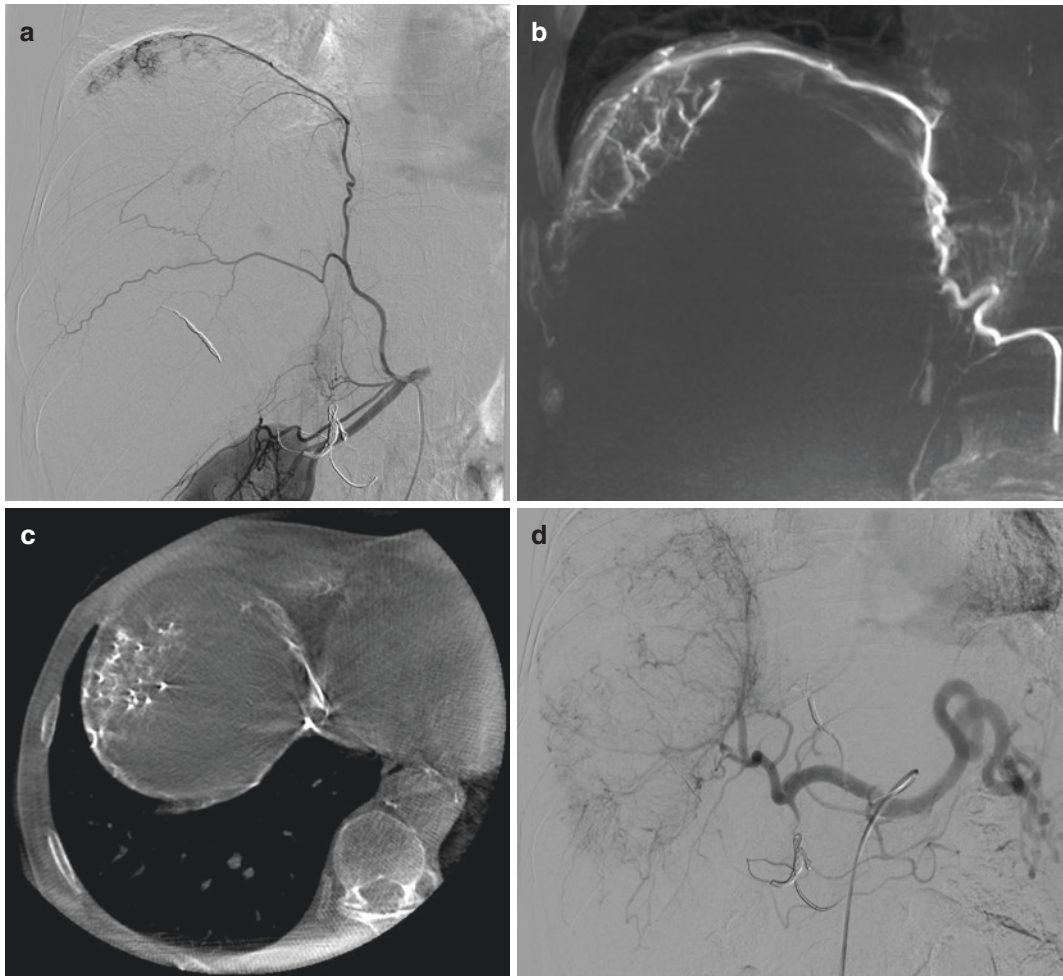


Fig. 2 Selective right renal artery DSA image (**a**) shows right phrenic artery arising from right renal artery and tumoral supply to the right superior lateral part of tumor. Cone-beam CT coronal (**b**) and axial (**c**) images demonstrate this supply. Control celiac injection DSA image (**d**) after embolization of right phrenic and also right gastric artery coil embolizations

extrahepatic arteries. Hepatic hilar peribiliary plexus could include numerous small vessels that could be unrecognizable during angiography and due to the progressive increase in intrahepatic arterial resistance which could reverse the hepatopetal flow to the hepatofugal flow resulting in nontarget embolization [67]. Therefore, measures for eliminating this hepatofugal flow should be taken.

In most of the cases, the cystic artery arises from right hepatic artery, and radioembolization could cause radiation-induced necrosis; therefore, whenever possible, microspheres should be given distal to the cystic artery origin, and if it is not possible, cystic artery could be embolized with Gelfoam on the day of radioembolization (Fig. 4) [68].

Although right gastric artery usually arises from proper hepatic artery, it has a high degree of variation. If microspheres pass through this artery, gastric necrosis, ulceration, and perforation could be seen. Therefore, embolization could be necessary (Fig. 5) [69]. Catheterization could be difficult for embolization, and sometimes embolization of this artery could be done through the left gastric artery [10].

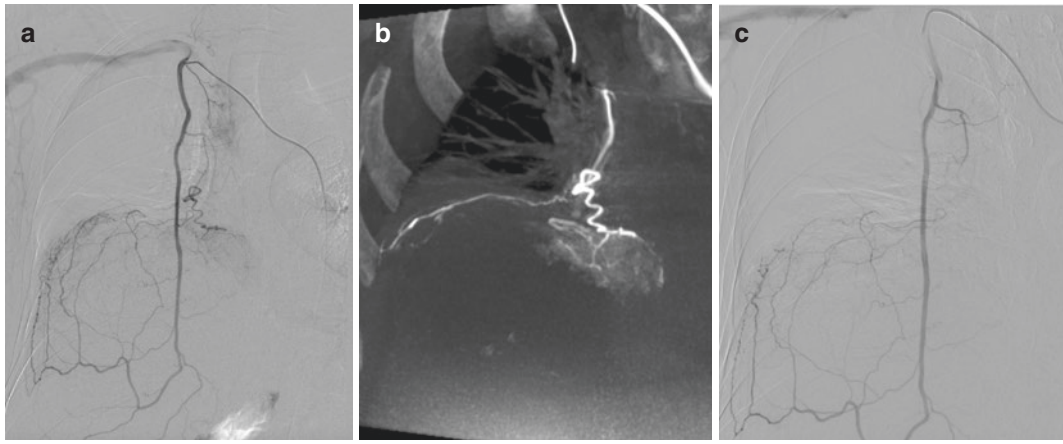


Fig. 3 Selective right internal mammary artery injection (**a**) and coronal maximum intensity projection cone-beam CT image (**b**) shows tumoral blood supply to the left medial superior part of the tumor. Control DSA image after superselective embolization with PVA particles shows cessation of blood supply to the tumor

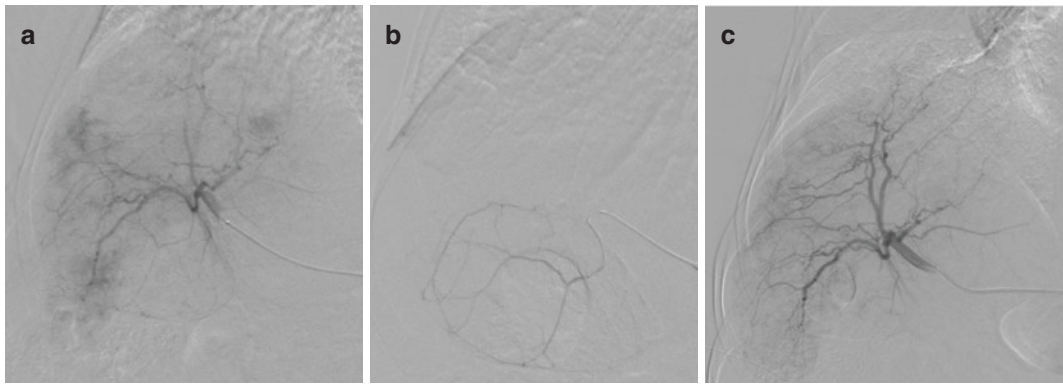


Fig. 4 Selective right hepatic artery injection (**a**) shows multiple hypervascular tumors and cystic artery originating from the right posterior artery. For right lobar therapy, cystic artery selectively catheterized (**b**) and embolized with Gelfoam just before infusion of ^{90}Y. Control right hepatic artery injection (**c**) shows embolization of cystic artery

In every mapping angiography pancreaticoduodenal arcade should be examined in order to prevent inadvertent pancreatitis, duodenal ulceration, or perforation [10]. When performing embolization in this arcade with a rich collateral vascular network, the possibility of collateralization and recanalization should be considered.

Falciform artery usually arise from the left hepatic artery and could lead to the development of localized, midabdominal wall burning sensation that can last for days or weeks following inadvertent radioembolization. When necessary, it could be embolized or an ice pack could be placed on the abdomen in order to cause vasoconstriction [70].

Intrahepatic communications between segments provide collateral flow in cases where there is occlusion or compromise of branch hepatic arteries. This feature is used for redistribution and consolidation of flow to tumors, thereby reducing the number of catheter positioning [66]. Embolizations are performed to alter the flow hemodynamics to optimize the administration point of microspheres. It is usually performed during the mapping angiography, and it is better to check them at the day of radioembolization due to the possibility of collateral develop-

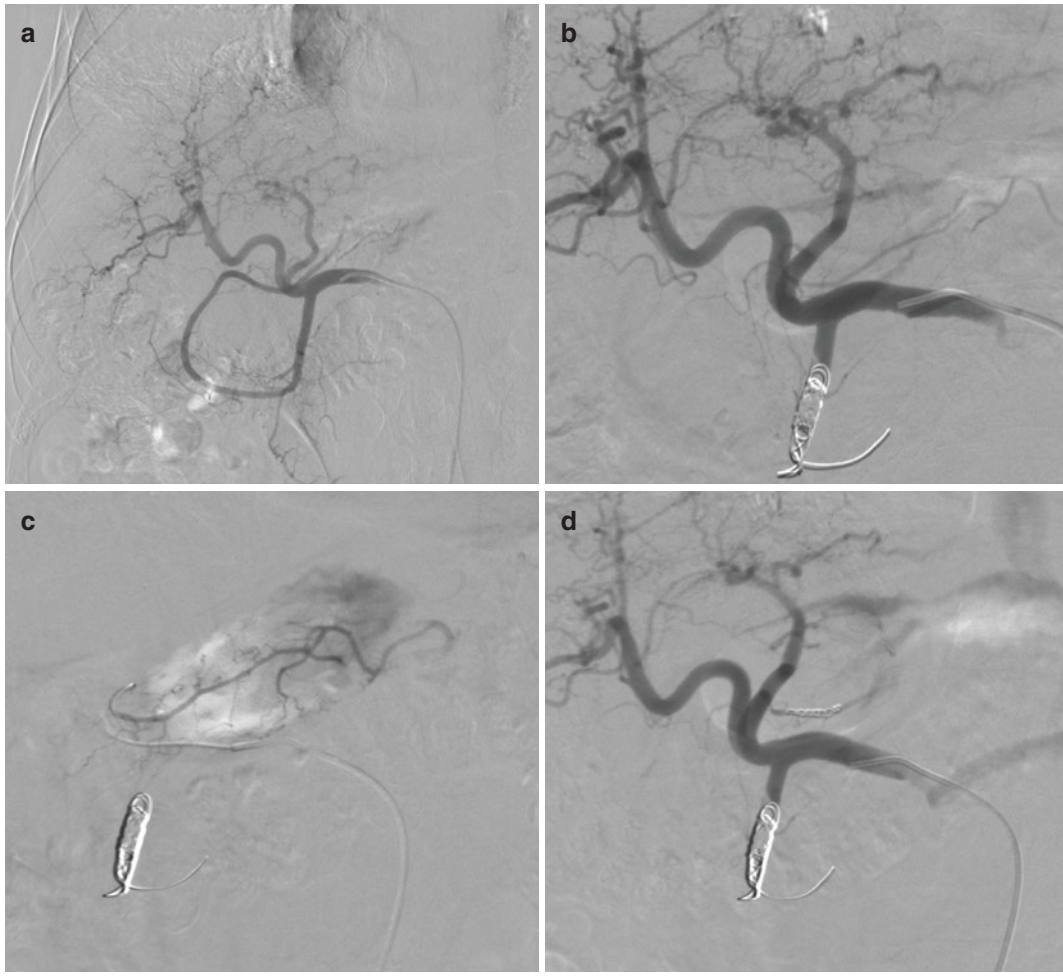


Fig. 5 Selective proper hepatic artery injection DSA image (**a**) shows bilobar disease. For truncal infusion, gastroduodenal artery coil embolized (**b**). Right gastric artery arising from proximal left hepatic artery superselectively catheterized (**c**) and embolized with coils (**d**)

ment and redistribution. If that is the case, additional embolizations might be required [10].

6 Mapping Angiography

Mapping angiography is performed to asses arterial supply of the tumor to be treated, to determine variant anatomy which could lead to nontarget embolization and embolize them, to consolidate and redistribute arterial supply of tumor to be treated which can also be done during radioembolization, and to calculate lung shunt fraction (LSF) and to simulate radioembolization by administering Tc MAA [4, 12]. The goal of radioembolization is to administer planned required dose to the tumor without damaging normal parenchyma and avoid nontargeted deposition of microspheres into important extrahepatic organs. In order to achieve these goals, hemodynamics of flow to the tumor can be modified by embolization of arteries to redistribute the intrahepatic collaterals to the tumor and to consolidate arteries to administer microspheres by simpler and safer route (Fig. 6) [10, 66, 71]. The assessment of preferential flow is important since it is the primary mechanism of microsphere distribution [4]. Preferential blood flow phenome-

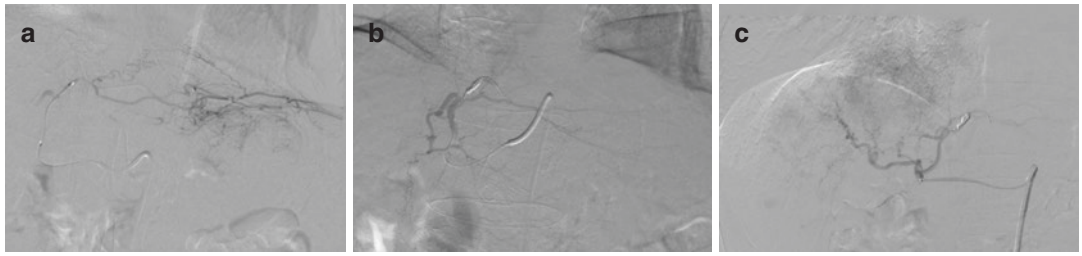


Fig. 6 Selective left hepatic artery injection (**a**) shows collaterals to the left diaphragm and stomach. These collaterals are embolized with coils to prevent nontarget embolization (**b**) for truncal infusion (**c**)

non that generates nonuniform deposition, or compartmentalization, of an embolic substance, allows delivery of ^{90}Y carrying microspheres through the hepatic artery to the tumor [72]. Therefore, one of the reasons of performing mapping angiography is to asses flow dynamics and plan interventions (like embolizations, etc.) to change the dynamics to properly administer and distribute the required dose to the tumors.

In order to optimize vascular dynamics, various permanent and temporary embolization materials, like coils, microplugs, balloons, and Gelfoam, could be used with an intention to decrease nontarget embolization of microspheres and facilitate antegrade flow to the target arteries [10, 12].

MAA is used to asses splanchnic and pulmonary shunting [73]. The size of the albumin microspheres is between 30 and 50 μm which is similar to that of glass or resin microspheres. Due to the density of MAA particles which is almost similar to that of resin microspheres, simulation is better for resin microspheres [74].

Systemic chemotherapies might affect the result of MAA study and eventually biodistribution of ^{90}Y microspheres. Also, they could increase the risk of liver toxicity [75]. Due to hypoxic effects of antiangiogenic drugs, they lead to poor uptake of 99 m Tc-MAA and in turn poor tumor targeting, and therefore they should be discontinued 8 weeks before mapping angiography [75, 76].

6.1 Shunt Reduction

HCC causes the development of functional arteriovenous shunts which is due to the vascular growth factors, neovascularity, complex process of angiogenesis, and the ongoing autonecrosis/remodeling occurring within tumor microvasculature. Depending on the magnitude of these shunts, microspheres could enter the pulmonary circulation through these shunts causing pneumonitis and fibrosis [7, 10]. Hypervascularity, tumor thrombus in portal and hepatic veins, CT or angiographic findings of shunting to portal or hepatic veins, large tumor burden, and infiltrative disease are among the main risk factors for shunting [7].

If the lung shunt fraction is greater than 20%, there is the possibility of nontarget pulmonary radiation deposition and radiation pneumonitis. The dose of 30 Gy to the lungs is generally accepted as the upper limit of single session of TARE, and 50 Gy is the total upper limit of cumulative absorbed lung radiation dose of repeated TARE [75]. If the hepatopulmonary shunt fraction ratio (HPSFR) is in 10–15% and 15–20%, the activity is decreased by 20% and 40%, respectively. TARE is not performed if the HPSFR is greater than 20% [7, 77].

Shunt reduction procedures, like low-dose TARE, bland embolization, TACE with beads larger than 300 μm, sorafenib administration, chemotherapy, hepatic vein balloon occlusion, variceal embolization, and segmental TARE, are employed in cases with elevated LSF [7, 77].

6.2 Patient Selection

The decision to select patients for radioembolization should be based on a multidisciplinary team that includes nuclear medicine specialists, hepatologists, medical oncologists, radiation oncologists, surgeons (experienced in liver trans-

Table 3 Selection criteria for TARE

Performance	Eastern Cooperative Oncology Group (ECOG) status ≤2
Life expectancy	Greater than 3 months
Purpose of procedure	Definitive, bridge to transplantation, palliative
Tumor biology or stage	Advanced-stage or aggressive tumor can be associated with poor prognoses
Liver reserve	Loss of functional liver reserve is associated with the prognosis and benefit of procedure
Hematological parameters	Granulocyte count $\geq 1.5 \times 10^9$/L, platelet $\geq 60 \times 10^9$/L
Renal function	Serum creatinine level <2.0 mg/dl
Liver function	Serum bilirubin level <2.0 mg/dl, liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) should not be above five times of normal level
Tumor size	Tumor involving more than 70% of the liver or 50% of the liver with <3 mg/dl serum albumin level may have poor prognoses
Pulmonary function	Respiratory function tests should be normal
History of EBRT	Cumulative toxic dose should be regarded

Modified from [4, 74]

plantation), and interventional radiologists [3]. Choosing the treatment modality for HCC depends on some factors such as tumor size, location, morphology (e.g., presence of portal venous invasion, etc.), accompanying comorbidities (e.g., underlying liver disease), and the presence or absence of extrahepatic disease [10]. Selection criteria for radioembolization procedure are summarized in Table 3.

6.3 Indications and Contraindications

The main indications for radioembolization therapy are reduction of size of intrahepatic tumors (downsizing), increasing future liver remnant (FLR) volume, bridging to liver transplantation for HCC, controlling the size of tumor and providing hypertrophy of FLR by radiation lobectomy before resection, delaying progression of advanced HCC (Fig. 7), palliation, and intent to cure [61]. Applicability in the setting of portal vein thrombosis or invasion, capacity for downstaging or bridging for liver transplantation, facilitation of liver resection by providing hypertrophy of FLR, and the lower incidence of postembolization syndrome are among the advantages of radioembolization [3, 78].

Contraindications of radioembolization are poor liver function (high bilirubin levels and elevated liver function tests, low serum albumin level), renal dysfunction, high lung shunt in mapping angiography, and extrahepatic disease except lymph nodes. Although the serum bilirubin level is required to be below 2 mg/dl, if the tumors can be treated superselectively, radioembolization could be applied to the patients with serum bilirubin levels up to 3 mg/dl. The indications and contraindications of radioembolization are shown in Table 4.

Care should be taken while performing TARE treatment to the patients with poor hepatic function. Total bilirubin is the most widely defined indicator of liver function, and the level of it is desired to be less than 2 mg/dl in patients receiving radioembolization treatment. In patients who undergo bilobar radioembolization therapy, less elevation of bilirubin levels should be a warning about the potential fulminant liver failure. However, patients with moderate hepatic failure can be treated if they are suitable for segmental therapy. Sudden changes from a chronic stable total bilirubin levels may cause sudden decompensation. At this situation, lab values should be rechecked within 10–14 days to assess whether this change is a normal fluctuation or represents a greater hazard [56]. Serum albumin levels provide valuable information for the hepatic function as well. Albumin will often reduce before the increase in total bilirubin, indicating worsening liver reserve and potential loss of liver function [12].

Not all of the HCC patients are eligible for resection or transplantation. Most of the patients are outside the established criteria for liver transplantation. Downstaging means making the patients eligible for resection or transplantation by reducing the size and the number of tumors and tumor marker levels. By this way, patient is brought within the established or expanded crite-

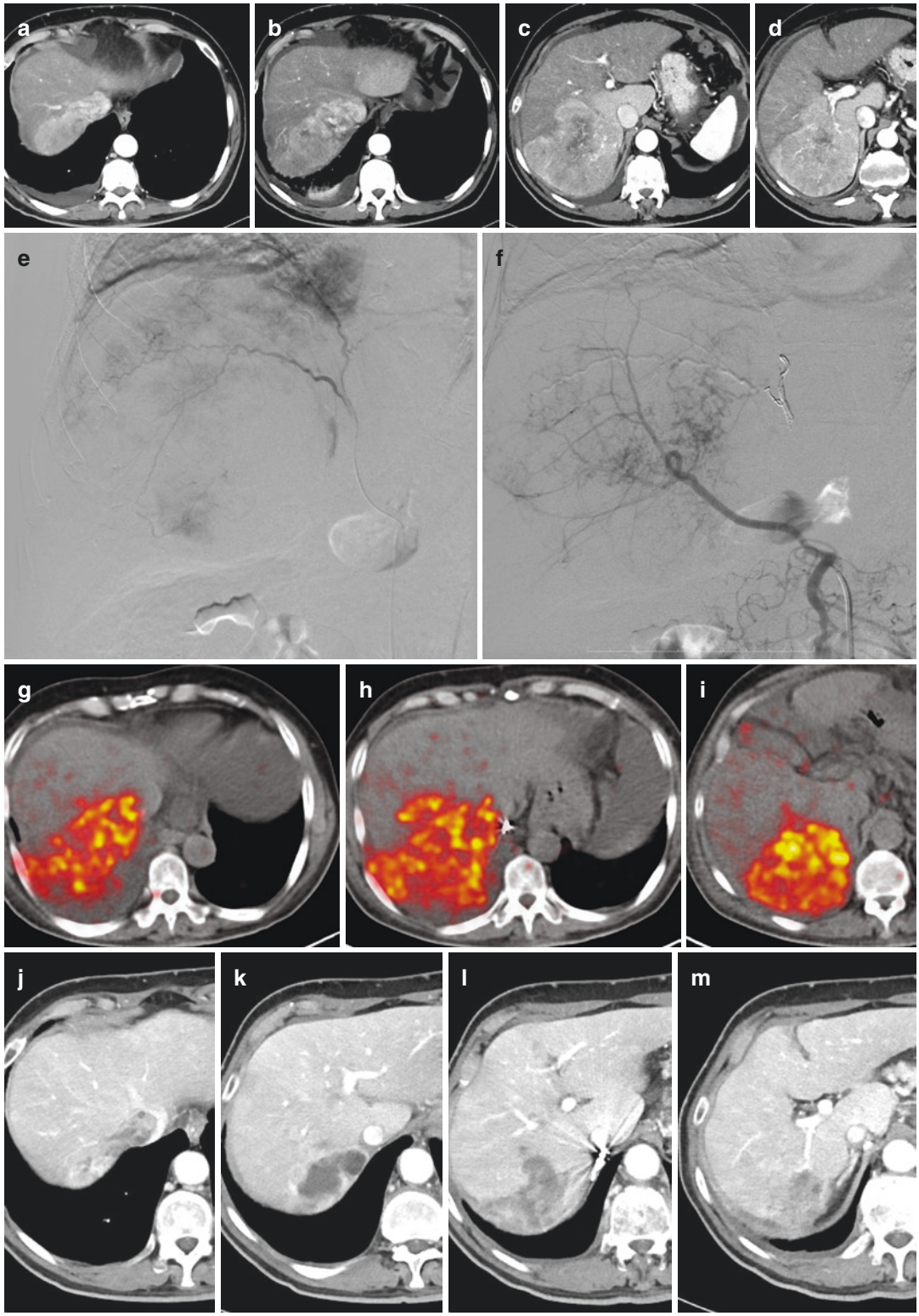


Fig. 7 Consecutive contrast-enhanced axial CT images (**a**–**d**) show huge tumor extending to the inferior vena cava. Selective right phrenic artery injection DSA image (**e**) shows significant tumoral blood supply. ^{90}Y infusion was performed from selectively catheterized replaced right hepatic artery after embolization of right phrenic artery for consolidation (**f**). Post-TARE PET images (**g**–**i**) show ^{90}Y deposition in tumor. Corresponding control contrast-enhanced axial CT images (**j**–**m**) show significant necrosis and decrease in the dimensions of tumor

Table 4 Indications and contraindications for TARE

Indications for radioembolization	
Downsizing	For reducing the tumor size
Increasing FLR	Before liver resection, for increasing the volume of contralateral lobe
Control of tumors	To prevent the increasing of tumor size
Bridging to transplantation	Make suitable the lesions to transplant which are not suitable
To delay progression or palliation	For advanced-stage tumors
Patients with macrovascular invasion	Minimal embolic effect of microspheres
Contraindications for radioembolization	
Poor laboratory values	Serum bilirubin level >2 mg/dl, serum albumin level <2 mg/dl, liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) above five times of normal level
Renal dysfunction	Serum creatinine level >2 mg/dl
High lung shunt	>30 Gy in single session or >50 Gy in total
Extrahepatic metastasis	Except lymph nodes

ria (Fig. 8). Downstaging by itself is not the only determinant factor for transplantation. In order to understand the tumor biology, there is a need for "test of time" to assess the recurrence or progression of tumor or development of distant metastasis (Fig. 9). Therefore, there should be a "bridging" period until the transplantation whether from cadaveric or live donor [5]. UNOS (United Network for Organ Sharing) and OPTN (Organ Procurement and Transplantation Network) require a progression-free period of at least 6 months from the date of listing [79]. TARE has an important role in both downstaging and bridging. Prolonged response to ^{90}Y might be considered having favorable tumor biology and lower recurrence rates [2].

TARE with "curative intent" is usually used for BCLC A patients and solitary tumors with diameters less than 5 cm in unresectable HCC by radiation segmentectomy using higher doses of radiation focally to induce complete pathologic necrosis (Fig. 10) [80].

Radiation lobectomy is another concept for ipsilateral tumor treatment and causing contralateral future liver remnant volume hypertrophy and function similar to portal vein embolization but at a slower rate [81, 82].

Same-day procedure can be applied to some patients with difficult arterial access (such as stenosis, tortuosity, or dissection), with allergy to the contrast, or requiring general anesthesia. This procedure should not be applied to the patients with a low GFR number, vascular invasion, infiltrative tumors, and/or tumor burden more than 50%. Especially patients with macrovascular invasion are more likely to have high degree of lung shunts, so same-day procedure should be strongly avoided [3].

In the presence of recurrence after liver resection, radioembolization is a good alternative treatment option for many patients. However, prior resection may reduce the functional capacity of the liver and can increase the risk of toxicity. It is known that small total liver volume is an independent risk factor of REILD [83, 84]. Decreasing liver volume will cause the absorbed radiation dose to increase relatively and further increase the risk of REILD, too [85]. In spite of all these, in the published series, there is no clearly defined increase of risk for REILD. But in these studies, empiric dose reduction and subtotal liver remnant treatments were applied. In this situation, there is no consensus that the standard ^{90}Y dose and therapy may need to be changed after liver resection [86]. So, a conservative treatment strategy should be applied whenever possible. In this condition, postoperative liver volume changes should be taken into account when calculating the patient dose. The aim of the treatment should be keeping the dose of normal liver parenchyma lower than 50 Gy while delivering a therapeutic dose to the tumor [83].

Lobar therapy is more appropriate for multifocal large tumors. Peripherally located solitary tumors are best treated with segmental approach. Tumors in central segments could have dual blood supply from segmental branches from both hepatic arteries.

Portal vein thrombosis is generally accepted as a contraindication for TACE since it is a sign

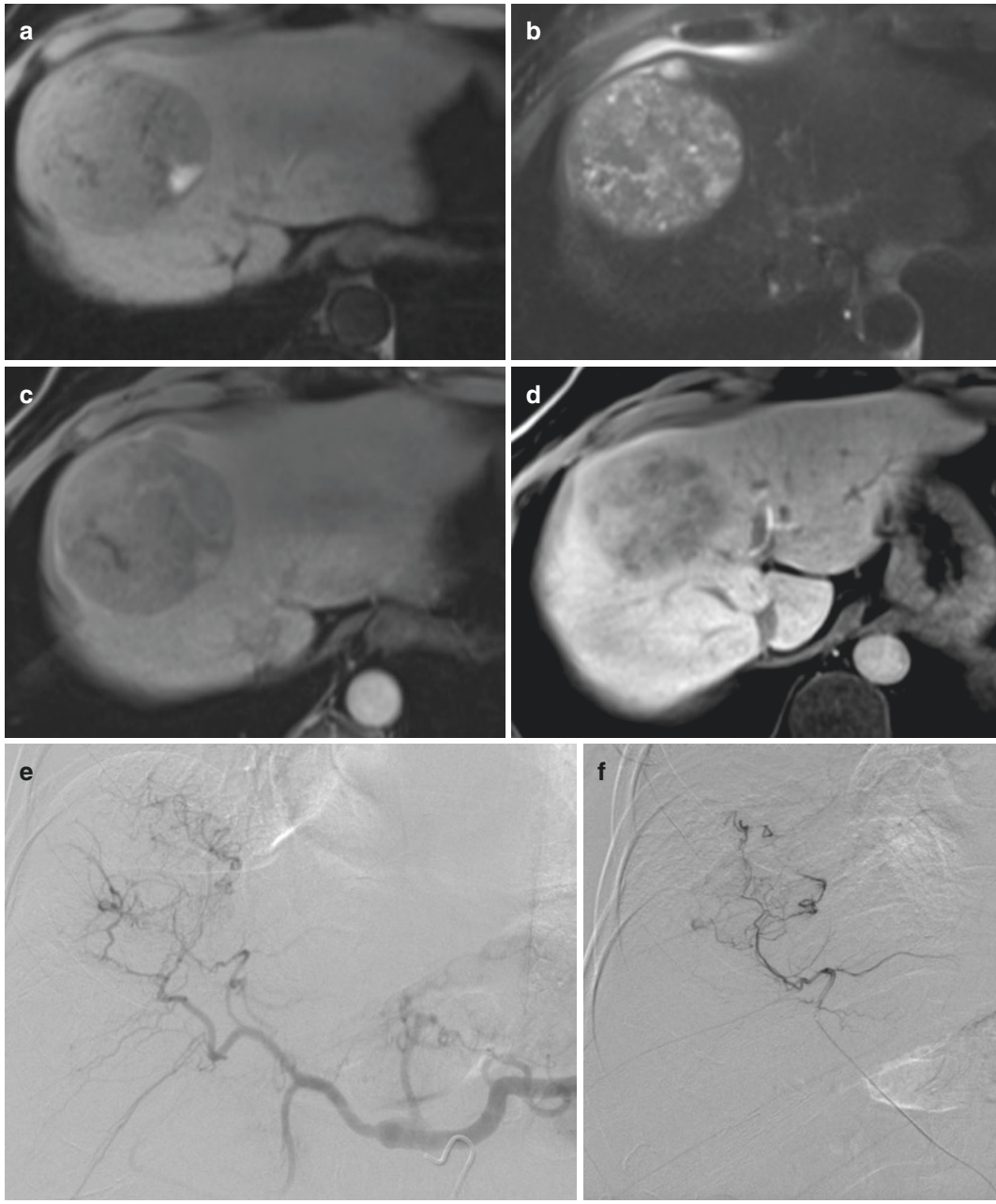
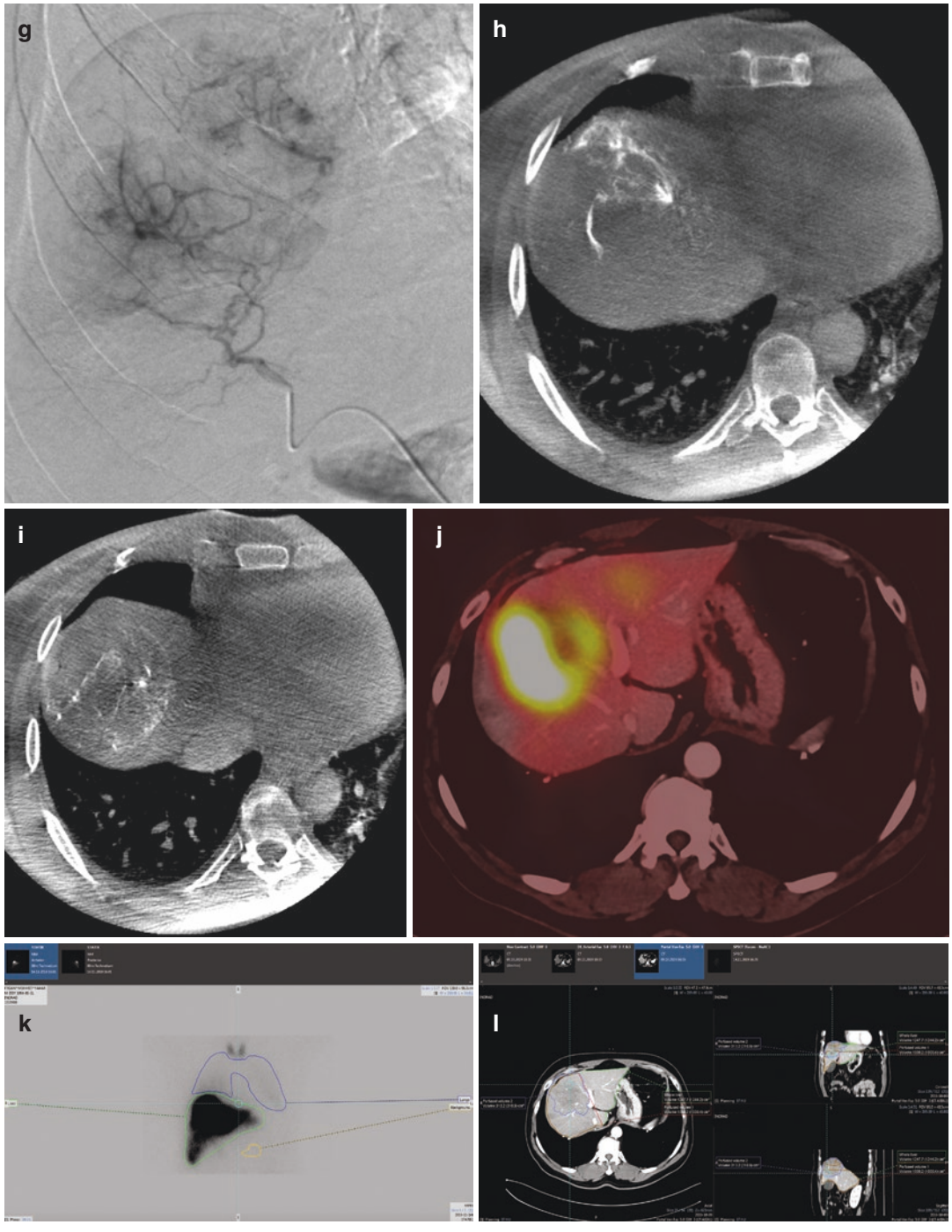


Fig. 8 Axial T1W (**a**), T2W (**b**), and T1W contrast-enhanced (**c**) and liver-specific contrast-enhanced (**d**) MRI images show an 8 cm HCC lesion in segment 7, 8, and 4. Celiac injection DSA image (**e**) shows tumoral blood supply from S4 and right hepatic artery. Selective S4 (**f**) and right hepatic (**g**) artery injection DSA images and cone-beam CT images (**h**, **i**) demonstrate blood supply from S4, S8, and S7 branches. Fusion Tc-MAA CT image (**j**) shows tumoral coverage. Planar image (**k**) shows the hepatopulmonary shunt ratio obtained from the hepatic artery perfusion scintigraphy data. Traces are drawn on radiological images to define the liver lobe volume, target volume of the lobe or segment, and tumor where the patient will be treated (**l**). The absorbed dose delivered to the target lobe (**m**) and the absorbed dose delivered to the tumor (**n**) are calculated. Just before the radioembolization, S4 (**o**), S5, and S6 arteries (**p**, **q**) were embolized with Gelfoam selectively to redistribute and consolidate flow to the tumor. Post-TARE PET CT image (**r**) demonstrates complete coverage of tumor. Control coronal CT images (**s**–**u**) complete necrosis and decrease in diameter of the lesion over 6-month period. After downstaging, patient had live donor liver transplantation. Explant pictures (**v**, **y**) show cirrhotic liver and protruding necrotic tumor



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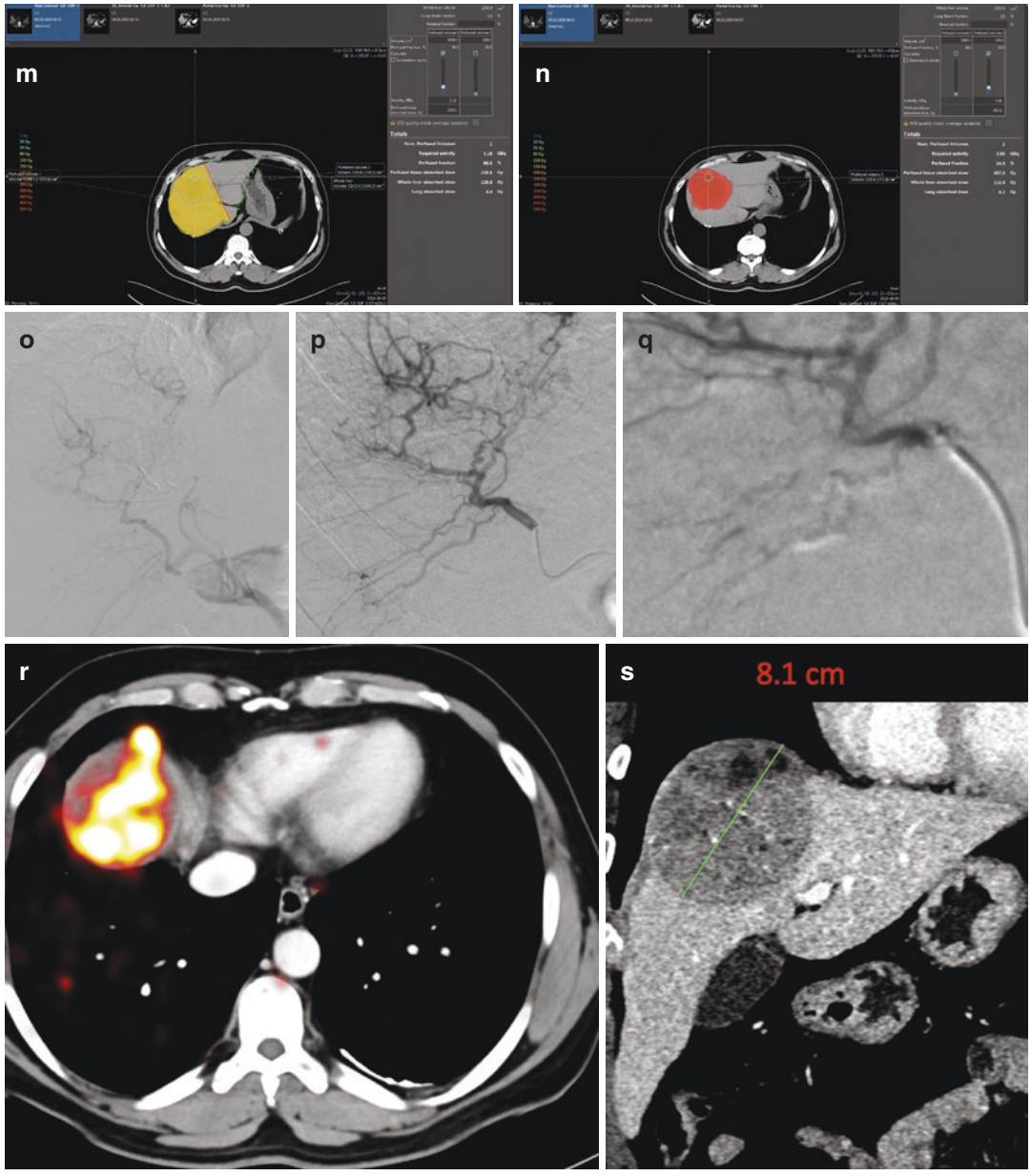

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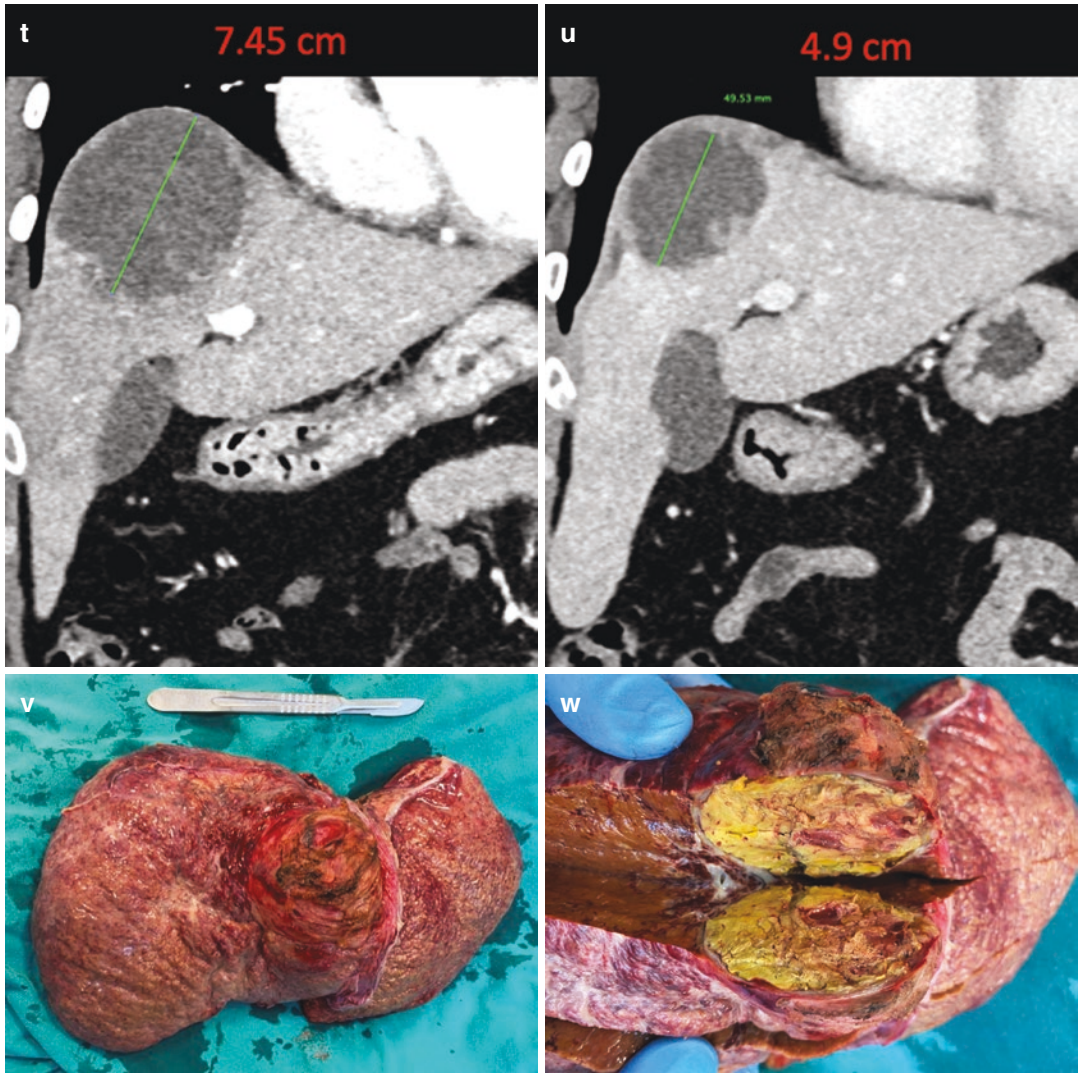

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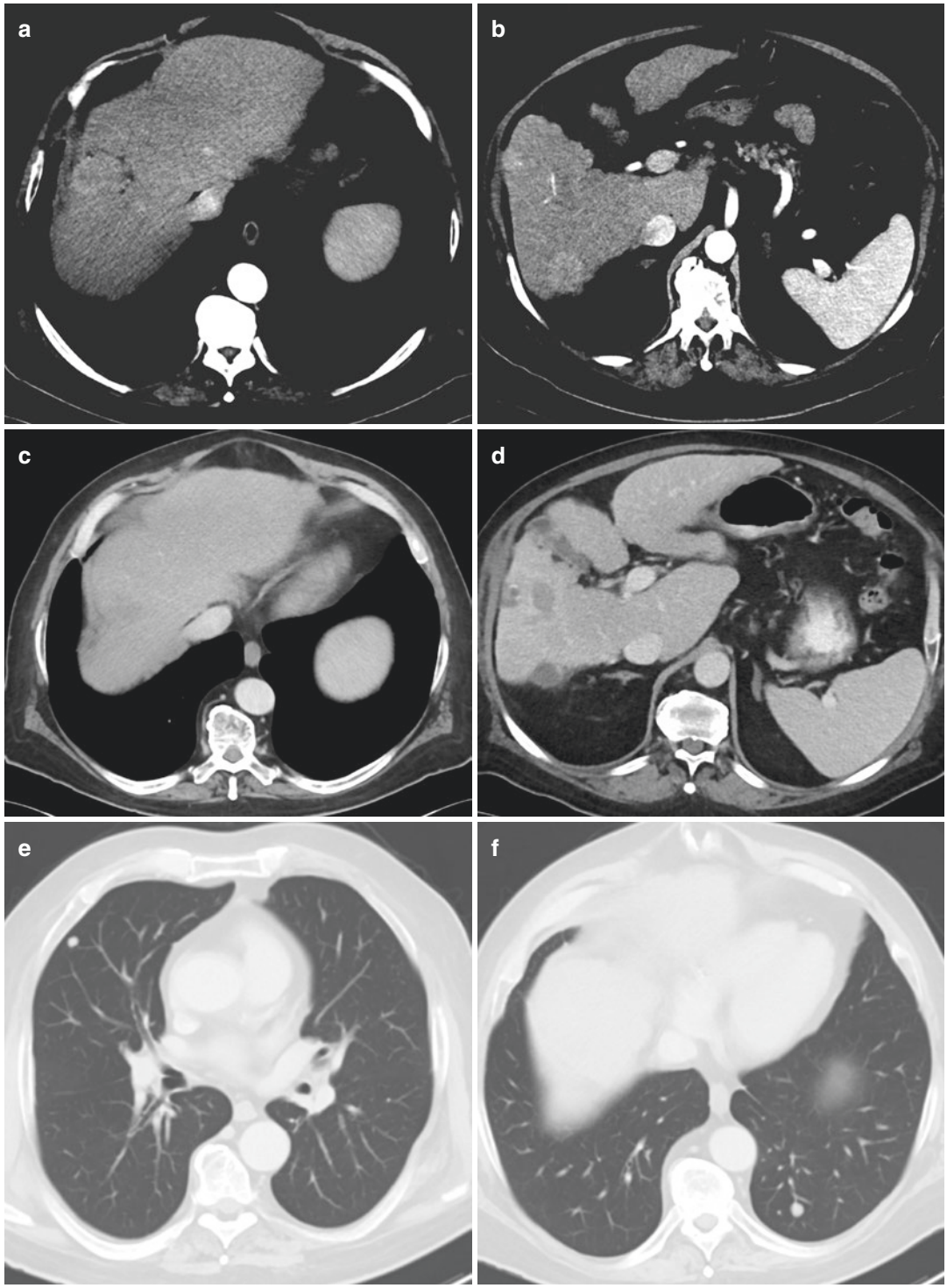

Fig. 9 Axial contrast-enhanced images (**a**, **b**) show multifocal HCC in the right lobe. Corresponding axial contrast-enhanced CT images (**c**, **d**) 3 months after TARE demonstrate necrosis of lesions. Although the patient is downstaged, metastatic lung nodules in both lungs (**e**, **f**) developed 5 months after TARE, and the patient was delisted from transplantation waiting list

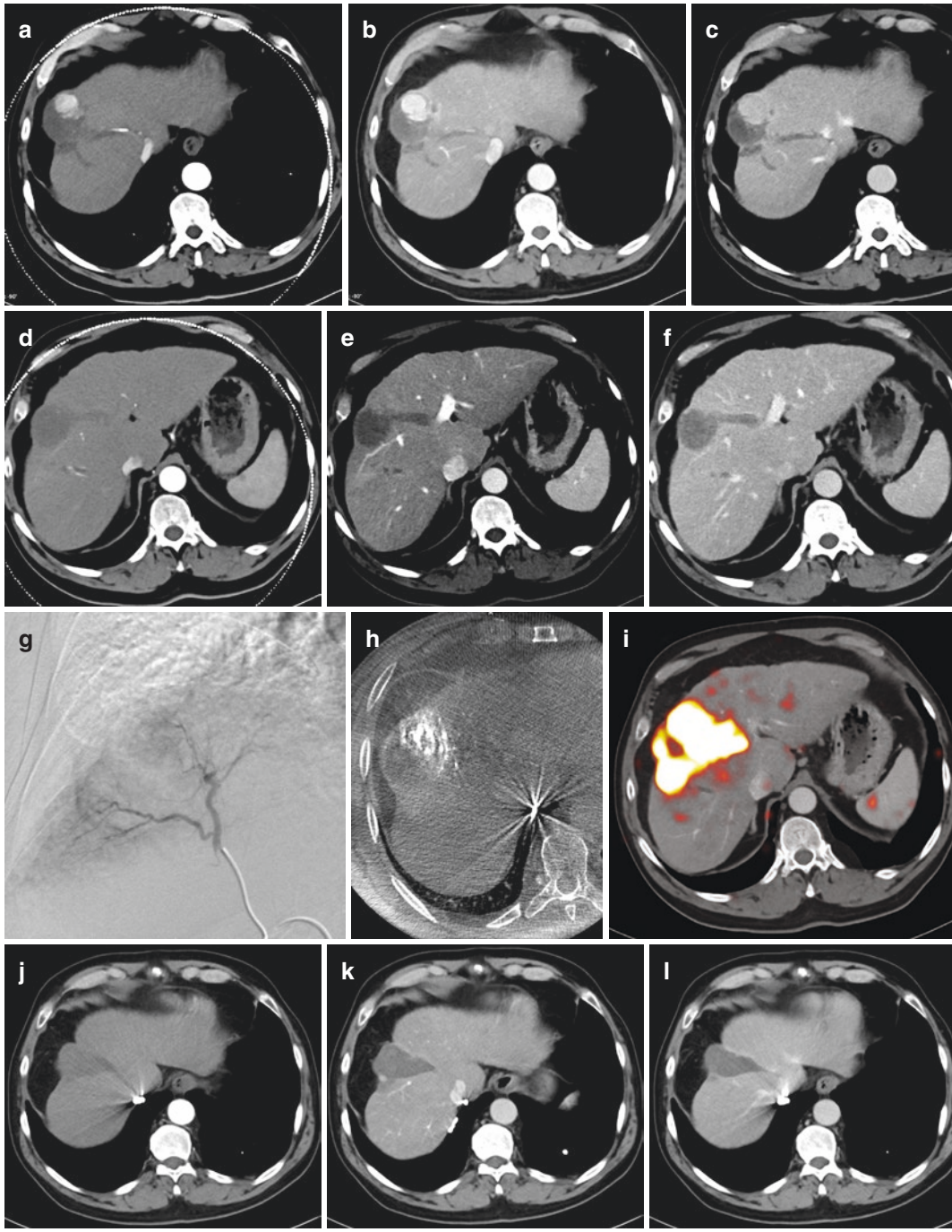

Fig. 10 Axial arterial (**a**, **d**), portal (**b**, **e**), and venous (**c**, **f**) phase CT images at two different levels show an HCC lesion of 4 cm in diameter located in segment 8 with hypervascular nodular part and necrosis. There is also extension to the middle hepatic vein. Superselective injection into the tumoral feeding artery DSA image (**g**) and axial cone-beam CT image (**h**) demonstrate wedge-shaped perfusion of the tumor. Post ^{90}Y TARE with glass particle PET CT image (**i**) shows complete coverage of tumor. Three months after TARE, corresponding axial CT images (**j**–**o**) at the same levels demonstrate complete necrosis of the tumor and regression of hepatic vein thrombosis. Coronal reformatted CT images before (**p**) and after (**q**) TARE show necrosis of the lesion and regression of the middle hepatic vein thrombus

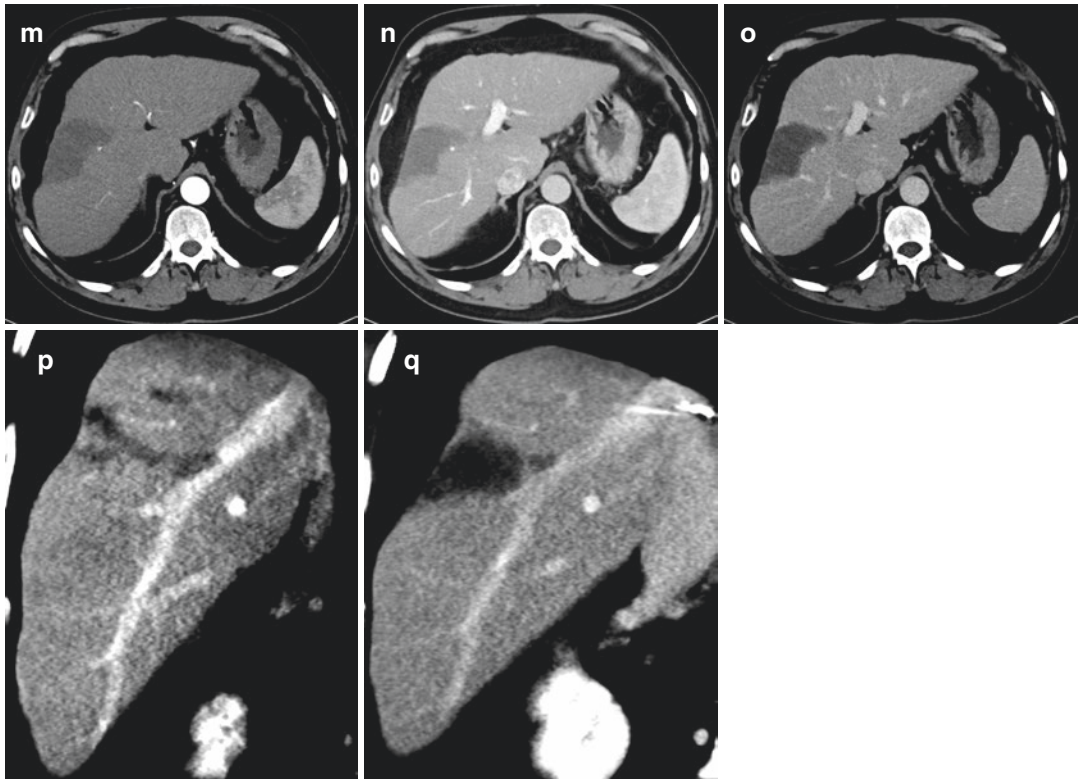

Fig. 10 (continued)

of progressive disease of extensive tumor growth, extrahepatic spread, or progressive functional impairment [6]. Although TACE could be performed by multiple segmental therapies in selected patients, TARE, which has a minimal embolic effect and shown to have favorable response, is a safe alternative in portal vein thrombosis cases, and regression of portal vein thrombus is possible (Fig. 11) [9, 12, 81].

While repeat treatments in unilobar disease appears to be safe, for the bilobar repeat treatments, alternating therapies could be employed [12].

6.4 External Radiotherapy and SBRT

As the liver has low irradiation tolerance, conventional external radiotherapy has a limited place in the treatment of hepatocellular carcinoma. Purpose of the use of external radiation therapy in HCC is mostly palliation of metastasis such as lymph node, bone, or soft tissue [87–89]. With the developments in technology and the use of three-dimensional conformal radiotherapy (3D-CRT), the use of radiation therapy especially in patients with unresectable HCC has increased. But, although using 3D-CRT, REILD is still a major complication. Especially, in specific locations such as dome of liver (due to radiation pneumonitis) or porta hepatis (due to the risk of major biliary and vascular damage), external beam radiotherapy has limitations [74].

While calculating the dose delivery, nontumoral tissue complications are an important parameter. To minimize the normal tissue complications, respiratory movement must be considered. Delivering the dose in fractions to the tumor may be useful in targeting the radioresistant and radiosensitive malignant cells at different sessions. But, more than one treatment is needed [90].

Due to the technological advances in imaging methods and planning of radiotherapy, stereotac-

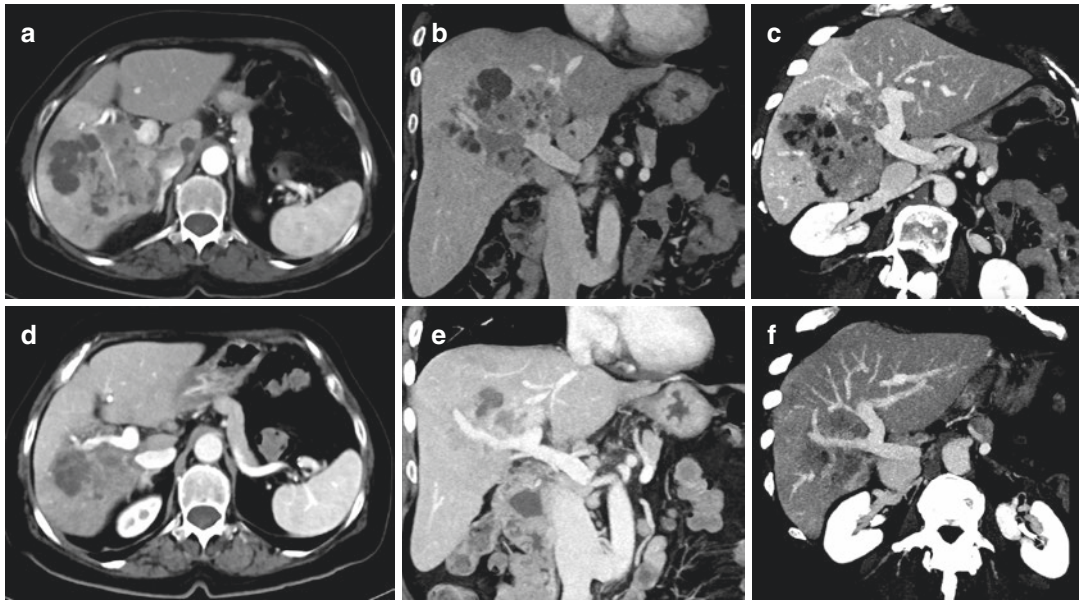

Fig. 11 Contrast-enhanced axial (**a**), coronal (**b**), and oblique reformat (**c**) images show right lobe tumor compressing and invading the right portal vein. Corresponding control contrast-enhanced images (**d–f**) show decrease in the size of the tumor and relive of right portal vein compression and improvement in invasion

tic body radiation therapy (SBRT) which delivers highly conformal radiation therapy with geometric precision has become possible. SBRT has become an effective treatment alternative for early and locally advanced HCC [91–93].

SBRT has recently been recognized as an effective treatment option for nonsurgical localized intrahepatic HCC and included in the 2019 National Comprehensive Cancer Network Guidelines and the practice guideline statement of the American Liver Disease Studies Association [94, 95].

SBRT is used for stabilization and regression in HCC patients as a primary treatment with increasing the median survivals from 11 to 25 months [96–98]. SBRT seems to be safe and efficacious local bridging therapy for patients with local or advanced HCC who are on the waiting list for LT. Also, it can be applied as a primary treatment and can be combined with other locoregional treatment modalities.

In these new techniques, toxic dose is delivered to the tumors while normal liver parenchyma is saved [99]. But, although these advanced targeting techniques are used, liver toxicity after treatment is still a major problem. So, radioembolization therapy after external radiotherapy should be performed carefully [100].

7 Complications of TARE

TARE is a relatively safe treatment procedure. While a small proportion of patients have experienced mild side effects such as self-limited exhaustion or abdominal pain, TARE is associated with low rates of serious complications. The complications associated with TARE can be divided into three major groups: extrahepatic, intrahepatic, and vascular complications.

7.1 Extrahepatic Complications

Extrahepatic complications of TARE (such as pulmonary, gastrointestinal, esophageal, or pancreatic complications) usually occur because of nontarget embolization and lung shunts which cause radiation-induced pneumonitis due to the high radiation dose to the lungs [101]. Collaterals

between hepatic artery and extrahepatic organs should be identified during mapping angiography and should be embolized prophylactically before TARE in order not to cause nontarget embolization.

7.1.1 Radiation-Induced Lung Disease

There is limited information about radiation-induced lung disease after radioembolization treatment. In a review of 515 patients by Kennedy et al., the incidence of RILD was found to be 4%. Of these, 75% was treated single session whole-liver therapy by using empirical dosimetry. In current practice, empirical dosimetry method is no longer used [51]. The effect of the microspheres on the lung parenchyma is due to arteriovenous shunts, which are commonly seen in HCC [102]. During radioembolization, some of the ^{90}Y passes to the lung because of these intratumoral arteriovenous shunts. Mapping angiography with 99mTechnetium MAA minimizes the risk of RILD by evaluating lung shunts and calculating treatment dose. The cases in the literature describe life-threatening disease which includes pathologically acute-subacute interstitial pneumonitis. In these patients, increasing dyspnea and restrictive lung disease developed in 1–6 months after radioembolization [103]. It is important to exclude the other reasons of dyspnea in patients who present with shortness of breath. There is no evidence-based treatment for RILD. Supportive treatment with oxygen supplementation and intravenous steroid can be performed [8].

7.1.2 Gastrointestinal Complications

Nontarget infusion of microspheres to the gastrointestinal organs might cause ulceration or perforation in these organs. In a review with 39 studies by Naymagon et al., mean incidence of gastric ulceration after radioembolization was found to be 4.8% [104]. Approximately 5 weeks after radioembolization, these patients experience abdominal pain, nausea, anorexia, and vomiting [105]. Ulceration of the stomach or duodenum which is induced by Yttrium-90 may not respond to medical therapy. So, surgery may be needed. To prevent radioembolization-associated ulceration, mapping angiography should be performed carefully and coil embolization should be done if necessary.

If the microspheres spread into the pancreatic vessels, radiation-induced pancreatitis may occur. This type of pancreatitis usually affects the head of the pancreas. This complication can be very painful for the patient and leads to a prolonged hospitalization, food restriction, and i.v. treatment.

Although it is theoretical, damage of attenuated radiation to the organs adjacent to the liver (such as the colon, duodenum, or stomach) may be possible as gastritis or enteritis. Another side effect of attenuated radiation may occur in the right hemithorax as pleural effusion [101].

7.1.3 Radiation-Induced Cholecystitis

Although it is rare, ^{90}Y carrying microspheres could enter into the cystic artery and perforators from the hepatic parenchyma or into the gastroduodenal artery branches that supply gallbladder and then cause mucosal injury and ischemia. In order to prevent radiation-induced cholecystitis, infusion distal to the cystic artery is advised. In cases where this is not possible due to necessary flow dynamics or anatomic location, prophylactic embolization, usually with temporary agents, could be employed. If radiation cholecystitis develops, most cases are followed conservatively. However, if there is perforation or emphysematous cholecystitis, then cholecystectomy or percutaneous cholecystostomy may be needed (Fig. 12) [101, 106, 107].

7.1.4 Bile Duct Complications

Intra- and extrahepatic biliary complications after TARE are associated with the embolic effect and necrosis of the biliary ducts due to radiation. Unlike normal liver parenchyma, the intrahepatic bile ducts have only single feeding artery which arises from the hepatic arterial branches as a vascular plexus (peribiliary capillary plexus) around the bile ducts. The diameter of the vessels in this plexus is the same as ^{90}Y embedded microspheres; therefore, after TARE, ischemia may develop in the bile ducts [108]. In a study with 327 patients who underwent TARE by Atassi

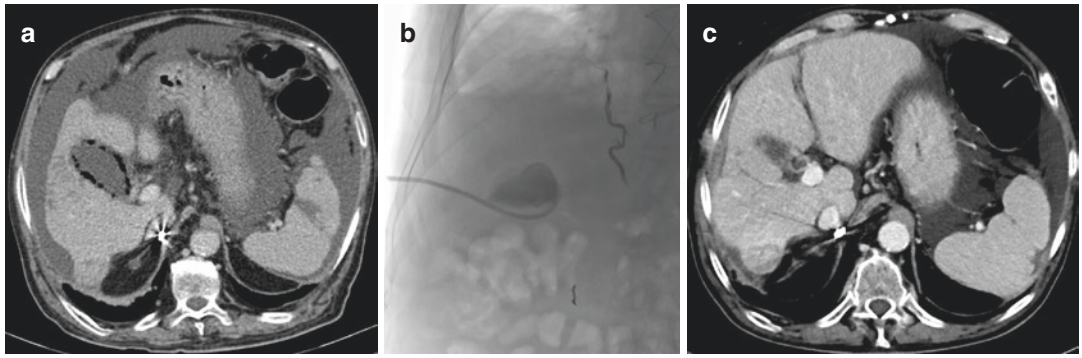

Fig. 12 Contrast-enhanced axial CT image (**a**) after TARE shows ascites and air in the gallbladder wall (emphysematous cholecystitis). Spot image (**b**) obtained after placement of percutaneous cholecystostomy shows the drainage catheter and embolization coils in right phrenic artery. Control contrast-enhanced axial CT image (**c**) after removal of drainage catheter shows resolution of cholecystitis and ascites

et al., the rate of biliary sequelae was approximately 10%. Less than 2% of patients needed biliary interventions. The most common biliary complications were biliary stricture and necrosis. Most of biliary interventions employed for these complications are drainage of bilomas and abscesses and percutaneous cholecystostomy for radiation cholecystitis. Biliary necrosis was less common in patients with primary HCC than in patients with metastatic liver lesions. This may be due to the hypertrophy of the peribiliary vascular plexus and history of chemotherapy of patients with metastatic tumors [109]. Damage in the bile ducts can take several forms such as biloma cavities or dilatation of the bile ducts. The reason of the biloma cavities can be the necrosis of the peripheral bile ducts with leakage due to the damage of vascular plexus which supplies bile ducts [110, 111]. Treatment of biliary damage after radioembolization is usually conservative. Percutaneous drainage for bilomas or abscesses, balloon dilation, or stents for strictures can be applied. Surgery may be necessary in very rare cases.

7.1.5 Radioembolization-Induced Liver Disease (REILD)

Sangro et al. [50] first used the term of REILD which refers to a collection of symptoms resulting from progressive liver decompensation related to radioembolization. Findings of the REILD are jaundice, ascites, high serum bilirubin levels, and low serum albumin level, and incidence of it is nearly 5% [85]. REILD typically presents with patterns of sinusoidal obstruction as veno-occlusive disease. Supportive treatment is applied to these patients. While the syndrome can be self-limited, liver failure and death can be seen in serious cases [50].

All transarterial embolic therapies could cause liver toxicity that its degree of damage depends on many factors like dose, particle size, baseline liver reserve, and tumor to liver perfusion. The calculation of actual dose of normal liver parenchyma is difficult since microspheres distribute inhomogeneously. Functional liver reserve and regenerative function are the two main determinants of liver toxicity. Cirrhosis, previous chemotherapies and locoregional therapies, resection, etc. all affect the functional reserve and regenerative capacity. In almost all the patients that underwent radioembolization treatment with ^{90}Y, some degree of liver toxicity is seen.

Liver-dependent factors such as infiltrative type of HCC (volume of the tumor 50% of total liver volume), serum liver transaminases levels greater than five times the normal value, serum albumin level <3 g/dl, and total serum bilirubin level >2 mg/dl strongly associated with a 3-month mortality. The bilirubin level is the best indicator for REILD [10].

Although serious liver toxicity associated with radioembolization is rare, if it develops, it

can be divided into early and late stages. Acute liver toxicity occurs in 2–16 weeks after treatment with no tumor progression or biliary obstruction [112].

Chronic toxicity can be seen in months or years after treatment. So, the rate of chronic toxicity is not known well yet. The mechanism of chronic toxicity is thought to be associated with radiation-induced fibrosis. It presents with atrophy of the liver and findings of portal hypertension [113, 114].

REILD is so rare after the first TARE therapy. If there is no lesion in the contralateral lobe, the second session can be tolerated as well. But the cumulative dose of the liver increases the risk, so prior whole-liver therapy is an important risk factor for REILD. If the tumoral lesions could be catheterized selectively, patients with large tumors can be treated safely even when they have other risk factors for toxicity [85]. Instead of whole-liver therapy, sequential lobar treatment can be performed to allow the contralateral lobe for regeneration with an interval of 4–6 weeks. However, caution should be exercised when considering sequential treatments because REILD may occur in 16 weeks after first procedure. So, the absence of clinical deterioration in 4–6 weeks after treatment should not be seen as conclusive evidence that additional therapy is safe [115].

For the mild cases, current standard of treatment includes diuretics and long-term high-dose steroids. For more serious and acute cases, long-term low-dose heparin, ursodeoxycholic acid, and pentoxifylline can be added to treatment [10].

7.1.6 Post Radioembolization Syndrome

In post radioembolization syndrome, symptoms such as fever, fatigue, nausea, vomiting, and anorexia are seen. The incidence of it has been defined as significantly less than the postembolization syndrome encountered after TACE. Post radioembolization syndrome is usually self-limited. Some patients may need symptomatic treatment and hospitalization. Single-dose steroids can be given preprocedurally [8].

7.2 Vascular Complications

TARE treatment has the same risks of vascular complications with other intra-arterial procedures. Hematoma or pseudoaneurysm at the access site or arterial dissections can be seen during therapy. The risk of vascular injury increases in patients who have previously received chemotherapy [18].

8 Radiologic Follow-Up

The aim of the follow up is the evaluation of the response or disease progression. Four-phase dynamic contrast-enhanced CT or multiphasic dynamic contrast-enhanced MRI is performed after 4–8 weeks following TARE procedure to evaluate the response to the therapy. To avoid the misinterpretation of reversible or transient findings, intervals of follow-up imaging is not performed earlier [56, 116]. The most common transient finding on follow-up CT images is reduced density at the site where microspheres accumulate. These findings are thought to be due to edema, congestion, or microinfarction in the treated areas [56]. After the first radiological study, patients are followed with scans every 3 months.

According to the guidelines of the World Health Organization (WHO) and the Response Evaluation Criteria in Solid Tumors (RECIST) group, the most important indicator of a successful treatment response is reduction in tumor size [117]. However, necrosis, cystic degeneration, hemorrhage, or edema can cause the increasing in tumor size. The European Association for the Study of the Liver (EASL) necrosis criteria are also used to evaluate necrosis that develops in tumors [118]. According to a recent study, use of combined size and necrosis criteria is more accurate than the use of size criteria alone in evaluating the response to ^{90}Y treatment [119]. Therefore, the indicators which can show tumor necrosis such as tumor vascularity, 18-fluorodeoxyglucose uptake on PET-CT, volume of tumor (viable tumor burden), diffusion weighted MRI, and serum tumor markers (serum AFP level) should be evaluated for tumor response. Functional MRI

may play a role in detecting tumor response earlier [120]. It may take 6–9 months to achieve maximum response (totally devascularization with no recurrence). Serial imaging together with laboratory examinations allows proper follow-up of treated patients for the response assessment. Follow-up imaging also helps to evaluate patients who were downstaged or in bridging period for the possible recurrences.

9 Conclusion

Interventional oncologic approaches broaden the treatment options for HCC. TARE is a safe and effective option for selected group of patients who are not suitable for surgery or other locoregional interventional treatments or patients with failed interventions. It has a proven effect in downstaging and bridging. TARE has an important role in every stage of HCC, and the technical developments and improvements in dose-related issues will positively affect the outcomes of patients with HCC.

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Intra-arterial Chemotherapy and Transarterial Chemoembolization in Hepatocellular Carcinoma

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1 Introduction

Transcatheter intra-arterial therapies are image-guided locoregional therapies mostly used in the interventional radiology for the treatment of patients with primary and metastatic tumours, most commonly localized in the liver [1]. These therapies include intra-arterial chemotherapy (IAC), transarterial embolization (TAE), transarterial chemoembolization (TACE) with or without drug-eluting beads (DEBs), and radioembolization using embolic particles loaded with a radioisotope, most commonly Yttrium-90. The main goal of these therapies is to cause an ischemic/hypoxic environment and, consequently, coagulative necrosis in the tumour by delivering selectively chemotherapeutic drugs to the tumour-feeding arteries. The anticancer effect of all embolization procedures is based on terminal arterial blockade and subsequent tumour ischemia. IAC consists of intra-arterial infusion of the chemotherapeutic drugs by selective catheterization of the hepatic artery targeting the delivery of high concentrations of chemotherapeutic drugs directly to the tumour. TACE (with or without drug-eluting beads) combines targeted chemotherapeutic drug delivery with simultaneous embolization of the tumour-feeding artery. Transarterial radioembolization integrates delivery of internal radiation to the tumour with minimal embolic effect unlike other embolization treatments. These therapies are accepted treatment modalities for providing survival benefit in selected patient populations. In this chapter, we describe the rationale behind of IAC, TAE, conventional transarterial chemoembolization (cTACE), and transarterial chemoembolization (TACE) with drug-eluting beads (DEBs) and provide a review of the existing medical literature. Transarterial radioembolization is beyond the scope of this chapter.

2 Intra-arterial Chemotherapy: Rationale and Overview

IAC is a minimally invasive percutaneous image-guided radiologic procedure with employing angiographic catheter as a conduit to achieve a higher local concentration of chemotherapeutic agents to the targeted unresectable tumour with fewer significant systemic side effects [2]. The rationale for regional chemotherapy is to maximize drug concentrations and tumour drug uptake in the target organ and minimize systemic toxicity [3]. For successful IAC, several important principles regarding tumour biology, drug pharmacology, and delivery systems must be fulfilled [4]. These concepts are that (1) locoregional delivery of chemotherapeutic agent leads to increased local concentration of the drug, (2) increased locoregional concentration of the drug leads to increased therapeutic response, and (3) locore-

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gional drug delivery leads to decreased systemic exposure of the used drug. Several variations of this technique are available, and no standard protocol has been uniformly adopted. Many centres have differed in the choice and/or dose of the anticancer agents used, treatment end points, and the schedule and/or interval of retreatment.

3 Intra-arterial Chemotherapy: Clinical Evidence of Safety and Efficacy

The results of IAC have been studied most extensively in patients with colorectal cancer and unresectable liver metastases. The role of TAC outside of metastatic colorectal cancer has been less researched. Okusaka et al. [3] published findings of the randomized phase III trial comparing TAC and TACE for the treatment of patients with unresectable HCC. In this prospective 161-patient study, there was no significant difference when the median overall survival time was compared between these two therapies. IAC produced less tumour necrosis than TACE, particularly in tumours more than 3 cm. The important thing is that the Barcelona Clinic Liver Cancer staging classification and treatment schedule [5], a worldwide used staging system for HCC management, does not include IAC in its algorithm of the treatment options for HCC. Also, the American Association for the Study of Liver Disease practice guidelines [6] did not recommend systemic or selective intra-arterial chemotherapy and warn not to use these treatment methods as standard of care. However, in Japan, TAC has traditionally been used to treat patients with advanced HCC with vascular invasion or multiple intrahepatic lesions or both [7].

4 Transarterial Embolization: An Overview

TAE (also known as bland embolization) is a minimally invasive image-guided procedure performed with the aim to restrict the tumour's blood supply by delivering particles which do not contain a chemotherapeutic or radioactive agent. In the context of treating an HCC tumour with TAE, polyvinyl alcohol particles or gelatin-based microspheres are used most commonly, although alcohol with ethiodized oil and gelatin sponge has also been described [8]. Deficiency of arterial flow results in an ischemic/hypoxic environment and, consequently, coagulative necrosis in the tumour. The embolic agent can also potentially incite a localized inflammatory reaction and focal angionecrosis [9]. The therapeutic end point of TAE is the stasis of flow in the arteries supplying the tumour with pruning of the distal branches of the treated artery. The targeted tumoral arterial supply is interrupted with an embolic agent, most commonly microparticles ranging from 40 to 120 μm in size [10]. Depending on the disease distribution within the liver, the treatment approach can vary including lobar treatment for multifocal disease or targeted segmental treatment for unifocal disease [8]. The most common associated risk is that of postembolization syndrome, the severity and duration of which might be correlated with the degree of healthy tissue ischemia and underlying liver function [11]. The use of novel intraprocedural technologies such as cone-beam CT is utilized to ensure complete tumoral coverage while avoiding nontarget embolization [12]. TAE is reserved for nonsurgical candidates with liver-dominant disease. Studies have demonstrated that HCC patients in stage B of the Barcelona Clinic Liver Cancer staging classification system derive the most benefit from this procedure followed by stage C [13]. Patients in BCLC stage A may undergo TAE to maintain eligibility for transplantation per the Milan and University of California, San Francisco criteria [14]. The contraindications for TAE include decompensated cirrhosis (Child-Pugh B8 or higher), significantly reduced portal venous flow, creatinine clearance <30 mL/min, high tumour burden, severe comorbidities, untreated oesophageal varices, and elevated liver function markers [15].

5 Transarterial Embolization: Clinical Evidence of Safety and Efficacy

Llovet et al. [13] reported that TAE confers significant survival benefit compared to best supportive care. Tsochatzis et al. [14] published results from a meta-analysis of six randomized controlled trials comparing TACE with TAE, and none of them revealed significant differences in overall survival. Lee et al. [15] summarized evidence from three studies revealing no significant differences in 3-year survival rates, adverse events, or RECIST responses. Kluger et al. [16] found that TAE patients were significantly less likely to require retreatment before transplantation than TACE patients. Malagari et al. [17], in the prospective randomized comparison of chemoembolization with doxorubicin-eluting beads and bland embolization with Bead Block (Boston Scientific, Marlborough, MA) for HCC, found significant improvement in time-to-progression in the DEB-TACE group, but no change in overall survival.

Since induced ischemia from embolotherapy could be the dominant contributor to tumour cell death and bland embolization does spare the cost of chemotherapy and its toxicity profile, TAE should continue to be offered to appropriately selected patients.

6 Transarterial Chemoembolizations: An Overview

TACE is a minimally invasive image-guided procedure performed with the aim to restrict a tumour's blood supply. According to the Society of Interventional Radiology guidelines [18], chemoembolization is currently defined as the infusion of a mixture of chemotherapeutic agents with or without iodized oil, followed by embolization with particles. During TACE, embolic particles with or without chemotherapeutic drugs are injected through an angiographic catheter directly into a tumour-feeding artery. There are two main mechanisms enabling TACE in patients with HCC. The carcinogenesis of HCC is a multistep process that leads to a gradual shift in tumour blood supply from predominantly portal to predominantly arterial circulation. Due to the predominately arterial feeding of HCC, transarterial embolization interrupts the tumour's blood supply and slows down or stops the growth of the tumour [19]. Additionally, targeted administration of chemotherapeutic agents allows delivery of a higher dose to the tumour's tissue while simultaneously reducing exposure for the liver parenchyma. After transarterial embolization, chemotherapeutic drugs are not washed out from the occluded tumour's vessels that results in a higher concentration of drugs within the tumour with a longer period of the exposure to the cytotoxic effect. An ischemic necrosis induced by embolization causes a failure of the transmembrane pump, resulting in a greater absorption of tumoricidal agents by the tumour cells. Thus, the concentration of the agents within the tumour can be 40 times greater than that of the surrounding normal liver parenchyma [20]. As a consequence of the above, TACE selectively targets the tumour while normal liver is relatively preserved. According to the Barcelona Clinic Liver Cancer (BCLC) staging system [5], TACE is the first-line treatment for intermediate-stage disease, which includes asymptomatic patients with well-preserved liver function and limited to liver large or multifocal tumours and without macrovascular invasion or extrahepatic spread. For these patients, TACE is recognized as a treatment with proven survival effect on survival [13, 21, 22]. The BCLC system also recommends a treatment migration concept in that TACE should be used in patients with early-stage HCC in whom the recommended treatments are not feasible or have failed [5]. The use of TACE was also supported by other staging systems such as the Chinese University Prognostic Index [23], the Hong Kong Liver Cancer staging system [24], and the Japanese Integrated Staging scoring system [25]. There are two TACE techniques [1, 26], namely conventional TACE (cTACE), which uses a mixture of a chemotherapeutic agent with Lipiodol, and TACE with DEBs (DEB-TACE) which will be reviewed further separately.

7 Critical Appraisal of Patient Selection for TACE

Patient selection is crucial for the success of TACE [26]. The exclusion of absolute contraindications should always be the first step in the assessment of patient suitability for TACE. Absolute and relative contraindications include features of decompensated liver disease, extensive bilobar tumour load and impaired integrity of the portal vein, as well as untreated large varices, huge tumour size, and severe comorbidities [27, 28]. However, patients with moderate to severe hepatic insufficiency can still be treated with TACE if embolization is performed segmentally or sub-segmentally, targeting a small volume of the liver. The presence of segmental or sub-segmental portal vein invasion is acceptable for cTACE if only injection of the drug/Lipiodol emulsion without particulate embolization is performed in the portion of the liver parenchyma deprived of portal venous flow and particulate embolization is delivered only into the tumour-feeding arteries. This approach ensures that non-tumoural liver tissue can still rely on adequate arterial flow [29].

Accepted absolute contraindications for TACE are summarized as follows [19, 26–28]:

- Eastern Cooperative Oncology Group [30] Patient Performance Status >1
- Decompensated liver cirrhosis (Child-Pugh class B, score >8) with jaundice, clinically significant hepatic encephalopathy, refractory to treatment ascites, and/or hepatorenal syndrome
- Impaired portal venous circulation due to portal vein thrombosis or high portal hypertension with hepatofugal blood flow
- Extensive tumour involving both lobes of the liver
- Main portal vein tumour thrombosis
- Untreatable intrahepatic arteriovenous fistula
- Impaired renal function
- Active systemic infection
- Uncorrectable bleeding disorder
- Previous shock related to contrast media

Accepted relative contraindications for TACE are summarized as follows [19, 26–28]:

- Presence of oesophageal varices with high risk of bleeding
- Tumour larger than 10 cm
- Severe comorbidities
- Incompetent papilla with aerobilia
- Biliary dilatation

8 Conventional Transarterial Chemotherapy: An Overview

cTACE involves the imaging-guided intra-arterial injection of a cytotoxic drug, such as cisplatin, doxorubicin, epirubicin, idarubicin, or mitomycin C, which is emulsified in the Lipiodol, into tumour-feeding artery thorough angiographic catheter. Lipiodol, also known as ethiodized oil, is a poppy-seed oil used by injection as a radiopaque contrast agent (Lipiodol® Ultra-Fluid; Guerbet, Villepinte, France). Intra-arterial injection of cytotoxic drug is followed by intra-arterial injection of an embolic agent, such as Gelfoam, polyvinyl alcohol, or acrylic copolymer gelatin particles [31]. During cTACE, Lipiodol carries and delivers chemotherapeutic agents to the tumour and causes embolization of the tumour microcirculation [32, 33]. cTACE is the current standard of care for large or multinodular tumours isolated to the liver for patients with preserved liver function and absence of portal vein invasion [34]. cTACE use has been reported in patients with more advanced HCC, such as microvascular or macrovascular invasion, or limited extrahepatic disease with adequately preserved hepatic function [35]. cTACE is also used in patients with early-stage HCC as a bridge to liver transplantation or for patients not eligible for liver transplantation, hepatic resection, and ablation [5]. cTACE is the recommended standard of care for the treatment of intermediate-stage HCC in most current international guidelines [34, 36]. A recent systematic review of cTACE efficacy that comprised a

total of 10,108 HCC patients found that the median overall survival was 19.4 months and that the 5-year survival rate was 32.4% [37]. Despite these facts, some important limitations remain. One of the issues of cTACE is the huge heterogeneity of the techniques and schedules used in clinical practice. Further differences exist with regard to the selectivity of TACE (lobar versus segmental versus super-selective), which has been reported to be an important determinant of procedure tolerance and efficacy [38]. To deal with these limitations, a worldwide expert panel published consensus technical recommendations in order to encourage cTACE standardization [29].

The most important recommendations of the worldwide expert panel [18] are summarized below:

- Eastern European Oncology Group [30] Patient Performance Status to be 0
- Multiphasic computed tomography (CT) or dynamic contrast-enhanced magnetic resonance imaging (MRI) of the liver as the preferred modalities for the treatment allocation
- Cone-beam CT use for the tumour visualization, targeting, and assessment of treatment completion
- Doxorubicin (50–75 mg/m^2) or cisplatin (50–100 mg/m^2) as the most proven chemotherapeutic agent
- Preparing water-in-oil emulsion (aqueous chemotherapy droplets in internal phase and Lipiodol in continuous external phase) to improve tumour deposition
- Gelatine sponge use with 100–300 microns-sized calibrated microspheres with the aim to occlude distal vessels with preservation of feeding segmental arteries
- Super-selective approach with microcatheter for treating a single tumour or small number of tumours
- Lipiodol opacification of the small arterioportal sinusoids as a predictive factor for tumour response and local recurrence [39]
- Assessing tumour viability using the mRECIST criteria [40]
- At least two cTACE procedures 2–8 weeks apart in order to ensure a presence or absence of the tumour response

9 Conventional TACE: Clinical Evidence of Safety and Efficacy

cTACE has been established as the standard treatment for intermediate-stage HCC without portal vein invasion in consequences of two randomized controlled trial studies, which used either doxorubicin [13] or cisplatin [21] mixed with Lipiodol. These studies represent the only level 1 evidence for intra-arterial therapies for HCC demonstrating the superiority of cTACE over best supportive care. Regarding the safety of cTACE, symptoms related to postembolization syndrome (fever, nausea, and abdominal pain) may be observed in up to 80% of patients and were generally mild, transient, and manageable. The most common complications included liver failure, cholecystitis, gastrointestinal bleeding, ascites, and encephalopathy. Treatment-related death rates varied between 0% and 6% [41]. It is less likely to see deteriorated quality of life after TACE [42]. A multicentre prospective Asian cooperative study on intermediate-stage HCC patients treated with cTACE reported a median survival time and 1- and 2-year survival rates of 3.1 year and 89.6 and 75.0%, respectively [43]. cTACE has been reported in patients with more advanced HCC, such as macrovascular invasion or limited extrahepatic disease with adequately preserved hepatic function. In the prospective non-randomized study, HCC patients with segmental or subsegmental portal vein invasion were treated with cTACE or conservative care according to the patient's preference. The 12- and 24-month OS rates for the cTACE and conservative groups were 30.9%, 9.2%, and 3.8%, 0%, respectively ($p < 0.001$) [35]. In the USA, cTACE is also used in patients with early-stage HCC as a bridge to liver transplantation or when liver transplantation, hepatic resection, and image-guided ablation are not possible [44].

10 Transarterial Chemoembolization with Drug-Eluting Beads: An Overview

DEB-TACE, a different transarterial drug delivery technique, involves the intra-arterial injection of DEBs loaded with various types of chemotherapeutic agents [45]. DEBs are non-resorbable embolic microspheres loaded with a chemotherapeutic agent with the ability of slow drug release, which should ensure high local and low systemic drug concentrations. DEB-TACE was primarily developed to enhance the delivery of the chemotherapeutic agent while minimizing systemic toxicity and to provide a standardized embolizing effect. Commercialized DEBs are composed of various hydrophilic ionic polymers that can bind to anthracycline drugs via an ion exchange mechanism. Several microsphere diameters are available, ranging from 40 to 900 μm. The unique properties of these beads, and therefore of this transarterial drug delivery system, allow the fixing drug doses and the ability to release the chemotherapeutic agents in a sustained and controlled manner. Different microspheres are available for DEB-TACE. DC Bead (BTG International, London, UK) is a relatively new drug delivery embolization system comprising a range of hydrogel microspheres that are biocompatible, hydrophilic, non-resorbable, and precisely calibrated. DC Beads are available in four different ranges – 70–150 μm, 100–300 μm, 300–500 μm, and 500–700 μm – with drug loadings varying from 5 to 45 mg/mL hydrated beads. HepaSphere (MeritMedical, MA) is a biocompatible, non-resorbable, expandable, and loadable microsphere. The HepaSphere beads are available in a range of sizes: 30–60 μm, 50–100 μm, 100–150 μm, and 150–200 μm. TANDEM microspheres (CeloNova Biosciences/Boston Scientific, MA) (Fig. 1a–c) are non-resorbable polymethacrylate hydrogel that are available in three sizes: 40 ± 10 μm, 75 ± 15 μm, and 100 ± 25 μm. LifePearl (Terumo Corporation, Tokyo, Japan) are polyethylene glycol embolization microspheres that can be loaded with chemotherapeutic agents (such as doxorubicin, irinotecan, idarubicin, and epirubicin). LifePearl microspheres offer a wide range of drug loading options, enhanced suspension characteristics, and tight calibration. LifePearl microspheres are available in three sizes: 100 ± 25 μm, 200 ± 50 μm, and 400 ± 50 μm. DC Bead LUMI (BTG International, London, UK) (Fig. 2a–e) is radiopaque, biocompatible, non-resorbable hydrogel beads, produced from polyvinyl alcohol-like conventional DC Bead, but incorporating a tri-iodobenzyl radiopaque moiety with a covalent bond. DC Bead LUMI is designed to be inherently radiopaque and thus perfectly visible under X-ray-based imaging modalities, such as CT, cone-beam CT, and fluoroscopy. DC Bead LUMI is available in three size ranges: 70–150 μm, 100–300 μm, and 300–500 μm.

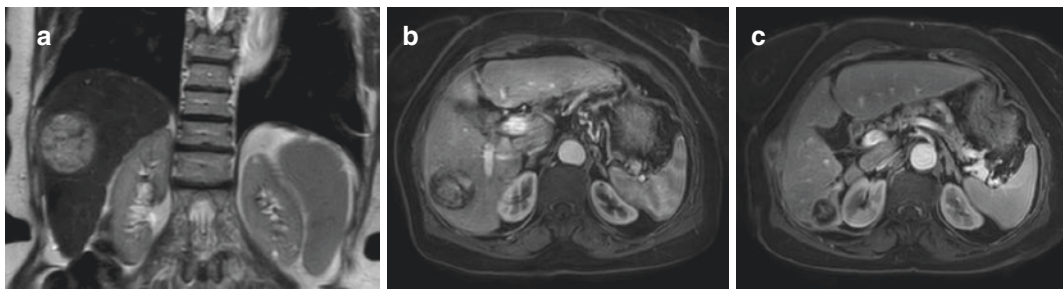

Fig. 1 (**a**) T2-weighted magnetic resonance imaging demonstrated histologically proven hepatocellular carcinoma in the right lobe of the liver in 73-year-old female patient later treated with DEB-TACE using TANDEM 40 μm microspheres loaded with doxorubicin. (**b**) Contrast-enhanced magnetic resonance imaging demonstrated hypervascular hepatocellular carcinoma in the same patient before treatment. (**c**) Contrast-enhanced magnetic resonance imaging demonstrated avascular and shrunken tumour in the same patient 3 years after the treatment. These findings were evaluated as complete response according to mRECIST criteria

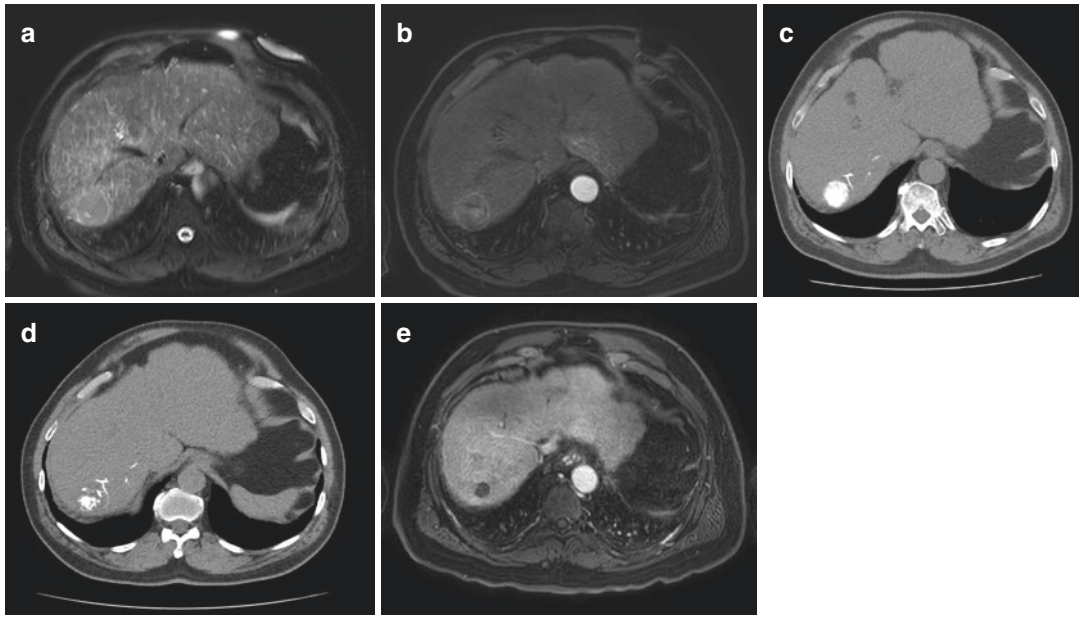

Fig. 2 (**a**) T2-weighted fat saturated magnetic resonance imaging demonstrated hepatocellular carcinoma in the right lobe of the liver in a 69-year-old male patient later treated with DEB-TACE using DC Bead LUMI 70–150 μm microspheres loaded with doxorubicin. (**b**) Contrast-enhanced magnetic resonance imaging demonstrated hypervascular hepatocellular carcinoma in the same patient before treatment. (**c**) Non-contrast computed tomography performed the day after the treatment with DC Bead LUMI demonstrated distribution of the radiopaque microspheres within the tumour in the same patient. (**d**) Non-contrast computed tomography performed 1 week after the treatment with DC Bead LUMI demonstrated distribution of the radiopaque microspheres within the tumour in the same patient. (**e**) Control contrast-enhanced magnetic resonance imaging demonstrated avascular and shrunken tumour in the same patient 19 months after the treatment. These findings were evaluated as complete response according to mRECIST criteria

11 Transarterial Chemoembolization with Drug-Eluting Beads: Clinical Evidence of Safety and Efficacy

Safety and efficacy of DEB-TACE have become the object of a number of studies. Generally, safety and efficacy of DEB-TACE were evaluated by the randomized European Precision V phase II trial in 212 patients with predominately intermediate-stage HCC [28], and a post hoc comparison showed a significant reduction in drug-related systemic and liver toxicity. Grosso et al. [46] published initial results of a multicentre trial that employed HepaSphere microspheres loaded with doxorubicin or epirubicin to treat 50 patients with unresectable HCC. The technical success was achieved in all cases, and no major complications were experienced. The authors evaluated tumour response 1 and 6 months after the procedure, observing an objective response rate of 84 and 77.4%, respectively, at the first and second follow-up time points. Therefore, they concluded that DEB-TACE using HepaSphere is a feasible, effective, and safe procedure. Malagari et al. [47] reported similar promising midterm outcomes using doxorubicin-loaded DC beads as treatment of 3–10 cm HCCs in 71 patients. Overall survival at 12, 18, 24, and 30 months was 97.05%, 94.1%, 91.1%, and 88.2%, respectively. Sustained overall survival was seen in 66.2% of patients. Despite postembolization syndrome being observed in all patients, the rate of severe procedure-related complications was just 4.2%. Therefore, authors stated that DEB-TACE with DC Bead is an effective and safe procedure in the treatment of HCC with high rates of response and

midterm survival. Spreafico et al. [48] reported a study with the aim to evaluate the short-term safety and efficacy of the 70–150 μm DC BeadM1 loaded with doxorubicin in 45 patients with HCC undergoing DEB-TACE as a primary therapy or as a bridge to liver transplantation. The authors reported an OR rate of 77.7% and a grade 3/4 adverse event rate as low as 1.5%. Moreover, pathology demonstrated that 35% of the treated nodules presented a coagulative necrosis area larger than 90% of their volume. Thus, the authors concluded that DEB-TACE with DC BeadM1 is an effective and safe procedure, providing either tumour downstaging or necrosis. Two different prospective studies that investigated the potential role of DEB-TACE, experiencing the use of both HepaSphere 30–60 μm [49] and TANDEM [50], reported good response rates in both cases: the OR was 68.9% in the HepaSphere study and 61.3% in the TANDEM study. Recently, Greco et al. [51] achieved encouraging results in terms of tumour response using 40 μm Embozene TANDEM particles with overall response of 72.6%. Richter et al. [52] in the MIRACLE I prospective multicentre study reported similar results using 75 μm Embozene TANDEM particles with a higher overall response as 95%. Balli et al. [53] demonstrated that superselective DEB-TACE with doxorubicin-loaded beads sized 40–75 μm was an effective and safe treatment method with prolonged time-to-progression and progression-free survival in early and intermediate stages of HCC. An analysis concerning the particles size was performed by Prajapati et al. [54] who retrospectively compared the overall survival, efficacy, and safety of small (100–300 μm) and large (300–500 and 500–700 μm) DEB-TACE beads in two groups of patients with unresectable HCC. The authors found that the use of 100–300-μm-sized particles was linked with significantly higher survival rate and lower complications than the employment of 300–500 and 500–700-μm-sized DEBs. In another retrospective comparative study, Balli et al. [55] reported a higher response rates, prolonged overall survival, and progression-free survival after DEB-TACE performed with doxorubicin-loaded microspheres sized below 100 μm than in above 100 μm patient group. In conclusion, these findings underline that implementation of DEB-TACE may be further increased by the adoption of small particle sizes.

12 Comparison of Conventional Transarterial Chemoembolization and Transarterial Chemoembolization with Drug-Eluting Beads

Both cTACE and DEB-TACE have been considered as the standard treatment for unresectable HCC. DEB-TACE ensures the loaded chemotherapeutic agent slowly releases to achieve a lower systemic drug peak compared to cTACE [56]. DEB-TACE was expected to improve the performance of conventional cTACE. Two retrospective studies [57, 58] have suggested the superiority of DEB-TACE, whereas other comparative studies have not confirmed this superiority. Idilman et al. [59] reported that no differences in survival or side effects were observed between the cTACE and DEB-TACE in their retrospective study. In a large comparative study of Western HCC patients, Facciorusso et al. [60] demonstrated that drug-eluting bead chemoembolization with 100–300 μm particles did not seem to improve survival in comparison with conventional chemoembolization, which in turn provided better tumour responses and time-to-progression. Moreover, the randomized controlled trial of DEB-TACE versus cTACE for HCC performed by PRECISION Italia Study Group [61] showed that adverse effect incidence and severity did not differ between the arms, except for post-procedural pain, more frequent and severe after cTACE ($P < 0.001$). The 1- and 2-year survival rates were 86.2% and 56.8% after DEB-TACE and 83.5% and 55.4% after cTACE ($P = 0.949$). Thus, the authors stated that DEB-TACE and the cTACE are equally effective and safe, with the only advantage of DEB-TACE being less post-procedural abdominal pain. Additionally, Karalli

et al. [62], in their retrospective real-life analysis, reported that DEB-TACE had better tolerability compared to cTACE, but overall survival did not differ between the two treatments. Zhang et al. [63] also demonstrated that compared to cTACE, DEB-TACE offered slightly better disease control rate and tolerability for HCC patients. However, DEB-TACE does not provide higher progression-free survival than cTACE.

Two meta-analyses [64, 65] and one systematic review [66] regarding comparability or superiority of cTACE and DEB-TACE were recently published. In the first meta-analysis performed by Facciorusso et al. [64], 4 randomized controlled trials and 8 observational studies with 1449 patients were evaluated. Non-significant trends in favour of DEB-TACE were observed as for 1-year (odds ratio: 0.76, 0.48–1.21, $p = 0.25$), 2-year (odds ratio: 0.68, 0.42–1.12, $p = 0.13$), and 3-year survival (odds ratio: 0.57, 0.32–1.01, $p = 0.06$). Meta-analysis of plotted hazard ratios confirmed this trend (hazard ratio: 0.86, 0.71–1.03, $p = 0.10$). Pooled data of objective response showed no significant difference between cTACE and DEB-TACE (odds ratio: 1.21, 0.69–2.12, $p = 0.51$). No statistically significant difference in adverse events was registered (odds ratio: 0.85, 0.60–1.20, $p = 0.36$). Based on these results, the authors stated that results of performed meta-analysis stand for a non-superiority of DEB-TACE with respect to cTACE in HCC patients. In the second meta-analysis regarding comparability of cTACE and DEB-TACE [65], no significant difference was found in overall response at 3, 6, 9, and 12 months, complete response, partial response, disease control rate, stable disease, overall survival, and complications between cTACE and DEB-TACE. The authors stated that DEB-TACE had similar therapeutic effects to those of cTACE. Furthermore, major complications in both therapies were similar; thus, the authors concluded that superiority of DEB-TACE over cTACE remains unclear, and further research with high-quality evidence is needed. However, in the recently published systematic review, Yang et al. [66] evaluated the effects of DEB-TACE, TARE, and cTACE in terms of overall survival, tumour response, and complications. The authors found that DEB-TACE had a better overall survival at 1 year ($p = 0.006$), 2 years ($p = 0.046$), and 3 years ($p = 0.035$) when compared with cTACE.

In conclusion, despite the theoretical advantages of DEB-TACE over cTACE, it is still controversial as to whether DEB-TACE is superior to cTACE in terms of efficacy. However, it seems that DEB-TACE shows at least similar clinical outcomes and less adverse events than cTACE. Further organized prospective studies are required to identify combination strategies and to develop better treatment approaches for patients with HCC.

13 Future Directions: Transarterial Chemoembolization and Systemic Therapy Combination

TACE has been established as the most widely used therapeutic intervention for patients with intermediate-stage HCC. Despite level 1 evidence of survival benefit for TACE in BCLC stage B, it remains a palliative treatment. This may be explained by the hypoxic environment created by the TACE procedure, which can induce neoangiogenesis by stimulating vascular endothelial growth factor and other angiogenic pathways, promoting revascularization and growth of residual viable tumour [5, 67]. In an effort to address this problem, many studies have been conducted combining TACE with systemic anti-angiogenic agents, most commonly sorafenib (Nexavar; Bayer AG, Leverkusen, Germany), based on its proven activity against advanced HCC. The GIDEON trial [68] was an observational registry of 3202 patients with HCC of BCLC A, B, and C stages treated with sorafenib alone or in combination with TACE. Adverse events were reported in 2732 (85.3%) patients overall, with no notable differences in the incidence of adverse events, regardless of TACE treatment history. Overall survival was

12.7 months in prior TACE patients, 9.2 months in non-prior TACE patients, 21.6 months in concomitant TACE patients, and 9.7 months in non-concomitant TACE patients. The authors stated that the combination of TACE with sorafenib appears to be a well-tolerated and viable therapeutic approach. The SPACE trial [69], a prospective randomized phase II trial in patients with BCLC stage B HCC, included 307 patients allocated randomly to DEB-TACE with sorafenib and DEB-TACE with placebo. There was no difference in TTP between the two arms (169 vs. 166 days in the sorafenib and placebo arms, respectively, $p = 0.072$). Overall the trial had a negative outcome with no impact on overall survival ($p = 0.29$). A further subgroup analysis from the SPACE trial suggested that patients with more advanced disease could benefit more than those with intermediate-stage disease [70]. In a phase III trial of TACE with sorafenib (TACE-2) [71], 313 patients were randomized to sorafenib or placebo with DEB-TACE 2–5 weeks later and additional TACE on demand. This trial reported no significant difference regarding a median progression-free survival (7.9 vs. 7.8 months in the sorafenib and placebo arms, respectively, $p = 0.94$) and median overall survival (21.1 and 19.7 months in the sorafenib and placebo groups, respectively, $p = 0.57$). In the phase III STAH trial [72], 169 patients were randomized to sorafenib alone or sorafenib combined with cTACE within 7–21 days of randomization. Compared with sorafenib alone, sorafenib combined with cTACE did not improve overall survival in patients with advanced HCC. However, sorafenib combined with cTACE significantly improved time-to-progression, progression-free survival, and tumour response rate. For combined treatment and sorafenib alone, median time-to-progression was 5.3 and 3.5 months, respectively ($p = 0.003$); median progression-free survival was 5.2 and 3.6 months, respectively ($p = 0.01$); and median overall survival was 12.8 and 10.8 months, respectively ($p = 0.290$). The authors stated that sorafenib alone remains the first-line standard of care for patients with advanced HCC. Kudo et al. [73], in the randomized, multicentre prospective TACTICS trial, compared the efficacy and safety of TACE plus sorafenib with TACE alone using a newly established TACE-specific end point and pretreatment of sorafenib before initial TACE. Patients in the combination group received sorafenib 400 mg once daily for 2–3 weeks before TACE, followed by 800 mg once daily during on-demand cTACE sessions until time to untreatable progression, defined as untreatable tumour progression, transient deterioration to Child-Pugh C, or appearance of vascular invasion/extrahepatic spread. Median progression-free survival was significantly longer in the TACE plus sorafenib than in the TACE alone group (25.2 vs. 13.5 months; $p = 0.006$). Overall survival was not analysed because only 73.6% of overall survival events were reached. Median time to untreatable progression (26.7 vs. 20.6 months; $p = 0.02$) was also significantly longer in the TACE plus sorafenib group. Overall survival at 1 year and 2 years in TACE plus sorafenib group and TACE alone group were 96.2% and 82.7% and 77.2% and 64.6%, respectively. The authors stated that TACE plus sorafenib significantly improved progression-free survival over TACE alone in patients with unresectable HCC. Meta-analyses [74, 75] of TACE in combination with sorafenib have reported improved time-to-progression in patients with a combination; however, the addition of sorafenib failed to improve significantly in overall survival compared to TACE alone. In conclusion, a number of clinical trials inquiring addition of sorafenib to TACE did not demonstrate any significant improvement of overall survival due to addition of sorafenib to TACE for patients with intermediate-stage HCC.

Another systemic agent (bevacizumab; Roche Diagnostics GmbH, Mannheim, Germany) was tested in combination with TACE. Pinter et al. [76], in a randomized phase II study investigating the addition of bevacizumab to TACE, reported no evidence of increased efficacy assessed by radiological response rate. Smolka et al. [77], in the study comparing TACE + bevacizumab to TACE alone, reported that bevacizumab did not change quantitative tumour response to TACE. Briefly, the combination of TACE with anti-angiogenic drugs has been disappointing in

terms of survival outcomes; however, due to potential safety, further studies are warranted in patients with advanced HCC.

14 Future Directions: Transarterial Chemoembolization and Immunotherapy Combination

A substantial body of evidence supports the development of immunotherapy to treat HCC. It is logical to combine TACE therapy with checkpoint inhibitors because the tumour burden will be less than in patients with advanced disease; checkpoint inhibitors will be administered in a immunogenic environment with probability of overcoming local tumour-mediated immune suppression; and both TACE and checkpoint inhibitors have been shown to stimulate immune responses against HCC [78]. Experiments in immunotherapy such immune checkpoint inhibitors are also underway. Several combination strategies of immunotherapies with thermal ablations and cryoablation were investigated in vitro and in animal models of HCC [79]. Recently, Marinelli et al. [80] investigated the safety of locoregional treatment (transarterial chemoembolization or Yttrium-90 transarterial radioembolization) combined with nivolumab for intermediate and advanced hepatocellular carcinoma (HCC). The authors reported that during a median follow-up of 11.5 months (range, 1.8–35.1), no grade III/IV adverse events attributable to nivolumab were observed. In addition, there were no nivolumab-related deaths, and 30-day mortality after LRT was 0%. Thus, the authors demonstrate that locoregional treatment performed concomitantly with nivolumab immunotherapy had an acceptable safety profile in patients with intermediate and advanced HCC. In conclusion, current evidences suggest that the combination of systemic treatments and TACE seems to be a relatively safe option for treating patients with advanced disease. More studies are necessary to produce solid data over a longer follow-up period.

15 Current Implementation of Transarterial Chemoembolization in the Middle East Countries

We aimed to report a brief summary about the TACE implementation in the Middle East countries based on literature screening from PubMed. Indeed, it is not possible to describe all articles; thus, only newest investigations from the last 5 years were included into this brief literature review. Zaky et al. [81] evaluated the short-term outcome of the decision, taken by the multidisciplinary tumour board for the treatment of HCC patients with surgical resection, local ablative therapy, cTACE, and palliative supportive care. The authors found that the management of HCC was better performed through a multidisciplinary team decision, and cTACE has a success rate of 33.3%. El Sherbiny et al. [82] investigated changes in Doppler parameters of portal pressure after interventional management of HCC, including TACE, and reported improved portal hypertension parameters after TACE. The authors recommended Doppler ultrasound use as a reliable and effective method of evaluation of portal hypertension after TACE for HCC. Abdelmaksoud et al. [83] evaluated the prognostic factors and management in patients with HCC with portal vein thrombosis. The authors reported a significantly worse prognosis in patients with more than two tumours, abdominal lymphadenopathy, and serum bilirubin >2 mg/dL. Additionally, specific treatment significantly increased survival compared to patients left untreated ($p = 0.027$). Thus, TACE was considered as a promising procedure for unresectable portal vein thrombosis-associated HCCs. Abdelaziz et al. [84] studied a combined ablation techniques and assess survival benefit comparing TACE with radiofrequency versus TACE with microwave ablation techniques. A higher tendency to provide complete response rates after TACE with microwave ablation comparing with TACE with radiofrequency ablation was reported ($p = 0.06$). This was particularly evident with lesions sized 3–5 cm ($p = 0.01$). Rates of complications showed no significant difference between the groups. The

authors concluded that TACE with microwave ablation led to better response rates with tumours 3–5 cm, with no difference in survival rates. Moustafa et al. [85] reviewed the factors influencing the development of an extrahepatic collateral arterial blood supply to HCC and described a systematic approach to enhance the ability to predict the presence of extrahepatic collateral arteries. They also describe the proper technique for TACE of each extrahepatic collateral artery and how to avoid potential technique-related complications. Abdella et al. [86] assessed the outcomes after TACE in patients with segmental portal vein thrombosis regarding Child-Pugh classification, radiological response, and 1-year survival. TACE succeeded to achieve disease control in 93.3%, 86.3%, 57.7%, and 44.4% of patients after 1, 3, 6, and 12 months, respectively. Post-TACE liver decompensation occurred in the form of ascites in 30%, jaundice in 10%, and hepatic encephalopathy in 3.3% within 1 month of TACE. One-month survival after TACE was 100%, 3 months was 96.6%, 6 months was 86.6%, and 1 year was 60%. Mean overall survival of the included patients was 17 months (s.e. = 1.59). The authors concluded that TACE seems an alternative option for unrespectable HCC with portal vein thrombosis in patients with preserved liver function. Hassan et al. [87] evaluated the frequency of regulatory T cells and serum levels of IL-6 and IL-10 before and after TACE. HCC patients had a significantly higher level of IL-6 and IL-10 when compared to the control group ($p = 0.0002$, $p < 0.0001$), respectively. However, after treatment, there was an elevation in the levels of IL-6 and IL10 followed by a decrease to the baseline levels. Patients with large tumours (≥5 cm) showed higher levels of both IL-6 and IL-10 than those with smaller tumours. Moreover, HCC patients showed a higher frequency of regulatory T cells in comparison with the controls ($p = 0.002$). No significant correlation was observed between the frequency of regulatory T cells and IL-10 before and after treatment ($r = 0.38$, $p = 0.30$). The authors concluded that HCC patients have significantly higher levels of IL-6 and IL-10 and a higher percentage of regulatory T cells than controls; the regulatory T-cell levels were altered after chemoembolization; and IL-6 have a potential in reflecting the patient's condition after treatment, thus helping in monitoring therapy. Khalid et al. [88] investigated the prognostic value of the albumin-bilirubin grade in patients undergoing TACE for unresectable HCC. The mean duration of survival at the last follow-up was of 12.1 ± 12.14 months (range 1–49). Univariate analysis showed serum albumin ($p = 0.003$), serum bilirubin ($p = 0.018$), Child-Pugh score ($p = 0.019$), albumin-bilirubin grade ($p = 0.001$), and presence of varices ($p = 0.04$) to be the main predictors of 6-month survival after TACE. On Cox analysis, only ALBI score ($p = 0.038$) showed statistical significant association. The authors concluded that albumin-bilirubin grade may serve as a surrogate marker in predicting the prognosis of HCC patients undergoing TACE. Hassan et al. [89] evaluated the role of diffusion-weighted magnetic resonance imaging in the detection of residual HCC after DEB-TACE. Diffusion-weighted magnetic resonance imaging had a sensitivity of 77.1%, a specificity of 60.7%, a positive predictive value of 71.05%, and a negative predictive value of 68%. The difference between the malignant and benign groups' ADC variables was statistically significant ($p < 0.003$). The ROC curve showed that the area under the curve is $C = 0.718$ with SE = 0.069 and 95% confidence interval from 0.548 to 0.852. The authors concluded that diffusion-weighted magnetic resonance imaging has limited diagnostic value in the assessment of viable tumour tissue after DEB-TACE in cases of HCC. Balli et al. [53] evaluated the effectiveness and safety of super-selective TACE with doxorubicin-loaded DEB sized 40–75 μm for HCC in early and intermediate stages according to BCLC staging system. Median follow-up was 22 months (range, 13–31), and 42 (93.3%) patients were followed up for more than 1 year. Overall complete response, partial response, and progressive disease rates were 53.3%, 33.3%, and 13.4% at 1 year and 22.2%, 26.7%, and 13.3% at 3 years, respectively. For target lesions, these rates were 60.0%, 26.7%, and 13.3% at 1 year and 28.9%, 6.7%, and 4.4% at 3 years, respectively. Median overall survival duration

was 24 months (95% CI, 20.9–31.9 months). At 1 year and 3 years, overall survival rates were 71.0% and 44.4%, respectively. The only statistically significant relationship with overall survival was presence of chronic liver disease, which worsened the overall survival rate ($p = 0.031$). Time-to-progression was 23 months (95% CI, 15.1–40.0), and progression-free survival was 28 months (95% CI, 6.2–39.8). Postembolization syndrome occurred in ten patients (22.2%). Transient grade I/II bilirubin and aminotransferase elevation was observed in 26 (57.7%) and 18 (40%) patients, respectively. The authors stated that super-selective DEB-TACE with doxorubicin-loaded beads sized 40–75 μm is an effective and safe treatment method with prolonged time-to-progression and progression-free survival in early and intermediate stages of HCC. Presence of chronic liver disease is the only significant factor that worsened overall survival ratios after DEB-TACE. Balli et al. [55] compared the efficacy and safety of super-selective DEB-TACE with doxorubicin-loaded microspheres sized below and above 100 microns for treatment of HCC. Although statistically insignificant, median overall survival (19 months vs. 32 months, $p = 0.190$) and median progression-free survival (13 months vs. 20 months ($p = 0.574$) were longer, and 1–3-year objective response rates (7.40% vs. 23.33%, $p = 0.330$) were higher in above-100-microns group than in below-100-microns group, respectively. No mortality or major complications were observed. Grade I/II adverse events were detected in all patients. Transient elevations in liver function tests (grade III adverse events) were similar in both groups (3.57% vs. 3.33%; $p = 0.980$). The authors concluded that super-selective DEB-TACE with doxorubicin-loaded microspheres sized <100 microns is an effective and safe method for the HCC treatment. Objective response rates are higher and survival durations are longer after DEB-TACE is performed with doxorubicin-loaded microspheres sized below 100 microns. Farid et al. [90] measured serum vascular endothelial growth factor levels before and after cTACE versus DEB-TACE and evaluated its efficacy in predicting response to therapy and tumour recurrence. Vascular endothelial growth factor level was higher than baseline after cTACE ($p < 0.001$) and DEB-TACE ($p = 0.004$). It was also significantly higher in patients with progressive disease ($p < 0.001$). Vascular endothelial growth factor level at cut-off values of 97.3, 149.8, and 104.1 pg/mL could discriminate disease progression from treatment success with area under ROC curves of 0.806, 0.775, and 0.771, respectively. The sensitivity was 88.9%, 88.9%, and 77.8% and specificity was 62.5%, 64.6%, and 66.7%, respectively. However, no relation to tumour recurrence in complete response group could be detected after 1 year. The authors concluded that vascular endothelial growth factor serum levels may predict response to therapy in patients treated by DEB-TACE or cTACE, but it has no relation to tumour recurrence.

This brief literature review demonstrates that various technical, methodological, clinical, and prognostic aspects regarding TACE in HCC are under investigation in the Middle East countries.

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Radiotherapy for Hepatocellular Carcinoma

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Abbreviations

3D-CRT	Three-dimensional conformal radiation therapy
ALD	Alcoholic liver disease
BED10	Biologically equivalent dose, alpha/beta = 10
CGE	Cobalt Gray Equivalent
CP	Child- Pugh
CR	Complete response
cRILD	Classical radiation-induced liver disease
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
FFLP	Freedom from local progression
GBD	Global Burden of Disease
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
ICPI	Immune checkpoint inhibitor
IL-6	Interleukin 6
IMRT	Intensity-modulated radiation therapy
LC	Local control
mRECIST	Modified Response Evaluation Criteria in Solid Tumors
NASH	Nonalcoholic steatohepatitis
ncRILD	Nonclassical radiation-induced liver disease
NTCP	Normal tissue complication probability
ORR	Overall response rate
OS	Overall survival
PD-1	Programmed cell death protein-1
PD-L1	Programmed Cell Death-Ligand 1
PR	Partial response
RFA	Radiofrequency ablation
RILD	Radiation-induced liver disease
RTOG	Radiation Therapy Oncology Group
SBRT	Stereotactic body radiation therapy
TACE	Transcatheter arterial chemoembolization
TKIs	Tyrosine kinase inhibitors
TRST	Treatment-related severe toxicity
VMAT	Volumetric modulated arc therapy

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1 Epidemiology and Risk Factors for Hepatocellular Carcinoma

1.1 Epidemiology

Liver cancer is the sixth most common cancer worldwide (reaching 4.7% of all cancers). It is the third most common cancer in Eastern

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Mediterranean countries and the most common cancer in Egypt (reaching 19.7%) [1–3]. Primary liver malignancies are the fourth most common cause of cancer deaths worldwide, the third most common cause of cancer deaths in Eastern Mediterranean countries, and the most common cause of cancer deaths in Egypt [1, 2].

The most common type of primary liver malignancies is hepatocellular carcinoma (HCC) (75–80%) followed by cholangiocarcinoma (15–20%) [4]. HCC is three times more common in males than in females [5]. This difference can be attributed to differences in exposures to toxins and viral infections, as well as a possible protective effect of estrogen-mediated inhibition of IL-6 production that is more important in females [6].

1.2 Risk Factors for HCC

HCC is a result of chronic liver diseases. Chronic hepatitis B viral infection (HBV) and hepatitis C virus (HCV) infection are the most common causes, followed by nonalcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), and exposure to dietary toxins such as aflatoxins and aristolochic acid.

Worldwide, HCV is the primary cause of HCC in North America, Europe, Japan, parts of Central Asia and northern Africa, and the Middle East, particularly Egypt [7, 8].

In the Middle East, a systematic review showed that HCC in cases from Iran, Lebanon, Turkey, and Yemen was mainly caused by HBV infection, while those of North African nations (Egypt, Tunisia, Morocco, Algeria, and Somalia), as well as Saudi Arabia, were mostly related to HCV infection. Egypt showed the highest rate of HCV infection among HCC cases, reaching 80% [9].

Data from the Global Burden of Disease (GBD) showed an increase in the incidence of HCV-related HCC by 16.1% in the Middle East and North Africa from 1990 to 2017, where Egypt showed the highest rate reaching 47.1% [10].

2 Management of HCC

The selection of the optimal treatment is complex and depends on multiple factors, including the location and extent of liver disease, the underlying liver function, and the physical status of the patient. Surgical management has shown the best survival outcomes, either by partial resection or via total hepatectomy with liver transplant.

2.1 Surgical Candidates

Partial hepatectomy is the treatment of choice in patients with good liver function, where lesions can be resected with negative margins, in the absence of portal hypertension or major vascular invasion, and acceptable remnant liver function. Patient with Child-Pugh (CP) score class A and selective cases of CP class B can be candidates for surgical resections, whereas CP class C is a contraindication. The 5-year overall survival (OS) is 61%, and the 5-year disease-free survival is 21% [11].

Liver transplant is the optimal treatment modality for patients who are candidates for surgery, presenting with unresectable tumors. Different selection criteria have been adopted and validated for the proper selection of patients who will undergo liver transplant. One of which is the Milan criteria (solitary lesion $\leq$5 cm or $\leq$3 lesions, all $\leq$3 cm), with 4-year OS after liver transplant reaching 85% [12]. An expansion of the Milan criteria has been conceived "Shanghai criteria" which includes the following parameters: solitary lesion $\leq$9 cm or $\leq$3 lesions with the largest $\leq$5 cm, a total tumor diameter $\leq$9 cm without macrovascular invasion, lymph node invasion, or extrahepatic metastasis. The use of Shanghai criteria yields a similar OS to the Milan criteria [13]. The adoption of liver transplant as a definitive treatment is not widely used in third-world countries (e.g., Middle East countries) mainly because of the paucity of donors.

2.2 Nonsurgical Candidates

In nonsurgical candidates, other local or systemic treatment modalities are utilized, taking into con-

sideration the liver function, the size and distribution of intrahepatic tumors, the vascular supply, and the patient's overall performance status. The choice of locoregional treatment falls into one of three categories: percutaneous ablation, transarterial embolization, or radiotherapy.

2.2.1 Locoregional Therapy

Percutaneous ablation uses high-energy electrical current or microwaves conducted into the tumor leading to tissue necrosis. Optimal outcomes are achieved in tumors <3 cm, that is distant from the major blood vessels, bile ducts, and abdominal organs, with a 5-year OS of 60.8% [14].

Transcatheter arterial chemoembolization (TACE) works through catheter based intra-arterial injection of chemotherapeutic agents in the tumor followed by obstruction of a preselected hepatic artery branch that feeds the tumor inducing ischemic tumor necrosis. The stimulation of vascular endothelial growth factor (VEGF) secretion increases vascular permeability and thus triggers higher intrahepatic chemotherapy deposition. TACE is ideal for patients with good performance status, normal liver function, and tumors smaller than 10 cm and in the absence of portal vein thrombosis (PVT). Prospective studies showed better survival with TACE when compared to best supportive care [15, 16].

2.2.2 Systemic Therapies

Historically, the use of the systemic chemotherapy treatment for HCC was limited because most patients who are not eligible for surgery or local therapy are also medically unfit to receive chemotherapy, and as such these patients were offered best supportive care.

Sorafenib Over the past few years, novel systemic treatments emerged including tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitor (ICPI). In 2008, sorafenib, an oral multikinase inhibitor, was approved as a first-line systemic treatment for advanced HCC, which was defined as no previous or disease progression after surgical or local therapy. Sorafenib improved median survival time from 8.9 months to 10.7 months [17].

Sorafenib and Chemotherapy The addition of chemotherapy [two chemotherapy protocols used (GEMOX or doxorubicin)] to sorafenib added no to minimal benefit with worse toxicity profile, and as such combination of sorafenib and chemotherapy is not recommended for treatment of HCC [18, 19].

Lenvatinib An oral multikinase inhibitor, was compared with sorafenib in a phase III non-inferiority trial for patients with advanced HCC who didn't receive any previous treatment. Patients who received lenvatinib had a median survival of 13.6 months compared to 12.3 months in patients who received sorafenib, with similar side effect profile [20].

Regorafenib An oral multikinase inase inhibitor, was approved as a second-line treatment option for patients with HCC who progressed while receiving sorafenib. Regorafenib improved median survival from 7.8 to 10.6 months when compared to placebo in the RESORCE trial [21].

Atezolizumab and Bevacizumab Atezolizumab [an ICPI that targets programmed cell death protein-ligand 1 (PD-L1)] and bevacizumab [a vascular endothelial growth factor-A inhibitor (VEGF-A)] combination was compared with sorafenib for the treatment of advanced HCC. The combination showed improved median progression-free survival (PFS) (6.8 months vs. 4.3 months) and improved 12-months OS (from 54.6 to 67.2%). According to the hepatocellular carcinoma-specific mRECIST criteria, objective response rate was 33.2% for patients who received atezolizumab and bevacizumab compared with 13.3% for patients who received sorafenib, and also 18 (5.5%) of the patients who received the combination of atezolizumab and bevacizumab achieved complete response (CR) compared with none of the patients who received sorafenib [22]. As such, this combination has been approved as first-line treatment for advanced HCC.

Nivolumab [an ICPI that targets PD-1] monotherapy has been studied in a the phase II check-

mate 040 trial that included patients with unresectable HCC who progressed on or were intolerant to sorafenib, with CP score A. Patients who didn't receive sorafenib and had no viral etiology had a 6-month OS of 89%, while patients who received or progressed on sorafenib had a 6-month OS of 75% and a median survival of 13.2 months. Based on these results, nivolumab (240 mg every 2 weeks) was approved as a second line of systemic treatment for HCC in 2017 [23].

Nivolumab and Ipilimumab The efficacy and safety of nivolumab and ipilimumab [an ICPI that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)] were investigated in another cohort of the checkmate 040 trial. The trial investigated three dosing protocols. Protocol A, in which patients received nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, administered every 3 weeks for four doses followed by nivolumab 240 mg every 2 weeks achieved a 32% investigator-assessed objective response rate compared with 15% for nivolumab alone [24]. Combination of nivolumab and ipilimumab was approved by FDA as second-line treatment in 2020.

3 Radiotherapy for the Management of HCC

The utilization of radiotherapy as a treatment option for HCC has evolved over the last decades. Initially, external beam radiation therapy was used as a palliative treatment for patients with multiple liver metastases, where the whole liver was treated with low doses of radiation that offered local control (LC) only. A prospective RTOG study published in 1993, comparing whole liver doses of 27, 30, and 33 Gy, showed high rates of late radiation-induced liver disease (RILD) with the 33 Gy arm [25]. With the utilization of newer radiotherapy techniques (three-dimensional conformal radiotherapy (3D-CRT) and intensity-modulated radiotherapy (IMRT)), partial liver treatment has emerged as these techniques allow for the delivery of higher doses of radiation to targets within the liver while minimizing radiation dose delivered to normal surrounding liver tissue.

3.1 Radiotherapy Techniques

3.1.1 3D-CRT and IMRT

3D-CRT and IMRT utilize 3D images obtained by computed tomography to allow for target localization, target delineation, and avoidance of surrounding normal structures, such as the normal liver tissue. IMRT is more advanced than 3DCRT as it uses modulated beams that allow for more improved target coverage, more conformal radiation dose distribution, and better radiation dose sparing of critical normal structures. A study by Yoon et al. compared doses delivered by 3D-CRT and IMRT in addition to patient outcomes. Higher doses were delivered utilizing IMRT compared with 3D-CRT (62.5 vs. 53.1 Gy). IMRT also provided significantly higher 3-year OS (33.4 vs. 13.5%) and PFS (11.1 vs. 6.0%) as compared to 3D-CRT. The authors concluded that IMRT is associated with better outcomes and less toxicity as it allows for higher doses to be delivered to the target while meeting liver and other surrounding normal tissue constraints [26].

3.1.2 Stereotactic Body Radiation therapy (SBRT)

The use of SBRT for the treatment of unresectable HCC emerged over the last few years as it can safely deliver high conformal ablative doses in fewer fractions of radiation with minimal liver toxicity. SBRT uses multiple radiation beams to concentrate the converging beams on the target lesion, and as such minimal dose is delivered to the surrounding normal liver tissue. The use of SBRT requires advanced tumor tracking, image guidance, and breath monitoring while delivering the treatment to achieve the best outcomes and to minimize morbidity [27].

A wide range of doses and fractionations has been used. Yoon et al. published a retrospective study of 93 patients with small HCC lesions who received definitive radiotherapy using SBRT with doses ranging from 30 to 60 Gy in three to four fractions. Results showed a 1- and 3-year OS of

86% and 53.8%, respectively, size-dependent 3-year LC reaching 100% in ≤2 cm tumors, 93.3% in 2.1–3 cm lesions, and 76.3% in larger lesions [28]. Another study published in 2013 by Jang et al. that used a median dose of 51 Gy (range, 24–60 Gy) in three fractions showed a dose-dependent response relation for LC and OS. The 2-year LC and OS reached 100% and 71%, respectively, for patients treated with doses higher than 54 Gy, and outcomes dropped to 64% and 30%, respectively, when doses less than 45 Gy were used [29]. Data from Michigan showed same outcomes after using SBRT or radiofrequency ablation (RFA) for primary liver cancer with freedom from local progression reaching >80% at 2 years for both, although the use of SBRT yielded better outcomes for lesions ≥2 cm [30].

One retrospective study compared SBRT to TACE in 188 patients. Infield control (IFC) and OS were higher in the SBRT group (77.5% vs. 55.6%; $P = 0.007$, and 55.0% vs. 13.0%; $P < 0.001$, respectively). The benefit was seen in recurrent cases only, with no difference in newly diagnosed cases [31]. A systemic review of 16 studies, which included 973 patients with 1034 lesions, treated with SBRT with a median biologic effective dose (BED10) of 100 Gy (range 59.5–180 Gy), with a median tumor size of 2.3 cm, showed a LC of 94%, 92%, and 93% at 1, 2, and 3 years, respectively, and median OS of 90.9%, 67.5%, and 73.4% at 1, 2, and 3 years, respectively. Grade 3 and above were low (5.3%), with no associated treatment-related mortality [32]. Table 1 below summarizes some published trials of SBRT in HCC. A randomized phase III trial of radiofrequency ablation (RFA) versus SBRT (36–54 Gy in three fractions) in small HCC (<5 cm) is currently ongoing (NCT03898921).

3.1.3 Combination of TACE and Radiotherapy

TACE has been the most commonly used modality in treating unresectable HCC. SBRT has recently emerged as a new effective modality with encouraging outcomes. Local recurrence rates are still high, thus the idea of combining the two modalities together to increase LC. In 2018, Buckstein et al. published a retrospective study of 103 patients with unresectable HCC treated with TACE and SBRT, where almost half of the patients had planned SBRT after TACE, while the others received salvage SBRT within 2 years after TACE. On follow-up imaging, 62.1% had a CR and 26.3% had a partial response (PR). Higher rates of CR were achieved with planned TACE + SBRT (79.6% vs. 43.5%, $P = 0.006$). One-year OS was 70.8% for planned TACE-SBRT vs. 61.5% for salvage ($P = 0.052$) [35].

A meta-analysis compared combined TACE-SBRT to SBRT alone as first-line treatment for unresectable HCC and found better 5y OS (95% CI 1.01–2.04, $p = 0.04$) and disease control (95% CI 1.02–1.16, $p = 0.02$) rates in the combined group [36]. Another systematic review of 25 trials showed better survival in patients treated with combined modalities compared to patients treated with TACE alone, with a median survival of 13.5 months and 22.7 months, respectively [37]. In this review, combined treatment was associated with significant increase in incidence of gastroduodenal ulcers, as well as an increase in alanine aminotransferase level and total bilirubin levels.

A phase III trial compared radiotherapy plus TACE to sorafenib alone. The trial showed improved PFS rate at 24 weeks in the TACE-RT group (55.6% vs. 7.4%) and significantly better OS (55 vs. 43 weeks). Eleven percent of patients in the TACE-RT group underwent curative resection due to downstaging [38].

There is an ongoing phase III trial (USA) that is comparing LC at 1 year using TACE vs. TACE + SBRT (five fractions) as a bridge for liver transplant eligible patients (NCT03895359).

3.1.4 Proton Therapy

Proton therapy is characterized by a sharp rise and fall in energy absorption, known as the Bragg peak. It is appealing in HCC, since it allows to treat liver tumors with higher doses while limiting the dose to the healthy liver. Proton radiotherapy is also associated with a decreased risk of nonclassic radiation-induced liver disease. Studies investigating proton therapy for HCC are summarized in Table 2. An ongoing phase III study (USA) is comparing photon to proton therapy in locally recurrent or unresectable HCC with OS as primary outcome (NCT03186898).

Table 1 Select published studies of SBRT in HCC

Study (author et.al)	Study design	Population and size of lesions	Intervention	Median follow-up	Primary outcomes	Results
Takeda et al. 2016 [33]	Phase II	101 patients Solitary HCC <4 cm, not candidate for surgery or RFA Median size: 2.3 cm	SBRT 35–40 Gy in five fractions	41.7 months	LC	3-year LC: 96.3% 3-year liver-related cause-specific survival rate was 72.5%, OS: 66.7%
Shen et al. 2019 [31]	Retrospective	188 patients Medium-sized (3–8 cm)	SBRT vs. TACE	26.6 months	IFC and OS	3y IFC higher with SBRT (77.5% vs. 55.6%; $P = 0.007$) 3y OS higher with SBRT (55.0% vs. 13.0%; $P < 0.001$)
Wahl et al. 2016 [30]	Retrospective	Inoperable, nonmetastatic HCC, small lesions. Total of 224 patients Median tumor size in SBRT was 2.2 cm and 1.8 cm in RFA group	63 had SBRT (83 tumors) Median dose 30–50 Gy (four to five fractions) 161 had RFA (249 tumors)	13 months for SBRT and 20 months for RFA	Freedom from local progression (FFLP)	1 and 2y FFLP: RFA: 83.6% and 80.2%, respectively SBRT: 97.4% and 83.8%, respectively
Jang et al. 2020 [34]	Prospective, phase II	65 patients Unresectable HCC, <10 cm Median tumor size: 2.4 cm	45 to 60 Gy in three fractions	41 months	Treatment-related severe toxicity (TRST) at 1 year after SBRT	1y TRST was 3% One patient developed RILD at 1 month LC: 97% at 2 years and 95% at 3 years OS: 84% at 2 years and 76% at 3 years

Another phase III study (Taiwan) is comparing LC at 3 years using proton therapy vs. RFA in treatment-naïve, medium- to large-sized HCC (3–7 cm) (NCT02640924).

3.1.5 Radiotherapy and the Immune Response

Radiotherapy affects the tumor and its microenvironment, as well as the immune cells. Lymphocytes are the most radiosensitive cells, followed by myeloid cells. Tumor cells escape the immune system by changing the expression in MHC molecules. When cells are irradiated, even with sublethal doses, the phenotype of tumor cells changes as well as the gene expression, and cells are more susceptible for T-cell-mediated immune response. New peptides are produced by the protein degradation triggered by radiation leading to an increased peptide pool. These peptides can be recognized by the resting T cells, leading to an antitumor immune attack [43]. Therefore, radiation is able to stimulate the innate and adaptive immune responses, thus improving local and distant tumor control [44].

3.1.6 Radiation and Immunotherapy

Multiple preclinical studies showed synergistic effects when combining radiotherapy with immune checkpoint inhibitors (ICI) (e.g., PD-1 and PD-L1 inhibitors) because of pro-immunogenic properties of radiotherapy as well as the role of ICPI in overcoming radiation resistance [45–50]. These preclinical study results show strong evidence of the benefit of adding radiotherapy to immunotherapy in addition to multiple ongoing clinical trials evaluating the effect of combined treatments.

In the clinical settings, the role of combined immunotherapy with radiotherapy in liver cancer was shown in multiple retrospective studies. Chiang et al. published a case series of five patients with unresectable HCC treated with SBRT followed by anti-PD1 antibodies; two patients had CR, while three others had PR, with a median reduction of tumor diameter of 38.7% (range: 30.5–84.4%) and a 1-year control rate and OS of 100% each [44].

There is a phase II ongoing trial studying the effect of treating unresectable HCC with durvalumab, tremelimumab, and SBRT (24 Gy in three fractions), with overall response rate (ORR) as primary outcome (NCT03482102). Another ongoing phase I trial is studying the effect of SBRT followed by nivolumab or ipilimumab with nivolumab in unresectable HCC (NCT03203304). Several other prospective clinical trials are currently ongoing to evaluate the combined approach of radiotherapy and immunotherapy in HCC (Table 3).

3.1.7 Radiation Toxicity and Adverse Events

Radiotherapy has been proven to be an effective modality in treating primary liver cancers, but careful patient selection is essential in order to decrease potential side effects. The majority of patients presenting with HCC have cirrhosis, with a small, dysfunctional liver, whereby adding radiotherapy could risk further liver injury.

The most common acute side effects are fatigue and nausea.

RILD is the most paramount complication of radiotherapy to the liver, with a potential of progression to fibrosis, cirrhosis, and liver failure. It typically occurs 2–8 weeks after completion of RT, with nonspecific clinical manifestations.

Table 2 Select studies of proton therapy for HCC

Study (author et.al)	Study design and population	Intervention	Results
Nakayama et al. 2009 [39]	Retrospective study 318 patients with HCC (CP class A and B, ≤3 tumor nodules)	Proton therapy Dose:55–79.2 cobalt Gy equivalent (CGE) in 10–35 fractions	1-year and 5-year OS rates were 90% and 45%, respectively Only 1.6% of patients experienced grade ≥ 3 toxicity
Sanford et al. 2019 [40]	Retrospective 133 patients, nonmetastatic, unresectable HCC, radiation-naïve	Proton vs. photon therapy	Median OS higher in proton arm (31 vs 14 months) 2-year OS: 59.1% in the proton arm and 28.6% with photons, related to decreased post-treatment liver decompensation
Hata et al. 2006 [41]	Retrospective, 19 patients, CP class C	Proton therapy Total doses of 50–84 Gy (median dose 72 Gy) in 10 to 24 fractions (median 16 fractions)	1-year OS and PFS rates were 53% and 47% at 1 year, respectively. 2-year OS and PFS rates were 42% each.
Bush et al. 2016 [42]	Prospective, randomized 69 patients, met Milan or San Francisco transplant criteria	TACE vs. proton (70.2 Gy in 15 daily fractions)	Pathologic CR after TACE/proton was 10%/25% Similar median survival of 30 months Trend for better 2-year LC (88% vs. 45%, $P = 0.06$) and PFS (48% vs. 31%, $P = 0.06$) favoring the proton beam treatment group

Table 3 Select ongoing studies of combined radiotherapy and immunotherapy in HCC

Clinical trial number	Phase	Country	Status	Eligibility	Intervention	Planned enrolment	Radiation dose	Primary outcome
NCT03482102	Phase II	USA	Recruiting	Locally advanced/unresectable or metastatic disease	Tremelimumab + durvalumab + radiation	70 participants	24 Gy in three fractions	ORR
NCT03203304	Phase I	USA	Active, not recruiting	Unresectable HCC	Arm 1: SBRT followed by nivolumab Arm 2: SBRT followed by ipilimumab + nivolumab	50 participants	40 Gy in five fractions	Adverse events
NCT03316872	Phase II	Canada	Recruiting	HCC showing progression after sorafenib	Pembrolizumab + SBRT	30 participants	Five fractions	ORR
NCT01730937	Phase III	USA	Recruiting	Locally advanced or recurrent HCC	Sorafenib tosylate with or without SBRT	50 participants	Five fractions	OS

There are two types of RILD, classic RILD (cRILD) and nonclassic RILD (ncRILD). cRILD occurs in patients without underlying liver disease, commonly presenting with fatigue, abdominal tenderness, increased abdominal girth, hepatomegaly, anicteric ascites, and an elevation of alkaline phosphatase out of proportion to other liver enzymes. cRILD is a result of a veno-occlusive disease with central vein thrombosis at the lobular level, causing retrograde congestion. This complication was more frequent in the era of old delivery techniques where a large volume of the liver was treated. With the development of the new delivery techniques with CT-guided images; conformal treatments such as SBRT, IMRT, and volumetric modulated arc therapy (VMAT); and respiratory control, the incidence of cRILD has decreased dramatically.

ncRILD is the most common type of RILD in the current era, affecting patients with underlying liver disease such as cirrhosis or chronic HBV infection. Patients present with jaundice, elevated serum transaminase (>5 upper limit of normal), or decompensated liver function. The pathophysiology is not clear, but it is believed to be a reactivation of hepatitis or altered regeneration of liver cells.

RILD is a diagnosis of exclusion after ruling out other viral causes of hepatitis. Imaging (magnetic resonance or computer tomography) might show changes in attenuation in a nonanatomic distribution. Liver biopsy is diagnostic.

The incidence of RILD has been correlated to baseline liver dysfunction or cirrhosis, hypofractionated treatment, concurrent chemotherapy, stage, and size of the treated lesion [51–53].

Partial liver tolerance to radiation has been described using the Lyman normal tissue complication probability (NTCP) model. The threshold for RILD is a mean liver dose of 30 Gy (2Gy/fraction), with a risk of 5% with dose of 32 Gy [52].

3.1.8 Liver Constraints for Primary Liver Cancer to Avoid RILD

Initial assessment of the liver function is essential before deciding on radiotherapy treatment especially that patients with primary liver malignancies most commonly have small cirrhotic livers, with large lesions. It is essential to deduct the tumor volume (nonfunctional cells) from the total liver volume.

Multiple dose constraints have been suggested in order to decrease the risk of adverse events. If the liver is treated with standard fractionation (2 Gy per fractions), it is important to keep the mean liver dose (liver minus gross tumor volume) below 28 Gy.

When using hypofractionated regimen, SBRT, in three or six fractions, mean liver dose should be kept below 13 Gy and 18 Gy, respectively [54, 55].

The most commonly used dose tolerance model for liver, when treating with three fractions of SBRT, is to have at least 700 mL of normal liver receiving ≤15 Gy. This was extracted from a phase I study, where liver metastasis was treated with SBRT, with escalating doses from 36 to 60 Gy, in three fractions, and showed no grade 3 or higher hepatotoxicity [56].

Few studies evaluated the outcomes of RILD. A retrospective study showed that patients who developed early RILD (within 2 weeks from completing radiotherapy) had a high mortality rate reaching 61%.

4 Conclusion

With the improvement in radiation technology, including image guidance, breath control, dose escalation, and partial liver treatments, high LC rates were achieved with minimal toxicity to the intact liver and organs at risk. Current evidences support the use of SBRT as a curative modality for unresectable HCC.

The combination of radiotherapy and ICPI in HCC is promising, although the data is limited to preclinical trials and small case series. More randomized clinical trials are needed in the future that would define the best schemes of dose fractionation, the optimal timing for radiotherapy, as well as the best systemic treatment combinations.

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Liver Transplantation in the Middle East

Sezai Yilmaz

Abbreviations

DDLT	Deceased donor liver transplantation
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LDLT	Living donor liver transplantation
LT	Liver transplantation
MELD	Model for End-Stage Liver Disease
MESOT	Middle East Society of Organ Transplantation
SCOT	Saudi Center of Organ Transplantation
UAE	The United Arab Emirates

1 Liver Transplantation in the Middle East

Solid organ transplantation has been on a long rough road until the procedure was accepted as routine practice. It caused great exhaustion to the pioneers for they had to take down many obstacles before perfecting both the surgical technique and the postoperative immunosuppressive treatment protocols. From the beginning of the 1950s, the experimental models for transplantation have been established and successful. Although there were seven unsuccessful attempt of clinical trials for liver transplantation (LT) from various centers, the first successful LT was performed by Starzl et al. in summer of 1967 with a 13-month survival in the posttransplant period [1].

There is no doubt that western countries pioneered this novel therapeutic modality, but currently 100,000 solid organ transplantations are being performed in many centers around the world, and currently, solid organ transplantation is the gold standard treatment especially for end-stage liver and kidney failure. There are standardized organ allocation and immunosuppressive protocols in North America and many European and Asian countries (such as Japan and Korea). In the present chapter, I examine the effort of countries of the Middle East for solid organ transplantation. Some of these countries started solid organ transplantation programs as early as the western countries. However, the social and cultural structure of these societies somewhat limited the progression of this complex therapeutic modality. We will try to summarize evolution of LT in some of the countries that are a hallmark in the region.

1.1 Definition of the Middle East and Its Implications from a Historical Perspective

Middle East is defined as the land that is located in the southern and eastern border of the Mediterranean Sea which also includes some

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North African countries (i.e., Tunisia, Algeria, and Morocco), the Arabian Peninsula, Cyprus, Iran, and the countries like Afghanistan and Pakistan. It is the region consisting of neighboring countries and covering the places where the Middle East, Asia, Europe, and Africa come very close to each other. The concept of the Middle East is based on the Eurocentric approach and is a concept that Britons began to use in the nineteenth century. In this definition, England and European countries are considered as the center; concepts such as East, Far East, Near East, and Middle East have been determined accordingly. From another point of view, Middle East is an arbitrary term used to describe a region in West Asia that extends to Egypt in North Africa. Currently the Middle East is considered to cover 18 countries (Fig. 1). Majority of the Middle Eastern countries (13 out of 18) are part of the Arab world. The most populous countries in the region are Egypt, Iran, and Turkey, while Saudi Arabia is the largest Middle Eastern country by area [2, 3].

It has been the center of civilization in the beginning of the century and once had been the leader in medicine, theoretical physics, mathematics, and nature sciences. Furthermore, this region was a pioneer in literature and arts as well [2, 3]. The Arabian Peninsula, after evolution of Islam until the Mongolian raid, was the center of most advanced medicine in the world. During this time, Europe had succumbed to the dark ages and there were very few, if any, attempts for medical research. The first medical center in the Middle East started in Jundi Shapur (Ahvaz) in Iran before the advent of Islam. The most influential physician at the time was Al-Harith ibn Kaladah, who was Arabic in origin and was trained in Jundi Shapur. The teachings of this center were further influenced by the Greek philosophy of medicine due to the conquest of Alexander the Great [2–4]. In the Abbasid era Jundi Shapur continued to be the greatest center of medicine. Baghdad was the next city that evolved as the center of excellence in the medical sciences. Baghdad contributed to the training of physicians such as Rhazes and Avicenna (ibn Sina) who are considered to be the founders of modern pharmacy and medicine that have been thought for centuries in European medical training [2–4].

After the dissolution of the Abbasid empire, scientific advancements in the Arabic world came to a halt. Nevertheless, there were many Arab physicians that were scattered throughout Europe who contributed to modern medical teaching in European centers. One example is in Salerno pioneered by Constantine from Carthage who was an Arabic slave at the time and led the school of medicine in Italy. In addition, Spanish Arabs

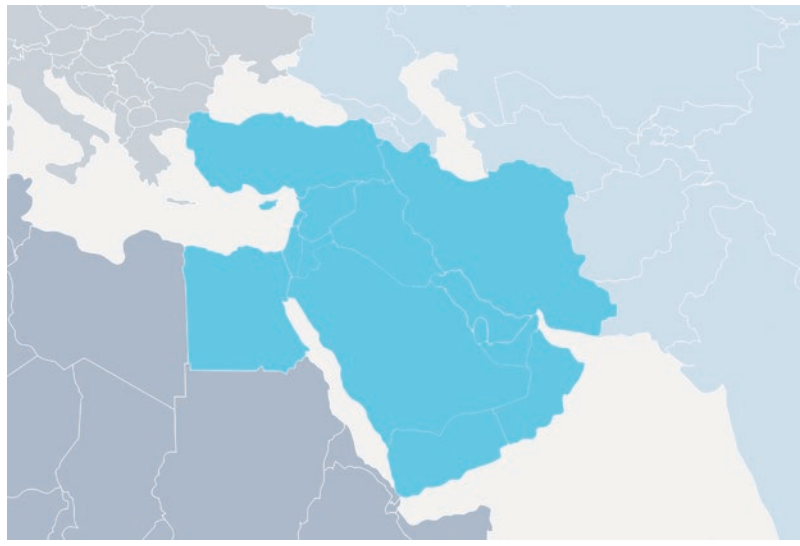

Fig. 1 The middle east map

forced out of Andalusia that escaped to Montpellier in France also practiced and thought about medicine in Europe. All these scientists knew Arabic, Greek, and Latin and translated the works of Avicenna, Hippocrates, and many other influential scientists and contributed to medicine in Europe. The Renaissance came and resulted in the flourishing of modern science and the arts in Europe. Meanwhile, in the Arabic world, the first modern Middle Eastern medical school was established in 1827 in Cairo, Egypt, which was followed by Lebanon and Syria [2–4]. Therefore, the Middle East was the world leader in art and sciences for nearly a thousand years before the industrial revolution in Europe, which in turn caused a great leap in medicine in western countries built on the foundations formed by Greek, Persian, and Arabic scientists.

Currently, there are many scientists that are occupied in the western countries but have Lebanese and Arabic ancestry. One of these prominent scientists is Michael Ellis De Bakey, born in Louisiana from Lebanese American parents and who became one of the famous cardiovascular surgeons. Another influential scientist famous in the transplant community is Sir Peter Medawar, known for his studies on skin grafts and who is the first scientist to determine the rejection phenomenon in skin grafts transplanted between different subjects and was nominated for Nobel Prize in Medicine in 1960. He was also born to a British family of Lebanese origin [2–4]. These historical and prominent examples show us the potential of the region and the inclination of the people to achieve great advancements in the field of science.

1.2 Facts About Organ Donation in the Middle East

The estimated population is about 400 million. Furthermore, nearly 60% of the population in the Middle Eastern countries are younger than 25 years of age. It is a multinational (also racially very diverse) geographic location that harbors many religions. The Middle East has a diverse ethnicity and religious background. Islam is the main religion in the Middle East. There are contradictory views among Islamic scholars and lawmakers on the legitimacy of organ donation from deceased donors. There are common features affecting organ transplantation in the Middle Eastern countries that include inadequate preventive medicine, uneven health infrastructure, poor awareness within medical community and lay public regarding the importance of organ donation and transplantation, and poor government support for organ transplantation. In addition, there is lack of team spirit among physician dealing with transplantation, lack of planning for organ procurement and transplant centers, and lack of effective health insurance [5–8]. In addition to all these factors, we should not forget religious factors that influence the necessary organizations in transplantation. The Amman declaration in 1986 was the first collective action to recognize brain death in Muslim countries [9]. This declaration paved the way for donation from deceased donors in the region. Following the declaration, LT, both from living liver donors and deceased liver donors, was started in several countries in the late 1980s. Turkey, Egypt, Iran, and the Kingdom of Saudi Arabia are pioneers of LT in the Middle East. Their collaboration in the Middle East Society of Organ Transplantation (MESOT), despite political conflicts, has developed a platform for promotion of transplantation and specifically LT in the Middle East.

Majority of the grafts required for LT in developed western countries are obtained from cadaveric donors. However, in Asian and Middle Eastern countries, grafts obtained from living liver donors are abundantly used. According to 2018 data, organ donation rates per million people are listed as follows (Fig. 2): 1.1 in United Arab Emirates, 2.9 in Qatar, 3.7 in Saudi Arabia, 6.7 in Kuwait, 7 in Turkey, 11.4 in South Korea, 14.3 in Iran, 20.3 in Sweden, 21.2 in England, 30.7 in America, 32.6 in Portugal, and 43.6 in Spain. These results clearly show the perspective regarding organ donation of the countries.

There are many reasons behind these differences between West and East. Among these reasons, the most striking ones are the differences in religious beliefs, cultural values, and education

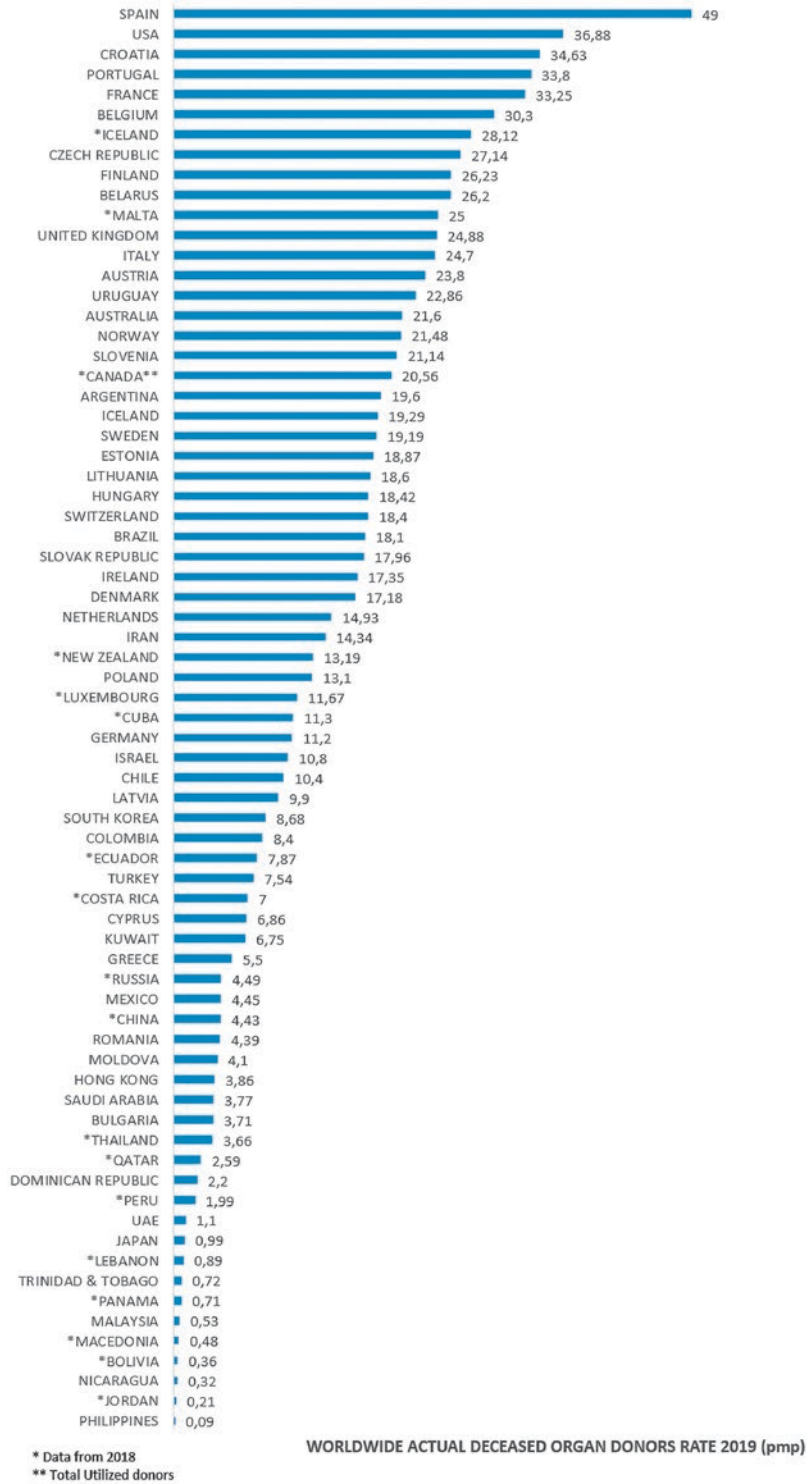

Fig. 2 Worldwide actual deceased organ donation rates in 2018

system. Also, insufficient state funds and deficiencies in the legislation on brain death also contribute to this difference. In western countries, effective organ donation campaigns are organized at the national level, religious beliefs are not very effective on individuals' lifestyles,

and awareness regarding organ donation that is a direct result of the education system are the major factors for higher deceased organ donation. On the other hand, in Asian countries, religious and cultural values are very influential on individuals' lives and decision-making process, and religious philosophies such as Buddhism, Taoism, Confucianism, Shintoism, Muslims, and Hinduism usually contradict cadaveric organ donation. The first modern fatwa on organ donation in the Muslim Middle Eastern countries was issued in 1966 by the Egyptian chief mufti. Later, in Malaysia (1969), Algeria (1972), Jordan (1977), Kuwait (1979), and Saudi Arabia (1982), the muftis issued fatwas on organ donation. The Islamic Fiqh Council of the Organization of the Islamic Council made decisions pointing out the importance of both live and cadaveric organ transplantation in its 1986 meeting. The first fatwa on organ transplantation and donation was published in 1980 in Turkey. In 1979, article 2238 entitled "Organ and Tissue Removal, Storage, Grafting, and Transplantation" was enacted. A while after the first law, in 1982, article 2594 was enacted on the use of organs and tissues of people who were irreversibly injured or died during accidents or natural disasters [10].

In a majority of the Muslim countries in the Middle East, fatwas that paved the way for organ donation were enacted more than half a century ago. The reason why these fatwas were not successful in increasing deceased organ donation in these countries where most of the people live according to religious rituals is still unclear. In our opinion, there are contradictions between the views reflected on the public and the official views of the muftis and other clergy or other possibility is due to the fact that there are ambiguities in the sociocultural structure of the people that are far beyond religious rituals.

Using a population estimate of around 83 million people, representing 95% of the sample size in Turkey, we have made two different national surveys. In the study conducted on religious officials, only 0.3% of 2350 clergymen stated that while they donated organs, 75.4% stated that they would not donate organs in the future. While 22% of the clergy stated that they would not donate organs due to their religious beliefs, 45.3% stated that there was no specific reason for not donating organs [11]. In a survey conducted on 3000 people representing the general population in Turkey, while 0.7% of the people viewed organ donation positively, 88.3% of them stated that they would not donate organs. While 28.5% of the general population stated that it was too early for organ donation, 17.9% were afraid of deterioration of body integrity, 11.5% were worried that their organs could be removed before death, and only 8.6% stated that they did not donate organs due to their religious beliefs [12]. In fact, when the responses of the participants in both studies are examined in detail and crosswise, it is clear that the most serious reason for not donating an organ is the individual's perception of the religious beliefs and sociocultural reasons.

2 Arab World and Commercial LT

The Arab world is composed of 22 countries in the League of Arab States founded in 1945. Thirteen of these countries are within the Middle East region. The first deceased donor liver transplantation (DDLT) in the Arab world was performed in 1990 at Riyadh Military Hospital in Saudi Arabia [13]. The first living donor liver transplantation (LDLT) was performed in 1991 at the National Liver Institute in Egypt [14]. Between 1990 and August 2013, 3804 LTs were performed in 11 Arab countries. Ninety-eight percent of these LTs were performed in Middle Eastern countries [15]. Unfortunately, five living donor deaths were reported in Egypt [16–20]. Only 20% of the transplants were DDLT. First meeting of the Pan Arab LT Society was held in Cairo in 2006. One of the main goals of the Pan Arab LT Society has been the creation and establishment of a Pan Arab LT Registry. It has held seven scientific congresses so far. However, up-to-date information is still not available.

Patients who need LT in Middle East usually seek commercial transplantation. Patients from Arab countries are still traveling to foreign desti-

nations to undergo LT with higher costs and inadequate postoperative care resulting in poor outcomes, which are known to be associated with commercial LT [21]. Liver donation from unrelated living donors is prohibited in some counties in the Middle East. However, unrelated living donors are major source of organ procurement in Middle Eastern countries. This has raised major ethical concerns based on informal reports on selling of livers from young health donors to elderly recipients. Relationship of living donor to recipient is an important concern throughout the region but especially in Egypt, considering the high poverty rates in the country and the fact that the largest percentage of LDLT is being performed by transplant centers in Egypt [22]. Consequently, the Egyptian parliament has recently enacted a law banning the sale of human organs and imposing restrictions on transplant operations for foreigners and stipulating jail sentences and fines for violation of the law. The absence of deceased organ donation in Egypt is troublesome but not from the perspective of the cultural barriers and the current political unrest [23].

In fact, patients from all over the world have begun to travel to China for organ transplantation. A combination of many factors, including easy accessibility, relatively low cost, short waiting time, and more liberal transplantation indications, has led a growing number of Saudi and Egyptian patients to seek LT in China. Only approximately 60 LTs are performed annually across all centers in Saudi Arabia. Suitable organ availability is significantly overwhelmed by the high number of patients on the waiting list which results in an increasing number of Saudi patients seeking LT abroad. In Egypt the situation is even more difficult, because transplants can only be performed from living donors. It is commonly stated that LT in China is quite affordable in comparison with the high cost of treatment in the USA and Europe. It is also possible to procure an organ in a relatively short time. Despite these attractive factors, the main growing concern is the uncertainty regarding the outcome. Between 2003 and 2007, 74 Middle Eastern patients had LT in China. Forty-six of them were Saudi and 28 were Egyptian. The waiting time in China prior to receiving liver transplant ranged from 5 to 20 days, with a median of 14 days. Average waiting time in Saudi Arabia ranges from 1 to 1164 days, with a median of 15 weeks. Duration of stay in China in the posttransplant period ranged from 10 to 70 days, with a median of 50 days. In patients who underwent LT in China, diffuse biliary strictures complicated the posttransplant clinical course. Diffuse biliary strictures can be a result of ischemia from hepatic artery thrombosis or extended warm ischemia times during graft preservation. However, the likely cause of these diffuse strictures was prolonged warm ischemia injury to the donor liver during procurement since the hepatic arteries of almost all of these patients were patent. Therefore, these strictures were likely due to suboptimal procurement of organs [24–26].

3 LT in Kingdom of Saudi Arabia

The Kingdom of Saudi Arabia is geographically the fifth largest state in Asia. It has a population of 32,775,000; age of most of the population is young (60% are below the age of 35 years), and approximately ten million resident are non-Saudi (mostly Middle Eastern and Asian). The per capita income is 20,812 US dollar, making the Saudi Arabia 38th in the world.

In Saudi Arabia, solid organ transplantation started in Riyadh Armed Forces Hospital in 1979. It was restricted to renal transplantation, and the foundations came from the establishment of the hemodialysis units or the patients with end-stage renal disease in 1971. After the transplant activities that started in the Riyadh Armed Forces Hospital, the National Kidney Foundation was established as a nonprofit organization to coordinate and register the hemodialysis activities and also organ allocation. Only after the approval statement of the Jurisprudence of Islamic Ulema did the transplant activities gained momentum, and in 1993, the National Kidney Foundation was reestablished as the Saudi Center of Organ Transplantation (SCOT). Responsibilities of SCOT are coordination of hemodialysis units and

coordination of organ donation from deceased donor (mainly donation after brain death) [27, 28].

There is a high demand for LT in the Saudi Arabia because of the high burden of liver disease in the country. In the early 1980s, hepatitis B virus (HBV) was epidemic, with a prevalence rate of approximately 8.3%. This high prevalence rate led to an increase in the number of patients requiring LT for end-stage liver disease and hepatocellular carcinoma (HCC) in subsequent decades. In 1989, the HBV vaccine was integrated into the expanded immunization program through which all newborn children throughout the country were vaccinated. This resulted in a significant reduction in the prevalence of the infection in the younger Saudi population which we expect will decrease the need for HBV-related LT. In addition, this decline was associated with a changing trend in the indication for LT from hepatic decompensation to HCC. The rate of LT for other indications including autoimmune and metabolic liver disease has been stable throughout the years. Progressive familial intrahepatic cholestasis and biliary atresia are the most common indications for LT among pediatric patients [27].

In the mid-2010s, the prevalence of hepatitis C virus (HCV) is approximately 1–2% with a predominance of genotype 4 infection, and HCV is currently the leading indication for LT. The indications for LT in Saudi Arabia are shifting from viral-induced liver disease to metabolic causes of end-stage liver diseases including obesity and hyperlipidemia which are extremely common in Saudi Arabia [29].

The need for LT in Saudi Arabia is estimated to be between 50 and 75 patients per million. Only 5–10% of the need is met by both LDLT and DDLT. Although transplantation from a non-relative is allowed, it comprises less than 2% of the transplants that are performed, annually. Each LT center has its own LT waiting list. Waitlist priorities are based on Model for End-Stage Liver Disease (MELD) scores, and more recently, the MELD-Na scoring system was adopted as the prioritization tool. The cadaveric donation is low (approximately three per million people), though the potential to increase this number seems to be high. The SCOT is a well- recognized national organ donation agency that oversees the donation process in the Saudi Arabia; it collaborates with the LT programs and the donating hospitals in expanding deceased organ donation [15]. The deceased donors are primarily expatriates (who compromise 30% of the Saudi population) residing temporarily in Saudi Arabia. The government offers financial compensation as an expression of gratitude for families who donate. According to SCOT, this is an expression of gratitude for the altruistic donation [27]. There have been ethical concerns that the cash payment to next of kin of the donors provided by the Saudi government and administered through SCOT may result in coercion to donate. The healthcare system in Saudi Arabia is funded by the government. The transplant is free for Saudi citizens, including the subsequent medical care and immunosuppressive medication. All patients are cared for in the respective transplant centers for life, without any charge. Saudi Arabia also accepts patients from Gulf countries (viz., Kuwait, Bahrain, and the United Arab Emirates) and occasionally takes cadaveric donors. Non-Saudis living in Saudi Arabia are not eligible for LT unless they have a living related donor as a financial sponsor.

The liver discard rate has been unacceptably high. Fifty percent of the livers were rejected due to suboptimal donor management. The same is true for documentation; the brain death protocol is completed in only 60% of the cases. In Saudi Arabia, where 212 liver transplants were performed in 2016, approximately one-fourth of the transplants are from deceased donors [27]. Despite all efforts by SCOT, the Ministry of Health, and transplant centers, the donation rate and the procedures are far from optimal. Further efforts can be made to support donation logistics and to improve the quality of donated organs.

4 LT in Egypt

HCV prevalence among the 15–59 years age group in Egyptian population is estimated to be 14.7%. Accordingly, Egypt has the highest HCV prevalence in the world [30]. In Egypt, the sole source of liver grafts is from living donors up to

fourth degree, as currently no legislation for deceased donor donation exists. DDLT is not yet implemented [31].

In Egypt, LDLT was performed in 1991 for the first time by the surgical team at the National Liver Institute, Menoufia University, with the help of Prof. Habib. The longest recipient survival was 11 months. This pioneer work led to efforts to pass a law legalizing cadaveric organ donation, culminating in the 1992 decree permitting cadaveric organs that were harvested from prisoners who had been sentenced to death. The surgical team at the National Cancer Institute, Cairo University, in 1992 performed two DDLT procedures, but unfortunately recipients died in the early postoperative period [32].

The regulations were enacted by the Egyptian Medical Syndicate. The programs started with the assistance and under-supervision of foreign (i.e., western) teams. The breakthrough was made in Dar Al-Fouad Hospital by initiating the LDLT program (August 2001), with the contributions of Prof. Tanaka, Kyoto University, Japan. This was followed by WadiEleneel Hospital (October 2001), National Liver Institute, Menoufia University (April 2003), and Maadi Armed Forces Hospital (September 2003). By that time, the number of centers performing LDLT (13 centers) increased, and altogether, 2500 procedures were performed and with associated improved results. Currently, there are 13 LDLT centers in Egypt, including 6 university centers, 2 military centers, 3 private centers, and 2 centers that belong to the Ministry of Health. The 93% of the patients who underwent liver transplant are adult and 7% are in the pediatric age group [32].

By the time LDLT developed and the number of cases increased, the Egyptian transplant surgeons start to face the problem of donor morbidity and mortality which leads to discussion about the law to accept the concept of brain death as a step to develop liver transplant from deceased donors. The law was raised in the Egyptian Parliament in an effort to pass the law, but it was very difficult at that time because there were many factors that influenced this concept. After the Declaration of Istanbul in 2008, the Egyptian Parliament approved the law in 2010. The main problem, from religious point of view, was the acceptance of the brain death concept. Many of Egyptian population believe that death occurs when the heart stops beating. So in case of clinical death with the heart still beating, they considered this victim still alive. In addition, it is believed that the humans are the product of God; therefore, how can you donate a part of your liver, which is not yours in the first place?

The potential number of donors for LDLT in Egypt is small, and this is mainly due to the high prevalence of HCV and schistosomiasis infection in seemingly healthy family members who are the potential donors for patients with end-stage liver disease. The law permits donation of the organs from up to third-degree relatives. Nonrelated living donation was accepted only when an independent ethical and legal committee approves that none of the patient's relatives are suitable as a right liver lobe donor. The legal age of consent for donation in Egypt is 18 years when the recipient is a parent, son, or daughter; otherwise, the lower age limit is raised up to 21 years.

Donor mortality rate is 1.66 per 1000 donors. This consisted of four donors [16–20]. The first one died 3 months after hepatectomy due to biliary leak followed by infection, septicemia, and multi-organ failure. The second one died 12 days after donation due to portal vein thrombosis. The third one was due to right subclavian artery injury during central line insertion, leading to massive right hemothorax. The fourth one died 1 month after donation due to hepatic insufficiency and hepatic failure. A major morbidity was also recorded due to hepatic insufficiency, and the donor needed LDLT that was performed 4 weeks after donation [32].

However, it is remarkably commendable that Prof. Kamel et al. at the University of El Shams in Cairo defined new criteria related to LT in HCC, with 5-year survival of more than 60% after LT in patients with HCC [33].

5 LT in Israel

The number of patients listed for LT has grown, but the number of deceased donors has remained constant, causing a lack of organs for LT in the

world. This situation is especially true in Israel, which has a population of 6.5 million and is not a member of the European pool.

Organ transplantation is widely accepted as the best therapeutic and often lifesaving option for many patients with end-stage organ failure. However, in Israel as in many parts of the world, the number of patients awaiting transplantation continues to increase, far exceeding the number of organ donors. A significant number of patients die every year while on the waiting list. This first survey of its kind in Israel, summarizing trends in the demographic characteristics of deceased organ donors, revealed an aging donor pool with fewer male donors and fewer dying from head trauma. This resulted in changes in organ utilization, in particular increased utilization of organs from older liver and lung donors, stable utilization rates for kidneys, and a marked decrease in heart utilization [34].

The Israeli Parliament passed the regulatory law on organ transplant and brain death in 2008, and it was fully implemented in 2010. It was developed in order to aid three major challenges to organ procurement and transplantation in Israel: (1) confusion regarding determination of death, (2) organ trafficking and unethical/illegal transplant tourism, and (3) the critical dearth of transplantable organs [35–37].

The major objective of the law was to attempt to strengthen brain death diagnosis to satisfy both medical and religious needs. Unfortunately, brain death as a medical criterion for death is not uniformly accepted within the Israeli population, particularly among the ultraorthodox community. A second objective was to prevent transplant tourism as both an illegal and unethical means of patients seeking solid organ transplantation. This has proven to have been successful, as demonstrated by the marked decrease in transplant tourism over the last 10 years. This was partly accomplished by the reduction in financial disincentives to living organ donors. The state now reimburses the expenses associated with being a living donor. In fact, over the last decade, live donation rates have doubled [38].

Perhaps the most controversial part of the law was a priority structure, favoring access to transplantation based on a point system. The highest priority was given to those whose first-degree relatives were deceased organ donors or those who themselves had been a previous living donor. The next level of priority was given to those who choose to register as a donor. The last priority level is for individuals with first-degree family members who have registered as donors. This is in the context of an opt-in system whereby donor families must still provide consent in order to proceed with the organ donation, regardless of registration status. Transplant surgeons argue that medical need alone should be the highest priority and that access to transplantation must not be based on a predetermined hierarchy [37, 38].

In the general population, the likelihood of requiring a lifesaving organ transplant is fivefold greater than the chance of being a deceased organ donor. This imbalance between supply and demand means that transplantation is highly dependent upon an adequate supply of both deceased and living organ donors [38]. In the studies conducted in Israel, the 1-year survival rate after liver transplants was reported as less than 75% [39–41]. There are efforts to improve these low survival rates.

6 LT in the United Arab Emirates

The United Arab Emirates (UAE) is an Arabian Gulf country located at the southeast end of the Arabian Peninsula with a population of about 9.3 million. It has made great strides in healthcare over the past several years and has among the highest life expectancy in the region. However, one of the key lacking areas of medical care in the country was the availability of solid organ transplantation. Collaborative efforts began a few years ago aiming to establish thoracic and abdominal solid organ transplantation from deceased donors in addition to continued development of the existing program on kidney transplantation from living donors. The UAE played an important role in efforts leading up to the declaration of Istanbul on organ traf-

ficking and transplant tourism in 2008, the groundwork for which was laid in a steering committee meeting organized by the Transplantation Society and the International Society of Nephrology in Dubai in December 2007. This landmark declaration helped establish a framework of ethical principles to guide the practice of transplantation worldwide. The legal definition of brain death in the UAE was confirmed in May 2017, paving the way for deceased donor organ transplantation [42].

The Cleveland Clinic Foundation was instrumental in the accelerated path to establishment of a multi-organ transplant center at Cleveland Clinic Abu Dhabi. Cleveland Clinic Abu Dhabi, which began clinical operations in early 2015, was established as a partnership between Mubadala Healthcare, Abu Dhabi, and Cleveland Clinic Foundation in Cleveland, Ohio. A meticulous and thoughtful collaborative approach, which began with identifying key operational needs, resulted in the establishment of transplant services within 2.5 years with the establishment of Cleveland Clinic Abu Dhabi. The first multi-organ procurement and transplant from a brain-dead donor in the UAE occurred at Al Qassimi Hospital in Sharjah on July 15, 2017 [42]. Also noteworthy was a regional organ sharing agreement with SCOT that enabled the utilization of deceased donor grafts for potential recipients in Saudi Arabia while awaiting full operational readiness for thoracic and LT at Cleveland Clinic Abu Dhabi. The overall organ donation rate remains low in terms of the number of organs recovered (3.66) and transplanted (3.57) per donor.

Shortage of deceased donors remains one of the primary challenges facing the transplant community in the UAE. This mandated establishment of LDLT to complement ongoing efforts to improve DDLT. An extensive collaborative effort, with Cleveland Clinic's main campus in Cleveland, Ohio, resulted in the first successful LDLT in the UAE on July 29, 2018. Since that time, an additional 13 LDLTs were performed (overall: four right lobe and ten left lobe grafts) over the past year and a half [42].

7 LT in Lebanon

From 1998 to 2014, 21 LTs were performed at the American University of Beirut Medical Center. Of these, 15 were in adults and 6 in children. Of the 21 transplants, 5 were LDLT, 4 children, and 1 adult. Five deaths occurred after transplant at a median of 9 days. Most of the deaths occurred in the first years of the program. This may be due to the numerous initial difficulties related to structuring, organization, and post-operative management [43].

In Lebanon, LDLT program was started as an alternative source of organs for transplant. However, LDLT is particularly complex because of the associated financial and social issues. Political insecurity and periodic conflicts have had a major impact on organ transplant, including LT. Cultural and religious factors are also major obstacles, as countries in the Middle East issue laws that have to agree with Islamic teachings. Many religious edicts (fatwas) have been issued stating that it is permissible by Islamic teaching to perform deceased organ donations. These statements have had a significant positive impact on the number of transplants performed. However, the Arab cultures are still sensitive toward the issue of possible loss of dignity of the dying process in the process of organ procurement. This obstacle continues to hinder the progress in transplants in the region. Hence, more work needs to be done to improve the rate of deceased donor organ donation. A problem we face related to the organ shortage is the inability to obtain another organ quickly in the event of major postoperative complications. In other parts of the world, urgently listed patients receive a liver transplant in a very short period, whereas in our country such patients sometimes die awaiting an organ. The political and security instability in the region is a major contributing factor to the shortage of donated organs. Up until 2013, the donation rate was quite good and improving; however, the Syrian political conflict has led to a vast number of Syrian refugees to be displaced to Lebanon, many of them wounded and requiring immediate hospitalization. Moreover, security in

Lebanon was also compromised during that same period. All of this resulted in an increase in hospital occupancy, and more attention was given to the trauma cases; hence, the focus on organ donors and organ transplant was decreased. Other problems are the lack of coordination in the region, limited support from governments and insurance companies, and public mistrust in the concept of organ transplantation [44, 45].

8 LT in Syria

By the beginning of 2011, the government of Syria was taking steps to initiate LT. For this purpose, efforts had included cooperation with well-known Brazilian and Iranian liver transplant centers, where specialized teams were sent for training. However, the trainers from those countries could no longer visit Syria because of the war. Since 2011, the Syrian conflict has destroyed much of the country's infrastructure. A project to initiate liver transplant came to a halt because of complex reasons but mainly because foreign trainers could not visit Syria. The Syrian conflict has affected all aspects of organ transplant, paralyzing new projects and negatively affecting existing programs.

Despite these adverse conditions, recently, a few LTs have been performed from living related donors. Additionally, need for LT of the Syrians living in the north of Syria and Syrian refugees in Turkey is provided for by the Turkish government, free of charge [46, 47].

9 LT in Jordan

With the scarcity of DDLT in Jordan, LDLT remains the only viable option for patients with ESLD. The first liver transplantation in Jordan has been performed in June 2004 at King Hussein Medical Center (KHMC) in corporation with a Turkish team. In July 2009, the first LDLT was performed by Jordanian team without assistance. Since then and until 2018, 98 LDLTs and 13 DDLTs have been performed in Jordan [48].

10 LT in Qatar

There was a significant increase in the number of cases where patients had opted to have their transplant surgery abroad and returned with serious complications. These factors combined to play a key role in the need for a strategic plan to promote organ donation and further develop transplant services in Qatar. The plan addressed the problem of lack of organ donation. In order to promote organ donation in Qatar, it was developed as a unique model that later on became to be known as the Doha Donation Accord and gained international recognition as the best representation of international guidelines as well as in accord with Qatari laws and regulations. The Accord is a model that subscribes to the legislative, human, and religious aspects of donation.

In Qatar, the transplant activity started with renal transplantation 1986, but it did not flourish until 2009 when the Doha Donation Accord (DDA) was developed. The Doha Donation Accord was the first milestone in the development of Qatar transplant services. Hamad Medical Corporation, under the leadership of our Managing Director and through effective teamwork across all departments, has created and developed a transplant center that is a very attractive option for many patients who previously would have to go abroad for treatment.

The launch of the Qatar Center for Organ Transplantation (QCOT) was on November 27, 2011. During the ceremony, the United Network for Organ Sharing (UNOS/USA) raised their flag in acknowledgment of the standard of excellence achieved by the Qatar center. Ten days after the launch of QCOT, HMC announced successful completion of the first liver transplant surgery in Qatar, with an excellent outcome for the recipient. Until 2019, four LDLTs and seven DDLTs have been performed in Qatar. The vision of QCOT is to become a regional center of excellence in multi-organ transplantation that brings to life HMC's vision of providing the highest standards of patient care, research, and education, supported by a wide international collaborative network [49, 50].

11 LT in Kuwait

Kuwait performed very limited number LT, but they were subsequently suspended because of logistical and technical reasons [15].

12 LT in Iraq

A program for LDLT has recently been developed in Iraq with a potential of performing 15 LDLTs per year [15].

13 LT in Oman

The LT program is in its beginning stages in Oman. The majority of the ESLD is due to congenital disorders such as familial intrahepatic cholestasis and cystic disease of the liver. This is due to the fact that consanguineous marriage rate is nearly 80% in Oman [51]. First LT (LDLT) is performed recently in October 2017 in an adult female with hepatocellular carcinoma; it was performed in Royal Hospital in Muscat [52]. There are many obstacles to be overcome by the Omani government in terms of organization and infrastructure that should precede before solid organ transplantation accelerates.

14 LT in Cyprus

There are two distinct races that occupy the Island of Cyprus. The Turkish Cyproid residents occupy the northern part of the island, and The Turkish Republic of Northern Cyprus has shown great progress in education and has concentrated well-trained personnel to this region. The Greek-occupied area has very scarce centers that have an intent to perform organ transplantation, but the activities are very few and currently very negligible.

In the Turkish Republic of Northern Cyprus, great effort is being made especially in the field of renal transplantation. The Human Cell, Tissue and Organ Transplant Related Rules Regulating Act was passed by the republic senate in 2014 [53], and many research is being performed to delineate the causes of end-stage renal disease among the Turkish population [54, 55]. Since the approval of Human Cell, Tissue and Organ Transplant Related Rules Regulating Act was approved, 25 renal transplantations have been performed. Eleven of the organs were procured from deceased donors, and 14 were living related renal transplantations. LT program has not started yet, but the procured organs such as the liver and heart were sent to the Republic of Turkey for successful transplantation [56, 57].

15 LT in Iran

In Iran, solid organ transplantation started in 1967 with renal transplantation. It was the first solid organ transplantation among countries of the Middle East [58]. Although this was the case, organ procurement and transplantation act was not established until 2000 [58]. A great progress in organ transplantation was obtained after the Supreme Religious Leader's recognition of the concept of brain death and organ donation from deceased donors (i.e., the fatwa or approval of the religious leadership). Transplantation of organs from the deceased donors increased exponentially [58–62]. The most controversial law that was adopted by the Iranian government to reduce the transplantations performed abroad was the approval of the compensated and regulated living unrelated donor renal transplant program approved in 1988. In this program, the living unrelated donors were compensated, and also the health insurance coverage was extended by the government and the third-party charity funds before and after the donation procedure [62]. Although it has been suggested that this approach eliminated the renal transplant waiting list, it may raise the concern of commercial organ transplantation despite the statement of the officials that every counter measure is taken [58, 60, 62]. Nevertheless, we believe the concept of altruistic donation both for deceased and living donors in organ and tissue transplantation and any form of compensation raises the ethical concerns in any scenario including the Iranian model.

Until this date, there is no established organ allocation system in Iran. There are seven zones in Iran and the organ procurement is strictly regulated; however, the allocation system of the procure organs is not very clear. The review by Malek-Hosseini et al. suggests that there is a steady supply of deceased donor organs to Shiraz Organ Transplant Center, but the activity of other centers in Iran is not very clear [62].

In the last 11 years, the etiology of end-stage liver disease has changed in Iran. Although HBV-related liver disease is the most common etiology of liver failure (nearly 20%), it is decreasing since 2006 due to nationwide vaccination program that has been adopted in 1992 for neonates and 2005 for adults. Currently, the incidence of nonalcoholic steatohepatitis (25%) and cholestatic disease (7%) is increasing among the patients in the waiting list. HCV seems to be the fifth most common cause of end-stage liver failure [60, 63].

First LT in Iran was performed in 1992. Since 1992, over 2000 LTs have been performed, and 90% of the organs were procured from deceased donors. The deceased donation increased after the approval of the senate of the law regulating the brain death and procurement and transplantation of the organs from the cadaveric donors in 2000. The deceased donation increase doubled (2.2–5.7 per million population) since 2009 in Iran, and living donor LT has stayed relatively stable [58, 60, 64–68].

16 LT in Turkey

By virtue of the personal efforts of Prof Haberal, the "Organ and Tissue Harvesting, Preservation, Inoculation, and Transplantation" Law no 2238 was enacted in 1979 in Turkey [69]. That law was composed of four chapters: general provisions, organ/tissue harvesting from a living donor, organ/tissue harvesting from a deceased donor, and punitive articles [10, 70]. That law was a well-designed one, and, to our knowledge, it was the world's second or third organ transplantation law. Later, the law no. 2594 on utilization of organs or tissues of persons severely and irreversibly injured or killed by accidents or natural disasters was enacted in 1982 [10, 70]. Following passage of laws no. 2238 and 2594, legal impediments on cadaveric tissue and organ transplantation have been eliminated. Thirty years after passage of these laws, however, the desired level of deceased organ procurement has not been achieved. One of the most significant reasons of this apparent failure was deep devotion to customs and religious beliefs regarding organ or tissue harvesting from dead corpses implementing desecration of the latter [10, 69, 70]. In an attempt to overcome this prejudice, the transplantation community applied to the Supreme Council of the Directorate of Religious Affairs to issue a fatwa for organ transplantation and Islamic rules. The Supreme Council issued a fatwa in 1980, stating that there are no restrictions in Holy Quran regarding organ transplantation and thus it would be permissible to make organ transplantation [69]. Currently, November 3–9th is celebrated as organ donation week, during which panels on organ transplantation are organized throughout the country. As social media networks have become widespread, campaigns for organ/tissue/blood donation have acquired a new dimension. The Radio and Television Supreme Council has obliged all nationwide radio and TV channels to broadcast public service ads that highlight the importance of organ donation. Two separate studies have been conducted in 2006 and 2011 to study the reasons of negative attitude toward organ donation [11, 12]. Their results indicate that concerns related to organ mafia have been eliminated, but socioeconomic factors were still operational. The support of the Ministry of Health to the centers for solid organ transplantation, mainly LT, has remained quite limited until the early 2000s. Thus, most of the data on the LT procedures performed until 2011 have been collected from the centers performing this procedure. The organ transplantation policy of the Ministry of Health has become a national policy, and a separate unit named "Department of Organ, Tissue, Cell, and Dialysis Services" has been established within the body of the General Directorate for Treatment Services. This unit has written a software program titled "Turkish Organ

and Tissue Information System." Functioning actively since January 2011, this software now includes information of both patients in the waiting list and those already transplanted [69].

The national organ sharing program was initiated in 1989 to coordinate national organ transplantation centers and fair allocation of deceased organ supply. Later, the Ministry of Health established the National Coordination Centers in 2001 for allocation of deceased donors [69]. Taking into account Turkey's geographical regions, population distribution, and transportation means, nine national coordination centers were established in nine big provinces. The remaining 72 provinces were subordinated to the nearest coordination center. The coordination centers present organs supplied by deceased donors first to urgent cases in their own region. In case of absence of any such candidate recipient, these centers provide organs for urgent cases in other regions [69]. To ensure proper functioning of the system, the Ministry of Health has prepared a directive titled "The National Organ and Tissue Transplantation Coordination System." In line with this directive, coordinatorships have been subjected to training on how the allocations would be done and which patients would be prioritized. Certain regulations have been put in place to regulate establishing and managing LT centers in Turkey. The Ministry of Health directive titled "Directive for Organ Transplantation Centers" should be complied with in order to establish a LT center in any hospital. LT centers in Turkey should perform a certain number of LT or harvesting procedures within a year. Centers unable to meet the specified number of such procedures first receive a warning from the council; they then lose their certificates when indicated by a failure of making improvements and fulfilling required specifications [10, 69, 70]. In Turkey, postgraduate training is required to operate a LT center. As such, it is required to work at least 2 years at a center performing more than 50 LT procedures a year or at least 1 year at a center performing more than 100 such procedures a year. It is equally acceptable to complete such training in Turkey or abroad [69].

16.1 History of LT in Turkey

The history of LT in Turkey starts in 1988. The first successful DDLT was performed by Prof. Haberal and his team [71] in 1988. The same team performed a successful segmentary (left lobe lateral segment) LDLT in a pediatric case in 1990 [72]. One month after that operation, a left lobe LDLT was performed in an adult patient for the first time in the world [73]. Haberal and his colleagues successfully transplanted a liver and a kidney harvested from a same living donor to an adult patient in the same session [10, 70, 71]. After Haberal had paved the way for LT, a number of Turkish surgeons began to be closely interested in LT. Prof. Sezai Yilmaz, by performing successful LTs in Inonu University, both made that institution one of the best transplantation centers around the globe and contributed to the foundation of a LT institute in Inonu University.

The history of LT in Turkey can be examined in three stages: the initial stage (1988–1996), the development stage (1997–2001), and the stage of rise and spread (2002–). This classification was made considering the number of LTs and centers performing these procedures. This classification scheme was first proposed by Prof. Sezai Yilmaz [69]. Apart from our apparent weakness in organ donation and deceased donor procurement, this classification points out that LT has gained momentum in Turkey since 2002 (Fig. 3).

16.2 The Current Status of LT in Turkey

As of October 2020, there are a total of 49 LT centers in Turkey. Twenty-three of these centers are state universities, 7 are foundation universities, 12 are private hospitals, and the remaining 7 are training and research hospitals of the Ministry of Health. Since the first LT performed in 1988, a total of 16,798 LT procedures have been carried out in Turkey. However, we could only access detailed data of the LT after January 2002. Between January 2002 and October 2020 which has been defined as the stage of rise and spread, 16,442 LT procedures were performed in total. In

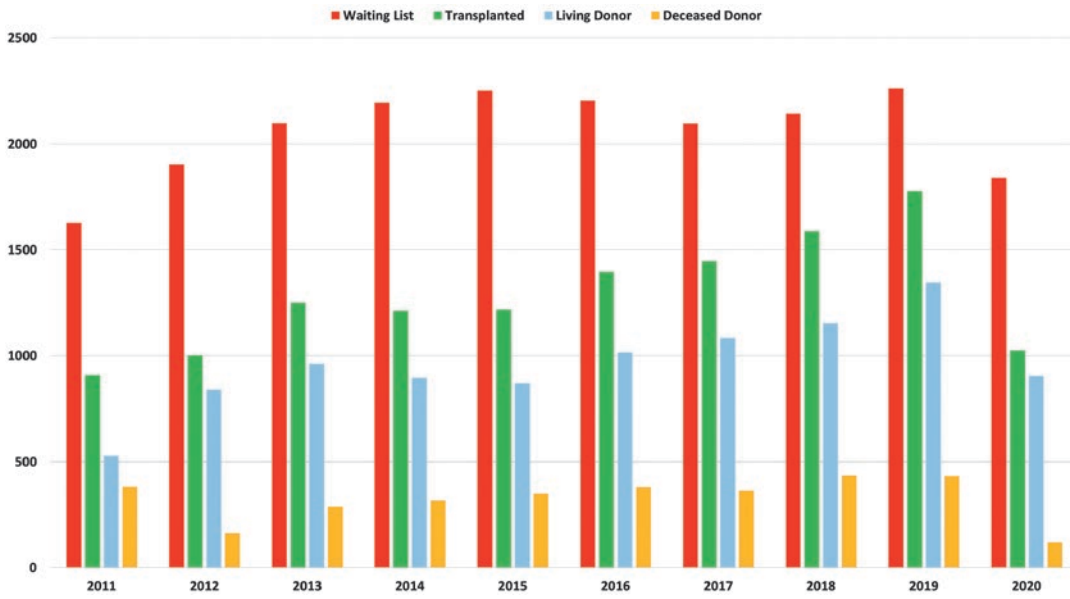

Fig. 3 The number of liver transplantations in Turkey by years

11,841 (72%) of the transplantations, living liver donors were used while 4601 (28%) procedures employed deceased donors. The number of LTs in Turkey by year has been in Fig. 3. In 4440 (25%) of 17,735 brain death cases occurring between January 2011 and October 2020, the families gave permission for organ harvesting and use. Among 20,583 patients in the waiting list, 12,587 (62.5%) had the chance to have deceased or living donor LT. While the overall 1-year patient survival rate in the 2011, 2012, and 2013 was calculated as 71.4%, 78.1%, and 80.5%, respectively, the 1-year survival rate is 90% in 2019.

16.3 LT Societies in Turkey

The first society regarding the transplantation activities that was established was the Turkish Transplantation and Burn Foundation. It began publishing a journal entitled *Dialysis, Transplantation, and Burn* in 1983, the last issue of which was published in January 2009. The second society related to transplantation was the Middle East Dialysis and Organ Transplant Foundation. Founded in 1984, this society was renamed as the Middle East Society for Organ Transplantation (MESOT) in 1987. Since 1988, MESOT regularly organizes congresses every 2 years, and the official journal of MESOT is experimental and clinical transplantation which is published monthly. The third society for transplantation is the Turkish Transplantation Society. It was established in 1990; this society was affiliated by the Transplantation Society and MESOT. The fourth society is the Turkish Transplantation Centers Coordinators Association that was established in 1994 and supported by the work of 55 transplantation centers in Turkey (liver, kidney, heart). The fifth, and the last, society is the LT Society which was founded in 2005; it specifically addresses physicians dealing with LT. The society is active in social media, and the society addresses important points in transplantation online from the platform established in the website.

16.4 Prospects for the Future of LT in Turkey

Among grafts used for LT in Turkey, only one-fourth are provided by deceased donors. This ratio is far below that of socioculturally devel-

oped western societies. The most effective way to broaden the deceased donor pool is to persuade public to donate organs when they are alive. This is because in countries like Turkey that have not completed their sociocultural development, it is not as easy as to ask relatives of deceased persons to donate their organs. An identification card should be provided to all donors that specify donated organs, blood group of the donor, and tissue specifications of the donor whenever possible. By this way, mourning family members will not have to give permission after death of the donor. To make organ donation more widespread, all physicians, nurses, and other ancillary healthcare staff should receive in-service training at least twice a year. In addition, meetings should be held in schools, colleges, dormitories, jails, large holdings, and social platforms under the supervision of transplantation coordinators to increase awareness of the importance of organ donation. Organ transplanted patients should be allowed to relay their experiences to public via these panels and meetings. Organ donation should definitely become an official state policy. Available organ transplantation societies in Turkey should not organize annual meetings engaged in activities only aimed at informing physicians, but these organizations should provide ordinary people with ample information about organ donation and transplantation via their webpages as well as social networks. They should also establish links in which healthy and diseased individuals may ask questions. The Ministry of Health should pool surgeons with postgraduate LT training and build more efficient and well-equipped centers instead of ones that performed in LT equipped centers in a few numbers annually.

Unfortunately, by now, there was no center studying on experimental studies about LT in Turkey. For the first time in Turkey, the "Research Center for Diseases and Transplantation of the Liver" was established in Inonu University Liver Transplant Institute in 2020. We already know that Prof. Starzl, to whom we express our gratitude, studied experimental solid organ transplantation models in a significant part of his career. Thus, the experimental research center with adequate technical equipment to study all aspects of LT (genetic, biological, pathological, ultrastructural, biochemical, and surgical) will make significant scientific advances in LT field. It should be kept in mind that LT had not improved to the survival in the world until the basic science experiments resulted in the development of cyclosporine and tacrolimus. Majority of the Nobel Prize nominations were given to the basic science research in the field of transplantation. Therefore, the future will start from the benches of the laboratories, and it will spread to the clinical application. For this reason, the transplant centers in Turkey should be prepared to form well-equipped research centers in order to tackle the current obstacles encountered in organ transplantation.

16.5 Simultaneous Five LDLTs in Turkey

LT, which was an experimental procedure five decades ago, is currently the gold standard treatment modality of end-stage liver disease. Together with the advancement in the immunosuppressive therapy, surgical techniques, and the patient care, survival following LT improved rapidly. A major challenge in LT field is the insufficient number of donors compared with the growing demand of transplant candidates. Many strategies to overcome the organ shortage have been developed including extended criteria donors and living donor LT. The adventure of LDLT, which started in the late 1980s, became the standard operation of LT centers after a decade. This was especially true for an Asian country because cadaveric donation is very scarce due to cultural problems. Similarly, the liver transplant centers in Turkey are performing LDLT with increasing frequency. Since LDLT provides equal or superior results for both chronic and acute liver failure, transplant surgeons are faced with an obligation to perform multiple LDLT procedures including both planned and emergency LDLT simultaneously. As a matter of fact, a few centers have published that they performed a very-high-volume liver transplants in 1 year in both adult and pediatric age groups

[74–76]. It is understood from these publications that these centers carry out more than one liver transplant per day. Today, high-volume transplant centers are defined as "the center of excellence" when they can achieve this low mortality. These centers are attraction centers for patients and physicians who will be trained in the field of LT. However, the important is to obtain sustainable performance. This is only possible with hard work. It is inevitable that these centers will be the focus of attention of the media.

Despite these advances in LDLT, many potential living donors cannot donate their organs to their relatives due to reasons such as blood group incompatibility and low graft weight and to a lesser extent due to immunologic problems such as pre-sensitization. "Swap" or liver paired exchange LT has been developed to provide more suitable organs to the recipients that would at most benefit from the transplanted graft. In this scenario, a suboptimal donor recipient pair swaps the organ to a suitable recipient and receives a more suitable graft in return. However, in order to overcome future ethical problems for the swapped donors and their matched nonrelatives, multiple LDLT procedures must be performed simultaneously in centers practicing "swap" LTs. Therefore, whole of the organizational schema, equipment and facilities need to be suitable for such transplant scenario.

Annually, 300 LTs are performed in our institute, and more than 3000 LTs have been performed since the beginning of our transplant program in 2002. More than 90% of these transplantations are LDLTs. In July 2018, we performed three simultaneous LDLTs on the same day under the supervision of Prof. Sukru Emre from Yale University in the USA, who is also a senior lecturer of our institute. Three simultaneous LDLTs were probably the first in the world. Until then, some days we had three, four, or even five LTs in different time periods on the same day. But not all of them were LDLTs; one or two of these could very well be DDLT. These multiple transplants were made due to emergency indications. In order for a liver transplant center to achieve these, it must have enough, highly experienced surgical and anesthesiology teams, facilities, and physical conditions including the operating room and intensive care units. Inonu University LT Institute physically includes 12 operating theaters, 3 intensive care units (each with a capacity of 12 patients), and 116 patient beds. In this institute, there are 25 liver transplant surgeons, 5 LT anesthesiologists, 5 intensive care specialists, 3 radiologists, 3 hepatologists, and 3 infectious disease specialists who are specialized in the care of transplant patients. These experiences and possibilities showed that we can do five simultaneous LDLTs. In June 2019, we performed five simultaneous LDLTs including one pediatric and four adult patients. All operations started at 8 a.m. and ended at 6:30 p.m. Donors and recipients were discharged without any problem on the 20th postoperative day at latest. After approximately 18 months of follow-up, all patients are healthy and alive.

Using an economic theory, Roth has managed to increase the spread of kidney donation, worldwide. This was a revolutionary development for patients with end-stage renal disease. The idea of a kidney swap won Alvin Roth the Nobel Prize for Economics in 2012. To date, dual cross-LDLT operations have been carried out in the world. However, Roth's invention is more advanced than binary swap kidneys. Roth received the Nobel Prize by suggesting triple swap kidney transplants. Simultaneous five LDLTs performed at our institute have been a rehearsal of at least triple or even five swap LDLTs that will be held for the first time in the world. Urgent transplant requirements of the patients and transplant scenarios such as swap LT can urge transplant centers to perform multiple simultaneous LTs. The transplant centers should be prepared and must be able to handle such challenging situations. This can only be achieved by experienced transplant centers with excellent equipment and advanced physical facilities.

17 Conclusions

What was once a myth soon became a reality. Thus, LT became the gold standard treatment modality in end-stage liver disease. Today many

centers in the world perform this complex procedure. However, some regions are more advanced in terms of allocation of the organs and advancement of the procedure. However, in geographic locale such as the Middle East, where the population is undereducated and dictated by the traditional culture, it is not easy for an innovative technique such as LT to evolve. Nevertheless, there seems to be some spark in certain countries of the Middle East. LT is a must for every country. It should evolve and be propagated in every country in the world in order to help these unfortunate patients. In the Middle East, collaborations between countries who have adapted an efficient transplant program and the less developed ones would help to accelerate the propagation and standardization of this complex surgical technique. The collaboration should exist in every area including postoperative patient care and last but not the least in the area of research. The physicians in the field must not forget that advances in transplantology succeeded because of the tremendous research efforts of the scientists in the field. Therefore, the future will start from the bench of the laboratories and spread to the clinic.

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Individual Patient Assessment and Therapy Decision-Making in a Live Donor-Based Liver Transplant Institute

Brian I. Carr, Sezai Yilmaz, Burak Isik, and Ramazan Kutlu

1 General Approach and Practical Decision-Making

Each newly diagnosed HCC patient will have an assessment of their hepatitis profiles, complete blood count, differential and prothrombin time and standard liver function tests, AFP levels, and CT scan of their chest and abdomen for assessment of tumor extent (maximum tumor diameter, number of hepatic tumor nodules and their location, presence and extent of PVT, assessment of liver contour for cirrhosis, and evaluation of indices of portal hypertension). For patients being considered for resection, an indocyanine green (ICG) test may be performed. For patients being considered for transplantation, an assessment of liver volume and search for a potential liver donor among the patient family are conducted and then a psychosocial assessment of both patient and selected donor.

The patient is then presented to the weekly Liver Tumor Board for whole team assessment while, the all the radiological examinations are projected for all team members to examine. Colleagues present are liver transplant surgical team members – both donor and recipient teams – diagnostic and interventional radiologists, radiation oncologists, hepatologists, medical oncologist, pathologists, psychosocial assessment team, tumor registry, and notetaker. Sometimes a definitive decision cannot be made at a first presentation, and interventional radiological procedures (like image-guided biopsies, mapping angiography) or PET scan assessment of possible lymph nodes are needed.

For patients being considered for resection, an indocyanine green (ICG) test may be performed. For patients being considered for transplantation, an assessment of liver volume and search for a potential liver donor among the patient family are conducted and then a psychosocial assessment of both patient and selected donor.

Patients with BCLC stages 0 or A and very early or early-stage HCC are then assigned to liver resection, local ablation (RFA or MWA), or liver transplantation, depending on the detailed topography of the HCC in relation to major vascular or biliary structures and the functional liver reserve, as only liver transplantation can be potentially curative for limited extent HCC in the presence of uncertain or poor liver function. Ablation is quite suitable for patients without

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portal hypertension (absence of ascites, splenomegaly, or varices and without thrombocytopenia) and with one to two small lesions. We do fewer resections these days as we have a very active liver transplant program, mainly with liver donors, and many of our Middle Eastern families have large families, often with a choice of potential donors to evaluate for liver size appropriateness, blood group compatibility, and absence of psychosocial issues. However, some patients who have excellent liver function, have several unilobar liver lesions, or don't have a live donor can still be considered for liver resection.

The majority of our patients are in intermediate BCLC stage B with multifocal lesions or the subset of advanced stage C that has PVT, but without metastases. For intermediate stage B patients, we offer TACE or increasingly, TARE, because of the small number of treatment sessions needed and the low incidence of side effects, provided their serum total bilirubin levels are <3.0 mg/dL. For patients with major branch PVT and absence of extrahepatic metastases, we offer TARE, due to its safety in this situation, and may also consider SBRT in selected cases. In patients with main stem PVT, their bilirubin is often abnormal and TARE with SBRT or external beam XRT cannot be offered, but only supportive care.

Most of our patients have active chronic HBV infection and are placed throughout their HCC treatments on nucleos(t)ide analog HBV therapy, as it can improve liver function and likely also has an HCC effect. Patients with chronic HCV infection have a postponement of any direct-acting antiviral (DAA) HCV therapy that they are not already on, till after their HCC treatments.

Our decision-making needs to be flexible and responsive to the changing developments over the patient's disease course. Especially for patients who have beyond-Milan criteria, i.e., HCC nodules >5 cm diameter or multifocality or even branch PVT, a positive transplant decision can be made after a treatment-induced AFP decrease close to normal levels, a tumor shrinkage, or an opening of blood flow has occurred in a previously thrombosed branch PVT.

Therapies being offered to BCLC stage C subsets with normal serum bilirubin levels are on the cusp of changing. The high response rates being reported in the new (2020 and 2021) drug combinations that include immune checkpoint inhibitors (ICIs), which approach 30% partial responses or even more, are beginning to appear to challenge the responses seen after TACE or TARE therapies. Therefore, at the time of writing, FDA-approved combination bevacizumab (Avastin) plus atezolizumab (Tecentriq) appears to be the first choice in the first line of therapy for nonsurgical HCC patients with advanced stage HCC (BCLC stage C) but may also begin to be attractive for the management of stage B patients who up to now have been offered regional therapies.

At all times and for all disease stages, general medical principles apply, including the need to aggressively treat pain and nausea and offer psychosocial support, as a matter of course and good oncology care. There is no overestimating the importance of the multidisciplinary team in evaluating the changing patient needs over the course of each step in the evolution of the disease process.

2 Therapeutic Choices in Less Wealthy Regions of the Middle East

The Middle East comprises a wide range of countries and a huge range of wealth and provision of services for public medical care, together with some that through recent warfare or political turmoil have had infrastructure damage. Clearly, the best choices have to be made based upon expected patient benefit and availability of medicines and services. Almost everywhere, analgesia and hydration can be offered, as needed. We, in Turkey, are in an intermediate category, with public health services available to everyone, but not always with the most expensive newer medicines. We are fortunate to work in a very modern liver transplant institute with surgical skills on offer that are the equal to anywhere in the world. Other counties are less fortunate than we are. Nevertheless, hepatic resection surgery is typically available. In places where RFA or MWA is not on offer for smaller lesions, percutaneous

ethanol injection (PEI) and acetic acid have cheap components (needle, syringe, alcohol) available almost everywhere and depend only on ultrasound guidance for placement of the needle tip. In several advance countries, cadaveric liver transplantation was not available for many years due to brain death and other ethical and religious issues. Yet many of those countries offered sophisticated and highly competent resection surgery, including in the presence of major branch PVT, which might be treated differently elsewhere. In a fairly advanced country like Turkey, we can offer sorafenib, but the much better ICIs are not available yet through our state insurance. The SHARP trial of sorafenib, which occurred 12 years ago, opened the way to minor responses and a few weeks of extra survival, compared to the preceding era. Combination ICI therapy now seems to be doing that, compared to sorafenib. Thus, medicine and especially oncology advances incrementally, but in this new era of targeted drugs and precision oncology, the rate of progress is increasing. One problem with the newer agents is their high cost. None of them are yet curative, unlike antibiotics, and yet for most of us, a few extra months of life seem worthwhile.

Part III

Country-Specific

Hepatocellular Cancer in Iran

Reza Malekzadeh and Hossein Poustchi

HCC is the second leading cause of years of life lost globally due to cancer, which clearly shows the high disease burden of liver cancer [1]. The survival rate of liver cancers in Asia is reported to be lower than in Europe and North America [3]. An Iranian study demonstrated the mean survival rate of 12.1 months, which is dependent on the tumor size, involved lymph nodes, metastasis, combination therapy of surgery and chemotherapy, and hepatitis B and C coinfection [4].

1 Incidence

In Middle Eastern countries, the prevalence of this cancer is lower compared to sub-Saharan Africa and some Far East countries [1]. Except for Egypt which has a high incidence of HCC, other Middle Eastern countries have a low incidence of HCC [5]. Iran is the second most common country in the Middle East with primary liver cancer report according to the Global Cancer Observatory in the year 2020. This report has changed significantly compared to 2010, in which Iran had the lowest incidence of primary liver cancers in the Middle East with 1.4 and 1.9 per 100,000 persons in male and female, respectively [5] (Table 1). Table 1 depicts the relatively close burden of hepatic cancers via the mortality to incidence ratio (MIR) in various countries of the Middle East, showing a homogenous survival of HCC in the Middle Eastern countries.

By a glance at the prediction of hepatic cancer in the Middle East in 2040, it is obvious that the incidence will be doubled with a stronger increase in males in this region (Fig. 1).

Based on the most recent (2016) reported national population-based cancer registry in Iran (INPCR) during (2014–2016), 7403 liver cancer cases were registered in the INPCR in 3 years [6–8]. The overall ASR of liver cancer in Iran (2014–2016) was 3.3 per 100,000 person-years. While 77.6% of all cancers were diagnosed through microscopic verification (MV%), only 34.6% (2560 cases) of the liver cases were confirmed by pathology [6–8]. Misdiagnosis in the registration process (metastatic liver cancers which were registered as primary liver cancers) may explain a percentage of this reported incidence rate, which demonstrates a false high percentage in Iran. If we only consider the pathology-confirmed cases as the absolute liver cancers in Iran, then the new measured ASR would be 1.19 on the national scale with the maximum report in Ilam by 2.35 and the minimum report in Guilan by 0.63. Based on the 2016 INCPR report, HCC rank 13th among all cancers in Iran in pathology-confirmed cases (Table 2), suggesting that primary liver cancers are not among the 10 most common cancer in Iran [8].

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There are several reasons for the low incidence of HCC in Iran. A high prevalence (>90%) of HBeAg negative HBV infection [9–11], comprising almost 95% of HBV infection in Iran [12, 13], resulted in the decline of the incidence of HCC in HBV-related liver disease. Very low prevalence [14] of HCV (<0.4%), low alcohol consumption due to religious beliefs [15] are also important reasons for the low prevalence of HCC at the moment. Suboptimal medical care for the cirrhotic patient and their short survival are other contributing factors; these patients do not liver long enough with cirrhosis to develop cancer.

As was expected, the age-specific incidence rate increases with aging in both genders (Fig. 2) [6–8].

Table 1 Population-based Cancer Registry Data for Middle Eastern countries incidence and mortality rates for primary liver cancers per 100,000 persons in 2020

Country	Incidence rate[a]			Mortality rate[a]	MIR[c]
	Male	Female	Both[b]		
Egypt	45.9	22.7	34.1	32.5	0.95
Iran	7.5	6.1	6.8	6.4	0.94
State of Palestine	8.3	4.9	6.5	6.4	0.98
Turkey	7.6	3.5	5.3	5.1	0.96
Saudi Arabia	6.8	3.3	5.2	5.1	0.98
Yemen	7.1	3.5	5.1	5	0.98
Qatar	5.7	4	5	4.9	0.98
Kuwait	5.4	4.1	5	4.7	0.94
Cyprus	6.8	2.9	4.8	4.2	0.87
Oman	5.8	2.5	4.4	4.1	0.93
Tunisia	4.7	4	4.3	4	0.93
Bahrain	4	3.4	3.7	3.6	0.97
Iraq	3.6	2.9	3.3	3.2	0.96
Jordan	3.6	2.5	3	3	1
Israel	4.2	1.8	2.9	2.6	0.89
Syria	3.2	2.5	2.9	2.8	0.96
United Arab Emirates	2.8	3.2	2.9	2.8	0.96
Lebanon	2.5	2.1	2.3	2.2	0.95

[a]The rates are adjusted for the standard world population
[b]The incidences in both sexes are sorted from the most common to the least common
[c]MIR: mMortality to incidence ratio

2 Etiology

2.1 Infections

Table 3 demonstrates the most common viral etiologies for HCC in the area; in Iran, HBV was reported as the main viral cause (Table 3)

Genotypes:

The most common genotypes of HBV and HCV are shown in Table 4. Although several studies reported genotype D as the most common genotype of HBV, a study in 2020 found genotypes B and F the most common ones in Iraq. However, the finding has not been confirmed by a larger sample size study yet. The most com-

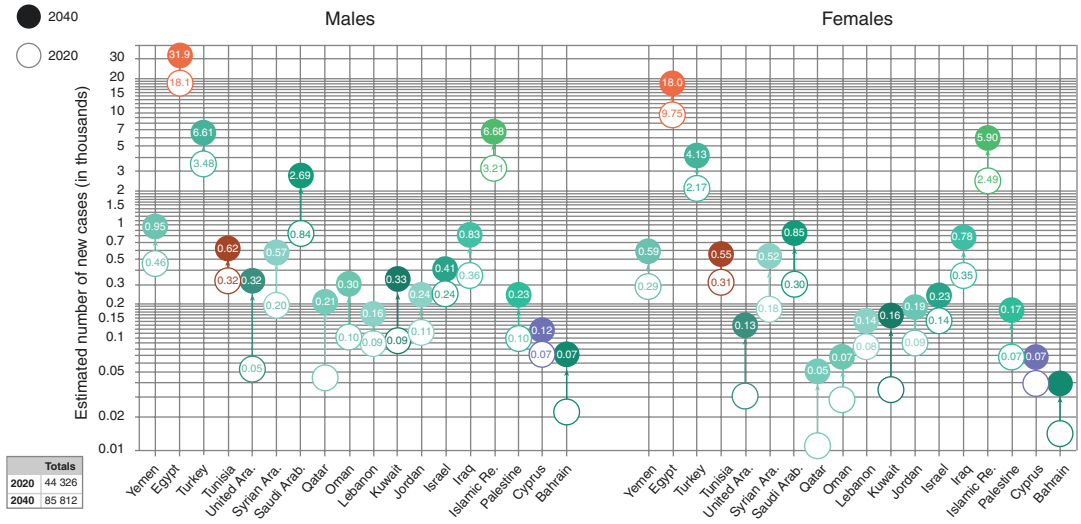

Fig. 1 Estimated number of new hepatic cancers in 2040

Table 2 Liver cancer rank in Iran among all other cancers INPCR based on microscopic verification (2016) [8]

Rank	Cancer site	Number			ASR (per 100,000 person-years)		
		Males	Females	Both genders	Males	Females	Both genders
1	Breast	248	13,787	14,035	0.63	33.62	17.12
2	Skin (nonmelanoma)	6821	4143	10,964	18.17	10.97	14.54
3	Colorectal	5386	4147	9533	14.4	10.82	12.58
4	Stomach	5184	2356	7540	13.85	6.09	9.92
5	Bladder	4876	1001	5877	13.11	2.59	7.79
6	Prostate	5650	–	5650	16.09	–	16.09
7	Thyroid	992	4151	5143	2.32	9.16	5.71
8	Leukemia	2349	1490	3839	6.25	4.04	5.14
9	Lung	2567	1001	3568	7.00	2.65	4.8
10	Non-Hodgkin lymphoma	1853	1194	3047	4.76	2.99	3.87
11	Esophagus	1537	1211	2748	4.04	3.22	3.63
12	Brain	1541	1026	2567	3.84	2.57	3.21
13	Liver	545	343	888	1.48	0.90	1.19

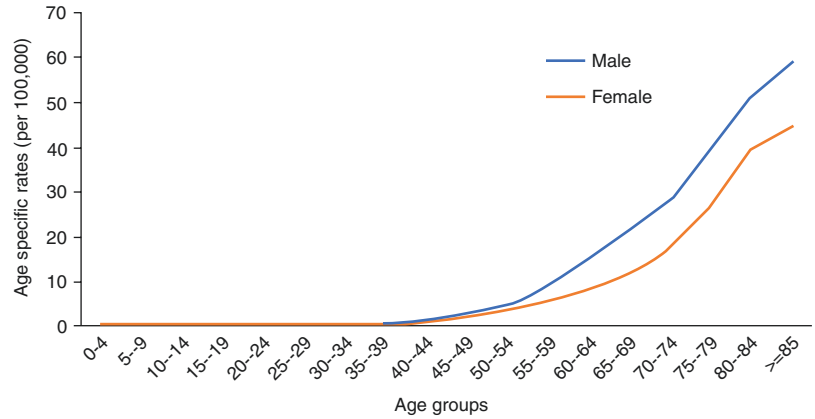

Fig. 2 Age-specific incidence rates (per 100,000) of liver cancer in Iran, 2014–2016

mon HCV genotypes in the Middle East were type 1 and 4. In 2020, Rezaee and colleagues found type 3a as the most common genotype in Iran [23], and they suggested the cause of a high level of 3a lies in the route of transmission. Type 3 is mostly transmitted with shared needles, while type 1 is transmitted among family members. However, this new finding in Iran suggests the probable change of dominant genotype in other countries in the area.

2.2 Non Alcoholic Fatty Liver and HCC

Following universal neonatal (1994) and adulthood vaccination against HBV (2008) and availability of very effective therapy for HBV and HCV in Iran, the incidence of infectious-related HCC has declined. Nonalcoholic fatty liver disease (NAFLD)-related HCC among countries in the Middle East is rapidly increasing following the epidemic of obesity and metabolic syndrome in this region [38–40]. Over 24% of Iranians suffer from obesity, a result that is as high as American reports [41]. Several studies have demonstrated an association between metabolic syndrome and type 2 diabetes mellitus as well as obesity, with HCC, suggesting that NAFLD is playing a significant role in the rising incidence of HCC [42–44]. This finding along with the 30% prevalence of NAFLD among Iranian [15] suggests that in the near future, NAFLD-related liver cancers will become the most common cause of HCC [15, 45, 46]. The main pathogenesis is most likely insulin resistance, related

Table 3 Most common viral etiology for HCC

Country	Cause of HCC	
	Most common	Least common
Egypt [16, 17]	HCV	HBV
Iran [5]	HBV	HCV
State of Palestine [18]	HBV	HCV
Turkey [17]	HBV	HCV
Saudi Arabia [17]	HCV	HBV
Yemen [17]	HBV	HCV
Qatar [19]	HCV	HBV
Kuwait [20]	HBV	HCV
Cyprus [20]	HCV	HBV
Oman [20]	HBV	HCV
Tunisia [21]	HCV	HBV
Bahrain [20]	HBV	HCV
Iraq [20]	HBV	HCV
Jordan [20]	HBV	HCV
Israel [20]	HCV	HBV
Syria [20]	HCV	HBV
United Arab Emirates [20]	HBV	HCV
Lebanon [22]	HBV	HCV

Table 4 Most common genotypes of HBV and HCV

Country	HBV [24–34]	HCV [23, 35–37]
Egypt	D	4
Iran	D	1,3
State of Palestine	D	4
Turkey	D	1
Saudi Arabia	D	4
Yemen	D	No reliable data
Qatar	D	4
Kuwait	D	4
Cyprus	D	1
Oman	D	1
Tunisia	D	1
Bahrain	D	4
Iraq	B	4
Jordan	D	4
Israel	D	1
Syria	D	4
United Arab Emirates	D	1
Lebanon	D	1

adipokine changes, stimulation of insulin-like growth factor, and oxidative stress which are the consequence of steatosis and hepatic inflammation [45]. The estimated proportion of HCC attributed to NAFLD in Iran at present time is 25–30% [45]. A subset of individuals with NAFLD develops NASH, a more serious form of liver damage. Population-based studies that have used serum levels of aminotransferases as a surrogate marker for NASH suggest that 3% of Iranian adults (about 25% of those with NAFLD) have NASH [15]. However, several studies demonstrate that HCC could develop in NAFLD subjects even without preexisting cirrhosis by 15–50% of cases being diagnosed without cirrhosis, and majority of these cases had concomitant metabolic syndrome and type 2 diabetes [47]. Unfortunately, HCC associated with non-cirrhotic NAFLD is less likely to be detected during surveillance and thus is more likely to be more advanced when compared to HCC in cirrhosis patients [46].

Once HCC develops in NAFLD cirrhotic patients, survival appears to be shorter than patients with viral etiology background. This may be due to the older age of NAFLD cirrhotic patients (mean age of 73), and factors such as having larger tumors (due to late diagnosis), greater likelihood to have comorbid heart conditions, and less likelihood to be diagnosed by surveillance in comparison to viral hepatitis cirrhotic patients [48]. But in comparing the survival of cirrhotic and non-cirrhotic NAFLD patients, the expected remaining years are equivalent or better in non-cirrhotic NAFLD patients [46], likely due to preserved liver function. We should mention that patients with concomitant HBV infection and obesity are more likely to develop cirrhosis, thus at higher risk for HCC with poorer survival [49].

Baseline alpha-fetoprotein is an easily obtained serum marker and provides both prognostic and surveillance value for HCC patients in stage 3 liver fibrosis with HBV or HCV infection. Use of this biomarker is quite cost-effective for HCC surveillance [42].

3 Diagnosis

The availability of gastroenterologists and imaging medical facility including sonography, CT scan, and MRI in all provinces and big cities

around the country along with clinical pathology laboratories made the diagnosis of HCC very feasible in Iran. Although a study in 2015 found the combination of MRI and DWI the most accurate diagnostic tool (94.79%) [50], ultrasonography is still utilized by some clinicians.

4 Treatment

Treatment by surgery, radiofrequency interventions, and liver transplant is also available in referral centers and is offered to Iranian patients with HCC. Approximately 20% of the patients are qualified for surgery. Most patients are being treated with radiofrequency or other ablation modalities, which have been shown to improve survival rates [51, 52]. Patients with cirrhosis and early HCC who are being diagnosed during screening are offered liver transplantation in Iran. The Shiraz Liver Transplant Center in Iran is one of the most active liver transplant centers in the Middle East region. HCC-related transplant have increased from 1% to 5.5% in Shiraz [54].

5 Prevention

The main strategy to reduce the burden of HCC is early diagnosis and treatment of chronic HBV and HCV viral hepatitis. Several studies in Iran have shown that even advanced fibrosis and early cirrhosis are reversible with effective therapy of the underlying viral infection [56, 72].

Two recent prospective studies demonstrated that statins can reduce the occurrence of HCC in patients infected by hepatitis B and C reported and show a positive role in decreasing the HCC risk in patients with NAFLD due to indirect effect on liver-related diseases [57–59].

A large pragmatic trial of polypill containing aspirin, statin, enalapril, and low-dose hydrochlorothiazide for prevention of cardiovascular disease (CVD) in an urban Iranian population with special focus on NASH has recently been completed and is expected to prevent HCC in addition to CVD in the near future [60]. Table 5 summarizes the strategies for preventing HCC.

Table 5 Strategies for prevention of HCC in Iran

1. Obesity management	Mediterranean diet Coffee consumption Adequate physical activity
2. Chemoprophylaxis	Treatment of the underlying viral hepatitis Aspirin use Statin use Antioxidant

6 HCC-Related Research Interests in Iran

Several studies on treatment of HBV and HCV for prevention and reversibility of advanced fibrosis and cirrhosis and large clinical trials for the elimination of HCV are among the most important contribution of Iranian reseachers. The clinical trial, named Enhancing Hepatitis C Linkage to Care (ENHANCE) aims to improve the HCV testing, linkage to care, and treatment of IV drug users in crowded cities of Iran. The pilot studies were conducted and showed the feasibility of this program [61–64].

Randomized controlled trials have shown that screening and prevention of HCC are feasible [65, 66]. We should consider it preventable cancer since the main etiologies of this cancer could be resolved. Effective HBV vaccination [67], concomitant with appropriate treatment of viral hepatitis, autoimmune hepatitis, NAFLD, and metabolic-related chronic liver diseases, can prevent cirrhosis and HCC [68–71].

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Hepatocellular Carcinoma in Kuwait

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1 Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality, with an estimated worldwide prevalence of 632,000 cases [1]. Data from the Arabian Gulf region indicates that liver cancer is the sixth most common cancer in the Gulf Cooperation Council (GCC) states, representing 5.2% of all cancers diagnosed between 1997 and 2007. Population-based cancer registry information distributed by Poustchi et al. proposed a yearly frequency of HCC per 100,000 in Kuwait of 8.1:3.6 for males and females. However, few studies addressed epidemiology and risk factors of hepatocellular carcinoma in this region. The fact that HCC incidence is not equally distributed throughout the world reflects the heterogeneous geographical distribution of the relevant environmental risk factors. Major differences in disease incidences exist among countries based on different etiologic factors of disease with more prevalence in the developing countries [2]. For example, the vast majority (>80%) of the cases of HCC occurs in the Far East and in sub-Saharan Africa, mostly as a consequence of chronic infection with hepatitis B virus (HBV), where the age-standardized incidence rates range between 28.5 and 48.8 per 100,000 males and 11.6 and 14.6 per 100,000 females. Arabian Gulf areas represent a population of around 40 million with very large expatriate communities. In Kuwait, the number expatriates is more than nationals.

2 Environmental Risk Factors

Chronic infection with the hepatitis viruses and alcohol abuse are the most important environmental risk factors for HCC, since these are the relevant etiologic factors for cirrhosis too [3]. In Asia and Africa, the dominant risk factor of HCC is chronic **HBV** infection compared with other low-risk areas like North America and Europe, where HCV infection accounts for the large proportion of cases [4]. In Kuwait, there is limited evidence on the national prevalence of HBV; however, prevalence is expected to be higher in those >30 years of age born before the introduction of the HBV vaccination program. There is also limited data on the burden of HBV-related hepatocellular carcinoma in the country.

HCV Chronic HCV infection is a leading risk factor in the majority of the resource-rich countries [5]. A meta-analysis of 21 case-control studies showed a 17-fold increased risk of HCC in

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HCV-infected patients compared with HCV-negative controls. HCV increases HCC risk not only by both promoting cirrhosis but also by causing specific genetic lesions to the infected liver cells [6]. In Kuwait, according to study by Shaaban et al., the prevalence of HCV infection is around 40%.

Alcohol has no direct carcinogenic effects on the liver, but chronic consumption of more than 50 g of alcohol per day is associated with an increased risk of cirrhosis in both sexes [7]. In contrary to western societies where alcoholic intake plays a major role in cirrhosis and subsequent development of HCC, data from Kuwait suggest only prevalence of around 3%. An interpretation for this comes mainly from the conservative Islamic nature of Kuwait society where the authorities prohibit alcohol intake by law.

Aflatoxin, a mycotoxin produced by the *Aspergillus flavus* contaminating the foodstuffs stored in warm, dump condition, is a relevant contributor of HCC of regional importance, being associated with increased risk of HCC in HBV-infected persons in parts of Africa and Asia [8]. However, there is insufficient data to shed light on the extent of exposure to this risk factor in Kuwait.

3 Emerging Risk Factors

3.1 Nonalcoholic Fatty Liver Disease [NASH Cirrhosis]

The association between NASH and HCC is strongly supported by case-control studies, but not by prospective studies [9]. However, clinical and epidemiological investigations, which link obesity and diabetes to HCC, support the role of NASH in HCC too. Diabetes mellitus is one of the main etiologic factors of nonalcoholic fatty liver disease (NAFLD)/NASH. Cohort studies prospectively evaluating patients for extended time periods showed diabetes to be a significant risk factor for HCC and not vice versa and to parallel duration of follow-up [10]. The WHO estimated that there are six Arab countries in the Middle East and North Africa which are among the ten countries in the world with the highest diabetes and prediabetes prevalence. According to the International Diabetes Federation in 2009, these countries are Saudi Arabia, Oman, Bahrain, Kuwait, UAE, and Egypt. In our study, more than one-third of our patients (40.5%) had diabetes as a risk factor for HCC at presentation.

Hemochromatosis Population studies showed a 1.7-fold increase in the incidence rates of HCC among individuals with hereditary hemochromatosis confirming preliminary observations in smaller studies in related population [11]. There is no data to shed light of impact of such risk factor on incidence of HCC in Kuwait. In conclusion, an increase in HCC incidence is expected in many areas of the world, including Kuwait, as a consequence of the accumulation of patients with cirrhosis due to virus hepatitis. HCV and diabetes mellitus are the main risk factors for HCC incidence among GCC citizens. Half of our patients were Egyptians, and those generally showed four times higher incidence for HCC between males to females compared to other Arab countries.

3.2 Manifestations

Patterns of manifestation – there is a range of clinical presentations for patients with HCC, from being asymptomatic to presenting with a life-threatening illness such as variceal hemorrhage [12]. Many patients who develop HCC have no symptoms specifically related to the tumor, especially for those who have been undergoing regular surveillance and have HCC detected at an early stage [13]. Symptomatic patients and patients with advanced lesions may present with mild to moderate upper abdominal pain, weight loss, early satiety, or a palpable mass in the upper abdomen [14–17].

Paraneoplastic syndromes – patients with HCC may occasionally develop a paraneoplastic syndrome that can manifest with the following features which (except for erythrocytosis) are generally associated with a poor prognosis [18]:

- *Hypoglycemia* – hypoglycemia, which usually occurs in advanced HCC, is thought to result from the tumor's high metabolic needs. Less than 5 percent of tumors secrete insulin-like growth factor-II, which can cause severe, symptomatic hypoglycemia sometimes early in the course of the disease [12, 13].
- *Erythrocytosis* – erythrocytosis in HCC is probably due to tumor secretion of erythropoietin (EPO) [19]. Although raised serum EPO levels may be present in up to 23 percent of patients with HCC, elevations in hemoglobin concentration or packed cell volume are uncommon, and most patients are anemic at diagnosis because of other effects of the tumor [20].
- *Hypercalcemia* – hypercalcemia can be present in association with osteolytic metastases, but it may also be seen in the absence of bony metastasis due to secretion of parathyroid hormone-related protein [21].
- *Diarrhea* – patients with HCC may infrequently present with intractable diarrhea and associated electrolyte disturbances (e.g., hyponatremia, hypokalemia, metabolic alkalosis) [22, 23].
- *Cutaneous features* – although skin changes are rare in patients with HCC, several cutaneous manifestations have been described; however, none is specific for the diagnosis [24].

These include:

- May present with a variety of cutaneous findings (e.g., scaly, violaceous papules overlying bony prominences of the hands) and is associated with solid organ malignancies.
- Foliaceus is a superficial blistering disease similar to pemphigus vulgaris, except that it rarely involves the mucous membranes. Blisters often appear as shallow erosions associated with erythema, scale, and crust formation, and the appearance may resemble severe seborrheic dermatitis.
- Sign of Leser-Trélat refers to the sudden appearance of multiple seborrheic keratoses, often with an inflammatory base in association with skin tags and acanthosis nigricans.
- Rotunda, which is characterized by multiple, round or oval, sharply demarcated scaling patches, has been reported in South African black patients with HCC [25].

Other clinical presentations – the following clinical presentations may be seen in symptomatic patients with HCC:

- Intraperitoneal bleeding due to tumor rupture. Tumor rupture is often associated with sudden onset of severe abdominal pain with distension and an acute drop in the hemoglobin and hypotension and is most commonly diagnosed by abdominal imaging. Computed tomography (CT) of the abdomen typically demonstrates a liver mass and free intraperitoneal blood [26].
- Obstructive jaundice caused by invasion of the biliary tree and compression of the intrahepatic duct or, rarely, as a result of hemobilia.
- Fever developing in association with central tumor necrosis.
- Pyogenic liver abscess (very rare) [27].

Extrahepatic metastases – extrahepatic metastases are present at the time of diagnosis in approximately 10 to 15 percent of cases, and they are more common in patients with advanced stage primary tumors (>5 cm, large vessel vascular invasion). Extrahepatic metastases occur as a component of disease recurrence after locoregional therapy in approximately 5 to 25 percent of patients [28].

The most common sites of extrahepatic metastases are the lung, intra-abdominal lymph nodes, bone [29], and adrenal gland, in that order. Brain metastases are rare overall (0.2 to 2 percent), although a higher rate has been reported in patients who have already developed metastases elsewhere or with locally advanced HCC [29].

3.2.1 Diagnosis of HCC

Most HCC patients are often diagnosed in an advanced stage with poor prognosis, due to absence of specific symptoms in early stages and lack of early diagnostic markers [30]. The diagnosis of HCC can be difficult and often requires

the use of one or more imaging modalities. Ideally, tumors should be detected when they are ≤2 cm in size so that all treatment options can be offered [31]. Some patients had incurable disease at the time of diagnosis [32]. In Kuwait, nearly half of the patient are at advanced stage at time of diagnosis with around 75% of our patient diagnosed based on characteristic CT scan finding.

3.2.2 Biomarkers

Alpha-fetoprotein Elevated serum AFP can also be seen in patients with chronic liver disease without HCC such as acute or chronic viral hepatitis, particularly in hepatitis C [33]. It is generally accepted that serum levels greater than 500 mcg/L in a high-risk patient are diagnostic of HCC (>400 ng/mL predicts for HCC with specificity greater than 95%) [34]. Patients with AFP levels >1000 mcg/L have an extremely high risk of recurrent disease following transplantation, irrespective of the tumor size [35]. AFP is elevated in 75% of cases. The level of elevation correlates inversely with prognosis. Around 25% of HCC cases in Kuwait have elevated AFP >400 at time of diagnosis.

4 Imaging Studies

4.1 Ultrasound

It is the modality of choice for HCC screening and surveillance because of its advantages and its high specificity that reaches over 90% for detecting HCC [36]. However, US sensitivity is limited in the background of cirrhosis and obesity and for detecting small HCC <20 mm [37]. It does not provide sufficient anatomic detail for planning surgical resection or ablation. A significant number of small lesions may not be detected with ultrasound screening (60% sensitive) [38]. US is also operator-dependent. Currently no alternative to US is appropriate for screening because of higher cost, radiation exposure (CT), and long exam times for CE-MRI (at least 30 min). Current practice guidelines do not advocate multiphasic CE-CT or CE-MRI for HCC surveillance. CEUS has the advantage over CT and MRI as it is less costly and it allows a dynamic contrast evaluation. It has shown excellent sensitivity for detection of hypervascular lesions [39]. CEUS has the same limitations as conventional US, such as operator dependence, limited sensitivity in obese and cirrhotic patients and for small lesions, and limited detection of deep liver lesions [40].

4.2 Computerized Tomography

Triple-phase CT has been found to be highly accurate in the diagnosis and characterization of HCCs but, like US, may miss smaller lesions. Pooled estimates reveal a sensitivity of 68% and a specificity of 93% [41]. Classic CT findings of HCC include a hypervascular pattern with arterial enhancement and rapid washout during the portal venous phase [42]. Multiphasic CT for HCC diagnosis should include four phases: (1) non-contrast phase in order to detect hyperdense structures (such as hemorrhage or changes related to locoregional therapy); (2) late arterial phase, corresponding to the peak of tumor enhancement; (3) portal venous phase (60–70 s postinjection) corresponding to the peak of portal venous and parenchymal enhancement within the liver and the most adequate for venous evaluation; and (4) the delayed venous phase (180 s postinjection) which increases detection of tumor capsule [43]. In tumors with a size between 1 cm and 2 cm, MRI was shown to be superior over CT (sensitivity: 84% vs. 47% for 1–2 cm) [44].

4.3 Magnetic Resonance Imaging

MRI provides an excellent method for characterizing HCC without radiation and the need for iodinated contrast. Technologic improvements have reduced scanning time and improved the specificity of the study. Pooled analysis demonstrated a sensitivity of 81% and a specificity of 85% [45]. HCC demonstrates a variety of features on MRI, depending on the tumor architecture, grade, and amount of intra-tumoral fat and glycogen [46]. MRI sensitivity is excellent for

lesions with a size ≥2 cm and 1–2 cm (100% and 84% in a lesion-by-lesion analysis). However, sensitivity falls to 29–43% for lesions with a size <1 cm [47]. CT and MRI have both limited detection of well-differentiated and small HCCs. Furthermore, approximately 40% of HCC are not hypervascular during the arterial phase, including early HCC, infiltrative HCC, and some poorly differentiated HCC, and the presence of washout can be absent in approximately 40–60% of small HCC [48]. The benefits of contrast-enhanced studies must be balanced against the risks if any anatomic or functional renal impairment is possible. Iodinated contrast for CT may worsen renal failure, and gadolinium enhancement on MRI has been linked to a syndrome of severe systemic fibrosis in a patient with renal failure [49].

4.4 Other Imaging Modalities

Several studies have suggested a role for [18F] fluorodeoxyglucose (FDG)-PET scanning for the detection of primary HCCs, tumor staging, assessing response to therapy, and predicting prognosis as an adjunct to CT [50]. The sensitivity of PET in diagnosis of HCC was 55% compared with 90% for CT scanning, although only PET detected some tumors (including distant metastases). Well-differentiated and low-grade tumors had lower activity on PET [51]. FDG uptake has been shown to be a prognostic marker for poorly differentiated tumor, microvascular invasion, shorter recurrence-free survival after curative treatment, and short survival in case of palliative condition [52]. FDG-PET might be a useful imaging modality for identifying extrahepatic metastases, although sensitivity is limited for lesions 1 cm or smaller [53]. With the emergence of quantitative imaging, the use of PET/MRI hybrid systems is promising [54]. Majority of hepatocellular carcinomas show high levels of PSMA expression on tumor vessels and on canalicular membrane of the tumor cells. Putative diagnostic, prognostic, and therapeutic value of PSMA in HCC warrants further clinically oriented investigations [55].

5 Biopsy

Biopsy is indicated in patients with HCCs that are larger than 2 cm with low AFP or in whom ablative treatment or transplant is contraindicated. Histological diagnosis via liver biopsy may, therefore, be necessary if HCC develops in a non-cirrhotic patient and if imaging studies are inconclusive for being compatible with HCC [56]. Liver biopsy is done under CT or US guidance with varying degrees of sensitivity (66–93% based on tumor size, operator experience, and needle size) and 100% specificity and positive predictive value [57]. The most common complication of liver biopsy is pain that, including mild discomfort, is reported by up to 84% of patients [58]. Severe complications correlated to liver biopsies, including perforation of gallbladder, bile peritonitis, hemobilia, pneumothorax, or hemothorax, are extremely rare [59]. Severe bleeding is usually evident within 2–4 hours and occurs in 1 out of 2500–10,000 biopsies; nevertheless, late hemorrhage, most likely due to clot dissolution, cannot be neglected [60]. The most quoted study about seeding risk is a meta-analysis that showed a rate of 2.7% in 1340 biopsies [61]. Adding three more recent series to this meta-analysis would obtain much lower rates of seeding, even less than 1% [62]. Histopathological subtypes of HCC according to the WHO include steatohepatitic variant, clear cell variant, macrotrabecular-massive variant, scirrhous variant, chromophobe variant, fibrolamellar HCC (fibrolamellar carcinoma), neutrophil-rich variant, and lymphocyte-rich variant. It has been recently reported that intrahepatic cholangiocarcinoma (iCCA) can be misdiagnosed as typical HCC in 4% of cases [63]. Given its rising incidence and poor prognosis, close attention is needed to differentiate iCCA from HCC [64].

Liquid Biopsy A liquid biopsy entails the analysis of tumor components released into the bloodstream [65]. Liquid biopsies could provide a valuable tool to overcome tumor heterogeneity, which is particularly pronounced in multifocal and advanced HCC, both at genomic and transcriptional levels [66].

6 Treatment of Hepatocellular Carcinoma

6.1 Liver Transplant and Hepatic Resection

Hepatic resection (HR) is a recommended approach for fit patients in whom Milan criteria for hepatic resection are fulfilled. It had a 5-year overall survival (OS) rate of 60–70%. Unfortunately, more than half of patients with primary HCC are diagnosed when their disease has reached the intermediate or advanced stages. Most of these patients developed multinodular tumors or macrovascular invasion. At a retrospective record of our patients in Kuwait, 61.2% of our patients demonstrated multifocal tumors at the time of diagnosis with liver resection only done in nine patients (8.3%). Additionally, 23.4% proved to have a macroscopic vascular invasion. This reflects the advanced nature of HCC in Kuwait society which limits many treatment options. Extrahepatic spread to the lymph nodes, lung, and bone is recorded in 21.6%, 9.9%, and 6.3% respectively.

7 Nonsurgical Treatment

A. Locoregional Therapies

Transarterial chemoembolization is an effective treatment for unresectable HCC patients with intermediate stage. However, it is contraindicated in those with decompensated cirrhosis, high tumor burden, tumor nodules ≥10 cm, and bile duct obstruction. In Kuwait, TACE services are offered widely through interventional radiology team bases at Amiri and Mubarak Hospital. Around 13% of HCC patients diagnosed between 2008 and 2017 received TACE to control their malignancy. TARE services are offered as well through Mubarak Hospital and Kuwait Cancer Control Center but in a narrow scale.

B. Ablation

Ablation leads to tumor tissue necrosis with 5-year survival between 38% and 60%. Ablative therapy is indicated for HCC patients with small liver cancer, Child-Pugh class A or B, up to three tumors each 3 cm or smaller in diameter. Radiofrequency ablation is widely practiced in Kuwait. Between 2008 and 2017, around 8% of HCC patients were subjected to RFA. Microwave ablation is also offered but in a narrow scale.

C. Pharmacological Treatments.

1. First-Line Systemic Therapy Sorafenib.

Sorafenib is an oral multikinase inhibitor approved for unresectable HCC since 2007, based on SHARP and ORIENTAL trial. During sorafenib treatment, associated toxicities were observed, including gastrointestinal upset, anorexia, hand-foot skin reactions, and fatigue with an overall 30% occurrence of grade 3–4 severity events requiring permanent discontinuation in approximately 28% of treated patients. In our retrospective data for HCC patients, 35% received systemic treatment with sorafenib, and they showed a better median overall survival of 9 months compared to 1 month only for those who did not receive sorafenib. Around 17% of our HCC patients who have HCV as their viral etiology and received sorafenib showed a median overall survival of 7 months, compared to 16% HCC with nonviral etiology and who received sorafenib showing a median overall survival of 12 months. At current, the majority of our patients can get access to treatment through patient help fund programs.

8 Lenvatinib

Lenvatinib, an oral multikinase inhibitor, is a potent inhibitor of VEGFR1–3. Lenvatinib was approved based on recent open-label, phase III, multicenter, non-inferiority trial (REFLECT). This trial demonstrated lenvatinib was non-inferior to sorafenib in OS in unresectable HCC with mOS of 13.6 months for lenvatinib group versus 12.3 months in the sorafenib group. In Kuwait, our patients with Child-Pugh A can get access to lenvatinib free of charge if holding Kuwait nationality.

9 Nivolumab

Nivolumab is a programmed cell death protein-1 (PD-1) immune checkpoint inhibitor. CheckMate 040 suggested overall objective response rate (ORR) of 15%, with a disease control rate (DCR) of 58% and an OS of 15 months in patients with aHCC with or without chronic viral hepatitis were demonstrated. CheckMate 459, a randomized, multicenter phase III study of nivolumab vs. sorafenib as first-line treatment in patients with aHCC, failed to show OS superiority for nivolumab after follow-up of 22.8 month. In Kuwait, nivolumab is not used as first-line treatment for patient with aHCC.

10 Second-Line Systemic Therapy

10.1 Regorafenib

Regorafenib, an oral multikinase inhibitor, targets angiogenic (VEGFR1–3, TIE2), stromal (PDGFR-β, FGFR), and oncogenic receptor tyrosine kinases (KIT, RET, and RAF) [125]. RESORCE study, a randomized, double-blind, parallel-group, phase III trial, 573 HCC patients who tolerated sorafenib were randomly assigned in a 2:1 ratio to receive regorafenib (oral dose 160 mg daily during weeks 1–3 of each 4-week cycle) or matching placebo (once daily during weeks 1–3 of each 4-week cycle). The results showed overall survival benefit with a hazard ratio of 0.63 (95% CI, 0.50–0.79; one-sided $P < 0.0001$) and median survival of 10.6 months (95% CI, 9.1–12.1) for regorafenib versus 7.8 months for placebo that the survival benefits from regorafenib were superior to placebo.

10.2 Cabozantinib

Cabozantinib is an oral multiple tyrosine kinase receptor inhibitor with activity against VEGFR1–3, MET, and AXL, and inhibition of c-MET and VEGFR decreases resistance of VEGFR inhibitor via c-MET axis. Based on phase III trial (CELESTIAL), randomized previously treated HCC patients to receive cabozantinib (60 mg once daily) or matching placebo, and the results showed the mOS was 10.2 months in the cabozantinib group and 8.0 months in the placebo group (HR for death, 0.76; 95% CI, 0.63 to 0.92; $P = 0.005$). Median PFS was 5.2 months in cabozantinib group and 1.9 months in placebo. This treatment is available in Kuwait as second line for HCC.

10.3 Ramucirumab

Ramucirumab is a recombinant IgG1 monoclonal antibody and VEGFR-2 antagonist approved for aHHC based on phase III trial (REACH), 565 patients were enrolled at second-line setting to receive ramucirumab with 8 mg/kg every 2 weeks, and 282 were assigned to receive placebo only. The result show improved mOS by 1.6 months for ramucirumab compared to placebo. Subgroup analysis showed that patients with elevated serum alpha fetoprotein (> 400 ng/mL) achieved a better OS benefit from ramucirumab treatment compared with placebo. The mOS in ramucirumab group was 7.8 months, which was significantly greater than 4.2 months in placebo group. This was confirmed by a randomized, double-blind, placebo-controlled, phase III trial (REACH-2) study. This study suggested that second-line treatment with ramucirumab significantly improved overall survival in HCC patients with higher α-fetoprotein level of at least 400 ng/mL. Moreover in Kuwait, ramucirumab is available for Kuwaiti patients. Recently, non-Kuwaiti patients can get access to ramucirumab through patient help fund programs.

10.4 Pembrolizumab

Pembrolizumab is a PD-1 monoclonal antibody that was studied in advanced HCC. KEYNOTE-224, a nonrandomized, multicenter, open-label, phase II trial, proved the efficacy and safety of pembrolizumab in patients with aHCC previously treated with sorafenib. KEYNOTE-240, a randomized, double-blind, phase III, followed to confirm this

finding. Unfortunately, the mOS was 13.9 months for pembrolizumab group versus 10.6 months in placebo group. The median PFS was 3 months versus 2.8 months for pembrolizumab versus placebo. It was showed that these differences did not reach statistical significance. As a result, this treatment is no more indicated in Kuwait.

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Insights on Hepatocellular Carcinoma in Saudi Arabia

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1 Background

HCC is one of the leading causes of cancer-related mortality. It is ranked as the fifth most common cancer and the third leading cause of cancer death globally. HCC accounts for 7.5% and 3.5% of cancer types in men and women, respectively [1]. In areas where hepatitis C (HCV) and B (HBV) viruses are prevalent, the incidence of HCC increases after the age of 20 years and reaches a peak at around 50 years of age. However, in most western countries, the incidence of HCC reaches its peak at 75 years of age [2, 3]. In Saudi Arabia, HCC is the sixth and twelfth most common cancer in men and women, respectively [4]. The age-standardized incidence rate (ASIR) for liver cancer in Saudi Arabia is 4.5 per 100,000 people [5]. When this number is compared with the global incidence rate of 5.3 per 100,000, the incidence rate of liver cancer in Saudi Arabia is between the highest and lowest global rates. However, most patients diagnosed with HCC in Saudi Arabia are in the late stages with poor prognosis and a higher mortality rate compared with other countries where early detection is more common [6, 7]. Hence, a robust screening program for HCC in regions where HBV and HCV are endemic such as Saudi Arabia should be a priority for healthcare providers. In Saudi Arabia, a study on hepatitis B screening observed that 7% of the children tested were positive for hepatitis B surface antigen (HBsAg) [8]. This has declined to less than 0.3% after introducing a compulsory vaccine for hepatitis A and B [9]. Due to the introduction of these compulsory vaccines, a lower prevalence of HBV is anticipated in the future. Other risk factors of HCC development, such as cirrhosis, obesity, aflatoxin B1, and others, also play an important role in the prevalence of the disease in Saudi Arabia.

2 Risk Factors for HCC in Saudi Arabia

2.1 Hepatitis B

Hepatitis B infection is considered the most important factor for HCC development in many countries. Globally, chronic hepatitis B causes more than half of HCC cases, with some regional differences, such as a high percentage of HCC caused by HBV in Korea [10].

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Many studies have investigated the epidemiological status of HBV in Saudi Arabia [11–17]; the prevalence of HBV infection in Saudi Arabia is slightly lower than the global prevalence [7]. A reduction in the prevalence of HBV has been noticed in the country over time, and thus, a low current rate is anticipated [18]. The estimation of HBV rate is mostly based on the findings of screening studies. However, these studies do not reflect the actual number, as some studies focused on high-risk groups such as IV addicts' abusers and human immunodeficiency virus-positive patients. A recent and more representative study in Saudi Arabia that included a large population of more than 70,000 participants who underwent premarital testing showed that the prevalence of HBV was 1.3 % [19].

Patients with chronic hepatitis B have a high risk of developing more serious diseases such as HCC [20]. In Saudi Arabia, the Systematic Observatory Liver Disease (SOLID) registry has data regarding the mortality from end-stage liver diseases [21]. Data has been collected from multiple centers around the country. One study showed that approximately 35% of HCC patients had HBV [22]. Other surveys found that 24–36% of HCC patients were HBV-positive [23].

In Arabic-speaking countries, the mortality of HBV-related HCC is doubled to more than 130% compared to the rest of the world between 1990 and 2010 [24]. A current study from the SOLID registry including screened persons between 2010 and 2015 concluded that HBV-positive people are still young, and the probability of developing HCC is very high in the future [25]. These findings suggest that there will be a rise in HCC cases in Saudi Arabia, while the prevalence of HBV will decrease. This high HBV prevalence was registered in the periods before the introduction of compulsory vaccines.

2.2 Hepatitis C

Hepatitis C is a major contributing factor for the development of HCC in developed countries. In Saudi Arabia, the prevalence of HCV infection is not well-known. A screening program of donated blood suggested that the prevalence of HCV in blood donors is approximately 0.4–1.1% [26]. Premarital testing showed that the prevalence of HCV was 0.33% [27].

An important connection between the prevalence of HCV infection and HCC cases in Saudi Arabia has been suggested previously. A study reported that 74% of HCC cases were HCV-positive [28]. Another study found that approximately 40% of HCC cases were HCV-positive in the western region of the country [29]; however, this study included many ethnicities, which may not reflect the actual HCV status in the Saudi population. In a more recent study from Riyadh, 64% of the HCC cases had an HCV infection with very advanced cirrhosis [30].

Although HCV seems to play a major role in HCC development in Saudi Arabia, screening and treatment plans using oral direct-acting antiviral agents that provide cure rates of up to 90% in HCV patients may eradicate HCC in the future. The drugs are available through the "Saudi special access programs" at different prices [31]. Therefore, a strategic plan should be established in Saudi Arabia according to the World Health Organization plan to eradicate HCV by 2030.

2.3 Cirrhosis

Cirrhosis is considered to be one of the main risk factors of HCC development, regardless of the cause. A cohort study showed that 50–70% of cirrhotic patients died from HCC [32, 33]. Factors such as gender, sex, age, and the period of cirrhosis are associated with the risk of HCC development [34]. In Saudi Arabia, a study involving more than 200 patients with HCC found that cirrhosis was the main cause of HCC development [28]. In this study, 70% of the patients were male, 48% had hepatitis C, 31% had hepatitis B, and 21% had cryptogenic cirrhosis. Another recent study showed that cirrhosis was found in 80% of HCC cases [30]. The previous data are consistent with the global reported numbers.

2.4 Aflatoxin B1

Aflatoxin B1 (AFB1), derived from some fungal species, is one of the HCC development factors in some African and Asian countries [35]. It is believed that this toxin has carcinogenic effects on the liver of highly vulnerable people, such as those with chronic hepatitis [36]. Studies suggested that the effect of AFB1 on HCC development is secondary to genetic variations [37]. In Saudi Arabia, a study showed that AFB1 levels were detectable in 79% of patients with liver diseases of unknown etiology [38]. A high AFB1 level was found in 4% of processed meat products sold in Riyadh [39]. No similar studies were carried out in the other regions. No study has examined the exposure of HCC patients in Saudi Arabia to AFB1.

2.5 Other Risk Factors

Several studies have shown an association between fatty liver disorders and an increase in the chance of developing HCC in obese people [40, 41]. Furthermore, the risk of developing HCC is high in the presence of other factors such as hepatitis C and diabetes [42]. It has been assumed that the prevalence of non-alcoholic fatty liver disease (NAFLD) in Saudi Arabia is 24% [43]. The number of diabetic patients in Saudi Arabia according to the International Diabetes Federation is more than 4 million. These cases represent approximately 20% of the population [44]. An increase in diabetes cases probably has an impact on many diseases such as HCC. In a study involving a Saudi population, 56% of HCC cases had diabetes, and 62% of the cases had both hepatitis C and diabetes [30]. These findings indicate that diabetes and obesity may play a role in the prevalence of HCC in Saudi Arabia.

Heavy alcohol consumption has been associated with an increasing incidence of HCC [45]. In Saudi Arabia, the role of alcohol consumption in HCC has been underestimated since its consumption is prohibited. However, this ban could encourage smuggling or the production of low-quality alcohol that could affect liver function. According to results from the Global Burden of Disease, 17% of the liver cancer cases in Saudi Arabia is as a result of alcohol consumption [7]. The role of alcohol in the prevalence of HCC in Saudi Arabia remains an area of potential future research.

3 Geographical, Gender, and Age Variations in HCC in Saudi Arabia

There is a variation in the incidence and mortality of HCC in different geographical locations in Saudi Arabia. According to the Saudi registry, in 2015, 376 liver cancer (LC) confirmed cases were reported, of which 77% were HCC cases. LC ranked 6th and 12th among Saudi men and women, respectively [46]. In addition, 72.9% of the cases were male, and 27.1% were female, with a male-to-female ratio of 2.6:1. The age-standardized rate (ASR) was 4 and 1.5 for males and females per 100,000, respectively. The case distribution in Saudi Arabia varied among the cities. Riyadh showed the highest ASR in which males accounted for 6.7 per 100,000, followed by the Eastern region and Tabouk that both registered an ASR of 4.6 per 100,000. The ASR in Najran and Makkah was 4.3 and 3.7 per 100,0000, respectively. For females, Riyadh also had the highest ASR (2.5 per 100,000), then the Eastern region (2.3 per 100,000), and Hail and Juof cities (2.1 per 100,000) [46]. The reason for this difference is unknown since the prevalence of HBV and HCV in these provinces is not high compared to other regions of the country [18, 47]. This could be caused by genetic variations or other confounding factors existing in the different regions of the country.

HCC in Saudi Arabia is more common in elderly people compared to youngers. For people of age 1–40 years, 40–50 years, 50–75 years, and older than 75 years, the confirmed HCC cases were in between 0.2 and 0.6%, 1.8 and 7%, 10 and 15%, and 27%, respectively, for the period between 2004 and 2014 [5]. These are compatible with falling of incidence of HCV and HBV in younger populations compared to older populations in the country.

4 Clinical Manifestations

The main features of HCC are right upper quadrant pain and weight loss. Other features such as weakness, ascites, and jaundice are nonspecific presentations of the disease. The features of HCC depend on the stage and presence of cirrhosis [48]. In Saudi Arabia, reported studies of HCC features in the country were not different from the documented features globally. A study in Saudi Arabia found that 91% of the patients presented with hepatomegaly, 76% complained of abdominal pain, 33% had splenomegaly, and 33% presented with abdominal swelling [49]. More than 90% of patients had abnormal liver function tests [50].

5 HCC Diagnosis

HCC diagnosis in Saudi Arabia, like elsewhere, includes a combination of radiological and laboratory investigations. Various imaging approaches, specifically cross-sectional imaging, are important in HCC management. These facilities permit the detection, classification, and staging of HCC cases and the establishment of a suitable treatment and follow-up. In Saudi Arabia, clinical imaging modalities for HCC diagnosis are accessible and available in all tertiary care centers. In addition, trained radiologists are available in most government hospitals, but are few in private hospitals [48]. The majority of tertiary centers have adopted western guidelines for the diagnosis of HCC [46].

6 HCC Management

Management of HCC patients in Saudi Arabia is conducted using different approaches according to the Saudi guidelines for the diagnosis and management of hepatocellular carcinoma that are continuously updated by the Saudi Association for the Study of Liver Diseases and Transplantation (SASLT) [48]. SASLT recommended that management of HCC should occur in a tertiary center which can offer multidisciplinary expertise [48]. It has adopted the American Association for the Study of Liver Disease (AASLD) guidelines for the management of HCC. AASLD has produced a viable algorithm for the management of HCC [51]. The management of HCC is dependent on the number of factors, including tumor stage and size. It is also dependent on the biochemical liver function and the general performance of the patient. The Barcelona Clinic Liver Cancer staging is an adopted prognostic model in Saudi Arabia [48].

Orthotopic liver transplantation (OLT) is considered the most effective method for HCC treatment, as the entire tumor tissue is removed and replaced with more functional tissue [52]. Although this option is available in Saudi Arabia, the shortage of organs for transplantation in the country limits the transplantation opportunities for many HCC cases. In addition, the country lacks cadaveric organ donors due to religious and cultural issues. Liver transplantation from living people has increasingly grown in the country in the last few years, but its role in HCC treatment is beyond the targeted plan [53]. Therefore, more efforts need to be made, especially to encourage donations from cadavers.

Hepatic resection (HR) is another curative option for patients with HCC without cirrhosis or portal hypertension. A small retrospective study found that HR offers 60% survival benefit [54]. HR remains a major surgical intervention and is only offered in tertiary centers. There is no existing database for patients who underwent HR, reflecting its rarity in practice.

Ablation is one of the therapeutic options for HCC. Two of the most widely used ablation interventions in Saudi Arabia are percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA). Both of them are available in tertiary centers in the country, but not in the centers of small cities and private hospitals. In addition, highly trained specialists are available in all hospitals, but the main obstacle is late referral of the HCC cases [54, 55].

Transarterial chemoembolization (TACE) is potentially dependent on the vascular invasion of HCC. TACE is available in tertiary hospitals in Saudi Arabia. In this procedure, the catheter is

connected to the artery that supplies the tumor, and a chemotherapeutic agent is injected. This allows a high concentration of chemotherapy agent to reach the tumor site. One hospital reported the radiological response of TACE which was used in 15 patients with HCC. There was a response in 26%, partial response in 13%, and no change in 33%. In addition, a recent retrospective study of 39 HCC cases treated with TACE showed that the median overall survival was 20 months, median progression-free survival was 9 months, and progressive disease occurred in 54% of the cases [56]. Although TACE is available only in large hospitals in the country, there is a lack of trained specialists who can perform TACE in majority of the hospitals.

Until recently, systematic treatment has not shown any promising outcomes in the management of advanced cases of HCC. The Saudi guidelines for the management of HCC in advanced stages have recommended the use of sorafenib in patients with advanced HCC, which offers modest survival benefits [48]. One study assessed the effectiveness of sorafenib for patients with HCC in Saudi Arabia [57]. The retrospective review included 212 HCC cases treated with sorafenib from 2007 to 2016 [57]. The median age was 68 years, and the patients with hepatitis C and hepatitis B were 44% and 30%, respectively. Although most of the patients were in the advanced stage of HCC, and the doses were reduced, the drug showed effective outcomes compared to the other studies in terms of safety and survival rates. Recent developments in systemic treatment of advanced HCC using a combination of atezolizumab immunotherapy and bevacizumab have shown better survival benefits compared to sorafenib [58]. However, access to new immunotherapy in Saudi Arabia is still very limited to some tertiary cancer centers.

7 An Overview of HCC-Related Research in Saudi Arabia

There have been noticeable recent advances in HCC-related research in Saudi Arabia. The studies primarily focused on understanding the basic molecular pathogenesis of the disease, studying the risk factors, validating new biomarkers, and conducting epidemiological studies. Although a few studies were designed to test the current therapeutic and diagnostic protocols for HCC in the country, most of them were retrospective in nature. In this chapter, some HCC-related research in Saudi Arabia will be highlighted.

7.1 HCC-Related Research on Risk Factors

HBV and HCV are among the main risk factors of HCC development in Saudi Arabia. Studies in the country have been conducted to understand the epidemiology and phylogenetic origin of the virus. Among these studies is a recent study conducted by Al-Qahtani et al. which included 319 HBV cases [59]. They showed that genotype D is the most dominant among the infected patients, and subgenotype D1 represented more than 90% [59]. A previous study in Jeddah included only 15 patients with HBV and showed that half of the cases had viruses with genotype D1, representing more than 70% of the genotype D. The remaining patients had HBV with the C genotype [60]. In the southern region of Saudi Arabia, HBV genotyping was conducted in 160 positive cases. Genotype D was found in 84% of the patients, followed by genotypes A and E with 11% and 4%, respectively [61]. Regarding HCV, the most common genotype of HCV is genotype 4. Genotype 4 accounted for 60%, while genotype 1 accounted for only 25% of the samples obtained from more than 600 HCV cases in Riyadh. Genotypes 2 and 4 were high in females, while genotypes 1 and 3 were high in males [62]. Though previous studies have shown the predominance of HBV and HCV genotypes in Saudi Arabia, further studies should identify the correlation between these genotypes and susceptibility to HCC treatment and development.

Genetic variations or mutations of the virus and the host play a role in the development, progression, and therapeutic outcomes of HCC [63]. In Saudi Arabia, several studies have been conducted in order to understand the implications of

the genomic diversity of the virus and the host on the clinical outcomes of HCC. An example of this is that a study in the country showed that genomic mutations of HBV/D1 were significantly linked to HCC development in Saudi patients [64]. Another study also showed that single nucleotide polymorphism SNP in mir30a of HBV was associated with a high susceptibility to cirrhosis and HCC development [65]. Moreover, another study reported that genomic screening of HBV showed viral mutations in F24Y, E64D, E77Q, A80I/T/V, L116I, and E180A from a Saudis-infected population that correlated with cirrhosis and HCC progression [66]. In addition, another study including Saudi patients showed that several mutations in the HBx gene of HBV were associated with progressive clinical stages of HCC [67]. Genetic diversity of HCV and its implication of HCC outcomes have been established in couple of documented studies conducted in Saudi population. A study revealed that the genetic variation in the DEPDC5 gene significantly influenced HCC progression in the HCV-infected Saudi population [68]. In addition, Toll-like receptor 3 (TLR3) is an important immune player against the virus; 563 HCV-infected Saudis were screened for eight *TLR3* SNPs, and rs5743314 was found to be highly linked to cirrhosis and HCC development [69]. Moreover, genetic variants of PARK2 have been shown to play a role in the development of HCC in HCV-infected patients in Saudi Arabia [70].

A retrospective study in Saudi Arabia was conducted to elucidate possible clinical and pathological differences between HCC cases caused by HCV and HBV. Infected patients with HBV were mainly male and younger age compared to HCV-infected patients. In addition, HCC patients previously infected with HCV presented with advanced cirrhosis. When making the preliminary diagnosis, HCC patients who contracted HBV did not fulfill the Milan criteria for liver transplant compared with patients with HCV-related HCC [30]. This significant difference shows the need for a different screening policy, but the treatment outcome and survival rate of HCC were not influenced by the type of the virus.

7.2 HCC Diagnosis-Related Research

There has been some work on validating HCC diagnosis using established diagnostic methods in Saudi Arabia. In addition, work has also been extended to discover approaches for diagnosing HCC in a noninvasive way. A previous study by a research group in Saudi Arabia on HCC proposed a technique to scan blood and urine samples using fluorescence emission spectra for the differentiation of various liver disorders, including HCC. It has been proposed that this method has a detection accuracy of 80%; however, no validation studies have been conducted to confirm this [71]. In addition, testing for new biomarkers of HCC in the Saudi population has been reported. Golgi protein-73 (GP-73) and prothrombin induced by vitamin K absence-II (PIVKA-II) showed high accuracy in detecting HCC compared to AFP [72]. A later study also confirmed the accuracy of GP-73 in the early diagnosis of Saudis with HCC compared to cirrhotic and healthy controls [73].

Serum alpha-fetoprotein (AFP) is a protein that is found highly in fetal blood and at a lower concentration in healthy adults. AFP is not a sensitive test for surveillance of HCC and has a poor positive predictive value [74]. The ability of AFP to be used as a marker of HCC diagnosis is controversial. A systematic review showed that it has a poor sensitivity for HCC screening at any level [75]. However, a recent meta-analysis concluded that AFP had a good accuracy for HCC diagnosis, and increasing the threshold levels to 400 ng/mL is strongly indicative of HCC, particularly in the context of an isolated nodule >2 cm in diameter [76]. In a multicenter study, AFP was used to diagnose HCC in 206 cases, 199 patients with cirrhosis, and 197 patients with hepatitis [28]. At the best AFP cutoff value, the sensitivity of HCV, HBV, and nonviral HCC was 73%, 65%, and 59%. At the same cutoff, the specificity was 36%, 30%, and 29% for HCV, HBV, and nonviral HCC, respectively. In addition, the study showed that similar sensitivity levels of 39%, 35%, and 32% and specificity levels of 96%, 98%, and 98% were achieved at cutoff levels of 102, 200, and

400 ng/mL, respectively. The positive likelihood ratios of AFP in the study were 2.8, 3.3, 9.9, 23.8, and 21.2 at >11.7, >20, >102, >200, and >400 ng/mL, respectively. The study summarized that in patients with cirrhosis, AFP had a very poor screening and diagnostic ability for HCC [28]. Nevertheless, the continuously rising AFP on serial measurements should prompt a vigorous and thorough radiological investigation.

Clinically, the diagnosis of HCC is based on multimodalities, which include clinical, biochemical, pathological, and radiological factors. Radiological investigations and biopsy should be recommended for patients with changing symptoms and/or increasing AFP levels. The Saudi Guidelines for the Diagnosis and Management of HCC recommend that development of a nodule with an abnormal texture in the liver of high-risk patients should prompt referral to a tertiary center [48]. Liver ultrasonography is recommended as the first modality of radiological diagnosis. It is cheap, easy, and quick to perform. However, it remains operator dependent. King Abdulaziz Medical City, Ministry of National Guard Health Affairs, has reported a case series of 235 patients. The most common radiological presentation was a single nodule measuring less than 5 cm [24]. Another case series of 363 patients from the College of Medicine, King Saud University, suggested that 55% of the patients had large multinodular tumors radiologically [6].

The Saudi Association of Gastroenterology has adopted the AASLD guidelines which recommend the use of a dynamic triphasic computed tomography scan or liver magnetic resonance imaging as confirmatory diagnostic tools. For lesions measuring 1–2 cm, two imaging modalities showed the "classical appearance of HCC" without a need for liver biopsy (if ALP >200 U/L) [51]. For lesions > 2 cm without high AFP levels, if two imaging modalities show the "classical appearance of HCC," biopsy is not required. However, if the lesion does not show the "classical appearance of HCC," biopsy is warranted. Sometimes, with lesions smaller than 1 cm, it can be very challenging to differentiate them from regenerative nodules. It is recommended that the imaging modalities are repeated at 3-month intervals.

7.3 HCC Treatment-Related Research

Compared with other tumors, the treatment of HCC is not well studied, and there are fewer randomized controlled trials to test various interventions and treatments. Surgical interventions are the most effective curative treatments. There are only four centers in Saudi Arabia where liver transplant is offered, three in Riyadh and one in Dammam [55]. In 2017, it was reported that about 2000 liver transplant operations were performed in Saudi Arabia. King Faisal Specialist Hospital and Research Centre (KFSH&RC) has performed 703 liver transplant operations. HCC accounts for approximately 25% of these patients [55]. KFSH&RC reported that downstaging strategies using drug-eluting bead chemoembolization did not improve overall survival before liver transplantation [77].

The lack of cadaver donors remains a global issue including Saudi Arabia. The liver donor transplant program started in 2001 to alleviate liver organ shortages [78]. The rational of liver transplant is to treat HCC as well as underlying problems of cirrhosis. Milan Criteria has been adapted to select those patients who are suitable for transplant [55]. Saudi Centre of Organ transplant is responsible for distributing organs equally between different hospitals in Saudi Arabia. Thus, the system is center- based regardless of the geographic location of the donors [53]. However, there is a problem with the donation system in Saudi Arabia, which could be a potential area of future research. Half of the donated livers were rejected due to a suboptimal donor management. Registration of brain death in Saudi Arabia is not robust enough; only 60% of brain death is documented [53]. An increase in the morbid obesity in Saudi Arabia has been one of the contraindications of the liver donation. It has been reported that out of 629 potential donors, only 87 became actual solid organ donors. This gives a conversion rate of 14% which away below developed nations which is 60%. To overcome organs shortages, KFSH&RC introduced split liver transplant. ABO-incompatible living donor liver transplant (LDLT) has also been initiated at KFSH&RC.

Radioembolization with yttrium-90 (Y90) microspheres is an optional treatment for HCC patients. This therapy has the advantage of being more specific to tumors compared to external radiation therapy. A retrospective study in Saudi Arabia of 28 HCC patients who received Y90 treatment showed MELD scores of 8.5 and 12 before and after the therapy, respectively [79]. Another recent retrospective study to evaluate the local efficacy and tolerability of Y90 therapy in 30 HCC patients in the country demonstrated similar findings with other studies having the same tolerability [80].A phase II trial of chronic daily VP-16 (etoposide) administration in unresectable HCC was performed at KFSH&RC between 1989 and 1991. VP-16 was administered at a dose of 50 mg/ m^2 daily p.o. for 21 days with 1 week off treatment. Etoposide has failed to show any activity in HCC [81].

8 Conclusion

In Saudi Arabia, HCC is the sixth and twelfth most common cancer in men and women, respectively. Hepatitis B and C are considered the main factors of HCC development in the country. A lack of robust screening program for early HCC detection in Saudi Arabia augments its mortality. For those patients whose life expectancy is very short, access to quality palliative care remain suboptimal. Regarding HCC-related research, to our knowledge, there is no oncology center in Saudi Arabia that has active clinical trials for HCC patients. Management of patients with HCC at specialist centers will facilitate designs and execution of clinical trials. It will also facilitate the inclusion of patients in international clinical trials of novel agents.

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Hepatitis C-Induced Hepatocellular Carcinoma in the Middle East

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1 Introduction

Hepatitis C virus (HCV) is a hepatotropic virus that belongs to the *Flaviviridae* family, discovered in 1989. It is a small, enveloped, positive-stranded RNA virus [1]. The virus has seven genotypes based on variations in the nucleotide sequence by >30% and 67 subtypes based on variations at the nucleotide sequence by less than 15% [2].

HCV is well-known to cause acute and chronic hepatitis. More than half of chronic liver disease around the globe is attributed to HCV [3]. Chronic viral hepatitis causes high mortality, and currently, they ranked the 7th leading cause of mortality around the world, with close to 50% of these deaths due to chronic hepatitis C (CHC) [4]. In 2013, 626,000 compensated cirrhosis, 137,000 decompensated cirrhosis, 16,100 hepatocellular carcinoma (HCC) cases, and 33,000 liver-related deaths were attributed to CHC. The number of deaths related to HCV complications has increased to around 399,000 deaths in 2015 [5].

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Based on the World Health Organization (WHO) reports in 2017, the estimated global number of patients with CHC was 71 million people [6]. Around one-third of these patients will progress to advanced fibrosis and cirrhosis, and close to 5% per year of those cirrhotic patients will develop HCC [5]. Not all patients infected with HCV will develop chronic hepatitis; up to 35% will clear the virus spontaneously, and the remaining 65% will progress to CHC, defined as persistent HCV RNA in the blood for more than 6 months [7]. Several studies have looked at factors associated with spontaneous clearance of acute HCV and identified age at acquiring the infection, female gender, ethnicity, and coinfection with other viruses such as HIV to be the most important factors [8–10]. Thomas et al. showed that the HCV clearance rate in non-black is higher with an odds ratio of 5 [8]. These ethnic differences in HCV clearance could be partly explained by the immune system response to different insults.

Genome-wide association (GWA) studies showed that HCV spontaneous clearance and treatment response are related to patients' genetic variations [11–14]. Different single-nucleotide polymorphisms (SNPs) in the IL28B gene in chromosome 19 coding for type III interferon INF-λ 3, as well as other SNPs in different chromosomes, were associated with HCV treatment response as well as HCV spontaneous clearance [15–17].

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There is marked variation in the natural history of CHC, ranging from minimal liver inflammation to progressive liver fibrosis and the development of complications such as decompensated cirrhosis and HCC [9, 18]. The natural history of CHC is affected by many factors, which can be divided into host-related factors such as age at acquiring HCV infection, coinfection with other viruses such as hepatitis B virus (HBV), human immune deficiency virus (HIV), presence of metabolic syndrome especially diabetes and non-alcoholic steatohepatitis (NASH), and concomitant alcohol abuse and to a less extent viral factors such as viral load and genotype [9, 18–22].

Despite the progression of CHC to advanced fibrosis, only a minority of CHC patients will develop HCC [23]. This progression is due to the complex interaction between HCV, the patient, and environmental factors that promote carcinogenesis. The mechanism of oncogenicity in HCV is different from HBV, where the viral DNA is integrated into the host chromosomal DNA. HCV is a cytopathic virus that replicates within the liver cell cytoplasm, promoting hepatocyte proliferation and inducing cellular inflammation. It also leads to mitochondrial damage and induces the production of reactive oxygen species (ROS). The induced inflammation and ROS production cause genomic mutations and instability, which is the nidus for the development of HCC [24].

HCC accounts for more than 85% of primary liver cancer (LC) [25]. In most cases, HCC develops on a background of liver cirrhosis except in chronic hepatitis B (CHB), where HCC can develop in non-cirrhotic liver. Liver cirrhosis due to chronic viral hepatitis is the leading risk factor for the development of HCC [26].

The risk of HCC is increased 15–20-fold in patients infected with CHC. Patients with liver cirrhosis due to CHC are at risk of developing complications such as decompensated cirrhosis at a rate of 3–6% per annum and development of HCC at 1–5% per annum [23].

Despite the availability of direct-acting antiviral agents (DAAs), HCV and its related complications will remain a significant global health issue in the coming years. This could be attributed to many newly discovered cases, a high number of unrecognized HCV-infected cases, and access to the new DAA [27].

2 HCV Epidemiology in ME

The Middle East (ME) is a transcontinental region that includes 18 countries from Asia and Africa (Fig. 1). The majority of those countries (13 out of 18) are part of the Arab world. The term "Middle East" may have originated in the 1850s in the British India Office. However, in 1902 the American naval strategist used the term the Middle East and it since then became widely known [28]. The ME has a total population of 371 million based on World Bank data in 2010.

The region demonstrates a wide range of anti-HCV and viremic prevalences and diversity in HCV genotype distributions. WHO estimates that there are at least 21.3 million HCV carriers in the Eastern Mediterranean countries, close to the number of carriers estimated in the Americas and Europe combined [29]. Country-level anti-HCV prevalence is classified into low (<1.5%), moderate (1.5–3.5%), and high (>3.5%) levels [29].

The reported prevalence of HCV in ME is affected by multiple factors. The reported prevalence in certain countries such as the Gulf Cooperation Council (GCC), a political and economic alliance of six Middle Eastern countries—Saudi Arabia, Kuwait, the United Arab Emirates, Qatar, Bahrain, and Oman—is affected by the population demography of those countries. More than one-third of the population of the GCC countries are migrants. Based on 2015 population statistics, the number of temporary worker immigrants in certain countries such as Qatar and UAE was more than 80% [30, 31]. Modeling prevalence demonstrated a 10% increase in HCV prevalence since 2007 in Qatar and UAE due to foreign workforce from endemic countries [30, 31]. Therefore, any epidemiological data that does not differentiate between nationals and temporary worker migrants will be affected by the HCV epidemiology profiles of the migrants' countries of origin.

Fig. 1 Countries that constitute the ME

The prevalence data from other Middle Eastern countries such as Turkey, Egypt, Iraq, and Jordan are less affected by immigration. However, most studies looking at HCV prevalence from ME used non-homogenous populations such as blood donors, hemodialysis patients, and multi-transfused patients. Those groups of patients do not represent the entire country population, and in fact, they are a high-risk population for HCV infection. In addition to that, older studies used anti-HCV as a marker of infection. The characteristic performance of anti-HCV testing using first- and second-generation EIA was not optimal [32].

The prevalence of HCV infection in Middle Eastern countries varies geographically. A low prevalence of HCV (<1.5%) was reported in Kuwait, Oman, Qatar, Iran, and UAE. At the same time, Iraq, Lebanon, Saudi Arabia, Turkey, and Syria had moderate prevalence (1.5–3.5%) and a high majority (>3.5%) in Egypt and Yemen. Table 1 showed the prevalence of HCV and the most common genotype among Middle Eastern countries [33–39].

Egypt is considered to have the highest prevalence of HCV in the world. Based on the Egyptian Demographic Health Survey (EDHS) that was conducted in 2008 on a large country representative sample, the prevalence of HCV among the age group 15–59-year-old was found to be 14.7%, 10% of those found to have chronic infection and genotype 4 was found in 90% of the patients [40]. In another large Egyptian Health Issues Survey (EHIS) conducted in 2015, including the younger age group, the seroprevalence of HCV was found to be 6.3% among the age group 0–59 years old. The investigators reported a significant reduction of 32% and 29% in HCV antibody- and HCV RNA-positive people [41]. The reported prevalence of HCV in certain Middle Eastern countries varies between different regions within the same country. In a population-based study in Iran by Merat et al., the prevalence of HCV in Iran was 0.3% in Tehran, 1.6% in Hormozgan, and 1.0%

Table 1 Prevalence of HCV and the most common genotype among Middle Eastern countries

Country	Population	Prevalence	Common genotype
Afghanistan	32,527,000	0.5%	Genotype 3
Bahrain	1,377,000	1.2%	Genotype 1
Egypt	91,508,000	6.3%	Genotype 4
Iran	79,109,000	0.2%	Genotype 1
Iraq	36,423,000	0.2%	Genotype 4
Jordan	7,595,000	0.3%	Genotype 1
Kuwait	3,892,000	0.8%	Genotype 4
Lebanon	5,851,000	0.2%	Genotype 1
Oman	4,491,000	0.4%	Genotype 1
Palestinian	4,668,000	2.2%	Genotype 4
Qatar	2,235,000	1.6%	Genotype 4
Saudi Arabia	31,540,000	0.3%	Genotype 4
Syria	18,502,000	3.0%	Genotype 4
Turkey	84,181,300	0.6%	Genotype 1
United Arab Emirates	9,157,000	1.3%	Genotype 1
Yemen	29,710,300	0.8%	Genotype 4
Cyprus	1,189,000	0.46%	Genotype 1 and 4

in the Golestan provinces [42]. Other studies reported a prevalence of 15.6% in Fars, 44.3% in Kerman, 29.6% in Zahedan, 59.1% in Hamadan, 71.3% in Gilan, and 76.7% in the northwest of Iran, representing an overall prevalence rate of almost 50% [43]. Similar findings were also reported by different researchers from Saudi Arabia with different HCV prevalence in different regions of Saudi Arabia [44].

Looking at risk factors for HCV transmission, reports from different Middle Eastern countries showed that medical practice especially before the era of blood and blood product screening such as blood or blood product transfusion in cases of thalassemia or hemophilia, hemodialysis, hospital instrumentation, and invasive procedures played a significant role in HCV transmission [45]. Alnaamani et al. showed that 41% of multi-transfused thalassemia patients from Oman were positive for anti-HCV. The majority of those patients were transfused before 1990 [46]. Age and level of education were also associated with HCV infection in certain Middle Eastern countries such as Yemen and Syria [47, 48]. Other risk factors for HCV transmission, such as intravenous drug abuse, piercing, and tattooing, as well as a risky sexual practice, contributed further to the spread of HCV among Middle Eastern countries [49]. Perinatal transmission from HCV-infected mothers to their newborn babies played a less important role as the risk of transmission is less than 5% unless the mother is coinfected with HIV. Intravenous drug abuse (IVDA) was found to be a major risk factor for HCV transmission in many Middle Eastern countries, including but not limited to Egypt, Lebanon, Oman, Palestine, Saudi Arabia, and Syria. Certain community groups such as prisoners and female sex workers are at increased risk of acquiring HCV infections, especially HIV-positive prisoners. The prevalence of anti-HCV among female sex workers in Lebanon, Libya, and Syria was higher than the general population [49, 50].

The majority of patients with positive anti-HCV in ME are chronically infected with HCV. The overall pooled mean viremic rate (positive HCV RNA) is 67.6% (95% CI, 64.9–70.3%). This figure is similar to that found in large population-based and nationally representative surveys [51]. Studies looking at the viremic rate at certain Middle Eastern countries reported the viremic rate to be 51.6% in Saudi Arabia and approximately 70% in Egypt and the UAE [5].

2.1 HCV Genotype and Subtype

HCV has 7 genotypes and 67 sub-genotypes based on variations at the nucleotide sequence by >30% and less than 15%, respectively [52]. There are several methods used to determine HCV genotypes; all of the methods use direct sequencing of certain regions of the HCV genome mainly (NS5, core, E1, and 5' UTR regions) using polymerase chain reaction (PCR) in combination with the phylogenetic analysis [53–56].

The distribution of HCV genotype varies between different regions of the world. HCV genotype 1 is the most prevalent worldwide representing 49.1%, followed by genotype 3 (17.9%), 4 (16.8%), and 2 (11%). The differences in HCV worldwide distribution are attributed to ancient world trade and human migration [57]. Genotype 4 is the most common in the ME,

accounting for 71% of all HCV-infected patients, followed by genotype 1, since most HCV-infected patients are from Egypt, where 90% of infected people have genotype 4 (Table 1) [58].

Analysis of HCV reports from ME showed that there are two main patterns of HCV genotype distribution. The first pattern represents most Arab countries where genotype 4 is the most common genotype except Jordan, Oman, Lebanon, Bahrain, and UAE, where genotype 1 is the most common [59]. Al-Busafi et al. reported the most common genotype in Oman to be genotype 1, representing 44%, followed by genotype 3, representing 35% [50]. The second pattern represents non-Arab countries (Turkey and Iran), where genotype 1 is the most common [59]. In Iran, genotype 1a is predominant for HCV, followed by genotype 3a and 1b, in addition to mixed genotypes [60]. Similar findings were reported from Turkey, where genotype 1b is the most common genotype representing >70% of HCV-infected people, followed by 1a. This HCV genotype distribution pattern in Turkey is similar to that reported from Eastern and Southern European countries [61].

3 Epidemiology of HCC in the ME

With an incidence of one million LC cases in 2016, LC is ranked as the seventh most common cancer worldwide. The majority of LC (75%) are HCC, followed by cholangiocarcinoma in 1–20% of cases [25]. HCC represents a leading cause of cancer-related mortality. More than 800,000 patients died in 2016 due to HCC. This high number of death ranked HCC as the fourth deadliest cancer [62]. Despite recent marked improvements in treatment modalities, the prognosis for HCC patients remains poor, with an average 5-year survival rate of approximately 5–6% [63]. Unfortunately, this is mainly attributable to a lack of access to medical facilities in many underdeveloped countries [64].

HCC is more common among males than females. The gender-specific age-adjusted incident rate (AAIR) ratio ranges from 1.3 to 3.6 worldwide. In high prevalence regions, the incidence of HCC rises after the age of 20 and peaks at 50 years of age [65].

There is marked variation in the prevalence of HCC in different parts of the world, with more than two-thirds of cases reported from East and South Asia as well as sub-Saharan Africa [66]. This is mainly due to differences in the prevalence of viral hepatitis, particularly hepatitis B and C, the predominant causes of liver cirrhosis, a known risk factor for HCC [67, 68]. The introduction of hepatitis B vaccination schedules in many countries has led to a marked reduction in HCC cases, with improvements in medical facilities and the designated screening programs similarly expected to help increase the detection and, therefore, reduce the incidence of HCC in these regions [69, 70].

The estimated risk of developing HCC in patients with CHC is 15–20 times higher than healthy persons, and this risk is further increased if CHC patients progressed to cirrhosis [71]. The age-standardized incidence rate (ASIR) of HCC in the Eastern Mediterranean countries based on the Global Burden Disease (GBD) study (2015) was 8.1 per 100,000 men and 4.7 per 100,000 in women [72]. The incidence and mortality of HCC in the Middle Eastern countries have increased based on the GBD 2015 [72]. However, such data should be interpreted cautiously. We have to keep in mind that cancer registries are incomplete in most Middle Eastern countries, and therefore the true incidence of HCC is underestimated. The absence of infrastructure and widespread medical facilities in countries affected by wars such as Iraq and Afghanistan will decrease the number of diagnosed cases as well as reported cases. Higher incidence rate and mortality related to HCC in other Middle Eastern countries could be attributed partly to improved healthcare facilities in certain countries as GCC; therefore, more cases are diagnosed and reported.

Cancer registry reports from certain Middle Eastern countries such as Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia, Bahrain, and Lebanon showed a lower incidence rate of HCC than high incidence countries in Southeast Asia and sub-Saharan Africa. The exception is Egypt,

where the incidence of HCC is considered high, most likely due to the high prevalence of HCV.

HCC is ranked the fourth most common cancer in Egypt and the second cause of cancer-related mortality in both genders [72]. The highest age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) of LC were observed in Egypt and Qatar in 1990 and 2017. There was a rise by close to 12% in the ASIR of LC in ME from 1990 to 2017. On the other hand, there was a decline in ASIR of LC in certain Middle Eastern countries such as Yemen, Lebanon, Oman, Syria, Turkey, Afghanistan, Bahrain, Iraq, and Qatar from 1990 to 2017 [73]. This decline in ASIR could be due to improvement in the health system in certain countries such as Oman, Bahrain, Qatar, and Turkey and underreporting in certain countries due to many factors such as wars in Yemen, Syria, and Afghanistan [73]. Based on the Saudi Cancer Registry (SCR) reported in 2014, LC was ranked the sixth most common cancer in Saudi men and the ninth in Saudi women [74]. The ASIR for LC was 4.5 per 100,000 populations, and the ASMR was 4.2 per 100,000 populations based on 2018 data from the International Agency for Research on Cancer (IARC). The ASIR of LC in Saudi Arabia and Kuwait was a bit higher compared to other GCC countries. The reported ASIR per 100,000 populations for Bahrain was 3.4, Qatar 4.1, United Arab Emirates 4.2, and for Oman 4.4 [75]. Alghamdi IG et al. looked at all the documented cases of LC from different regions of Saudi Arabia between 2004 and 2014 and reported 300 patients (9.1%) per year. Old age, mean age of 75 years old, Saudi males were commonly affected [76]. Al-Naamani et al. reported the characteristics of HCC among 284 patients from Oman and showed the mean age of presentation was 61.02 ± 11.41 years. Most of the patients (67.6%) were male. The majority had liver cirrhosis (79.9%), with the most common etiologies being CHC (46.5%) and CHB (43.2%) [77]. The highest ASIR and ASMR of LC among both genders were reported from Egypt at 49 and 3.8 per 100,000 populations, respectively [78].

HCC is not common in certain countries such as Lebanon, ranking 14th among both males and females with an ASIR of 3.5 and 2.2 per 100,000, in both males and females, respectively [79]. Different studies from Iran reported different incidence rates from other regions of Iran. This discrepancy in the incidence rate is most likely related to the prevalence of various risk factors and the accuracy of diagnosis and registration of cancer in different regions [80, 81]. In a meta-analysis by Hassanipour et al., LC's incidence rate among Iranian men and women was 1.66 and 1.2 per 100,000 populations, respectively. This incidence rate is lower when compared to Asian countries [82]. The Turkish ministry of health report in 2003 showed the incidence of HCC was 0.83 per 100,000 populations. There was no difference in the annual incidence of HCC in Turkey between 2000 and 2003 [83]. Based on the Turkish multicenter study of 222 patients with HCC, Alacacioglu et al. showed that the median age of HCC patients was 62 + 11.3 years. The majority of the patients (76.9%) were males with liver cirrhosis (74.2%) [84].

4 Risk Factors of HCC in ME

The main challenge still present in ME is the high prevalence of chronic viral hepatitis. Therefore, prevention of infection with hepatitis B and hepatitis C is the key to reduce the burden of viral hepatitis complications such as HCC in ME. The major risk factors for HCC are the presence of cirrhosis due to any etiology and HBV infection. Most HCC arises on the cirrhotic liver [85–87].

The marked improvement in the management of liver cirrhosis and screening for HCC over the last few decades has increased liver cirrhosis patients' survival and therefore increased the chance of development of HCC [88–90]. However, screening and diagnosis of early-stage HCC may lead to better treatment outcomes [91].

Other risk factors, such as aflatoxin B exposure, are important in certain parts of the world, especially Asia and Africa [92]. Liver cirrhosis due to hepatitis C and NASH are common risk factors for the development of HCC in the developed world [93, 94]. Studies and reports from ME showed marked variation in the etiology of

HCC. Chronic viral hepatitis is still the most common etiology of liver cirrhosis and HCC in the ME.

The ASIR of HBV-related HCC in ME decreased markedly from 1990 before the era of HBV vaccination to 2017; however, despite the marked reduction in HBV infection incidence, it remains the second common cause of HCC in ME. This is most likely due to the complications of HBV-infected people before the era of vaccination [73]. HBV-related liver cirrhosis is the most common etiology of HCC in Lebanon and Turkey, followed by HCV and alcohol-related cirrhosis, respectively.

CHB is associated with higher risks for HCC [95]. There is a closer relationship between HBV infection and the development of HCC. CHB patients can develop HCC at any stage of their disease; however, most develop HCC after reaching cirrhosis [96]. The risk of developing HCC is 100-fold higher for patients infected with HBV compared to those who are not infected [96]. Previous studies demonstrated that male gender, old age, high serum HBV DNA, pre-core and core promoter mutations, and the presence of cirrhosis are risk factors for the development of HCC among CHB patients [97–99].

The majority of Middle Eastern countries have introduced HBV vaccination, reaching 90% coverage before the age of 1 year. Unfortunately, this is not complemented by highly viremic pregnant women's treatment neither administration of hepatitis B immunoglobulin to babies of HBV-infected mothers. Moreover, the rate of HBV diagnosis in ME is low (6%); besides, only 2% of HBV treatment-eligible patients were treated [100]. All of the above obstacles will reduce HBV elimination activities in Middle Eastern countries.

CHB patients coinfected with HDV are at higher risk of acceleration to cirrhosis and, therefore, developing HCC [101]. Coinfection with HDV leads to accelerated fibrosis and cirrhosis in more than 70% of cases [102]. The reported prevalence of hepatitis D virus (HDV) among CHB patients in Jordan was 23% [103]. The prevalence of HDV among patients with HCC differs between different Middle Eastern countries. The reported prevalence in Jordan was 67%; however, patients with HDV coinfection were older than those with HBV mono-infection [103]. The reported prevalence of HDV in Turkey ranges from 18.8% to 23.0% of HBsAg-positive HCC [104, 105]. These findings suggest an association between HDV and HCC.

Non-alcoholic fatty liver disease (NAFLD) is becoming the leading cause of cirrhosis and HCC worldwide. The world prevalence of NAFLD is estimated at around 25% [106]. The prevalence of NAFLD in ME is approximately 30%, which is considered one of the world's highest prevalence [106]. Despite the limitations of the studies quoted by Younossi et al., the high prevalence of DM, obesity, and metabolic syndrome is reported in multiple publications [107]. GCC states reported the highest prevalence of obesity in the world among the young age population. This population will most likely carry their obesity and their associated NAFLD into adulthood and develop complications such as liver cirrhosis and HCC [108]. NASH-related HCC had the highest ASIR in ME, and the development of HCC on the non-cirrhotic liver was also described in patients with NASH [109].

For a long time, CHC is the most common cause of HCC in ME, followed by CHB. Chronic viral hepatitis accounted for approximately 70% of all HCC in ME [6]. Studies looking at the epidemiology of HCC in ME reported that the majority (70%) of patients with HCC from Egypt, which has the highest prevalence of HCV worldwide, were found to have markers for HCV infection [110, 111]. Similarly, more than one-third (39.5%) of HCC patients from Saudi Arabia were found to be positive for anti-HCV [34, 76]. The pathogenesis of HCV-induced HCC will be discussed later in this chapter.

The mortality related to HCC in ME is declining in the young population, most likely due to HBV vaccination and the high cure rate of HCV associated with DAA treatment [100]. Thus, future trends in HCC in Middle Eastern countries are expected to come down and to have a favorable outcome than other parts of the world, such as East Asia and Africa [112].

4.1 Risk Factors of HCC Among HCV-Infected Patients

The incidence rate of HCC related to CHC in the ME has increased by 16% over 17 years, from 1990 to 2017 [73]. The interaction between CHC and the host immune system is complex. This interaction involves multiple factors leading to the development and growth of HCC. The vast majority of patients with CHC develop HCC on a background of cirrhosis. However, approximately 15% of CHC patients who develop HCC have no cirrhosis [113–116].

Multiple host and, to a lesser extent, viral factors play roles in developing liver cirrhosis and HCC. Host factors such as chronic excessive alcohol consumption, NASH, and coinfection with HBV and HIV play major roles in accelerating liver fibrosis [98]. Poynard et al. showed that patients with CHC who consume more than 50 g of alcohol daily had a 34% higher rate of progression to advanced fibrosis compared to non-drinkers regardless of the age and duration of infection [9]. Similar findings were reported by researchers from Japan who showed that patients with HCV who consume more than 65 g of alcohol daily for more than 5 years have RR, 3.04; 95% CI, 1.31–7.09; and $P < 0.01$ of developing HCC [117, 118]. Further evidence of the additive risk of development of HCC in patients with HCV who consume a large amount of alcohol for many years was shown in the Dionysos study by Donato et al. from Italy [71].

NASH is a common risk factor for the development of HCC among CHC patients. There is a complex relationship between NASH and CHC. CHC, especially genotype 3, is known to induce fatty liver through the direct cytopathic mechanism, and NASH tends to accelerate liver fibrosis progression in patients with CHC [119]. The prevalence of fatty liver among patients with CHC varies from 40% to 80% [120]. However, the reported prevalence of NASH among CHC patients is much lower, around 4–10% [121–124]. Bedossa et al. showed that patients with CHC and NASH tend to have advanced fibrosis compared to patients with CHC alone. A higher grade of fibrosis and cirrhosis will predispose CHC to the risk of HCC [123].

HBV is a carcinogenic virus. Patients with CHB are at risk of developing HCC at any stage of their liver fibrosis. CHC coinfected with CHB tends to have a higher incidence of HCC of 6.4% as compared with an incidence of 2% for CHB alone and 3.7% for HCV infection alone [125]. Zampino et al. evaluated the development of HCC among patients with viral hepatitis. They found that 14% of HCV coinfected with HBV developed HCC compared to 2% and 4% among HBV and HCV mono-infection, respectively [126]. A similar synergistic effect was reported by Bevegnu et al., who showed 36% of HCV coinfected with HBV patients developed HCC compared to only 6% of CHC patients and 11% of CHB patients [127]. This additive risk of development of HCC in coinfected patients was also demonstrated in Asian studies. Oh et al. reported a hazard ratio of 115 for developing HCC in coinfection, 17 in HBV mono-infection, and 10.4 in HCV mono-infection [128]. Certain factors such as alcohol consumption, genetic factors, and NASH presence could contribute to the higher risk of development of HCC in HBV/HCV coinfected patients [71, 129, 130].

HIV is common among CHC patients. Almost one-quarter of HIV patients are coinfected with HCV [131]. Liver disease is a major cause of non-AIDS-related deaths, accounting for 16% of mortality among HIV-infected patients [132]. The introduction of highly active antiretroviral therapy (HAART) in the mid-1990s to treat HIV has markedly changed the natural history of HIV infection. The improvement in the survival of HIV patients led to a significant reduction in diseases related to the human immunodeficiency syndrome. Prior studies demonstrated accelerated liver injury in HIV mono-infection and HIV coinfected with HCV [133, 134]. Puoti et al. looked at an Italian cohort of 41 HIV patients who developed HCC and demonstrated an unfavorable association between HIV/HCV coinfection and HCC behavior (infiltrating tumors and/or extra-nodal metastasis at presentation (OR = 11.8; $P < 0.001$). HIV infection was independently associated with poor survival (hazard

ratio, 1.63; $P = 0.015$) [135]. Similar findings were reported by Beretta et al. in 2011, who demonstrated a shorter survival rate among young patients coinfected with HIV and HCV who develop HCC [136].

Most of the studies mentioned above were observational studies with a small number of patients, and there was no adjustment for other risk factors such as alcohol intake and the presence of NASH. In addition to that, these studies were performed before the era of DAA, where a significant number of patients were not treated for HCV using interferon-based therapy. The above findings were not demonstrated by Marcon et al., who looked at 399 Brazilian patients with HIV coinfected with HCV and compared them to 405 monoinfected with HBV or HCV. One-third of HIV-negative patients developed liver cirrhosis compared to 16.5% of coinfected ($P < 0.001$). HCC was diagnosed in 10 HIV-coinfected patients compared to 26 monoinfected with HBV or HCV [137].

One of the crucial factors for the development of HCC in patients with CHC is type 2 diabetes mellitus (DM-2). DM-2 is characterized by hyperglycemia, hyperinsulinemia, and insulin resistance. DM-2 is becoming a major cause of morbidity and mortality around the globe. The prevalence of DM-2 in ME has increased enormously over the last few decades. DM-2 has been found to increase the risk of different types of malignancies, such as breast, endometrial, renal, and GI cancers such as colon, pancreatic, and liver cancers [138–142]. DM-2 may induce liver fibrosis and HCC through different mechanisms, including hyperglycemia, hyperinsulinemia, insulin resistance, and activation of insulin-like growth factor (IGF) signaling pathways. These mechanisms are known to have proliferative effects such as insulin and insulin-like growth factor 1 and oncogenic effects such as hyperglycemia [143]. In addition to that, DM-2 also predisposes patients to NASH, which may progress to cirrhosis in up to 5% of cases [144]. Rousseau et al. looked at the 3107 male cancer cases and 509 population controls, used information on diabetes and several covariates collected by interview, and found that the risk of HCC was increased among people with diabetes and adjusted OR was 3.1 (95% CI: 1.1, 8.8) [145]. Similar findings were reported by El-Serag et al., who performed a meta-analysis of 13 cohort studies and 13 case-control studies and found that DM is associated with an approximately 2.5-fold increased risk of HCC [146]. The risk of developing HCC is significant in the presence of other HCC risk factors, such as chronic viral hepatitis, high alcohol consumption, and liver cirrhosis.

5 Pathogenesis of HCV-Induced HCC

The exact mechanisms of development of HCC in patients with CHC are unknown despite the establishment of the relationship between CHC and the formation of HCC [147, 148]. Chronic viral hepatitis is the leading cause of HCC. HCV has become an important risk factor for HCC in regions with intermediate-incidence areas such as the ME [73]. More than half of HCV-infected patients will develop cirrhosis, and 14.4% are predicted to develop HCC [149]. Different mechanisms of HCC development have been postulated to explain the causal relationship between HCC and HCV. Since the discovery of HCV in 1989, multiple studies using animal models revealed that HCV changes many cell signaling pathways involved in cell proliferation, migration, and transformation. Many of these changes will ultimately lead to chronic inflammation and fibrosis. The interaction between the host, the virus, and other additive mechanisms leads to the development of HCC (Fig. 2).

5.1 Viral-Related Mechanism

An extensive network of tubules and flattened sacs within the hepatocyte cytosol called the endoplasmic reticulum (ER) plays an essential role in lipids and protein biosynthesis, therefore maintaining hepatocyte function in the liver. CHC infection poses significant stress on the ER through alterations of protein synthesis, degrada-

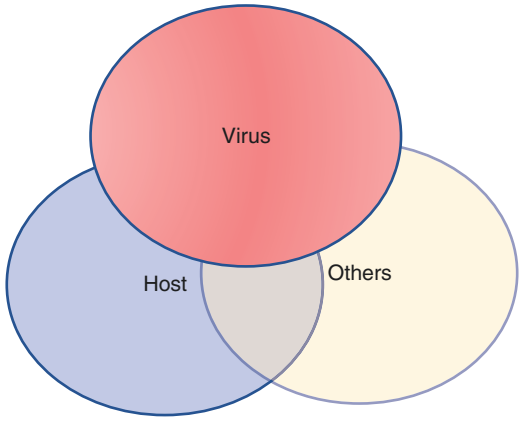

Fig. 2 Interaction between viral, host, and other additive mechanisms

tion, or folding. CHC infection, especially genotype 3, is associated with fatty liver. The accumulation of fatty acids and cholesterol within the infected hepatocyte may inhibit protein degradation, adding more stress to the ER. Due to these reasons, the stressed ER can activate specific signaling pathways leading to inflammation, cellular injury, fibrosis, and cirrhosis [150–152]. In addition to the stress imposed by CHC on ER, continuous viral replication increases hepatocyte's DNA damage, genomic instability, and ultimately death of hepatocytes through different forms such as apoptosis, necrosis, and autophagy [153].

Autophagy is the process by which the cells remove misfolded or aggregated proteins and clear damaged organelles and eliminate intracellular pathogens. Autophagy is essential for maintaining the cellular source of energy during extreme cellular stress. Uncontrolled autophagy has been linked to the development of HCC [154].

The stress produced by HCV infection is maintained and amplified by certain inflammatory cytokines and interferon (IFN). Infected liver cells respond to continuous stress imposed by CHC through unfolded protein response, antioxidant response, and induction of integrated stress response that enhances the transcription of many genes required for survival [155]. Analysis of whole-exome sequencing of HCV-related HCC tumors demonstrated downregulation of many suppressor genes, mutations in cancer driver genes, and genomic instability in the majority of cases [156, 157].

5.2 Host-Related Mechanisms

The innate and adaptive immune responses are both activated during HCV infection. Activation of both responses leads to the recruitment of many inflammatory cells. Production of interferons (IFNs) and certain cytokines by the immune system during HCV infection is a characteristic of early host immune responses [158]. HCV develops different mechanisms that impair the cytotoxic and immunoregulatory activities of immune cells to avoid early host immune responses and develop chronic infection [159].

CHC infection is characterized by immune-mediated inflammation with the production of chemokines, metabolites, and growth factors that promotes liver regeneration and fibrosis. The repeated cycles of liver cell death and regeneration could drive the formation of HCC [160, 161].

5.3 Additive Mechanisms

More than one etiology of liver cirrhosis can be found in the same patients leading to accelerated fibrosis, cirrhosis, and finally development of HCC. Coinfection with hepatitis B doubles the risk of development of HCC compared to each virus alone [162]. Similarly, patients with HCV and HIV coinfection are at higher risk of developing liver cirrhosis and HCC [163].

Components of metabolic syndrome such as obesity, type 2 diabetes, and associated NASH have been found to accelerate HCV-induced liver cirrhosis and HCC [164, 165].

The mechanisms involved in the HCV-induced HCC are similar to other etiologies leading to liver fibrosis, cirrhosis, and HCC. ER stress leads to chronic inflammation, cell death and regeneration, fibrosis, and the development of HCC. Activation of oncogenes and loss of tumor suppressor can play some role in the development of HCC; however, the development of HCC

post-HCV eradication is not fully explained by the previously mentioned mechanisms.

Multiple studies demonstrated different immune-related changes post-HCV treatment using interferon-free therapy. A rapid decline in HCV RNA leads to memory re-differentiation and reduced lymphocyte activation, restoration of HCV-specific CD8+ T cell function, modulation, and normalization of NK cell function [166–168]. These changes lead to loss of immune response to neoplastic cells and therefore HCC recurrence after DAA treatment.

6 Strategies to Prevent HCV-Induced HCC

Many patients with HCC are asymptomatic when tumors are at an early stage. Therefore, unfortunately, the majority are diagnosed at advanced stages, which is associated with a dismal prognosis [169]. One study from KSA showed that most of the patients were diagnosed at an advanced stage (53% had cancer of the Liver Italian Program score (CLIP) of 4–6 (advanced stage), and 55% had large multinodular tumors) [170]. A similar finding was shown in other country-specific reports from Qatar [171] and Oman [77]. Despite advancements in the treatment modalities of different types of cancers, HCC is considered a highly refractory cancer to therapeutic interventions [172]. It is well-known that prevention and early detection are the most rational and effective ways to substantially impact cancer-related outcomes rather than starting treatment at an advanced stage [173]. This highly supports the importance of considering prevention and early detection of HCC in high-risk patients [172]. Like any other preventive diseases or cancers, HCC prevention constitutes three levels of interventions (Table 2). The primary prevention focuses on preventing and eliminating exposure to HCC-related risk factors at an early stage using lifestyle and dietary changes, vaccination (under research), and avoiding exposure to environmental factors or carcinogens in an etiology-specific manner. Next is secondary prevention, which focuses on early detection or chemoprevention of HCC in high-risk patients. Lastly, tertiary prevention focuses on the prevention of the progression or recurrence of HCC [174].

Table 2 Levels of prevention against HCV-related HCC

Level	Description	Examples
Primary prevention	Prevention of new HCV infection	Screening blood products Universal precautions to prevent blood contamination in healthcare settings Education programs for high-risk patients Treatment of HCV-infected patients
	Prevention of fibrosis progression to cirrhosis	Treatment of HCV-infected patients Modifications of other related factors (i.e., treatment of HBV, HIV, lifestyle modifications in NASH)
Secondary prevention	Prevention of progression of cirrhosis or regression of cirrhosis	Treatment of HCV-infected patients Chemoprevention
	Early detection of HCC to improve treatment outcomes	HCC screening (liver ultrasonography with/without alpha-fetoprotein)
Tertiary prevention	Prevention of tumor progression or recurrence after curative treatment	Antiviral therapy for HCV Locoregional therapies Chemoprevention Close monitoring

Modified from Hoshida et al. [174]

6.1 Primary Prevention

Primary prevention of HCV-related HCC includes prevention of new HCV infection either through vaccination (under research) or prevention of transmission. Although research efforts are still ongoing, unlike HBV infection, vaccines for HCV infection are currently unavailable. The development of an effective HCV vaccine is challenged by several viral factors, including the lack of a neutralizing antibody and the diversity of the viral genome [175, 176]. Therefore, the primary

prevention against HCV transmission depends mainly on universal precautions, especially through contaminated blood or blood product transfusion, sterilization of medical instruments, and intravenous injection use (Table 2).

As we mentioned earlier, several reports from different Middle Eastern countries showed that healthcare-related exposures were most frequently reported, followed by exposure to intravenous drug use [177]. Blood and blood product transfusion, hemodialysis, surgical and other invasive medical procedures, dental practice, and medical injections were identified as key healthcare-related exposures [177]. The other important primary prevention method is eradicating HCV infection using antiviral therapies. Effective treatment of HCV is associated with preventing transmission of the virus as well as preventing progression to advanced fibrosis/cirrhosis and development of HCC [178]. Apart from Egypt, most Middle Eastern countries have not yet implemented a national HCV screening program [179]. In 2018, the Egyptian Government initiated a national HCV screening campaign, and all those with confirmed HCV infection are enrolled in government-subsidized treatment program using DAA regimen [180]. In Saudi Arabia, premarital screening for HCV is mandatory for all nationals [181]. However, one pooled analysis of 2500 prevalence measures, including 49 million tests, in the MENA region showed that the population risk of HCV exposure depends on whether the type of epidemic is generalized (like Egypt and Pakistan) or concentrated (like the rest of the countries). This study demonstrated that major gains could be achieved through targeted, cost-effective screening programs that factor in the epidemic type and are tailored to each country [182].

6.2 Secondary Prevention

Secondary prevention of HCV-related HCC aims at preventing HCC development in high-risk patients through direct anti-HCV therapy, treatment of additive factors such as HBV and HIV, lifestyle modifications in NASH patients, and elimination of alcohol intake. Over the last decades, there were several trials, including phase III trials, on chemoprevention therapies targeting inflammation, fibrogenesis, and carcinogenesis. Unfortunately, these trials have demonstrated limited efficacy and utility of those therapies [183–185]. In addition, conducting clinical trials of those identified candidate chemopreventive agents, such as statins, anti-diabetic drugs, aspirin, and dietary agents such as coffee, vitamin E, and fish oil, is limited by the long duration of cancer development that requires long-term, costly studies [176]. Thus, well-designed, prospective, population-based cohort studies might overcome those obstacles and provide the best evidence for the chemo-preventive effectiveness.

The eradication of HCV in patients using anti-HCV treatment lowers but does not eliminate HCC risk in already established advanced fibrosis or cirrhosis [172, 176, 186–190]. More data from IFN-based therapy demonstrated a beneficent long-term clinical effect in patients who achieve SVR, including a reduction in the risk of HCC [191–194]. One recent large population study from Canada included more than 8000 patients treated with older IFN-based therapy between 1990 and 2013 and showed that SVR prevents HCC. However, those with cirrhosis and age ≥50 years remain at higher HCC risk and will require continued monitoring for HCC [186]. Furthermore, different studies using post-therapy liver biopsy have observed that HCV eradication can lead to long-term histological regression of the grade of fibrosis or reduction in the rate of fibrosis progression in more than 50% of the patients [193, 195, 196]. Although we can believe that the same will happen in patients treated with DAA therapy, more studies have to be demonstrated [197]. A recent real-world cohort study from Portugal showed DAA-induced SVR is associated with a low risk (but does not prevent) HCC occurrence or disease progression [189]. In the same study, using post-SVR transient elastography to assess fibrosis improvement, there was an improvement in liver stiffness after SVR. This improvement may result from a decrease in necro-inflammatory activity after SVR, which may lead to overesti-

mation of fibrosis regression [198–200], a fact that has also been demonstrated by matched elastography-biopsy comparison [201].

Regarding patients with fibrosis stage 4 (F4), those with an "early regression" (decrease in hepatic stiffness ≥30% 24 weeks after the end of therapy) had higher baseline elastography values, which supports the role of inflammatory activity reduction in this context [202]. Another recent study using the FIB-4 index also showed that achieving SVR post-DAA therapy is associated with decreased fibrosis progression in more than half of the patients [203]. Different studies showed that SVR might lead to sustained progressive improvement in the degree of portal hypertension and, probably, in the degree of liver fibrosis through either direct (reduction in hepatic venous pressure gradient (HVPG)) or indirect measurement (improvement in platelet counts) [199, 200, 204].

Secondary prevention also includes early detection of HCC and therefore increasing the likelihood of curative treatment. Screening using a "one-size-fits-all" approach, i.e., regular 6-monthly ultrasound with or without a-fetoprotein (AFP), in populations with HCC risk is recommended by all current international societies [205–207], including the Saudi Gastroenterology Association [208]. A series of studies and reports indicate that HCC screening is cost-effective and associated with improved early tumor detection, curative treatment rates, and overall survival when available to more than 34% of patients at risk [209–211]. HCC screening is practically challenging to implement in clinical practice as only one in five patients received surveillance before HCC diagnosis (low application rate of <20%) [172, 212, 213]. The low utilization rate of HCC screening was not shown to be related to patient adherence, as one study showed only 3% of patients with HCC failed to complete surveillance despite orders [213]. Provider-related factors, including failure to recognize liver disease or cirrhosis, failure to order screening tests, and time constraints, were identified as more influential factors compared to patient adherence [213, 214]. HCC surveillance was more likely among patients seen by hepatologists compared to non-specialists (odds ratio of 6.1) [213]. Population-based interventions such as mailed outreach invitations could improve the surveillance rate to approximately 45% [215]. With the currently available resources, the large number of the target population is another limitation, given that cirrhosis is estimated to affect around 1–2% of the world population [216]. The extent of HCC risk for emerging populations, such as NAFLD without cirrhosis and HCV patients post-sustained virologic response (SVR), is yet to be determined. The most proper screening methods and surveillance internals for these populations will need to be clearly defined [212]. All of the above issues highlight the limitations of the current one-size-fits-all HCC surveillance strategy, which assumes a similar risk of HCC across all patients with the same clinical condition (e.g., HCV-related cirrhosis). This strategy may result in an over- or underestimation of HCC risk for each patient [217, 218]. Thus, a more precise determination of individual HCC risk is critical for implementing practical and feasible HCC screening and to enable optimal allocation of the limited resources, as detailed below.

6.3 Tertiary Prevention

The tertiary prevention aims to prevent HCC progression or recurrence after curative therapy of initial HCC, such as surgical resection, ablative treatment, or liver transplantation. However, despite the advances in surgical techniques and ablation technologies, the 5-year survival rate and recurrence rate are around 50% and 70%, respectively [219, 220]. The incidence of post-curative therapy HCC recurrence in cirrhosis is approximately three times more frequent than the first HCC. Therefore, it is vital to identify effective tertiary prevention interventions rather than secondary prevention [221].

HCC recurrence post-curative therapy has been classified as early (within 2 years of curative treatment) or late (>2 years after curative treatment) [222–224]. Early recurrence has been attributed to undetected micrometastasis and is associated with both tumor and surgical factors,

such as large tumor size, macroscopic vascular invasion, multinodularity, and close resection margins. On the other hand, late recurrence has been attributed to de novo second primary tumor development and is associated with host factors, such as background liver diseases, hepatitis virus load, and cirrhosis status.

To date, similar to secondary prevention, adjuvant chemoprevention therapies have not proven effective as tertiary prevention [224–226]. Although several early studies, including randomized control trials, explored the potential benefit of vitamin K2, retinoid, different systemic chemotherapy agents, and lately sorafenib, none of the studies reported successful [226].

Like primary and secondary prevention, tertiary prevention could also theoretically be achieved through anti-HCV therapies. One may expect that effective anti-HCV therapies may only reduce late HCC recurrence, as demonstrated by the Italian randomized control trial using adjuvant IFN-α therapy in HCV-related HCC [224]. However, IFN-α plus ribavirin therapy also reduced early recurrence within 1 year in another Taiwanese nationwide cohort study [227]. In another randomized control trial performed in Japan, 49 patients with HCV-related HCC were administered IFN-α therapy after complete ethanol ablation. Among the treated patients, 14 (29%) demonstrated SVR. Compared with the 25 patients who did not receive adjuvant IFN-α therapy, the rates of first recurrence were similar, but the rates of second and third recurrence were lower in those receiving adjuvant IFN-α treatment [225]. Therefore, IFN-based therapy has been shown to improve outcomes following curative HCC therapy.

Whether the high rates of SVR achieved with DAA regimens have a beneficial or deleterious effect on the risk of recurrence following resection or ablation of HCC has been debated, following the publication of a large number of generally small-scale, retrospective studies with contradictory results [228–237]. Systematic review and meta-analysis of Waziry et al. in 2017, including 13,875 patients to assess HCC occurrence (26 studies) and recurrence (15 studies) post-IFN therapy versus DAA-based SVR, found no evidence to support the higher rate of occurrence and recurrence of HCC post-DAA compared to IFN therapy [238]. In addition, a retrospective US cohort study including 797 patients with HCV-related HCC who achieved a complete response to resection, local ablation, transarterial chemo- or radio-embolization, or radiation therapy has shown that DAA therapy was associated with a significant reduction in the overall risk of death [239]. Therefore, there are no conclusive data that DAA therapy is associated with increased or decreased risk, differential time to recurrence, or aggressiveness of recurrent HCC in patients with a complete response to HCC therapy. Thus, DAA therapy should not be withheld from such patients. According to the recent EASL guidelines, DAA therapy can conveniently be deferred 4–6 months in patients without cirrhosis or with compensated, Child-Pugh A, cirrhosis to consolidate treatment and confirm a response to HCC therapy in patients treated with curative intent [240].

Treatment of patients awaiting or post-liver transplantation (LT) has also been controversial. The impact of DAA on delisting for HCC progression or recurrent HCC post-LT has not been well characterized. In a retrospective cohort study of 149 LT candidates with HCV infection and HCC at a single center, patients treated with DAAs for their HCV infection had a lower risk of waitlist dropout due to tumor progression or death compared to the patients who had not been treated [228]. Post-LT treatment of HCV was reported to be cost-effective in patients with HCC [241]. In patients with HCC, without cirrhosis, or with compensated cirrhosis, with an indication for LT, pre- or post-LT antiviral treatment indications are similar to those in patients who do not have HCC [240, 241]. In patients with HCC awaiting liver transplantation with an HCV infection in centers with long waiting times, HCV treatment should be initiated before liver transplantation to facilitate locoregional therapies to reduce waiting list dropouts due to tumor progression.

7 HCC Risk Prediction Models

HCC risk scores are used to enable precise HCC risk prediction. Such scores could identify a subgroup of individuals at high risk, maximizing the cost-effectiveness of screening tools and concentrating the efforts and resources, especially in resources-limited countries [172], in other words, applying HCC risk scores to those who are most likely to benefit from prevention and early detection, i.e., individual personalized risk-based HCC screening [172].

HCV-related HCC is previously discussed in this chapter. The risk of developing HCV-related HCC was shown in different studies to be significantly associated with the presence of cirrhosis, older age, male gender, excessive alcohol consumption [117, 118], coinfection with HBV [125–127, 129] and HIV [131–136], presence of obesity [164, 242, 243], diabetes status [139, 141, 143, 144, 146], and NASH [119–124].

Other epidemiologic and clinical studies have reported more demographic, clinical, lifestyle, genetic, and pharmacological factors that further affect or modify the likelihood of HCC [212]. The combination of different readily available clinical risk factors has been evaluated by various trials to develop HCC risk-predictive scores. However, their performance is somewhat limited, and they are yet to be adopted in clinical practice (Table 3) [212]. Molecular biomarkers have also been actively explored like AFP "a-fetoprotein," AFP-13, and DCP "des gamma-carboxy prothrombin," and some of them were combined with the clinical scoring systems to improve their HCC risk prediction [172, 244]. While the promise of these candidate molecular biomarkers is clear, some significant challenges and obstacles limit their clinical translation, like assay development and implementation and regulatory approval.

8 Antiviral Therapy and HCC

Before 2011, IFN-based therapy, with or without concomitant ribavirin, was the mainstay of treatment for those infected with HCV, with success rates ranging between 5% and 50%, depending on the genotype, stage of liver disease, and duration of therapy [259–261]. The addition of ribavirin improved outcomes but was poorly tolerated by most patients due to severe adverse effects. The management of HCV has transformed over the past decade, with SVR rates above 90% following the introduction of an IFN-free DAA-based regimen even in patients with cirrhosis [262–264].

Before using DAAs, IFN-based regimens were used in specific subgroups of patients, with significant histopathological improvements seen following successful treatment. Nowadays, it is harder to assess post-SVR histopathological changes as we are no longer required to perform pre-treatment biopsies as we were within the IFN era. However, when evaluating histopathology within 2 years of treatment, though there is a suggestion of fibrosis regression, persistent inflammatory activity has been observed despite the absence of the virus [265]. IFN-based therapies have shown that SVR is consistently associated with gradual regression of fibrosis and lower risk of HCC in retrospective studies [266, 267].

One of the goals of achieving SVR is a reduction in the incidence rate of HCC. Studies from the era of IFN-based therapy demonstrated clearly that attaining SVR was associated with a lower rate of development of HCC in 0.5–1% per year [186–188]. There was an increasing number of reports in 2016 describing a higher incidence rate of HCC post-DAA compared to old treatment with interferon-based therapy [229, 230, 268, 269]. However, one has to keep in mind that most of these studies were observational studies, including a small number of patients, with a short period of follow-up and no control arm. In addition to that and with the high safety profile of DAA compared to interferon, older patients with cirrhosis and other risk factors for HCC that were not eligible for treatment with IFN were included in most of those observational studies using DAA. The meta-analysis by Morgan et al. in 2013, including 30 observational studies, of which 18 studies had adjusted effect estimates, found that SVR after HCV therapy at any stage of fibrosis was associated with reduced risk for

Table 3 Clinical risk prediction models for HCV-related HCC

Risk models	Study design	No. subjects	Race/ethnicity	Cirrhosis	Variables	Validation
Singal et al. [245]	Prospective-retrospective, cohort	442 + 1050[a]	White, black, Hispanic	100% + 41%[a]	23 clinical variables	External
REVEAL-HCV [246]	Prospective-retrospective, cohort	1095 + 572[a]	Asian	1.4% + 7.0%[a]	Age, ALT, AST/ALT, HCV RNA, cirrhosis, HCV genotype	External
Ganne-Carrie et al. [247]	Prospective-retrospective, cohort	720 + 360[a]	n.a.	100%	Age, past alcohol abuse, platelet, GGT, SVR	Internal
Lok et al. [248]	Prospective-retrospective, cohort	1005	White, black, Hispanic	40% (Ishak 5/6)	Age, race, platelet, ALP, esophageal varices, smoking	No
El-Serag et al. [249]	Retrospective, cohort	5586 + 5760[a]	White, black	100%	AFP, ALT, platelet, age	Internal
Motosugi et al. [250]	Retrospective, case-control	66:66[b]	Asian	n.a.	LSM by MRE	
Chang et al. [251]	Retrospective, cohort	1252 + 627[a]	Asian	45% (F3/4)	Age, sex, platelet, AFP, advanced fibrosis, HCV genotype 1b, SVR	Internal
Ikeda et al. [252]	Retrospective, cohort	1056	Asian	10%	Age, AST, platelet before IFN treatment	No
scoreHCC [253]	Retrospective, cohort	871	Asian	30%	Age, AFP, platelet, advanced fibrosis	No
Wang et al. [254]	Retrospective, case-control	21:355[b]	Asian	33.8% (F3/4)	LSM, advanced fibrosis, diabetes	No
ADRESS-HCC [255]	Retrospective, cohort	17,124 + 17,808[a]	White, Hispanic	100%	Age, diabetes, race, etiology, sex, Child-Pugh score	External
Velazquez et al. [256]	Prospective, cohort	295 + 168[a]	n.a.	100%	Age, HCV, prothrombin time, platelet	Internal
VFMAP [257]	Retrospective, cohort	1808	Asian	13%	LSM, fast plasma glucose, sex, age, AFP	No

n.a. not available/applicable. *"Prospective-retrospective"* indicates the retrospective analysis of prospectively collected cohort in the past [258]. *ADRESS-HCC* age, diabetes, race, etiology of cirrhosis, sex, and severity of liver dysfunction-HCC, *AFP* a-fetoprotein, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CPA* collagen proportionate area, *GGT* c-glutamyltransferase, *HbA1c* glycated hemoglobin, type A1c, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *IFN* interferon, *LSM* liver stiffness measurement, *MRE* magnetic resonance elastography, *REVEAL-HCV* Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HCV, *SVR* sustained virologic response, *VFMAP* virtual touch quantification, fast plasma glucose, sex, age, and AFP

[a]Training + validation

[b]Case-control

HCC (relative risk for all, 0.24 [95% CI, 0.18 to 0.31]) [270]. The second systematic review and meta-analysis of Waziry et al. in 2017, including a total of 13,875 patients to assess HCC occurrence (26 studies) and recurrence (15 studies) post-IFN therapy versus DAA-based SVR, found no evidence to support the higher rate of occurrence and recurrence of HCC post-DAA compared to IFN therapy [238]. A third and recent 2019 meta-analysis by Rutledge et al. includes 138 studies with many patients (n = 177,512) looking at the development of HCC post-DAA compared to those of IFN-treated and untreated populations. This study also showed no evidence of increased HCC in patients who achieved SVR by DAAs than those treated with IFN [271].

Multiple other studies and meta-analyses showed similar evidence to the above-quoted studies. Therefore, achieving SVR will reduce the risk of HCC development but will not eliminate it. Patients with advanced fibrosis and liver cirrhosis will be at risk of developing HCC, and continuous surveillance for early HCC detection is highly recommended.

8.1 Post-SVR Surveillance

HCC recurrence is exceptionally high, reaching up to 70% after 5 years of resection. HCC can occur even quite 10 years after SVR. The 1–4% yearly incidence of HCC post-SVR is higher than other cancers [176]. Therefore, prevention of HCC development in patients with liver cirrhosis could represent the foremost effective way of improving patient survival [174]. Retrospective evaluation of patients who developed HCC post-SVR showed several HCC-associated risk factors, most of which are similar to the risk factors in patients with active HCV infection. The foremost important risk factor for HCC development post-SVR is the presence of advanced liver fibrosis [252]. Other important risk factors that might lead to the development of HCC post-SVR are old age, high alcohol intake, coinfection with HBV or HIV, and the presence of metabolic syndrome [272]. Viral factors resulting in irreversible changes in cellular signaling due to epigenetic activation or imprinting continue to drive carcinogenesis even after achieving SVR [273, 274]. Hassany et al. from Egypt prospectively have analyzed the occurrence of HCC in patients with liver cirrhosis who have achieved an SVR after IFN-free treatment with no history of HCC, and they have found that the HCC occurrence rate is 6.3% within the first 2 years [275]. Due to the continuous risk of HCC formation among patients with liver cirrhosis despite SVR, a regular, twice-yearly screening using ultrasound abdomen is highly suggested by all international guidelines, including the Saudi Association for the Study of Liver Diseases and Transplantation [276–279].

9 Management of HCV in Patients with HCC

9.1 DAAs in HCV-Related HCC

The use of DAAs has improved the disease outcomes among HCV-infected patients with SVR rates surpassing 95%. Nonetheless, recent studies on the efficacy of DAAs in patients with HCC demonstrated lower SVR rates at 60–90% than those without HCC [280, 281]. This might be explained by different possible mechanisms [280, 282–284]. For example, it has been proposed that there is a suboptimal penetration of DAAs to the HCV-infected tumor cells due to altered morphology and the nature of the tumor blood supply, which is, for the most part, the hepatic arterial branches when compared to the portal venous system [283]. However, the data to assess the efficacy of DAAs among the HCC cohort is limited because the initial DAA studies have excluded patients with HCC [285]. Other factors such as the DAA treatment regimens (e.g., SOF/RBV) used or inadequate duration of therapy may explain the suboptimal result report among patients with HCC. Hence, further trials are needed to evaluate SVR rates in this cohort of patients with the new generation of DAAs, prolonged duration of therapy, and treatment combinations.

10 HCV Treatment Considerations Based on HCC Therapy

10.1 DAAs and Locoregional Therapies

Locoregional therapies (LRTs) (e.g., percutaneous radiofrequency ablation, microwave thermal ablation, and ethanol injection) are used as curative interventions in the early stages of HCC and as palliative interventions (e.g., Transarterial chemoembolization (TACE) and Transarterial radioembolization (TARE)) in the intermediate to advanced-stage HCC [206]. There is limited evidence on the best HCV treatment approach in

patients with HCC that is amenable to LRT, and more research is required. Based on the little available evidence, several important points may be considered when deciding whether or not to treat those patients with DAAs and when. Initially, since LRTs are mainly used in patients with well-compensated liver disease, achieving SVR may significantly improve the liver function in those with decompensated liver disease and increase the eligibility for LRT [286]. Besides, as we referenced above, since SVR rates are decreased in patients with HCC, it is preferred in those with compensated liver disease to treat the HCC with LRTs before treating with DAA therapy [281, 287, 288].

10.2 DAAs and Liver Transplantation

There is much speculation regarding timing and the likely effect of HCV treatment in patients with HCC, especially in those under consideration for liver transplantation [283]. Based on the available data, there are undoubtedly several advantages and disadvantages of treating HCV before or after liver transplantation for HCC, which ought to be considered on an individual basis (Table 4) [282, 283, 285].

10.3 DAAs and Systemic Therapies

There are few available studies on using the DAAs in HCV patients with advanced HCC on systemic therapies. Sorafenib, an oral multi-kinase inhibitor, was the first breakthrough targeted therapy used to treat advanced HCC. Although sorafenib's impact on median overall survival does not extend life expectancy beyond 1 year, it is yet to be superseded a decade after the landmark SHARP trial [205, 206, 289]. Sorafenib was shown in vitro to effectively block HCV replication through different mechanisms [290–293], which has not yet been demonstrated in human studies [289, 294]. One study has shown that HCV infection is predictive of a more significant overall survival benefit with sorafenib than other liver disease causes [295]. Another interesting study by Kawaoka et al. has reported that HCV eradication before sorafenib treatment for HCV-related advanced HCC could improve the median time to treatment failure, post-progression survival, and overall survival [296]. However, a recent single-center study by Lin et al. has shown that untreated HCV patients, i.e., no DAA group, were more likely to have advanced-stage HCC and more likely to be treated with sorafenib [234]. A study by Beste et al. showed a remarkably lower rate of SVR in patients treated with sorafenib (59%) compared to patients who underwent surgical resection (78.9%) [284]. The favorable response to DAA therapy in the post-resection group could be explained by the inactive nature of HCC in this group who are more likely to have compensated liver disease.

Newer drugs like lenvatinib, a multi-kinase inhibitor, in the first-line and regorafenib, cabo-

Table 4 Advantages and disadvantages of treating HCV before or after liver transplantation for HCC

	Advantages	Disadvantages
HCV treatment pre-transplantation	May improve liver function Prevention of recurrence post-transplantation Prevention of post-transplant complications such as fibrosing cholestatic hepatitis May prevent the need for transplantation	Improved liver function may affect list priority in deceased donor liver transplantation (the majority of Middle Eastern countries use liver donor liver transplantation) Lower SVR rates Possible resistance-associated variants HCV- positive donors less favorable post-SVR Speculative DAA associated with HCC recurrence
HCV treatment post-transplantation	Improved SVR rates HCV-positive donors considered with improved wait times No concern for DAA and HCC recurrence	Worsening of pre-transplant liver function Fibrosing cholestatic HCV post-transplant (<5%)

zantinib (both multi-kinase inhibitors), and ramucirumab (an anti-VEGF mAb) within the second-line are incorporated into new guidelines [205, 206] and are started to be used in practice; however, the evidence about their potential interactions with DAA agents is still minimal. Although two small studies have shown no adverse effects of combining DAAs with sorafenib, either in terms of SVR rate or antineoplastic effect, more well-designed studies are required to assess the interaction between targeted DAAs and systemic therapies [297, 298].

10.4 Timing of HCV Treatment in Advanced HCC

As previously discussed, the timing of HCV treatment when considering curative options has been the source of some debate. The decreased efficacy of DAAs seen in the context of HCC offers a compelling argument for treating HCV after treatment of the tumor. In advanced HCC, the possibility of a cure is marginal, and then delaying HCV treatment is not practical. In patients where life expectancy is significantly limited, the risk/benefit ratio of treating HCV must be considered. The AASLD guidelines recommend palliative measures in patients with limited life expectancy within 12 months as those patients are unlikely to benefit from HCV eradication [205]. This includes patients with decompensated liver disease and advanced HCC. HCV eradication is the preferred option for those with a better prognosis before initiating sorafenib treatment in advanced HCC patients. This approach has better outcomes, including better overall survival, as mentioned above [296]. Therefore, the decision concerning the management of concomitant HCV and advanced HCC should be made on a case-by-case basis, considering the overall prognosis and potential benefit.

Advanced stages of HCC occur commonly and with increasing frequency in developing countries, including Middle Eastern countries, where it is also associated with a bad prognosis. This increasing burden of HCC could be explained by different factors, including the absence of exiting HCC screening programs, delayed presentation, delayed referral to a specialist, the limited number of specialists, and the limited treatment options offered in most countries [299]. Therefore, the timing of HCV treatment in advanced stages in resource-limited countries will also be factored into the presence of the different treatment options for HCC and the availability of DAA therapy, among other factors.

11 HCV Elimination in the ME: Opportunities and Challenges

HCV is one of the main etiological factors of HCC worldwide, and hence an effective control of this infection may reduce the disease burden of HCC. In October 2003, a national viral hepatitis therapy program was launched in Taiwan. This program has been shown to significantly reduce the burden of end-stage liver disease [300]. A total of 157,570 patients with CHB and 61,823 patients with CHC were treated with antiviral therapy from 2004 to 2011. There was a 22% reduction in mortality from chronic liver diseases and cirrhosis, a 24% reduction in HCC mortality, and a 14% reduction in HCC incidence in 2008–2011 compared with the 4 years before launching the program, 2000–2003 [300].

In 2015, only 20% of HCV infections were diagnosed globally, and only 7% of CHC eligible patients were treated [301]. In 2016, the 69th World Health Assembly endorsed the Global Health Sector Strategy (GHSS) for the elimination of viral hepatitis worldwide by 2030 [302]. This was followed by the WHO global service coverage targets using five key interventions in prevention and treatment to eliminate viral hepatitis as public health threats by 2030. The WHO target is a 90% reduction in the incidence of chronic HBV and HCV infections and a 65% reduction in mortality by 2030 (Table 5) [302]. Implementation of this strategy would prevent 7.1 million deaths globally between 2015 and 2030 [301].

Table 5 WHO's targets for the elimination of viral hepatitis [302]

	Target area	Baseline 2015	2020 targets	2030 targets
Impact targets leading to elimination	Incidence: new cases of chronic HBV and HCV infections	6 and 10 million infections	30% reduction	90% reduction (900,000 infections)
	Mortality: HBV and HCV deaths	1.46 million deaths	10% reduction	65% reduction (500,000 deaths)
Service coverage targets	*Prevention*			
	Three-dose HBV vaccine for infants (coverage %)	81%	90%	90%
	Prevention of mother-to-child transmission of HBV: HBV birth dose vaccination or other approaches (coverage %)	38%	50%	90%
	Blood safety: donations screened with quality assurance	89%	95%	100%
	Safe injections: percentage of injections administered with safety-engineered devices in and out of health facilities	5%	50%	90%
	Harm reduction: number of sterile syringes provided per person who injects drugs per year (coverage%)	20	200 (50% coverage)	300 (75% coverage)
	Treatment			
	HBV and HCV diagnosis	<5% of chronic hepatitis infections diagnosed	30%	90%
	HBV and HCV treatment	<1% receiving treatment	5 million people will be receiving the HBV treatment	80% of eligible persons with chronic HBV infection treated
		<1% receiving treatment	3 million people have received HCV treatment	80% of eligible persons with CHC infection treated

Abbreviations: *HBV* hepatitis B virus, *HCV* hepatitis C virus

The estimated incidence of acute HCV in ME based on the dataset used for the WHO 2017 Global Hepatitis Report [301] provided by the Center for Disease Analysis (CDA) Foundation's Polaris Observatory is 18.5 per 100,000 patients. As of 2015, only around 20% of HCV infections in the ME were diagnosed, and less than 10% of those diagnosed patients were treated (Fig. 3). Meanwhile, the estimated daily death due to HCV is more than 14 deaths, with nearly 7000 patients progressing to decompensated cirrhosis or HCC every year [179]. To achieve the WHO elimination 2030 targets (90% diagnosed and 80% treated), almost 100,000 people would need to be diagnosed and treated each year from 2016 to 2030 [179]. Therefore, more efforts are urgently required to achieve the goals for eliminating viral hepatitis and HCC in the ME.

Each country of the ME region should have its elimination plans and strategies tailored to meet its need to achieve high efficacy and cost-effectiveness of HCV eradication [303]. There are different ME country-based characteristics concerning HCV epidemiology, clinical practice, healthcare system, availability, and accessibility of diagnostic and treatment methods. For example, the screening of patients with CHC is considered appropriate only for high-risk rather than the general population in Saudi Arabia, where the prevalence of the viral infection is low in the general population [181]. Also, the simple aspartate aminotransferase-to-platelet ratio index (APRI)

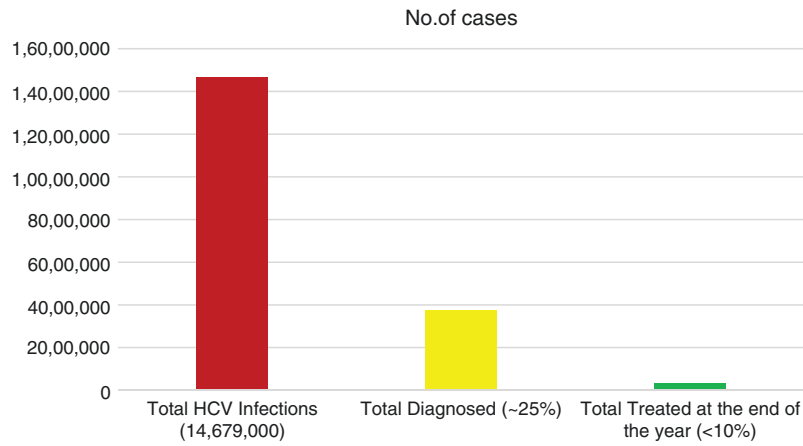

Fig. 3 Hepatitis C cascade of care in the ME in 2015. HCV hepatitis C virus

is considered as an alternative noninvasive test to assess the presence of cirrhosis in resource-limited countries. In contrast, transient elastography may be the preferred method in areas where they are available, and the cost is not a major constraint. Clinical guidelines for prevention, diagnosis, and treatment of CHC have been published by many national and international organizations, including the American Association for the Study of Liver Diseases [276], Asian Pacific Association for the Study of the Liver [304], and European Association for the Study of the Liver [277]. WHO has also published the guidelines with specific recommendations for low-income and middle-income countries [305].

There are different approaches to WHO's targets for viral hepatitis elimination across the ME [179]. These include steps such as the establishment of local strategies for viral hepatitis elimination, the building of databases, implementation of screening programs, starting awareness campaigns, and micro-elimination programs in selected high-risk groups such as hemodialysis patients, patients with thalassemia and sickle cell disease, and intravenous drug users and prisoners.

Certain Middle Eastern countries such as Qatar and Egypt are becoming role models to achieve the WHO's hepatitis C elimination goals by 2030 [306, 307]. Qatar, which is considered one of the low HCV prevalence countries, started a national program for HCV control and elimination in December 2014. This program's main goal was to prioritize and proactively manage HCV with the ultimate aim of viral hepatitis elimination by 2030. The program was based on four main pillars: primary prevention, early detection, clinical management, and continuous monitoring [307]. It was accompanied by a strong political will toward universal access to viral hepatitis-related services for all nationals and non-nationals. Important outcomes of this program were the improvement of HCV information systems, epidemiological surveillance, and patient registration. In 2016, a follow-up screening campaign was conducted, which included 7665 participants (21% were Qataris). The prevalence showed a reduction from 2% to 0.82% among the total population and from 0.8% to 0.2% among Qataris [307].

Egypt is considered at the top of the countries with the highest prevalence of HCV infection worldwide [306]. The economic burden of HCV in Egypt in 2015 was estimated to be 3.81 billion. This is expected to increase exponentially as HCV-infected individuals progress to more advanced diseases, i.e., decompensated cirrhosis, HCC, and liver-related mortality [306]. In response to this major issue, through its National Committee for the Control of Viral Hepatitis (NCCVH), Egypt started a national treatment program for Egyptian HCV-infected patients in 2007. Mass HCV treatment program had started using IFN-based therapy between 2007 and 2014 and then shifted to DAA therapy from 2014 to date. By September 2018, about 2.5 million

patients had been tested and treated with different combinations of DAAs [180]. In October 2018, the Egyptian Ministry of Health began a national population-based screening program. By September 2019, around 52 million adult individuals will be screened after excluding those treated or tested before [180]. The NCCVH anticipates that 2.5–3.0 million newly diagnosed HCV individuals will be eligible for free treatment. Therefore, Egypt has taken a great step toward HCV elimination. The Egypt HCV elimination strategy is considered a care model for other high HCV prevalence countries to use in their battle against HCV [180].

Despite this progress, and with only 10 years to go until the 2030 deadline is reached, more commitment from most Middle Eastern countries is required to achieve HCV elimination. The diagnosis and treatment of HCV infection are still not up to the ME region level to be considered on track for elimination. As per a report from the CDA foundation, no Middle Eastern countries in 2017 were considered "on track" for achieving the WHO elimination targets. However, after 2017, most countries started to treat all HCV patients regardless of their fibrosis stage, which is an essential step toward improving patient access to treatment. Furthermore, there are ongoing efforts to implement national strategies in some countries, like Saudi Arabia, Qatar, the United Arab Emirates, and Kuwait.

There are significant barriers to HCV elimination in ME. These barriers vary between the countries of the ME and need to be addressed to achieve HCV elimination. A major gap in response to the epidemic remains the lack of reliable prevalence data in many countries and regions, including Middle Eastern countries [6]. The availability of those accurate and verifiable HCV prevalence data for the region and each country allows the establishment of better elimination strategies. These strategies must be tailored to the population's needs and allow easy monitor of progress and impact of interventions.

Moreover, budget limitations and low HCV awareness among the general population are major challenges for HCV elimination in low- to middle-income countries like Egypt [306]. The HCV diagnosis rates depend on the cost of the investigations and HCV awareness among the general population [306]. In many countries worldwide, including Middle Eastern countries, most known HCV cases have been treated and cured, and there are no more new HCV patients to treat [308]. Most of the cases in many Middle Eastern countries, including Saudi Arabia, are unfortunately still undiagnosed. Therefore, there is an urgent need for a screening program to increase diagnosed patients [181]. Diagnostic resources, including the capacity to process large volumes of screening tests and the availability of confirmatory tests, remain obstacles for elimination in many countries globally. In several Middle Eastern countries, including Egypt, Saudi Arabia, and Oman, access to affordable low-cost generic DAAs has been supported and successfully incorporated within their national treatment plans [179, 181, 306, 309].

Furthermore, challenges for successful implementation still exist even when elimination strategies have been established within national healthcare plans. In most countries, HCV treatment is provided by specialists, which may increase the waiting list and decrease treatment access for many patients. This was justified in the IFN-based therapy era when the treatment was associated with long therapy duration, severe adverse effects, and low cure rates. However, the current therapies are easy to prescribe and administer and of short period with almost few if any side effects and high cure rates. Many studies have shown that general practitioners can successfully treat most patients without compromising SVR [310, 311].

HCV elimination has been proven to be highly cost-effective and cost-saving across various health settings [312]. The WHO 2030 deadline for eliminating HCV, which has been deemed ambitious by many, is achievable, provided strong global support and commitment. The Middle Eastern countries need more expanded efforts to increase screening, diagnosis, and treatment. These efforts must be matched with firm political intention, sustainable funding, improved linkage to care and access to cheap high effective generic DAAs, raising awareness, eliminating stigma, and improved point-of-care access to

include general practitioners [313, 314]. Perhaps then the region can get firmly on track toward HCV elimination.

After 5 years of progress and assessment, the recent CDA foundation 2020 report on tracking countries' progress toward WHO global targets has raised concerns about the limitations of the existing targets [315]. These impact targets compare a country's progress relative to its 2015 baseline when most countries did not have existing hepatitis epidemiology data. These targets also penalize those countries that started their programs before 2015 and those with a young population or a low HCV prevalence. Therefore, the Polaris Observatory collaborators have proposed that WHO simplify the exiting hepatitis elimination targets and change to absolute targets, shown in Table 6 [315]. They also recommend allowing countries to achieve these targets with their service coverage initiatives that will have the maximum impact. Using this proposed model, more countries are expected to achieve all the new targets relative to the existing ones (Table 7).

12 Projection of Future HCV-Related HCC Trends in ME

The projections of the future burden of HCV-related HCC can inform prevention strategies aimed toward reducing HCC occurrence. With the ambitious WHO 2030 elimination targets, in

Table 6 Simplified hepatitis elimination targets using absolute targets [315]

Primary objective	Reduce the incidence		Reduce mortality	
2030 target	Reduce HCV new chronic cases to ≤5 per 100,000 (excluding the new cases from immigration)	Reduce HBsAg prevalence among 1-year-olds to ≤0.1%	Reduce HBV and HCV mortality to ≤5 per 100,000	Demonstrate HBV and HCV year-to-year decrease in new HCV- and HBV-related HCC cases
Measure options	Conduct two national surveys (minimum 1 year apart) and estimate incidence between the two by age group	Conduct HBsAg surveys in 1-year-olds in multiple regions in the country and maintain prophylaxis measures	Establish/use the national registry for HCC, decompensated cirrhosis linked to patient and death registries attributed cause, and adjust for underreporting	Establish/use the national registry for HCC, decompensated cirrhosis linked to patient and death registries attributed cause, and adjust for underreporting
	Conduct two surveys (minimum 1 year apart) in high-risk groups accounting for >80% of new infections and estimate incidence rate	Conduct HBsAg surveys among 1-year-olds in high prevalence regions/populations and maintain prophylaxis measures	Establish/use the national HCC registry. Estimate annual decompensated cirrhosis to HCC incident ratio in ≥1 major center. Use HCC and cirrhosis survival studies to estimate overall mortality by year	Establish/use the national HCC registry. Estimate annual decompensated cirrhosis to HCC incident ratio in ≥1 major center. Use HCC and cirrhosis survival studies to estimate overall mortality by year
	Use modeling to estimate incidence	Use modeling that considers the impact of prophylaxis to estimate the incidence and maintain prophylaxis measures	Use modeling to estimate HBV- and HCV-related HCC and cirrhosis mortality	Use modeling to estimate HBV- and HCV-related HCC and cirrhosis mortality over time

Abbreviations: *HBsAg* HBV surface antigen, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus

Table 7 Comparison between existing WHO elimination targets and the recently proposed absolute targets by 2030 [315]

Middle Eastern countries reaching the existing WHO elimination targets by 2030						
HBV 90% reduction in incidence	HBV ≤0.1% prevalence among 5-year-olds	HBV 65%reduction mortality	Countries meeting all HBV targets	HCV 90% reduction in incidence	HCV 65% reduction in mortality	Countries meeting all HCV targets
None	Egypt	None	None	Egypt	Egypt	Egypt
	Iran					
	Kuwait					
	Lebanon					
	Oman					
	Qatar					
	Saudi Arabia					
	Turkey					
	UAE					
Middle Eastern countries reaching the absolute HBV and HCV elimination targets by 2030						
HBV _0.1% HBsAg prevalence among 1-year-olds		HBV reduces mortality to ≤5 per 100,000 and decreases in new HCC cases	Countries meeting all HBV targets	HCV reduces new chronic infections to ≤5 per 100,000	HCV reduces mortality to ≤5 per 100,000 and decreases in new HCC cases	Countries meeting all HCV targets
Egypt		None	None	Egypt	Egypt	Egypt
Iran				Saudi Arabia	Turkey	Turkey
Kuwait				Turkey		
Lebanon						
Oman						
Qatar						
Saudi Arabia						
Turkey						
UAE						

Note: Blue countries/regions—those not achieving the absolute targets for a similar category in Table 4; only countries/regions analyzed by Polaris Observatory are listed; *UAE* United Arab Emirates, *UK* United Kingdom

most countries, the incidence and prevalence of HCV case are expected to decrease due to a combination of a prevalent aging population, availability of treatment, and a reduction in risk factors secondary to improvements in the safety of blood products and harm reduction programs for injection drug users [316, 317]. However, the morbidity and mortality attributable to HCV are expected to increase as the current infected population progresses to advanced stages of liver fibrosis. In most countries, the increased disease burden will likely not be controlled without significant changes in the overall treatment paradigm, including increases in screening, diagnosis, and treatment. This suggests that countries should assess different strategies to help decide how to manage the expected increase in their HCV-related disease burden, including HCC.

13 Future Research Needs

There are significant needs for more epidemiologic and clinical research on HCC worldwide. In the DAA treatment era, HCV-related HCC remains a major health problem in the coming one to two decades. The development of a vaccine remains an essential target for achieving global control and eradication of HCV. There is also a critical need for more basic research on carcinogenesis in CHC and identifying more risk factors, i.e., viral, cellular, immune, and host-genetic factors that add to the development of HCC. Identification of the steps leading to the progression of CHC infection to cancer would help develop means of prevention, early detection, and treatment. Focusing on ME, more population-based studies are needed to understand

the current and future contribution of HCV to HCC in the region. These studies should examine known and suspected risk factors and collect appropriate biologic samples to assess HCC markers that help early detection of HCC. Such studies could additionally provide essential data on the risk factors for the progression of CHC to HCC. The relationship between HCV and other conditions like obesity, diabetes, and NAFLD needs further research, particularly given the high prevalence of these conditions in the region and the large number of HCC cases in which no specific risk factor can be identified. Post-SVR HCC is an important emerging issue, with pressing unmet needs for the clinical strategy of early tumor detection and intervention and identifying HCC molecular mechanisms for therapeutic target and biomarker discovery. Long-term clinical trials on the impact of post-DAA SVR on HCC development and recurrence are also required.

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Hepatocellular Carcinoma in the Middle East: An Overview

Sanaa Kamal

Abbreviations

ALD	Alcoholic liver disease
ASIR	Age-standardized incidence rate
ASMR	Age-standardized mortality rate.
H.H.	Hereditary hemochromatosis
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IARC	International Agency for Research on Cancer
MENA	the Middle East and North Africa
NAFLD	Non-alcoholic liver disease
NASH	Non-alcoholic steatohepatitis
SEER	Surveillance Epidemiology and End Results database

1 Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver malignancy and is the fifth most common cancer in men and is the seventh among women. The number of new HCC cases in 2018 was 841,080, and this represents 4.7% of all new cancer cases [1]. The incidence of HCC varies according to the geographic region, the prevalence of risk factors specific for HCC, and the accessibility to therapeutic options for such factors. The incidence rates vary between 5.1 per 100,000 person-years in Europe and 17.7 per 100,000 person-years in Eastern Asia [2]. The incidence rates of HCC peak at about the age of 50 years [3, 4]. The prevalence of HCC is exceptionally high in East/Southeast Asia, Egypt, several African countries and, historically, in southern Europe [3, 4]. HCC is the third cause of cancer death worldwide, resulting in 781,631 deaths in 2018 [2, 5, 6].

Epidemiological differences and etiologic factors of HCC vary across countries and geographic regions. Chronic hepatitis B- and C-related cirrhosis are responsible for approximately 80% of all liver cancer deaths in developed and developing countries [3, 5, 6]. Other risk factors such as high alcohol consumption, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH) associated with obesity and diabetes and cholestatic liver diseases such as primary biliary cholangitis are increasingly emerging as contributors in the evolution of HCC in Western countries [3, 5]. Hereditary hemochromatosis and hepatic iron overload states secondary to hemoglobinopathies can increase the risk of the development of HCC [7, 8]. Less common risk factors for HCC include inherited disorders: glycogen storage disease and alpha-1-antitrypsin deficiency [9, 10]. Aflatoxins are metabolic products of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* that develop in maize, oilseeds, and dried

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fruits. Aflatoxins are associated with HCC, and the International Agency for Research on Cancer (IARC) has recognized them as human carcinogens. In tropical and subtropical developing countries, exposure to high concentrations of aflatoxins causes acute hepatitis [11, 12]. Chronic exposure to aflatoxins results in genotoxicity, mutagenicity, and immunotoxicity [12].

2 Epidemiology of Hepatocellular Carcinoma in the MENA Region

The Middle East is a flexible geographic term that does not have a specific standardized definition. A more inclusive definition is the term, Middle East and North Africa (MENA), which includes Egypt, Libya, Tunisia, Algeria, Morocco, Syria, Lebanon, Jordan, Saudi Arabia, United Arab Emirates, Kuwait, Bahrain, Qatar, Oman, Iraq, Yemen, Sudan, Palestinian territories and Israel, Turkey, and Iran. MENA is a densely populated region with a total population of 456,707,404 in 2019–2020, which accounts for approximately 7% of the world's population. The MENA region comprises various ethnic groups, including Arabs, Persians, Turks, Kurds, Berbers, Jews, and Armenians, who follow Islam, Christianity, or Judaism. The countries in the MENA region have diverse economies. High-income countries with strong economies in the region include Saudi Arabia, United Arab Emirates, Kuwait, Turkey, and Iran, where vast oil reserves, industry, and tourism stimulate economic growth. Other countries, such as Yemen, Sudan, Syria, and Libya, have suffered from prolonged periods of wars and civil unrest, which adversely affected economic development and devastated healthcare infrastructure [13].

2.1 Incidence of HCC in the MENA Region

Hepatocellular carcinoma represents a public health problem and an economic burden in several MENA countries due to the high prevalence of HBV and HCV infection in the region, limited HCC screening, and paucity of preventive measures and therapeutic interventions in some countries. The incidence of HCC has changed over time, not only across MENA countries but also within individual countries, due to variations in environmental exposures, lifestyle patterns, screening procedures, and risk factor management efficacy. HCC burden also differs between high-income countries (HICs), low-income countries (LICs), and lower-middle-income countries (LMICs). Overall, the 2018 HCC incidence rates have not shown a significant decline in MENA countries despite improved HBV and HCV preventive and management strategies [1, 2] (Table 1).

As in other world regions, HCC is predominant among males (Fig. 1). In 2018, the number of new HCC cases in the MENA region was 37,184 (25,699 males and 11,485 females) with a cumulative risk of 2.18%, and the reported deaths were 36,601 (25,267 males and 11,334 females) with a cumulative risk of 2.16% [1, 2] (Table 1). Comparing the overall liver cancer age-standardized incidence rates (ASIR) data in the MENA region from 1990 to 2018 revealed a rise from 5.12 per 100,000 individuals to 5.83 per 100,000 in 2018, significantly lower than the incidence rates in Southeast Asia (17.5) [14, 15]. However, breaking down the results according to the incidence rates in individual MENA countries revealed three patterns, namely, countries that showed increased incidence rates over time, countries with stable incidence, and countries with decreased HCC incidence rates (Fig. 2). Egypt has exceptionally and persistently high incidence and HCC-related mortality rates, making liver cancer rank the first malignancy in Egypt [1, 16–18]. Although Egypt launched an ambitious nationwide program to treat all HCV patients with DAAs, the incidence of HCC is still high. Active treatment resulted in reductions of new hepatitis C virus infections. However, HCC still emerges due to the aging of cirrhotic patients infected with HCV several years ago and the extensive HCC screening programs that contribute to more diagnosis of HCC cases [17–19]. However, sev-

Table 1 Hepatocellular carcinoma new cases, mortality, 5-year prevalent cases in MENA countries (2018)

Country	Total population	Number of new HCC cases				Deaths Mortality rank				Number of prevalent cases (5-year)
		Number	Cum. risk	Rank[a]	(%)[b]	Number	Cum. risk	Rank	(%)[c]	
Egypt	102,334,403	25,399	3.8	1	19.7	25,084	3.77	1	29.4	19,361
Saudi Arabia	33,554,333	905	0.55	9	3.7	852	0.52	4	8.1	701
Iran	82,011,737	3492	0.48	11	3.2	3439	0.48	7	6.2	2671
Sudan	41,511,523	942	0.43	7	3.7	906	0.43	5	5.3	830
Turkey	81,916,866	4362	0.52	15	2.1	4307	0.52	9	3.7	3127
Qatar	2,694,843	42	0.45	11	3.3	40	0.44	6	6	32
UAE	9,541,612	97	0.47	15	2.1	96	0.45	8	4.6	82
Yemen	28,915,286	620	0.51	7	4.7	595	0.51	5	6.5	535
Bahrain	1,566,994	30	0.37	12	2.9	22	0.29	12	3.6	108
Syria	18,284,423	380	0.34	18	1.6	380	0.29	18	1.4	514
Lebanon	6,093,510	227	0.38	18	1.3	216	0.37	11	2.4	163
Jordan	9,903,798	188	0.31	16	1.7	178	0.30	10	3.1	142
Iraq	39,339,754	539	0.32	12	2.1	538	0.32	7	3.7	443
Kuwait	4,197,129	121	0.6	8	3.4	114	0.6	4	6.9	90
Oman	4,829,946	117	0.51	10	3.5	111	0.5	5	6.6	98
Libya	6,470,857	191	0.54	12	3.0	147	0.44	7	4.4	148
Tunisia	11,659,175	355	0.29	12	2.2	356	0.30	11	3.5	317
Algeria	42,008,056	563	0.17	21	1.1	544	0.16	17	1.8	486
Morocco	36,191,813	428	0.14	24	0.81	411	0.13	18	1.2	524
Israel	8,452,843	336	0.28	17	1.3	397	0.35	11	3.2	253

[a]Rank with respect to other cancers
[b]Percentage from all cancers
[c]Percentage from all cancer deaths

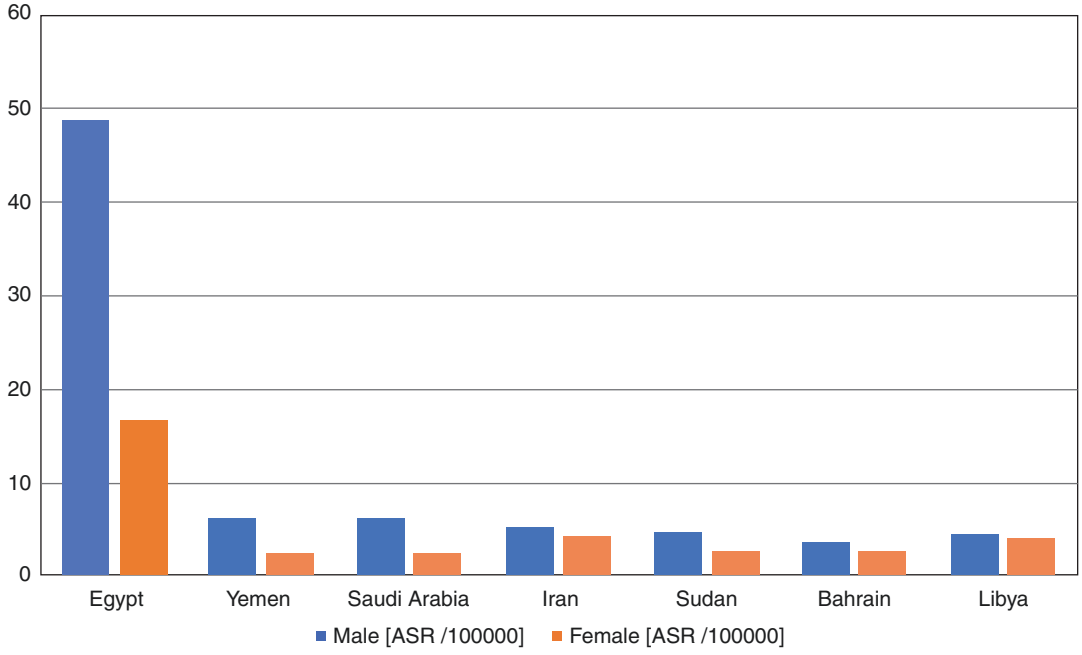

Fig. 1 Incidence of HCC in males and females in some MENA countries

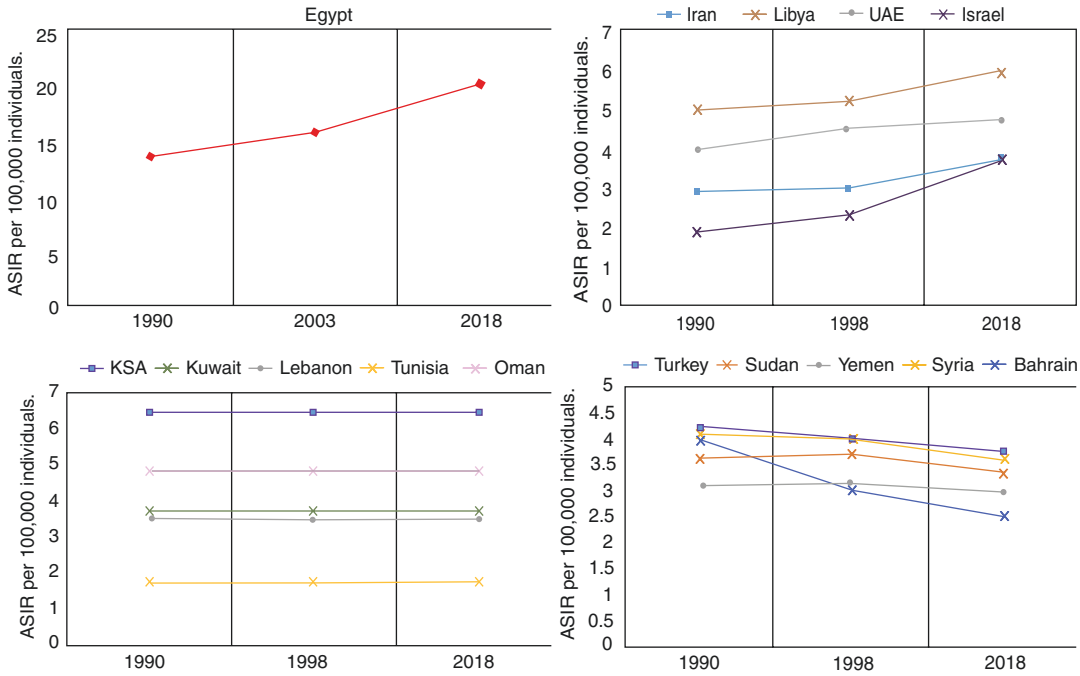

Fig. 2 HCC incidence trends 1990–2018 in MENA countries

eral reports from Egypt demonstrated de novo emergence or recurrence of HCC in HCV patients treated with DAAs [20, 21]. Further studies are required to characterize the magnitude and explanation of HCC occurrence in these patients.

In Israel, the age-standardized HCC incidence rates rose gradually from 1.93 and 1.22 for Israelis and Arab men in 2000 to 3.13 and 3. 63 for Israelis and Arab men in 2018 [1, 22]. The Islamic Republic of Iran, Libya, UAE, and Algeria had moderately increasing trends over time [1, 14]. The HCC incidence rates in Saudi Arabia, Kuwait, Bahrain, Oman, and Tunisia have not shown significant changes [1, 14]. In Turkey, the HCC incidence rates decreased from 4.2 to 3.5 per 100,000 individuals between 1990 and 2018, suggesting adequate control of risk factors of HCC [1, 14, 23]. Epidemiologic data in 2018 [1, 14] demonstrated HCC decline in Yemen, Syria, and Sudan (Fig. 1). However, one should be cautious in interpreting this decline that might result from underreporting rather than an actual HCC case decrease. For years, the three countries have been suffering from civil unrest and conflicts that damaged the healthcare infrastructure.

2.2 Mortality due to HCC in the MENA Region

Overall survival of patients with HCC varies substantially across the MENA countries. Mortality data in the MENA region showed increasing HCC-related mortality trends in MENA countries, confirming the poor outcome of HCC in MENA countries (Table 1). The ASMR per 100,000 individuals was 5.31 and 5.94 in 1990 and 2018, respectively [1, 2, 14, 15]. The highest ASMR is reported from Egypt, with 25,084 deaths and cumulative risk of 3.77%. In Egypt, the HCC ASMR increased from 13.79 in 1990 to 20.47 in 2018. HCC ranked the first cause of mortality-related cancer and was responsible for 9.1% of all-cause mortality in Egypt [1, 2, 14, 15, 18–20]. HCC was the fourth cause of cancer-related mortality in KSA and Kuwait and the fifth in Sudan, Oman, Libya and Yemen, respectively, respectively [2, 14, 15] (Table 1).

3 Risk Factors of Hepatocellular Carcinoma in the MENA Region

As in various world regions, hepatocellular carcinoma in the MENA region has several risk factors, including cirrhosis caused by chronic viral hepatitis, alcohol consumption, NAFLD, male gender, exposure to hepatotoxins such as aflatoxin ingestion, exposure to inorganic arsenic and some chemicals, and metabolic factors such as iron overload states and obesity and exogenous (oral contraceptive pill) or endogenous hormones [24]. The attributable population fraction (PAF) of risk factors for HCC varies in different MENA countries.

3.1 Chronic Hepatitis B and C Infections

The MENA region includes some of the countries most affected by viral hepatitis worldwide, and the burden of HCC in a given country parallels the prevalence of HBV or HCV. Various socioeconomic factors and treatment policies contribute to the high incidence of HBV and HCV infections. The MENA region demonstrates diversity in hepatitis B and C genotype distributions and transmission modes of both viruses [25, 26]. Hepatitis C- and hepatitis B-related cirrhosis and chronic hepatitis B are responsible for 80% of HCC cases [27].

3.1.1 Hepatitis B Infections in MENA Countries

Chronic HBV is a significant risk factor for hepatocellular carcinoma worldwide. Patients with chronic hepatitis B have a 25–40% lifetime risk of developing. Chronic hepatitis B results in ongoing inflammation that may progress to liver fibrosis and cirrhosis, which is an essential risk for HCC. HBV can also cause HCC in the absence of cirrhosis by integrating HBV DNA into the host genome, inducing genomic instability, and direct insertional mutagenesis of diverse cancer-related genes. Several factors increase HCC risk among patients with chronic HBV, such as male sex, older age, higher levels of HBV viremia, HBeAg positivity, HBV genotype D, longer duration of infection, coinfection with HCV, human immunodeficiency virus (HIV), or hepatitis D virus [28, 29].

The World Health Organization (WHO) estimates that the prevalence of chronic HBV infection in the MENA region ranges from low intermediate (2–4%) in most MENA countries to high intermediate (5–7%) in Sudan. The HBV prevalence rate is 5.1% in Yemen, 3.6% in Algeria, and 3.5% in Kuwait [26]. In Saudi Arabia, a cross-sectional study on 74,662 Saudis recruited from the general Saudi population showed that the prevalence rate of HBV was 1.3%, which is lower than older studies that estimated the prevalence to be 3.2% [30]. Chronic HBV infection is the risk factor for HCC in Iran, Saudi Arabia, Lebanon, UAE, Oman, Qatar, Turkey, and Israel [1, 25, 26, 28, 30] (Fig. 3). HBV genotype is also crucial in determining the risk for HCC since HBV genotype D patients carry a higher risk for HCC than patients with genotype A. HBV genotype D is the prevalent genotype in Turkey, Iran, Saudi Arabia, UAE, and Bahrain [3, 25, 31–33].

Most MENA countries implemented nationwide obligatory HBV vaccination, and about 68% of the countries achieved the target, which is HBV immunization of 80% of the newborns [34, 35]. HBV vaccination resulted in the decline of new HBV cases. However, the armed conflicts and unstable political conditions in Yemen, Syria, Libya, and Sudan hindered adequate HBV immunization in these countries.

3.1.2 Hepatitis C Infections in MENA Countries

Globally, the World Health Organization (WHO) estimates that in 2017, there were 1.75 million new HCV infections in the world (23.7 new HCV infections per 100,000 people) and 71 million people suffer from chronic HCV infection, with a significant number of these developing cirrhosis or hepatocellular carcinoma [27]. Despite the availability of safe, effective direct-acting antiviral therapies for chronic HCV, successfully eradicating HCV from several MENA countries is

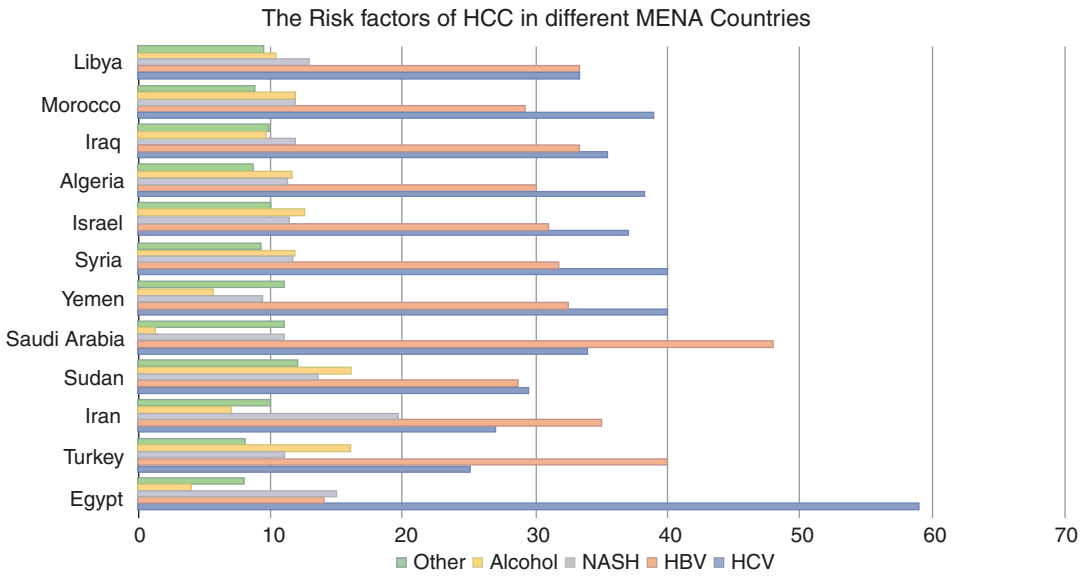

Fig. 3 Risk factors for HCC in MENA countries

challenged by limited testing and diagnosis, barriers for effective treatment of chronic HCV, late presentation, and poor compliance to therapies [36, 37].

HCV-related cirrhosis was by a significant risk factor for HCC in Egypt, Sudan, Libya, Syria, Iraq, Yemen, Algeria, Bahrain, Jordan, Kuwait, Morocco, and Tunisia. Chronic HBV infection is the risk factor for HCC in Iran, Saudi Arabia, Lebanon, UAE, Oman, Qatar, Turkey, and Israel [15–18] (Fig. 3).

The modes of HCV transmission also vary among high-income and middle-income countries and low-income countries, particularly those devastated by armed conflicts, civil war, or political instabilities such as Yemen, Syria, Libya, Iraq, Sudan, and the Palestinian territories. In low-income countries and countries with political instability and poor health infrastructure, HCV transmission frequently results from inadequate infection control policies, unsafe injections, and exposure to infected blood and blood products in healthcare and community settings.

Injection drug use (IDU) and sharing drug injection equipment. In some MENA countries (Egypt and Sudan), specific traditional practices such as circumcision, home deliveries, and scarification contribute to HCV transmission [26, 38].

The current escalation of immigration from or through the MENA countries particularly in Libya, Morocco, and Algeria towards northwestern countries due to economic, social, or political reasons or civil wars or natural disasters contributed to the elevation of HBV and HCV infections since immigrants often originate from countries with a high burden of viral hepatitis. The overall prevalence of HCV in African immigrants was 7.6% though it varied according to the immigrants' origin, ranging from 5.7% to 10.0% [39]. Therefore a suitable strategy has to be taken to deal with this emerging situation.

Thus, implementing strategies to reduce HCV transmission and adopting programs for early diagnosis and treatment of hepatitis C infections are critical for reducing the burden of HCV-related liver diseases, such as decompensated cirrhosis and HCC in patients with HCV infection.

3.2 Non-alcoholic Liver Disease (NAFLD) and HCC in the MENA Region

Non-alcoholic fatty liver disease (NAFLD) is a significant cause of chronic liver disease worldwide. NAFLD is defined as the liver's fatty infil-

tration in the absence of alcohol abuse or other causes of hepatic steatosis [40]. The spectrum of NAFLD encompasses steatosis, steatohepatitis, and fibrosis to cirrhosis. According to the extent of steatosis and liver injury hepatic histology, the categories of NAFLD include non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) [40, 41].

Reports have associated HCC with NAFLD, obesity, and impaired glucose tolerance, suggesting that NAFLD was a major underlying cause of HCC. Overnutrition and sedentary life contribute to obesity, which may directly predispose HCC and promote glucose intolerance and chronic liver disease, which represent and additional HCC risk [42, 43]. In a study that followed 195 cirrhotic NASH patients for 4 years, 25 patients developed HCC at the end of the study with a cumulative incidence of 2.6% [44]. A US-based population study of 4406 HCC patients showed that 59% of HCC cases were related to NAFLD, compared to 22% associated with HCV and 12% in association with ALD [45].

NAFLD's burden in the MENA region is unclear due to the scarcity of studies from the region. NAFLD prevalence in Kuwait, Saudi Arabia, south of Iran, and north of Iran was 33.3%, 16.6%, 21.5%, and 43.8%, respectively [46, 47]. In Sudan, a study showed a 20% NAFLD prevalence among the studied group [48]. NAFLD prevalence in Egypt ranges from 16% to 50% among pediatric and adult populations, respectively [49–51]. The prevalence of NAFLD in Turkey is between 48.3% and 60.1% [52] (Fig. 4). NAFLD is closely related to the high prevalence of diabetes mellitus type 2 and obesity in MENA countries, particularly oil-producing countries, due to reduced physical activity, unhealthy diet, and aging. The Middle East and North African countries are countries with a high prevalence of type 2 diabetes. The prevalence of type 2 diabetes is 21.1%, 20.2%, 20.2%, 20.0%, 19.9%, 19.2%, 19.1%, and 16.5% in Kuwait, Lebanon, Qatar, Saudi Arabia, Bahrain, UAE, Sudan, and Turkey, respectively [53, 54].

To date, studies investigating the role of NAFLD, obesity, and metabolic syndromes as risk factors for HCC in the MENA region are lacking. However, a study from Egypt reported that 33% of patients with chronic HCV had associated type II diabetes mellitus (D.M.). Cirrhosis was more prevalent among diabetic HCV cases,

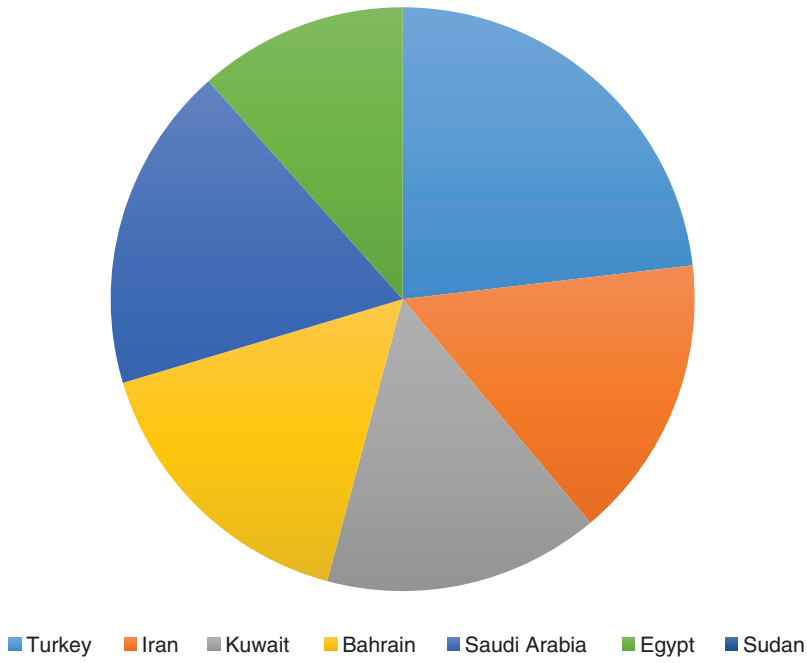

Fig. 4 Prevalence of NAFLD in some MENA countries

and the fibrosis score was higher in diabetic HCV patients than in nondiabetic HCV cases. Given the progressive rise of NAFLD and the expected decline in viral hepatitis in the MENA region, it is critical to conduct epidemiological studies to assess the potential contribution of NAFLD, obesity, and diabetes in the evolution of HCC in these countries.

3.3 Alcohol Consumption as a Risk Factor for HCC in MENA

Heavy alcohol consumption and alcoholic liver disease are not significant risk factors for HCC in MENA countries, given that Islam, the predominant religion in MENA nations, prohibits alcohol drinking. However, MENA countries embrace a population of different ethnic backgrounds and religious beliefs. Studies from some MENA countries showed that alcohol contributed to a percentage of HCC (Figs. 3 and 5).

3.4 Aflatoxins and Chemicals as Risk Factors for HCC in the MENA Region

Aflatoxins are toxic and carcinogenic chemicals produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and infect crops such as maize, peanuts, and nuts. Aflatoxins are Group 1 human carcinogens and potent hepatocarcinogens. Exposure to high doses of aflatoxins causes acute hepatitis, while chronic exposure causes HCC [11]. Aflatoxin exposure synergizes with chronic hepatitis B virus (HBV) infection to increase HCC in countries and populations with both risk factors. High aflatoxin exposure and HBV are prevalent in many parts of the developing world, particularly in Asia and Africa [55, 56]. The population-attributable risk of aflatoxins to HCC in the MENA region is unclear due to the rarity of such studies.

Egypt has a high prevalence of HCC, which is mostly due to HCV-related cirrhosis. However, the widespread use of pesticides and contamination of some cereals by aflatoxin B1 in rural areas

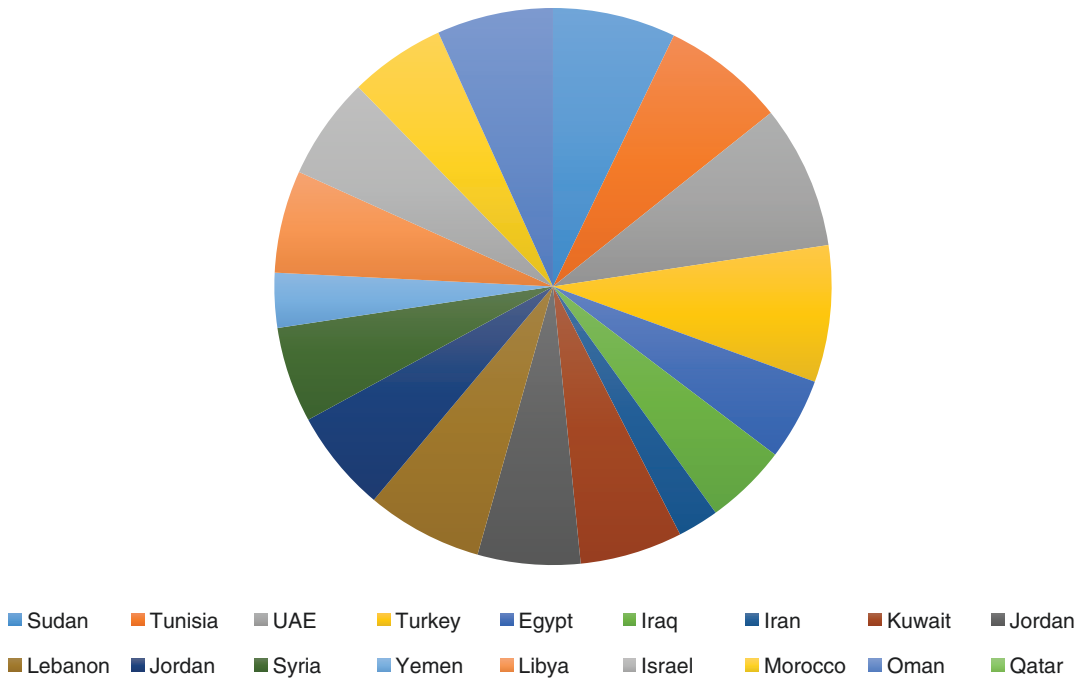

Fig. 5 The contribution of alcohol as a risk factor for HCC in some MENA countries

contribute partly to the burden of HCC in this country, in which agriculture is an important occupation of Egyptians [57–59]. Dietary aflatoxin exposure in Egypt is 7–57 ng/kg bw/day, which is relatively high [60]. Co-occurrence of aflatoxin and HCV in the Egyptian population has been shown in various epidemiological studies to be common [58, 59]. The interactions between HCV and aflatoxin and the role of both factors on the evolution and high prevalence of HCC in Egypt are intriguing and warrant further studies.

3.5 Iron Overload States as Risk Factors for HCC in the MENA Region

Hereditary hemochromatosis (H.H.) is the most common autosomal recessive disorder with a prevalence of 1 in 300 to 500 individuals. H.H. types 2, 3, and 4 are seen worldwide. In contrast, Type 1 form is mostly seen in Caucasian people of northern European descent. H.H. is characterized by an increased intestinal iron absorption, resulting in a progressive iron accumulation in the liver, heart, and pancreas, leading to progressive dysfunction [61]. HCC is a long-term complication of H.H. Patients with genetic hemochromatosis are 23 times more likely to have HCC than healthy individuals [62]. In a Swiss cohort, HCC occurred in 9% of all H.H. patients [63]. The prevalence of H.H. in the MENA region is unknown. No data is available on H.H.'s potential role as a risk factor for HCC in MENA countries.

Thalassemia is an inherited disease with worldwide distribution. However, the burden of β-thalassemia is exceptionally high in the MENA region, particularly Iran, Lebanon, Syria, Egypt, KSA, UAE, and North Africa. Consanguinity in several MENA countries contributes to its high prevalence in the region. Hemoglobinopathies, particularly thalassemia, are associated with iron overload due to repeated blood transfusion, which is the principal treatment of thalassemia patients. Several studies showed an increase in the incidence of HCC in patients with thalassemias, mainly in Italy and Greece [64]. A study showed that HCC was the most frequent malignancy in 3652 Greek thalassemic patients diagnosed between 1985 and 2018. An Egyptian study showed that patients with acute HCV and thalassemia have low rates of spontaneous resolution of HCV infection, and the majority develop chronic HCV, accelerated hepatic fibrosis, cirrhosis, and HCC in thalassemia patients with chronic HCV. Thus, HCV prevention and early treatment are critical for the prevention of HCC in this population [65].

4 Hepatocellular Carcinoma in Selected MENA Countries

4.1 Hepatocellular Carcinoma in Yemen

Yemen has been in armed conflict and political instability since 2015 and is suffering the worst humanitarian crisis in the world. The war has devastated the economy, destroyed the healthcare infrastructure, and resulted in severe food and clean water shortage. Yemen is considered the poorest country in the Middle East and North Africa (MENA), where 80% of the population are "at-risk" of hunger and disease. The COVID-19 crisis in 2020 further deteriorated the socioeconomic conditions and devastated the public infrastructure. Thus, updated data about the incidence and prevalence of liver cancer in Yemen are scarce, given the ongoing war and political instability.

In 2018, 620 new cases were reported from Yemen and 595 mortalities with a cumulative risk of 0.51%. The age-adjusted death rate is 3.18 per 100,000 population. HCC is the seventh frequent cancer in Yemen and represents the fifth cause of cancer-related mortalities [1, 14, 66]. An 8-year survey (2001–2008) showed that HCC is more prevalent in Yemeni men, mostly farmers, with an age range of 26–75 years (mean 53.5 ± 13.9 years). The overall mortality rate within 6 months of hospital admission was 24.3%.

Chronic hepatitis B virus infection (48.2%) and hepatitis C virus infection (38.2%) were the most frequently identified risk factors among Yemeni patients with HCC. Qat chewing and smoking were not statistically significant risk factors.

4.2 HCC in Libya

Libya is the second-largest country in North Africa. Libya used to be the wealthiest country in North Africa, a significant oil producer country with a small population. However, the ongoing political conflict and the weak security conditions have taken a severe toll on the Libyan economy. oil production, and healthcare infrastructure. The Libyan economy has been seriously affected by the intensifying conflict, which suffocates economic activity, the oil fields' closure, the decreasing oil, and the COVID-19 pandemic in 2020. These factors resulted in a significant deterioration of health services, a rise in communicable and non-communicable diseases, and poor outcomes of all cancers. In the absence of a strong government and the security gap, Libya became a transit point for illegal immigration towards Northern European countries and a final destination for African illegal immigrants.

According to the latest WHO data published in 2018, HCC deaths in Libya reached 228 or 0.73% of total deaths. The age-adjusted death rate is 6.13 per 100,000 population [2, 14]. A study conducted in Banighazi showed that HCC represented 5% of cancers in this region and ranked 12th [67].

Chronic HBV and HCV infection are an important risk factor for HCC in Libya [2]. A national study conducted in Libya showed that HCV was most prevalent among intravenous drug users (7.4%), followed by thalassemia patients receiving repeated blood transfusion (2.7%) and those who had a surgical operation (2.3%) or hospital admission (1.9%). Genotype 1 was the most frequent among all regions (19.7–40.5%), reaching the highest value in the Tripoli region, followed by genotype 4, which was more prevalent in the South (49.3%) and West (40.0%) regions. Genotype 3 was higher in Tripoli (21.3%) and East (15.9%) regions, while genotype 2 was common in North (23.6%) and South (22.5%) regions [68–70].

4.3 HCC in Syria

Syria's health system has been severely disrupted since the eruption of armed conflict in 2011. More than 50% of the country's public hospitals are not functioning. Up to 70% of the health workforce has fled Syria resulting in severe shortages in health facilities and qualified health personnel and failure of healthcare services and disruption of vaccination programs. Preventable diseases, particularly measles and HBV, reappeared in Syria due to the drop of vaccination coverage from 95% in 2010 to less than 40% in 2013 [71]. A retrospective study conducted between April 2014 and December 2015 on 171 Syrian refugee children aged between 0 and 18 years showed that about 5% were HBsAg and anti-HBc total positive and anti-HBs negative. HBV genotype D was the predominant type [72–74].

According to 2018 data [1], 380 new HCC cases occurred in Syria. The age-adjusted death rate is 5.25 per 100,000 of population or 0.39% of total deaths. In Syria, HCV, HBV, and alcohol contributed to 32, 14, and 19% of HCC cases, respectively [75].

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Current HCC Clinical and Research in Egypt

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1 Incidence and Risk Factors

1.1 Incidence

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. Egypt is the most populous country in the Middle East and the 14th most populous country in the world. The health authority in Egypt considers HCC as the most important health problem due to its increased incidence by twofold over a decade [1]. Using the incidence data of GLOBOCAN 2018 collected from Aswan, Damietta, and Minia cancer registries in Egypt, liver cancer showed high incidence (19.7% of the total cancer cases) and high cancer-related mortality (32.35% of the total cancer deaths) [2, 3]. This increased incidence of HCC in Egypt may be attributed to advancements in screening and diagnostic tools, as well as increased survival rate among cirrhotic patients, providing more time for HCC progression. This is in addition to increased incidence and complications of hepatitis C virus (HCV) infection, one of the most important risk factors for HCC in Egypt.

In global and Egyptian populations, HCC incidence shows gender variation. It represents the second and the sixth most common cancer in men and women, respectively [4]. Biological and environmental reasons play key roles in this gender variation. Biological reasons are attributed to sex hormones [5] as well as the difference in epigenetic and immune response, but an Egyptian study showed no clear relation between sex hormones levels and HCC [6]. The environmental reasons for HCC gender variation may be attributed to higher rate of men exposure to liver carcinogens (e.g., occupational exposure to chemical compounds, tobacco smoking, and alcohol) as well as other infectious risk factors especially hepatitis viral infection (both hepatitis B virus "HBV" and HCV) [7, 8].

In Egypt, as urban residents have better access to medical facilities, higher HCC incidence among urban population was detected, while HCC incidence among rural residents is undercounted [4, 9].

1.2 Risk Factors

Both HBV and HCV represent the major infectious risk factors for HCC in Egypt. Variability in the frequency of both HBV- and HCV-associated HCC in Egyptian patients is due to the variability in and the selectivity of the population studied (geographic region) as well as inconsistent viral testing methods used (Table 1).

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Table 1 Summary of studies reported the frequency of HBV and/or HCV infection among HCC Egyptian patients

Institution	Study period	Total study subjects/age	HBsAg, N(%)	HCV Ab, N(%)	HCV/HBV co-infection, N(%)	Notes (bilharziasis infection/treatment/ residency/other notes)	Reference
Ain Shams University hospitals	Jan. 2009 to Dec. 2011	1313/ range = 21–>80 years.	33 (2.51)	1199 (91.32)	35 (2.67)	Tartar emetic injection detected in 66.9% of the patients. 75% of patients came from rural area	[10]
Tanta Cancer center and the Gharbiah Cancer society	Dec. 2007 to Jan. 2009	148/range = 18–81 years	–	132 (89.2)	–	Patients was from rural area.	[11]
National Cancer Institute, Cairo University	1993 to 1999	403/range = 18–80 years	70 (17.5)	260 (64.4)	45 (11.2)	More than 97% of patients came from around the Nile River. Bilharziasis was prevalent in 71.4% of patients	[12]
Tanta University hospitals	Mar. 2009 to Feb. 2012	281/mean = 53.7 years	26 (9.25)	186 (66.19)	29 (10.32)	Patients were from rural area	[13]
National Liver Institute, Menoufia university	–	32/range = 42–72 years	15 (46.9)	22 (68.8)	12 (37.5)	–	[14]
National Liver institute, Menoufia university	–	60/–	19 (31.66)	31 (51.66)	–	–	[15]
National Cancer Institute, Cairo University	Jul. 1995 to Jan. 1996	33/mean = 55.2 years	5 (15.2)	25 (75.8)	–	Anti-Schistosoma IgG was detected in 21.2% of cases	[16]
Tropical medicine Department in Tanta University Hospital	Jan. 2005 to Jan. 2015	1440/–	47 (3.26)	1366 (79.38)	–	Patients were from rural area. 79.38% of HCC patients have anti-schistosomal antibodies	[7]
National Cancer Institute, Cairo University	1999–2009	132 (females)/ mean = 52.2 years	85 (64.4)	107 (81.1)	–	Among Egyptian women, multiple pregnancies increased the HCV-related risk for HCC	[17]
National Cancer Institute, Cairo University	–	236/mean = 54.1 years	18 (7.62)	204 (86.44)	–	63.5% of HCC cases are from rural area	[18]
National Cancer Institute, Cairo University	–	131/range = 18–80 years	95/129 (74)	99/131 (76)	–	About 28% of HCC patients came from urban area	[19]
National Cancer Institute, Cairo University	–	70/–	15 (21.4)	20 (30)	28 (40)	–	[20]

HBsAg hepatitis B surface antigen, *HCV Ab* hepatitis C virus antibody

1.2.1 Hepatitis B Virus (HBV)

Prevalence in Egypt

Between 1980 and 2007, HBV prevalence in Egypt was reported to be 6.7% among the general population and 25.9% among HCC patients [21]. In 2009, there was a decline in the seroprevalence of HBV among the general population, from 2.3% to 0.9% [22]. A cross-sectional analysis of the Egypt Health Issues Survey (EHIS) 2015 gathered from men and women aged 15–59 (15,777 samples) showed a decline in the HBV infection among general population (1.4%) [23]. The discovery of the HBV vaccine in the 1980s [24] and the application of universal infantile hepatitis B vaccination in 1992 were the main reason for this marked decline [25, 26]. Similar to the decline of HBV in the general population, a single-center study reported a decline in HBV among HCC-infected patients from 38.6% to 20.5% as a comparison between two consecutive period time (1993–1997) and (1998–2002), respectively [1]. Another single-center study reported that the percentage of HBV among HCC-infected patients over 10 years (2005–2015) was 3.2% [7].

Globally and in Egypt, transmission of HBV infection is mixed (horizontal and vertical) [8]. According to EHIS 2015 analysis, the most powerful driver of HBV infection in Egypt is sharing a household with a HBV-infected person [23].

HBV Genotype in Egypt

HBV genotypes are associated with differences in geographical distribution, clinical outcome, and the response to the antiviral treatment. Generally, there are eight genotypes [A–H] of HBV. In 2011, HBV genotype D (HBV/D) has been reported to be the most prevalent among Egyptians which is close to that in other Mediterranean countries [27]. Another study showed the presence of HBV genotype E among Egyptian healthcare workers [28].

1.2.2 Hepatitis C Virus (HCV)

Prevalence in Egypt

Egypt represents one of the top global burden with HCV. The annual rate of HCV-associated HCC is 3.36% [29, 30]. The prevalent HCV infection was caused by a large national treatment campaign of intravenous anti-schistosomal injections (tartar emetic) between the 1950s and 1980s. The target population of this treatment campaign was children and young adults with an average of nine injections/patient, which was reduced to six after 1975. This mass campaign led to increased HCV transmission due to poor sterilization technique, multiple injections over a time period, as well as a major mistake of reusing equipment. Until the 1990s, HCV infection was not known in medical sciences. So, absence of clinical symptoms in nearly 80% of HCV-infected patients treated in this campaign complicated the issue [29, 31–33]. The HCV seroprevalence detected in 1996, 2008, and 2015 by DHS (the Demographic Health Survey) was >40%, 14.7%, and 10%, respectively. This decline in the percentage of HCV seroprevalence between these time points was attributed to the aging of patients infected in the national campaign for schistosomiasis treatment 50 years ago [29].

In addition to the parental anti-schistosomal treatment, there are other risk factors for HCV transmission inside healthcare settings (e.g., blood transfusion) and outside healthcare setting (e.g., sharing personal equipment at home and also during circumcision performed by local healers and barbers in rural areas) [34, 35].

HCV Genotype in Egypt

Out of the seven HCV genotypes, genotype 4 is the most prevalent HCV infection in Egypt, and it represents 92.5% [36–39].

1.2.3 Chemical Compounds

Chemical compounds are an environmental (non-infectious) risk factor for HCC. The occupational activities of many individuals include exposure to a variety of chemical compounds. The liver plays the principal role in the processes of detoxification, metabolism, and excretion. Consequently, HCC can be the result of the adverse effects of many chemical compounds (both organic and inorganic). In Egypt, about one fourth of the population work in agriculture, rais-

ing their risk for HCC due to pesticide exposure [8]. Using carbamate and organophosphate compounds in agriculture is an additive HCC risk factor among rural males in addition to other well-documented risk factors (HBV and HCV) [18]. A study in the mid-delta area showed that both pesticides and fertilizers (phosphate and ammonium sulfate) were suggested to be independent HCC risk factors among residents [4].

1.2.4 Aflatoxin B1 (AFB1)

Aflatoxins are toxic metabolites of certain fungi (*Aspergillus flavus* and *A. parasiticum*) and have a well-documented role as a potent hepatocarcinogen. During both cultivation and storage post-harvest, they contaminate food commodities, especially in countries with hot and humid climates. In an Egyptian case-control study, HCC patients had a significant high percent of serum aflatoxin compared to the controls and a two-fold increased risk [40].

Many studies were done to identify the aflatoxin level in food products (local and imported samples) in different Egyptian governorates. Samples were positive for aflatoxins. Both the area of collection and the season of the year are two important factors affecting the aflatoxin level in these samples [40].

The most toxic naturally occurring aflatoxin is aflatoxin B1 (AFB1) that is classified as a group 1 human carcinogen according to the International Agency of Research on Cancer [41]. It has mutagenic effect due to single-base substitution in codon 249 of tumor suppressor *p53* gene. This point mutation has been detected in many Egyptian patients with AFB1-associated HCC [42]. In a case-control study, the prevalence of AFB1 was detected in 17% of HCC cases compared to 9.4% of controls. Also, there was a significant high level of AFB1 in HCC patients with both multiple lesions and tumor size >5 cm [43]. Aflatoxin-albumin (AF-alb) adduct is a validated biomarker of aflatoxin exposure. In a pilot study on 46 Egyptian HCC patients, the prevalence of AF-alb adduct was detected in 67.4% of HCC cases [44].

1.2.5 Other HCC Risk Factors

Limited numbers of epidemiological studies investigated the prevalence and association of other global HCC risk factors [8] among Egyptian patients. Family history is one of HCC risk factors, and a single study that included 103 HCC Egyptian patients reported that 21.4% of patients have a family history (1st- and 2nd-degree relatives) of HCC [45]. Also, diabetes is another HCC risk factor. Out of the five studies that showed the prevalence of diabetes among HCC Egyptian patients [4, 7, 45–47], only Ziada et al.'s study confirmed that type 2 diabetes raises HCC risk by two- to three fold [45]. For tobacco smoking, its association with HCC risk among Egyptian patients showed conflicting results [4, 45, 48].

2 Screening and Surveillance

In 2011, the Egyptian Society of Liver Cancer (ESLC) published the Egyptian Guidelines for management of HCC. It recommends for high-risk patients a screening every 4 months using both abdominal ultrasonography (US) and α-fetoprotein (AFP).

High-risk patients include:

1. All cirrhotic hepatitis patients with HBV infection, HCV infection, NASH, alcoholic cirrhosis, and genetic hemochromatosis.
2. Non-cirrhotic patients with HBV infection (carrier)

The recommended routine screening helps in the discovery of HCC in 33–50% of patients [49, 50], while symptomatic presentation accounted for the rest. Though an Egyptian study documented the effect of HCC surveillance in doubling the chances for the available curative options [51], but till now, there is no HCC surveillance program implemented in Egypt.

3 Diagnosis and Treatment

3.1 Diagnosis

In Egypt, there are many local factors that may affect disease presentation. Although there are seven types of HCC treatment centers [8], their

geographical distribution all over Egypt should be reassessed based on the difference of HCC patients' flow from different Egyptian governorates. Also, both the socioeconomic status of the HCC Egyptian patients and the lack of uniform health insurance that cover all Egyptians are additional factors affecting the disease presentation. As such, HCC patients could present with advanced disease due to the unavailability of complementary high-quality diagnostic tools that ensure standard and equal level of care among Egyptians in all Egyptian governorates.

Abdominal pain and jaundice represent the most common clinical presentations of HCC patient [49]. These clinical presentations are different from that in Western and Asian reports and indicate advanced disease stage which is accountable for accelerated liver function deterioration and poor survival outcome. In addition, fatigue, ascites, and, less frequently, cough and encephalopathy are clinical presentations in untreated HCC patients [52].

According to the Egyptian Guidelines for HCC, the diagnosis of HCC is based on tumor size, AFP level, and the triphasic spiral CT scan abdomen or a dynamic contrast MRI ≥1.5 tesla, whenever recommended.

Both chest/pelvis CT with contrast and bone scan are done for screening HCC metastasis if there is clinical suspicion or symptomatic patient.

3.2 Treatment

An accurate HCC staging at initial diagnosis would be helpful for both determination of the treatment options and the overall disease prognosis. A study that included 2000 HCC Egyptian patients (between January 2010 and December 2012) was done to compare four different staging systems for predicting prognosis and survival. The Barcelona Clinic Liver Cancer (BCLC) staging system was the most optimum prognostic system in HCC Egyptian patients, while the Cancer of the Liver Italian Program (CLIP) score is the best in HCC patients not amenable for treatment [50].

There are two therapeutic modalities in HCC: curative treatment and tumor control treatment. Curative treatment includes surgical options [*surgical resection and liver transplantation*] as well as ablative electrochemical therapies [*radiofrequency ablation (RFA), microwave ablation (MWA), and percutaneous ethanol injection (PEI)*]. The tumor control treatment includes non-ablative treatment [*catheter-based embolic therapies and non-catheter-based therapy*] and systemic therapies.

Low percentage of patients were diagnosed at an early stage (19.4–32.4%) and were candidates for curative treatment [1, 53]. All curative treatment modalities are available for HCC Egyptian patients [49, 54–56]. For liver transplantation, 19 Egyptian medical centers are currently licensed to perform liver transplantation partially funded by Egyptian government. Between August 2001 and August 2019, HCC cases represent 27% of the total number of living donor liver transplantation (LDLT): 4225 LDLT (personal communication). Most of the patients who received curative treatment showed significantly high survival [49]. This finding sheds light on the importance of early detection of HCC through screening and surveillance of high-risk patients implemented by the Egyptian MOH.

For non-curative treatment, transarterial chemo-embolization (TACE) is the most common tumor control treatment for HCC patients [49, 54, 55]. Also, systemic single or combined cytotoxic chemotherapy drugs are used under Egyptian national insurance as tumor control option. Molecular targeted therapies and immune checkpoint inhibitors are other systemic therapies available in the Egyptian market (cabozantinib, lenvatinib, sorafenib, regorafenib, nivolumab, pembrolizumab, ramucirumab) [40], but they are expensive therapies whose usage is limited to patients who can afford the cost as they are not available under Egyptian national insurance.

The overall survival of HCC Egyptian patients was reported to be nearly 80% at 6 months, nearly 55% at 1 year, and nearly 20% at 2 years [49, 53]. The median overall survival of untreated HCC patients is 2.3 months [52].

In addition to the most common prognostic factors reported, there are other local factors associated with poor prognosis of HCC among Egyptians. It includes the lack of the state of the

art in treatment for HCC covering all Egyptian patients (e.g., lack of health insurance that allows access to treatment, limited certain therapeutic modalities available for HCC patients, and limited resources or access to proper treatment especially molecular targeting therapies and immune checkpoints inhibitors).

4 Preventive Strategy for HCC

The Egyptian Ministry of Health and Population (MOHP) has a critical role in the HCC prevention through primary prevention (early prevention of HCC risk factors) and secondary prevention (treatment of risk factors at an early stage). The most successful strategy to reduce HBV incidence was the compulsory application of Egyptian infantile HBV vaccination program in 1992 [25, 26]. Also, Egyptian MOH exerted great efforts in HCV eradication. In 2006, the Egyptian Ministry of Health (MOH) established the National Committee for Control of Viral Hepatitis (NCCVH). NCCVH put the national treatment strategy for controlling HCV infection. To facilitate application of this strategy, a national network was established between specialized centers in HCV treatment. The main challenge was the high cost of the antiviral medication that should be provided for free or at reduced price to attract HCV-infected patients. In 2014 and with the introduction of direct-acting antivirals (DAAs), NCCVH succeeded to get DAAs at reduced cost after negotiation with manufacturing companies. After 3 years and with local production of DAA generics, the total number of HCV-infected patients treated under this program was more than 2 million patients [38, 57, 58].

In early 2018, a successful nationwide HCV screening and treatment program was launched by Egyptian MOH. Out of 49.6 million persons screened over a period of 7 months, 2.2 million HCV-seropositive persons were identified and referred for treatment in a government-subsidized treatment program. The total cost of this national program including screening, evaluation, and treatment was $207.1 million [58].

A major threat to the preventive strategies for both HBV and HCV is the instability in the Mediterranean region, due to wars and the Arab Spring, and the resulting immigration to Egypt. This immigration represents a challenge that may affect the epidemiological trends of HBV- and HCV-related HCC. Screening for immigrants is the most convenient solution for this challenge [8].

HCC risk factor prevention health program is another approach for HCC prevention and is highly recommended by the World Gastroenterology Organisation's global guidelines [59]. As well-trained healthcare provider has a vital role to identify patients at risk and refer them for screening and surveillance [60]. Also, two Egyptian studies used education-based intervention program showed promising results among the rural population (high risk) [61, 62].

5 General Overview of HCC-Related Research

On PubMed, the total number of publications about HCC in Egypt is 544 articles [63]. Many Egyptian studies identified some molecular abnormalities in HCC [64, 65]. In the era of advanced technology in molecular biology, other molecular abnormalities can be identified in a wide-scale survey of HCC Egyptian patients. This is highly required for implementation of personalized therapy concept and for improvement of the overall survival of HCC patients. Research biobank can help in the availability of HCC biological specimens for this objective.

5.1 Research Biobank

Research biobank is the organized collection and storage of human biological material for research purposes. It is considered invaluable resource for many types of medical research (e.g., genomic research, proteomics research, precision medicine). The introduction of research biobank concept is recent in Egypt, and its establishment requires both training and funding. That is why there are currently only eight research institu-

tions in Egypt housing biobank repositories. There are continuous efforts in other centers to establish additional repositories. All of these research biobanks are disease-oriented. A tangible research outcome of these biobanks especially in HCC research is not yet attained.

5.2 Clinical Trials

In Egypt, the concept of clinical trials was challenged over many years ago due to the lack of public awareness. That is why the overall number of HCC clinical trials registered on clinicaltrials.gov is low (ten interventional clinical trials). Except for one, all of these clinical trials are phase III. It is predictable that the public awareness about the importance of clinical trials was elevated after the COVID-19 pandemic in 2020. Also, on August 24, 2020, the Egyptian parliament as the legislative branch approved a national law to regulate clinical medical research in Egypt and the higher authority attestation on it was done on December 23, 2020. A change in the overall status of clinical trials in Egypt is expected.

The overall status of HCC research requires a focused attention especially in the era of precision medicine. The presence of a national HCC research program with well-determined strategy and goals will optimize resource allocation which is highly important in a low-income country like Egypt. In addition, it will identify gaps in knowledge and optimize collaboration between HCC Egyptian centers and peer international HCC institutions. Government health administration plays a pivotal role in this critical issue.

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Hepatocellular Carcinoma in Turkey: A Review of Disease Epidemiology and Treatment Outcomes

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1 Introduction

Globally 841,000 new liver malignancies were reported according to the World Health Organization (WHO) data representing 4% of all new cancers worldwide in 2018 [1]. Overall 8.2% of cancer deaths were due to liver malignancy [1]. According to the most recent Turkish Ministry of Health Cancer Statistics data, total of 440,810 malignancies were reported in Turkey between 2012 and 2016 [2, 3]. Of these, 5794 were liver malignancy, representing 1.3% of all malignancies in Turkey between those years, much less proportion compared to the global data [2, 3]. According to WHO reports, liver malignancy is the 17th most common malignancy in Turkey. Herein we summarize available literature on hepatocellular carcinoma (HCC) demographics and available disease features and therapeutic outcomes described in Turkey.

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2 Methods

We performed a literture review in MEDLINE with key words "HCC, Turkey" "HCC, outcomes, Turkey" "liver resection, HCC, Turkey" "Liver transplant, HCC, Turkey".

3 Results

A large Turkish HCC review was reported by Can et al., and they reviewed a total of 963 patients diagnosed with HCC in their study [4]. The distribution of the patients by provinces is as follows: 181 from Ankara (capital city), 40 from Diyarbakir, 185 from Izmir, 103 from Van, 16 from Malatya, 100 from Antalya, 50 from Isparta, 13 from Sivas, 98 from Istanbul, 50 from Bolu, 83 from Kayseri, 25 from Elazig, and 20 from Gaziantep. This is quite homogeneous sampling except that the north of the country was not represented. They reported that 205 (21%) of the 963 patients were women, with a male/female predominance of 4.8:1 and a median age of 61 years. The etiologic risk factors for HCC were hepatitis B in 555 patients (57.6%), 453 (81%) in men and 102 (19%) in women, again with male predominance, hepatitis C in 159 (16.5%), (14.9% and 22.4%, with a higher incidence in women), and chronic alcohol abuse (more than 10 years) in 137 (14.2%) (16.8% and 4.9%, higher in males). The Child-Pugh score paralleled with advanced disease stage and also a high level of AFP. They also

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investigated tumor stage between genders. There were 193 (20%) patients at Stage I, 248 (25.8%) at Stage II, 261 (27.1%) at Stage III, and 261 (27.1%) at Stage IV with more than half of patients being in advanced stage. Of the women, 44 (21.5%) had Stage I disease, 54 (26.3%) had Stage II disease, 51 (24.9%) had Stage III disease, and 56 (27.3%) had Stage IV disease. Of the men, 149 (19.7%) had Stage I disease, 194 (25.6%) had Stage II disease, 210 (27.7%) had Stage III disease, and 205 (27%) had Stage IV disease. No difference was found between men and women, in terms of tumor stage classification (p: 0.855). They also could not find association between tumor stage and disease etiology [4]. They also reported that 67.3% of the patients had high AFP levels (>20 ng/dL), and high AFP level was parallel to advanced-stage disease (p = 0.037) [4]. They concluded the viral etiology (hepatitis B and hepatitis C infections) in the Turkish population is the most common etiology in HCC development, followed by alcohol abuse [4]. The Child-Pugh classification and AFP levels were found to be prognostic factors in Turkish HCC patients [4]. There was no survival data in this report. It should also be noted that distribution of the patients reported here is not reflective of actual population data, for instance, Istanbul is the largest city of Turkey, representing almost 20% of the entire population; therefore, we could comment that there is some selection bias in this report.

Another report by Akkiz et al. reviewed a large cohort of HCC patients (n = 1332) from several collaborating Turkish institutions [5]. They reviewed baseline features and tumor parameters such as maximum tumor diameter (MTD), portal vein thrombosis (PVT), and α-fetoprotein (AFP) levels. The predominant etiological factor was HBV (60.86%), followed by HCV (20.72%). The mean maximum tumor diameter was 5.89 cm, with approximately one-third of patients having tumors 6.5 cm. The mean AFP level was 5686.54 IU/mL; 42.13% of patients had normal AFP laboratory values, and 41.97% of patients had AFP values >100 IU/mL. The mean albumin was low at 3.09 g/dL, and the mean total bilirubin was elevated at 2.96 mg/dL. A comparison of patients with and without PVT showed significantly larger tumors; greater multifocality, blood AFP, and C-reactive protein levels; and, interestingly, lower HDL levels in the patients with PVT [5]. They also evaluated the distribution of HCC features based on cities. They included six cities, mostly southeast area of Turkey and the capital city with the following estimated populations: Ankara 5.5 M, Adana 2.2 M, Diyarbakir 1.6 M, Hatay 1.6 M, Mardin 890 K, and Mersin 1 M. This is not a homogeneous representation of the country given there are 84 cities in the entire country, and their population did not include Istanbul, with 15.5 M population, and third largest city Izmir 4.3 M. Despite the limitations, it is worthwhile to review their data: Highest mean MTD was higher in Mersin, whereas Ankara and Hatay had lowest MTD (7.0 cm vs. 5.03 and 5.33 cm, respectively). Mersin had 40.74% incidence of PVT, and Hatay had lowest incidence of PVT (20.69%) consistent with MTD finding. Multifocality was highest in Diyarbakir (53.49%) and lowest in Mersin (17.02% of patients). Mean AFP levels were highest in Mersin (10,109 ng/mL) and lowest in Mardin and Ankara (2885 and 3254 ng/mL, respectively). There were also large regional differences in underlying liver disease. Cirrhosis was present in 88.29% of patients in Diyarbakir but only in 62.92% of the patients in Hatay. HBV was the most common etiology with 73.95% in Diyarbakir, but the HBV rate was 40.24% in Hatay. HCV incidence was highest in Hatay (30.49%) and lowest in Diyarbakir and Mardin (8.37% and 7.81%, respectively). HDV was found in 17.13% of the patients in Diyarbakir but <10% elsewhere [5]. One of the shortcomings of this review is that they do not mention the differences of early diagnostics, differences to access to medical care, and social cultural and even religious barriers to seek professional medical care, which are known to be quite different among different regions in Turkey, despite the universal and socialized healthcare.

Alacacioglu et al. reviewed 221 patients with HCC from 5 hospitals in Turkey [6]. They reported that 44.4% of the entire cohort had HBV (98 patients, 5 had alcohol abuse and 2 had hepatitis D also) followed by HCV (47 patients;

21.3%) (five of them also had alcohol abuse) as the underlying cause of HCC (6). Other etiologies were as follows: hepatitis B and C coinfection (11 patients; 5%), chronic alcohol abuse (more than 10 years) (13 patients; 5.9%), and cryptogenic cirrhosis (nine patients; 4.1%). No etiologic cause could be identified in 43 patients (19.5%). The single nodule was the dominant tumor pattern (153 patients, 69.2%) [6]. The most common tumor diameter was <5 cm in 100 (45.2%) patients. Extrahepatic metastasis (lung, bone, and adrenal gland) was present in 12 (5%) patients. Stage IV tumor according to TNM classification was present in 58 (26%) patients. The HCC patients with HBV had mostly Stage III and IV disease (41.8% and 26.5%, respectively), whereas HCC patients with HCV had shown mostly similar distributions according to disease stage (Stage I, 12.8%; Stage II, 29.8%; Stage III, 27%; Stage IV, 29.7%). The AFP levels were >400 ng/dl in 48 (21.7%) patients, which was considered as diagnostic for HCC. Only 31 (14%) patients received surgical therapy (resection, liver transplantation). One hundred and ninety (86%) patients received palliative therapy or no therapy. The overall survival (OS) was 14 months. Patients aged <60 years and female gender had longer survival compared with those aged >60 years and male gender (median OS: 15 vs. 12.6 months, $P = 0.619$ and 17.6 vs. 11.7 months, $P = 0.057$, respectively). The OS of patients with cirrhosis and nonviral hepatitis was shorter with respect to those with no cirrhosis and nonviral hepatitis (median OS: 13.9 versus 19.1 months, $P = 0.286$ and 10.7 versus 15.3 months, $P = 0.797$) although none showed statistical significance [6]. The OS of the individuals with normal AFP levels was also longer than that with high AFP levels. They concluded that the viral etiologies (hepatitis B and C infections) in Turkish population are the top two leading etiologies of HCC development [6]. The Child-Pugh classification, AFP levels, TNM classification, female gender, and treatment status were prognostic factors in HCC patients, whereas viral disease versus other etiologies had similar OS [6]. Quarter of the patients were Stage IV, and the vast majority of the patients (>80%) had noncurative treatments; therefore, there seems to be a selection bias in this report. They highlighted that due to the lack of national HCC screening program in Turkey, patients are diagnosed at advanced stages [6]. This holds true as of 2020.

Similar to above report, Dogan et al. reported clinicopathologic characteristics and risk factors for HCC in Turkey [7]. They retrospectively reviewed 98 HCC patients between 2004 and 2011. Median age was 61 (range, 16 to 82). Majority (80.6%) was male, and number 1 etiology was HBV (60.2%) followed by HCV (15.3%) and alcohol-related liver disease (15.3%). Seventy-two (73.5%) were at advanced stage, and 54 (55.1%) had elevated serum alpha-fetoprotein (AFP). Median OS was 7.0 months (range 0–145 mo), and median OS was significantly longer in female patients ($p < 0.024$). Median OS was significantly lower in HBV patients compared to other etiologies ($p < 0.016$) [7]. Distribution of TNM stages was as follows: Stage I was 10.2%, Stage II was 13%, Stage III was 57.8%, and Stage IV was 12.3%. In other words, 73.5% of the patients were diagnosed at advanced stage (Stages III and IV) consistent with former reports. Only nine patients (9.2%) had undergone surgery. Chemoembolization is performed in 14 (14.3%) patients. Twenty (20.4%) patients had cisplatin, interferon, adriamycin, and 5-fluorouracil combination chemotherapy protocol, eight (8.2%) patients had single agent adriamycin, five (5.1%) had 5FU and folinic acid, four (4.1%) patients had UFT, and six (6.1%) patients had been treated with tyrosine inhibitor sorafenib [7]. They reported no statistically significant difference between treatment type for median OS [7]; however, there was no adjustment for tumor stage or the degree of liver disease; therefore, it is difficult to compare outcomes based on treatment type alone.

They concluded that HCC is a highly lethal tumor and generally diagnosed in advanced stage (Stages III and IV) in Turkey [7]. As a consequence, very few patients underwent definitive surgical treatment in this cohort [7]. Leading causes were HBV, HCV, and alcohol consumption in descending order, similar to other reports [4–7].

We found one report regarding the molecular features of HCC in Turkey. Ozdemir et al. investigated prevalence of codon 249 mutation of p53 in HCC, cirrhosis, and chronic hepatitis B (CHB) patients [8]. This mutation is induced by aflatoxins, products of *Aspergillus flavus*, which could be seen in potential food contamination. They reported that the codon 249 mutation of p53 is found in one out of 50 HCC (2%) patients. They concluded although codon 249 mutation of p53 is rare, it does exist in HCC patients in Turkey [8].

4 HBV and HCV in Turkey

According to the WHO, Turkey has intermediate (2–8%) endemicity for HBV [1, 2]. A report by Ozkan stated that the estimated number of HBV carriers in Turkey is about 3.3 million, with an overall HBV prevalence of 4.57%; thus, both prevention and therapy of HBV-infected patients are urgent medical need of Turkey [9]. He stated that even a very conservative assessment means that 10% of the carriers would need treatment, which means that 330,000 chronic HBV cases would be eligible for treatment in Turkey alone [9].

There was no leading cause of HBV being endemic in Turkey; however, the Ministry of Health report listed below groups as high-risk groups for HBV infection in Turkey. Some known examples are:

- Healthcare workers.
- Hemodialysis patients.
- Vertical and horizontal transmission within family.
- Substance abusers.
- Multiple sexual partners/sexual habits.

According to a study carried out by TURKHEP in 2010, hepatitis B virus carriage (HBSag+) is 4% in Turkey (TURKHEP, 2010). In the same study, the hepatitis C virus carriers in Turkey (anti-HCV+) were reported to be 0.95% [10]. In 1998, the universal infant immunization program changed the HBV epidemiology in Turkey and has become mandatory at birth since 2003 and is covered by the universal healthcare. This has resulted in an apparent trend towards reduced disease levels. However, prevalence of HBV infection is still high in adolescent and young adults. We will likely continue to see ongoing decrease in the following decades. Igde et al. reviewed a total of 101,648 patients of all ages at a tertiary level hospital in Samsun, at the north coast of Turkey, between 2014 and 2016. HBsAg and anti-HB seropositivity was found to be 4% and 38.3%, respectively [11]. They concluded that catch-up immunization programs, education, and follow-up policies in addition to routine infant immunization are needed to further decrease the HBV infection rates in Turkey [11].

Aygen et al. reviewed the Turk-Hepatitis Registry (HEP-NET) Project data, which included real-life cohort of hepatitis patients from 15 centers in Turkey [12]. In the project, 10,165 hepatitis patients from 10 different hospitals were evaluated. According to the results, HBV/HCV coinfection was detected in 99 patients. The mean age of the cases was 40.9 + 21.7 years; 56.6% of them were males and 43.4% were females. The most important risk factor was hemodialysis (25%) in this group followed by dental therapy, surgical procedure, and blood transfusion [12, 13].

Tozun et al. reviewed participants from urban and rural areas of the predetermined 23 EUROSTAT NUTS 2 region (n = 5460) (mean (SD) age, 40.8 (14.7) years) [14]. They reported that the seropositivity rates for hepatitis B surface antigen (HBsAg), anti-HCV, anti-HBs, and anti-HBc total were 4.0%, 1.0%, 31.9%, and 30.6%, respectively [14]. Among HBsAg-positive cases, 94.5% were anti-HBe positive, 70.2% were HBV-DNA-positive, and 2.8% were anti-HDV total positive; 99.1% of HBV infections were of genotype D. Close contact with a hepatitis patient (OR 3.24; 95% CI 2.25–4.66; $p < 0.001$), living in the southeastern region (OR 2.74; 95% CI 1.7–4.45; $p < 0.001$), male gender (OR 1.77; 95% CI 1.28–2.46; $p < 0.001$), married status (OR 1.62; 95% CI 1.02–2.57; $p = 0.038$), education less than high school (OR 1.53; 95% CI 1.04–2.26; $p = 0.03$), orodental interventions (OR 1.54; 95% CI 1.01–2.35; $p = 0.047$), and a history of non-

disposable syringe use (OR 1.4; 95% CI 1.01–1.96; $p = 0.045$) were significant determinants of HBsAg positivity. Age ≥50 years (OR 2; 95% CI 1.09–4.3; $p = 0.026$) was the only significant predictor of anti-HCV positivity. Study revealed an HBsAg positivity in 4% and anti-HCV positivity in 1% of the adult population, and at least one-third of the population has been exposed to HBV infection in Turkey [14].

5 Comparison of HCC Treatment Modalities

Akcam et al. compared outcomes of patients who underwent liver resection ($n = 38$) vs. OLT ($n = 28$) for localized HCC [15]. A total of 66 patients (with a median age of 62) who met the study criteria were analyzed. Postoperative complications (13.2% vs. 28.6%, $P = 0.45$) and perioperative mortality rates (7.9% vs. 10.7%, $P = 0.2$) were similar. While Child-Pugh Class A patients were more prevalent in the resection group (78.9% vs. 7.1%, $P = 0.0001$), the rate of patients who met the Milan criteria was higher in the OLT group (89.3% vs. 34.25, $P = 0.0001$) [15]. Recurrence rates were 36.8% in the resection group and 3.6% in the OLT group at the end of the median follow-up period (32 vs. 39 months, respectively). The HCC-related mortality rate was significantly higher in the resection group (39.5% vs. 10.7%, $P = 0.034$). However, a subgroup analysis of patients who met the Milan criteria revealed similar rates of recurrence and HCC-related mortality (15.4% vs. 8%, $P = 0.63$). Based on logistic regression analysis, number of tumors ($P = 0.034$; odds ratio, 2.1) and "resection"-type surgery ($P = 0.008$; odds ratio, 20.2) were independently associated with recurrence [15].

Gokcan et al. retrospectively analyzed 12-year data of 115 patients with biopsy-proven HCC [16]. Most patients had cirrhosis due to hepatitis virus infection. Median follow-up time was 17 months (1 month–9.5 years) after the diagnosis of HCC. The nodule was single in 43 (37.4%) patients, there were 2–3 nodules in 30 (26.1%), and there were >3 or diffuse nodules in 42 (36.5%) patients. Distribution of treatment modalities was as follows: 23 (20%) patients had liver transplantation, 15 (13%) had HCC resection, 12 patients (10.4%) had radiofrequency ablation (RFA), 26 patients (22.6%) had transarterial chemoembolization (TACE), 2 (1.7%) had alcohol ablation, and 37 patients (32.2%) had no treatment. Tumor sizes of nine patients (39.1%) in the transplanted group exceeded the Milan criteria. Mean survival was 72 ± 6.9, 78.8 ± 12.5, 19.5 ± 2.8, 20.6 ± 4.2, and 16.0 ± 5.9 months in those that received transplantation, resection, RFA, TACE, and no treatment, respectively ($p < 0.001$) [16]. Survival was significantly worse in patients >63 years old ($p = 0.001$), with serum albumin level ≤3.4 g/dL ($p = 0.01$), and with diffuse HCC ($p < 0.001$). They concluded survival was significantly better in patients who underwent liver transplantation or surgical resection [16].

6 Outcomes of Liver Transplantation for HCC in Turkey

Liver transplantation (LT) is the only curative treatment for HCC in patients with underlying chronic liver disease. Living donor liver transplantation (LDLT) is more common in Turkey compared to deceased donor liver transplantation (DDLT). According to the Ministry of Health reports, there were a total of 7423 liver transplants performed in Turkey in the last 5 years (2015–2019), of which 5456 (73.5%) were LDLT and 1967 (26.4%) were DDLT [17]. In this section, we summarized relevant studies about the outcomes of LT in HCC patients in Turkey.

Regarding living donation, there is no scientific report as to why the rates are so high; however, based on our observations, we could hypothesize that this is multifactorial: (1) lack of a robust deceased donor system, (2) close family ties, and (3) cultural and religious beliefs and perceptions. In other words, we do not know if living donation rates would remain the same in the event of having a robust and uniform deceased donor organization across the country. This could

be another area of research and quality improvement project for Turkey.

Balci et al. examined the outcomes of patients who received LDLT for HCC comparing the impact of up-to-seven criteria and Asan criteria (AC) with Milan criteria (MC) [18]. Between July 2004 and July 2009, of the 175 consecutive LDLT cases, there were 45 consecutive patients with HCC. Forty patients who completed 12-month follow-up were enrolled. In search for the highest number of expansion, they selected AC as the extended criteria. Patients were divided into having tumors within MC, beyond MC within AC, and beyond criteria (BC) groups. With a median follow-up of 46 months, overall 1-, 3-, and 5-year survival was −90%, −81%, and −70%, respectively. In patients within AC, estimated mean survival was 49.8 vs. 40.5 months for BC group (P = 0.2). Disease-free survival was significantly higher in patients within AC compared with BC group: 48.0 vs. 38.6 months (P = 0.04). Preoperative AFP level >400 and poor tumor differentiation were factors adversely effecting recipient survival. On multivariate analysis, the presence of poor tumor differentiation (P = 0.018 RR: 2.48) was the only independent predictor of survival [18]. They concluded that extension of tumor size and number to AC is feasible, without significantly compromising outcomes; however, the presence of poor tumor differentiation was associated with worse outcomes after LDLT [18].

Ince et al. reported 215 patients who underwent predominantly live donor liver transplant for HCC at their institute in Malatya over 12 years [19]. There were 152 patients within and 63 patients beyond Milan criteria. Patients beyond Milan criteria were divided into two groups according to presence or absence of tumor recurrence. Recurrence-associated factors were analyzed. These factors were then applied to the total cohort for survival analysis. They identified four factors, using multivariate analysis, that were significantly associated with tumor recurrence. These were maximum tumor diameter, degree of tumor differentiation, and serum AFP and GGT levels. A model that included all four of these factors was constructed, the "Malatya criteria." Using these Malatya criteria, they estimated disease-free survival and cumulative survival, for patients within and beyond these criteria, and found statistically significant differences with improved survival in patients within Malatya criteria. Survival of the patients within the newly defined Malatya criteria compared favorably with beyond Milan extended criteria and highlighted the usefulness of serum AFP and GGT levels in decision-making [19].

A single-center study by Unek et al. assessed the validity of the Milan and University of California San Francisco (UCSF) criteria and examined the long-term outcome of LT in Turkish patients with HCC in Turkey [20]. They reviewed cases between 1998 and 2009, and 56 of 356 OLTs were performed for HCC. Based on explant pathology, patients were categorized into three groups: Milan + (n = 34), Milan -/UCSF + (n = 7), and UCSF- (n = 14). Median follow-up period was 39.5 (1–124) months. The 5-year overall survival rates in the Milan +, Milan -/UCSF +, and UCSF groups were 87.7%, 53.6%, and 33.3%, respectively (P < 0.001). Within these groups, tumor recurrence was determined in 5.8%, 14.3%, and 40% of patients, respectively (P < 0.011). Additionally, the presence of microvascular invasion within the explanted liver had a negative effect on the 5-year disease-free survival (74.7% vs. 46.7%, P < 0.044). They concluded the Milan criteria are reliable in the selection of suitable candidates for OLT for the treatment of HCC [20]. They commented that UCSF criteria can be safely utilized for LDLT [20].

Yaprak et al. performed a clinicopathological analysis of risk factors that affected survival after LT [21]. Out of 389 LTs performed from 2004 to 2010, 102 were for HCC patients. Data were collected retrospectively. Variables were as follows: age, gender, preoperative alpha-fetoprotein (AFP) levels, Child-Pugh and MELD scores, prognostic staging criteria (Milan and UCSF), etiology, number of tumors, the largest tumor size, total tumor size, multifocality, intrahepatic portal vein tumor thrombosis, bilobar disease, and histological differentiation. One hundred and

two patients were evaluated. The 5-year overall survival rate was 56.5%. According to the UCSF criteria, 63% of the patients were within and 37% were beyond UCSF ($P = 0.03$). Ten patients were excluded (one with fibrolamellary HCC and nine because of early postoperative death without HCC recurrence), and 92 patients were assessed. The mean age of the patients was 56.5+/−6.9 years. Sixty-two patients underwent living donor liver transplantations. The mean follow-up time was 29.4+/−22.6 months. Fifteen patients (16.3%) died in the follow-up period due to HCC recurrence. Univariate analysis showed that AFP level, intrahepatic portal vein tumor thrombosis, histologic differentiation, and UCSF criteria were significant factors related to survival and tumor recurrence. The 5-year estimated overall survival rate was 62.2% in all patients. According to the UCSF criteria, the 5-year overall survival rate was 66.7% within and 52.7% beyond the criteria ($P = 0.04$). Multivariate analysis showed that AFP level and poor differentiation were independent factors [20]. They concluded prognostic criteria related to tumor biology (especially AFP level and histological differentiation) should be considered and poor differentiation and higher AFP levels are indicators of poor prognosis after LT [20].

Egeli et al. reviewed elderly HCC patients and LT outcomes [22]. The study reviewed 535 LT patients, of which 77 (14.4%) were over 60 years of age. The median follow-up period was 86.7 (1 to 247) months. The elderly group's survival rate was significantly lower than that of the younger group ($P = 0.002$). In elderly patients, survival rates of 1, 3, 5, and 10 years were 67.4%, 56.4%, 53.8%, and 46.1%, respectively [22]. They concluded appropriate selection in the preoperative stage provides successful survival results in elderly patients [22].

Polat et al. reviewed patients between 2011 and 2018, and 165 of 749 LTs for HCC cases performed at their center were evaluated retrospectively [23]. Survival, demographic characteristics and etiology, preoperative alpha-fetoprotein (AFP) level, Model for End-Stage Liver Disease (MELD) score, prognostic staging, and morphologic and histologic properties were evaluated. As a result, 139 cases of 165 were living donor liver transplantation (LDLT). The mean age was 57.7 ± 7.3 years, the mean follow-up period was 27.8 ± 20 months, and 41 patients (24%) died before follow-up. Recurrence of HCC was detected in 23 (14%) cases. Overall survival was 85%, 71%, and 64% for 1, 3, and 5 years, respectively. The 1-, 3-, and 5-year survival within vs. beyond Milan criteria was 90%, 80%, and 76% vs. 75%, 66%, and 44%, respectively. In the University of California San Francisco criteria, it was 86%, 76%, and 70% vs. 76%, 60%, and 30% compared with 1-, 3-, and 5-year survival. While histopathological poor differentiation and AFP elevation affected the course negatively, they reported that good differentiation did not have a significant effect on survival. They concluded that poor differentiation, lymphovascular invasion, and an increased number of nodules significantly affected survival in both within and beyond cases [23].

7 Summary

In the light of these data, we can conclude that, despite national immunization program, HBV remains the leading cause of HCC in Turkey, followed by HCV, alcohol-related liver disease, NASH, and cryptogenic liver failure. Despite limited data, there are variations in the country, not only in terms of etiology of HCC but also distribution of stages of the disease. Depending on the series, 50% or more of the HCC patients present in advanced stage; therefore, minority of HCC cases undergo curative treatments or transplantation. Liver transplantation is widely performed in Turkey with a dominance of living donor liver transplantation. There is a significant data gap regarding national outcomes data in Turkey. Social and cultural differences may play a role in the access to medical care which also require further research. Lastly, the lack of national HCC screening programs and a national HCC database magnify the burden of HCC in Turkey. We do believe there could be several steps to be taken in order to construct such database:

1. Create a Turkish National HCC Task Force Committee: The need of a national database and specific goals about HCC should be prepared by professional leaders, including but not limited to surgeons, hepatologists, oncologists, epidemiologists, and infectious disease experts to prepare a task force and present this to the Ministry of Health.
2. Seek government and private sources to finance such a national scale project.
3. Continuous growth of the task force by recruiting experts to take the ownership and to continue to lead the field in Turkey.

In the same context, multi-institutional collaborations would greatly enhance such goals and should be encouraged within each institute that delivers tertiary care for HCC.

As Turkey has universal healthcare, cost could be the main determinant of the decision-making process in healthcare. Therefore collegial work with the Ministry of Health would be a key factor of a national project. Also private sector attention could expedite such ideas by providing additional financial source.

As far as follow-up, every opportunity should be used for HBV/HCV and HCC screening. This could be done via several approaches:

1. Continuous medical education for general practitioners: For instace, regardless of the cause of a medical visit for a patient, patients should be offeref to be checked for viral serology since HBV is still the leading cause of cirrhosis in Turkey.
2. Supportive programs and initiatives directing both public and healthcare providers through the Ministry of Health.
3. Adding a “health curriculum” at different levels in school education, which should include but not limited to vaccinations, safe sexual practice. As mentioned above, a new Turkish National HCC Task Force would be a tremendous momentum to initiate such moves and create public and state and private attention.

While we are aware this is a huge undertaking, we believe such task force(s) would not only enhance national strategies to fight against a specific disease burden but also improve the patient care and outcomes in long term in Turkey.

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Targeting c-Met and AXL Crosstalk for the Treatment of Hepatocellular Carcinoma

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1 Introduction

Over the decades, successful strategies have led to significant improvement in prevention and treatment of cancer due to increased early diagnosis rates and the development of more effective therapeutics. According to cancer statistics, cancer-related deaths have been continuously declining between 1990 and 2019 in the United States, and projection studies estimate less mortality in 2020 [1]. A similar trend is expected to occur in most other countries including Turkey. However, the projections should be reevaluated due to COVID-19 pandemic which has caused delayed treatment and surgery and pause of activity in research labs and slowed clinical trials. Considering the rates before the global pandemic, long-term declines in the number of deaths related to lung, colorectal, prostate, and breast cancers resulted in an overall improvement in cancer mortality. Only a few cancers have increased incidence and mortality rates, and liver cancer is one of the outstanding.

1.1 Hepatocellular Carcinoma in the World and the Middle East and North Africa (MENA) Region

Worldwide, liver cancer ranks sixth in incidence (fifth in males) and fourth in cancer-related deaths (second in males) [2]. MENA region consists of many countries spreading in three continents in the Middle East and North Africa and is a highly populated part of the world with a population over 500 million. Turkey is considered as one of the countries in the MENA region. Based on GLOBOCAN 2018, in Turkey, liver cancer ranks the 15th of all cancers with 4362 new cases [2]. It also ranks ninth in mortality rates with 4307 deaths. Estimated number of deaths in Turkish female population in all ages is 1560 and ranks ninth, whereas in males the estimation is

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2747 and ranks 8th. Based on a report by Turkish Ministry of Health evaluating cancer statistics of Turkey in 2016, liver cancer incidence is higher in males; it ranks tenth in all age groups [3].

According to Global Burden of Disease (GBD) data of 1990–2019 from the Institute of Health Metrics and Evaluation, the age-standardized incidence, prevalence, and mortality rates of both sexes of liver cancer patients comparing MENA region and Turkey are given in Table 1. The data is extracted from and graphs are formed in GBD results tool [4]. Based on this evaluation, liver cancer incidence, prevalence, and mortality rates in Turkey are below the average of the MENA countries; however, all metrics are in rise since 1990 in both Turkey and the MENA region.

Primary liver cancer is the most common type of liver cancer and is classified into subtypes based on the origin of initiating cells: hepatocellular carcinoma, cholangiocarcinoma, hepatic angiosarcoma, fibrolamellar carcinoma, and hepatoblastoma [5]. Among them, HCC alone accounts for 90% of primary liver cancer [6]. Risk factors for HCC development are mostly non-hereditary and include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, aflatoxin exposure, excess alcohol consumption, non-alcoholic fatty liver disease (NAFLD), obesity, and diabetes [7]. Chronic HBV and HCV infections have been by far the major etiological agents that result in HCC and still remain as the most important risk factors both globally and in the MENA region as well [8, 9]. Chronic hepatitis C infection is the major cause of HCC in the MENA region in which HCV infection is the underlying etiology of 70% of HCC in this area [10]. In Turkey, similarly, the most important risk factors for HCC development are hepatitis B and C infections. In contrast to overall situation in MENA region, HCC in Turkey is mostly due to

Table 1 GBD data of age-standardized liver cancer incidence, prevalence, and mortality numbers and rates of Turkey, North Africa, and the Middle East

		Year	Value	Upper	Lower
Incidence					
Number	North Africa and Middle East	1990	10734.18	11996.56	9445.18
	North Africa and Middle East	2019	27545.69	33840.58	22112.90
	Turkey	1990	1349.21	1640.10	1079.85
	Turkey	2019	2767.82	3413.37	2204.34
Rate	North Africa and Middle East	1990	3.11	3.48	2.74
	North Africa and Middle East	2019	4.53	5.56	3.63
	Turkey	1990	2.26	2.74	1.81
	Turkey	2019	3.40	4.20	2.71
Prevalence					
Number	North Africa and Middle East	1990	11683.90	13022.01	10302.00
	North Africa and Middle East	2019	33485.32	40969.85	27179.33
	Turkey	1990	1460.58	1773.30	1163.89
	Turkey	2019	3750.23	4670.19	2959.21
Rate	North Africa and Middle East	1990	3.39	3.77	2.99
	North Africa and Middle East	2019	5.50	6.73	4.47
	Turkey	1990	2.44	2.97	1.95
	Turkey	2019	4.61	5.74	3.64
Death					
Number	North Africa and Middle East	1990	10913.41	12227.10	9575.47
	North Africa and Middle East	2019	26432.40	32610.87	21210.64
	Turkey	1990	1376.97	1665.60	1104.03
	Turkey	2019	2536.79	3120.69	2009.00
Rate	North Africa and Middle East	1990	3.16	3.54	2.78
	North Africa and Middle East	2019	4.34	5.36	3.48
	Turkey	1990	2.30	2.79	1.85
	Turkey	2019	3.12	3.84	2.47

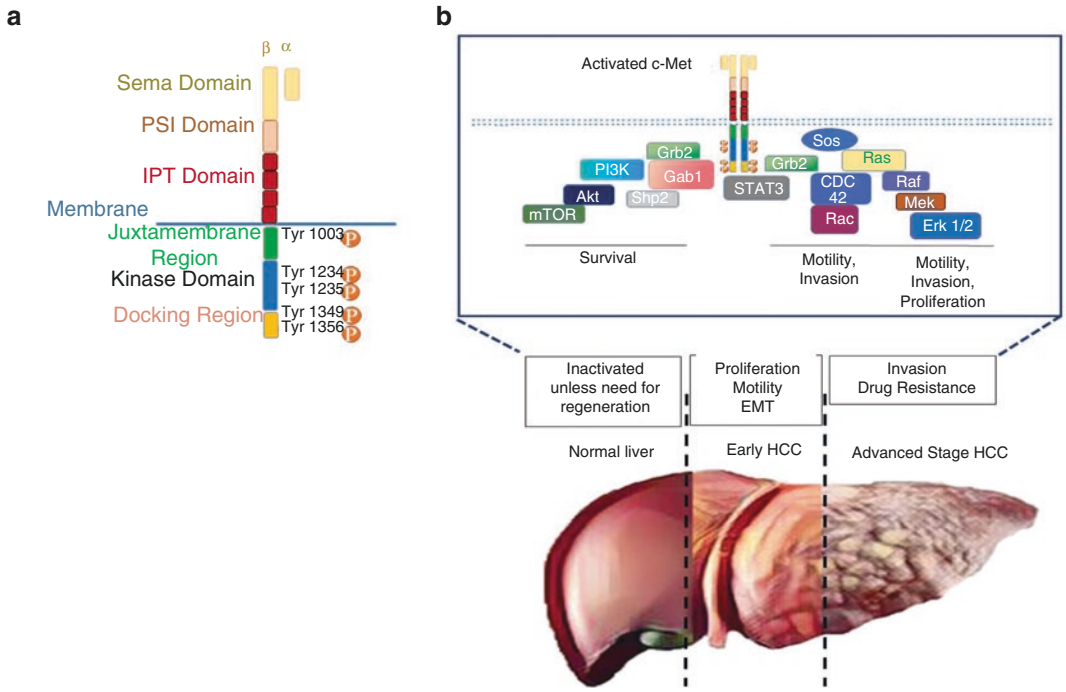

Fig. 1 c-Met structure and signaling in HCC. (**a**) The extracellular region of c-Met consists of semaphorin (Sema) domain, a cysteine-rich plexin-semaphorin-integrin (PSI) domain, and four immunoglobulin-plexin-transcription (IPT) domains. Through the linkage of a transmembrane helix, the intracellular juxtamembrane region harboring Tyr1003 is connected to the extracellular domains. The tyrosine kinase domain, harboring Tyr1234–35, serves the catalytic function and is flanked between juxtamembrane and docking regions. Multidocking sites Tyr1349 and Tyr1356 reside at the tail of the C-terminus and are elementary for recruiting adaptor proteins. (**b**) The dimerization of c-Met leads to transactivation of tyrosines 1234–35 and hence autophosphorylation of Tyr1349 and Tyr1356. This initiates the downstream signaling through the interaction of proteins containing SH2, phosphotyrosine binding (PTB), and c-Met binding domains. Phosphorylation of Tyr1003 serves an autoinhibitory function. Main cascades activated downstream are PI3K/Akt, Stat3, CDC42/Rac, and MAPK. Tumorigenesis associated mechanisms of proliferation, survival, and aggressive phenotype are associated with motility, portal invasion and drug resistance, which are initiated through the activation of these regulatory molecules at various stages contributing to the development and progression of HCC.

HBV infections with 57.6% (81% in males and 19% in females), while HCV infections account for 16.5% (14.9% in males and 22.4% in females) [11]. In the same etiological study, excess alcohol consumption was implicated in 14.2% (16.8% in males and 4.9% in females) of HCC patients. Age-standardized liver neoplasm mortality rates are on the rise (21.9% increase based on data of 2008–2014), and there is a growing positive correlation between obesity prevalence and liver malignancy in Turkey [12]. The contribution of non-alcoholic steatohepatitis (NASH) to HCC is also increasing in the MENA countries; the rates increase from 8.64% to 10.94% between 1990 and 2017 [10]. In Turkey, the contribution of NASH also increased from 8.13% to 10.42% between 1990 and 2017. Due to successful antiviral therapies and preventive vaccination strategies, hepatitis-infected patient numbers are expected to drop significantly in the near future, leaving their place to NASH and obesity epidemic in the MENA region and globally [8–10].

HCC is diagnosed in late stages due to lack of early symptoms and early diagnostic tests and limited number of screening programs [13]. Since there is yet no treatment option reversing advanced stage HCC, treatment options for the lately diagnosed HCC patients are limited and the prognosis of the disease is poor. The Barcelona Clinic Liver Cancer (BCLC) classification is a

well-defined staging system to achieve the most suitable treatment option for HCC patients. Based on this algorithm analyzing nodule size and number, the presence of vascular invasion and extrahepatic metastasis, Child-Pugh score, and patient status, patients diagnosed in early stages of HCC are ideal candidates for ablation, resection, or transplantation and receive curative therapy where the median overall survival is more than 60 months and 5-year survival rates are 40–70% [14]. Child-Pugh score and alpha-fetoprotein (AFP) levels are important prognostic factors for HCC; advanced disease stage is correlated with high AFP levels and high Child-Pugh score [11]. The median overall survival for HCC patients in Turkey is 14 months and is parallel with low Child-Pugh score and AFP levels [15].

Most of the patients are diagnosed with unresectable HCC (at intermediate, advanced or terminal stage), who are eligible only to receive chemoembolization and sorafenib as a first-line treatment [14]. The overall survival rates are 20 months in the intermediate stage when patients receive chemoembolization and 10.7 months in the advanced stage where patients receive sorafenib [16, 17].

1.2 First- and Second-Line Therapeutics Approved by FDA for Use in Liver Cancer

Sorafenib is an oral multi-tyrosine kinase inhibitor and was approved by the US Food and Drug Administration (FDA) in 2007 for patients with unresectable HCC, after reports of the SHARP trial showed that it increased the median overall survival (OS) rate of HCC patients to 10.7 months, 3 months longer than the placebo group [17]. Regorafenib, a covalently modified form of sorafenib, was approved by FDA in 2017 as second-line treatment in HCC after RESORCE trial, where it improved the median survival for 2.6 months compared to placebo [18]. Lenvatinib, another oral multikinase inhibitor, has gained the approval of FDA as a first-line treatment for unresectable HCC in 2018 based on the REFLECT trial [19]. Lately in 2020, the encouraging results of IMbrave150 trial showing better overall and progression-free survival outcomes when compared to sorafenib have led to the use of the combination of atezolizumab and bevacizumab for patients with unresectable or metastatic HCC tumors who have not received prior systemic therapy [20]. Other drugs received approval from FDA as second-line treatments in HCC care: **(1)** Small-molecule multi-target tyrosine kinase inhibitor cabozantinib which showed improved median overall survival and progression-free survival in CELESTIAL trial was approved in 2019 [21]. **(2)** Ramucirumab, a monoclonal antibody targeting vascular endothelial growth factor receptor-2 (VEGFR-2), was approved in 2019. The use of Ramucirumab showed improved OS of HCC patients with increased AFP levels in REACH-2 trial (the first positive phase 3 trial of biomarker-selected patient population with HCC) and was approved in 2019 [22]. **(3)** Based on KEYNOTE-224 phase 2 clinical trial, immune checkpoint inhibitor pembrolizumab was granted accelerated FDA approval in 2018 [23]. **(4)** Another immune checkpoint inhibitor nivolumab received accelerated approval from FDA in 2017, whereas the use of nivolumab in combination with another checkpoint inhibitor ipilimumab also gained FDA approval as second-line treatment in advanced HCC patients [24].

In patients with unresectable advanced stage or metastatic cholangiocarcinomas displaying fusion or rearrangement of fibroblast growth factor receptor2 (FGFR2), pemigatinib was approved in 2020 based on findings from FIGHT-202 trial [25]. Many if not all these drugs are also approved in Turkey.

Despite a high number of approved therapeutics, liver cancer patients still need new treatment options for longer survival. The family of receptor tyrosine kinases has pivotal roles in the development and progression of liver cancer. Accordingly, RTK targeting constitutes a major therapeutic option for HCC.

2 Receptor Tyrosine Kinase Pathways in Liver Cancer

Several RTK pathways are activated by genetic and/or epigenetic alterations during the course of liver cancer development and progression.

These pathways contribute to proliferation and survival of tumor cells via activation of phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling through (1) extracellular matrix (ECM) remodeling by expression and secretion of matrix metalloproteinases (MMP) 2 and 9; (2) persistent angiogenesis to induce the transformation of hepatic sinusoids enabling sinusoidal capillarization and epithelial-mesenchymal transition (EMT) and gain of plasticity through upregulation of mesenchymal markers and downregulation of epithelial markers; (3) alteration of intracellular cytoskeleton to gain more motile and invasive character via increasing focal adhesion kinase (FAK), RhoA, and Rac expressions and activations; and (4) reprogramming of the glucose and lipid metabolism through alterations in the levels of hexokinases, glucose transporters, fatty acid transporters, pyruvate kinases, etc. [26].

Epidermal growth factor receptor (EGFR) activation is one of the earliest events to be reported in HCC development. EGFR overexpression and overactivation are common mechanisms observed in the development of HCC and are associated with drug resistance in patients who receive prior treatment [27–29]. VEGF and its RTK member receptor VEGFR are known to contribute to HCC as well. Supporting the hypervascular nature of HCC tumors, VEGF and VEGFR are highly expressed both in the tumor and the niche [30]. Immunohistochemical analysis of 107 HCC patients showed that VEGF-A, VEGFR-1, VEGFR-2, and VEGFR-3 were higher in peritumoral tissue whereas VEGF-C isoform was higher in the HCC tumors; further analysis also identified that VEGF and VEGFR expressions have a prognostic value in HCC [31]. Owing to the angiogenic potential of the VEGF pathway, its expression leads to the formation of new vessels, allowing the development of tumors and homing of tumor cells in metastatic sites [30]. It also aids extravasation and intravasation through alterations of cell-cell junctions that leads to changes in vascular permeability and also allows the tumor cells to penetrate into the liver niche [30].

Transforming growth factor receptor (TGFR) activation is also associated with HCC; in early development phase of the disease, its ligand TGF-β is considered as a tumor suppressor; however, on the contrary in advanced stages of HCC progression, it acts as a tumor promoter [32, 33]. The most well-recognized role of the activation of this pathway is the triggering of EMT-associated events. Wnt and b-catenin pathways are frequently altered in HCC [34, 35].

Moreover, RTK dysregulations on Wnt/β--catenin and Hippo pathways are common in HCC development and progression. The activated RTK members FGFR, EGFR, and tropomyosin receptor kinase A (TRKA) are shown to phosphorylate Wnt co-receptor LRP6 and also β-catenin and therefore activate the canonical Wnt signaling [36]. The interplay of Hippo pathway and RTKs is shown in many studies as well. When activated, many RTKs (including c-Met, RET, FGFR, etc.) are shown to directly or indirectly regulate the Hippo pathway in many cancer types, including HCC [37]. The crosstalk is present in both ways; the regulation of many RTKs is found to be affected by Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) complex of Hippo pathway.

Considering the RTK family, hepatocyte growth factor (HGF) receptor c-Met and AXL belong to the family of RTKs and are the two receptors whose pivotal roles have been extensively studied lately in liver cancer.

2.1 c-Met and its Role in Liver Cancer Progression and Treatment Response

2.1.1 c-Met Signaling Pathway

c-Met is expressed in normal and malignant cells and is the only known receptor of HGF/Scatter Factor (SF). As a well-defined pleiotropic factor, HGF promotes diverse biological processes including scattering, differentiation, motility and invasion, proliferation, and survival through activating its receptor c-Met. Developmental phenotypes were investigated in conditional knockout mice, organ-specific deletion models, and over-

expression studies since complete knockout of HGF or c-Met is embryonically lethal [38]. The phenotypes are found to be similar for both HGF and c-Met affecting the embryonic development of many organs including the liver, kidney, lung, pancreas, muscle, prostate, breast, bone marrow, and retina [38]. Regeneration capacity of liver, kidney, and gestational pancreatic β-cells is also found to be seriously impaired in many studies; these results highlight the essential and imperative function of c-Met activation in normal development of the organism [39–44].

c-Met is encoded from *MET* proto-oncogene located in 7q21–31 in humans [45]. The encoded product is a 170 kDa single chain precursor polypeptide which is then glycosylated and cleaved at amino acids 307–308 by proteases in the Golgi, resulting in N-terminal spanning 40 kDa α chain and C-terminal spanning 140 kDa β chain [46]. The disulfide linkage between α and β chains allows the receptor to form the mature monomer that is translocated to the membrane.

The extracellular structure of c-Met (Fig. 1a) constitutes **(1)** semaphorin (SEMA) domain in α chain and N-terminal of β chain interacting with the serine proteinase homology (SPH) domain of HGF with low affinity, **(2)** cysteine-rich plexin semaphorin integrin (PSI) domain, and **(3)** four immunoglobulin-plexin-transcription (IPT) domains which are immunoglobulin-like regions found in plexins and transcription factors responsible for interacting with HGF's N-terminal and first kringle domain with high affinity [47, 48]. The juxtamembrane domain of the receptor lies in the β chain, under the IPT subdomains, which is followed by the catalytic kinase domain and multidocking site.

When HGF is secreted by stromal cells or in an autocrine manner, the ligand is bound to c-Met monomers on the extracellular membrane, keeping them in close proximity and stabilizing homodimerization [49]. The dimerization triggers transphosphorylation of Y1234–25 on the kinase domain of each receptor revealing the kinasing potential for phosphorylation of Y1349 and Y1356 [48]. These autophosphorylation tyrosines serve as docking sites for adaptor proteins carrying src homology 2 (SH2) domains, including src homology region 2 domain-containing phosphatase-2 (SHP-2), son of sevenless (SOS), p85, signal transducer and activator of transcription 3 (STAT3), and grb2 associated binding protein 1 (Gab1) [50].

Downstream signaling is conveyed through three main paths (Fig. 1b):

1. MAPK pathway: The interaction of Ras with the scaffold complex which may comprise Gab1-growth factor reduced-bound protein 2 (Grb2)-SOS-src homology 2 domain containing (SHC) allows Ras to be activated by guanosine triphosphate (GTP) loading [50]. Activated Ras recruits rapidly accelerated fibrosarcoma (Raf) kinase, resulting in the sequential activation cascade of Raf-mitogen activated protein kinase kinase (MEK)-mitogen activated kinase (Erk).
2. PI3K/protein kinase B (Akt) pathway: The phosphorylation of phosphatidylinositol (4,5)-biphosphate-2 (PIP2) forms PIP3 which carries Akt to the membrane that leads to its phosphorylation on S473 and T308. The activated Akt is translocated to the nucleus where it controls many key molecules including mTOR, GSK3β, MDM2, and Myc to regulate cell survival and metabolism [51].
3. STAT3 pathway: STAT3 can bind to active c-Met and becomes phosphorylated, followed by translocation to the nucleus. As a transcription factor, it is involved in expressional control of genes involved in mitochondrial activation, angiogenesis, and invasion [52].

The activation of these pathways has several biological consequences related to regeneration and wound healing in normal cells but aggressive behavior in tumor cells, such as proliferation, survival, motility, invasion, and alteration of cell metabolism. Therefore c-Met signaling is tightly controlled in cells. One mechanism is the regulation of c-Met inhibition through dephosphorylating the activatory tyrosines by phosphatases PP2A and PTP-1B [53]. Also an autoinhibitory loop is activated, when c-Met heterodimerizes, through the phosphorylation of Y975 and Y1003. The phosphorylation of these residues initiates

downregulation cascade involving E3 ubiquitin ligase that ubiquitinates and targets c-Met for degradation [54]. Despite the control mechanisms, c-Met overactivation is achieved through the steps of carcinogenesis. Canonical mechanisms involve HGF binding, whereas non-canonical mechanisms are ligand-independent and involve mutations in kinase domain rendering the receptor overactive, deteriorations in downregulation loop, and activation through crosstalk and heterodimerization with other receptors [55–59].

c-Met has been shown to interact with many cell surface molecules including integrins, mucins, plexins, CD44, caveolin-1, and other receptor tyrosine kinases [58, 60, 61]. Since RTKs share varying degrees of homology in their kinase domains, heterodimerization and transactivation are common [62]. Interaction of c-Met with epidermal growth factor receptor (EGFR) is the most extensively studied in HCC, colorectal cancer, and lung cancer. These studies have shown that EGFR-c-Met activation leads to elevated motility, proliferation, survival, invasion, metastasis, and drug resistance in cancer cells [63–66]. Similarly, interaction with human epidermal growth factor receptor 2/3 (Her2/3) heterodimerization leads to increased PI3K/Akt signaling and hence aforementioned responses [66, 67]. Interaction with RON increases colony formation, whereas insulin-like growth factor receptor (IGFR) and RET lead to increased motility and invasion [66, 68, 69]. In the presence of HGF, activation of c-Met also results in heterodimer formation with insulin receptor (IR) and IR activation to transmit intracellular signals of insulin in diabetes [62].

2.1.2 c-Met Alterations in Cancer and HCC

It is not surprising that loss of regulation on such a potent pathway leads to tumor development and carcinogenesis in several tissues. Aberrant c-Met signaling due to c-Met/HGF overexpression or overactivation is found in liver, colorectal, thyroid, lung, gastric, ovarian, breast, head and neck, and renal tumors [70–80].

c-Met overexpression is a very common mechanism in cancer and could arise from genetic alterations, most frequently occurring through *MET* gene amplification as observed in varying levels in renal, colorectal, lung, adenocortical, gastric, and HCC tumors [51]. Point mutations are not common as gene amplification except for hereditary or sporadic forms of papillary renal cell carcinoma and are very limited in non-small cell lung cancer and head and neck cancer [51, 81]. In a Turkish cohort, the sequencing of regions coding for non-kinase and kinase domains of c-Met identified no mutations in non-small cell lung cancer patients [81]. Guichard et al. have analyzed 125 HCC tumors by high-resolution copy number and 24 HCC tumors by whole-genome sequencing and could not detect any activating point mutation regarding *MET* gene [82].

Another mechanism of overexpression is through cellular triggers inducing c-Met transcriptional overexpression including; HGF overexpression which upregulates MET expression and enzymes responsible for c-Met protein maturation, oncogene activation, loss of tumor suppressors, and loss of control by noncoding RNAs and environmental transcriptional regulators like hypoxia [83, 84].

Elevated c-Met expression is detected in 35–65% of breast cancer patients, 15–80% of renal cancer patients, 25–60% of pancreatic cancer patients, 35–70% of non-small cell lung cancer patients, and 35% of colorectal cancer patients [85–89]. In a cohort of advanced intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer patients, 34.1% of the patients have overexpressed c-Met protein [90]. A high expression level of c-Met, 76.7%, was also indicated in extrahepatic cholangiocarcinoma patients who received curative resection and adjuvant chemoradiotherapy [91]. Also overexpressed c-Met positivity was detected in 45% of intrahepatic cholangiocarcinoma patients with an overexpression ratio of 11.7% and in 68.4% of extrahepatic cholangiocarcinoma patients with a ratio of 16.2% [92]. c-Met overexpression is also common in HCC; an early study identified two- to tenfold increase in *MET* expression in half of the HCC patients who received surgical treatment [93]. Another study also identified 31.6% of

patients with higher *MET* expression and 69.6% with higher c-Met protein level compared to peritumoral tissue [94]. c-Met overexpression in HCC is correlated positively with intrahepatic metastasis, recurrence and poor prognosis, portal vein invasion, and advanced tumor stage [95–97].

In studies concerning MENA countries, c-Met RNA and protein expression was found to be increased in papillary thyroid carcinoma (PTC) and associated with more aggressive character, advanced disease stage, and activated Akt in a cohort of 536 Middle Eastern PTC patients [98]. c-Met expression and activation were also identified in a Turkish HCC cohort and associated with motile, invasive, and mesenchymal character [61]. Also in both this study and our analysis of cancer dependency map (DepMap) portal regarding HCC cell lines, c-Met is determined to be associated with less differentiation (Table 2).

2.1.3 Targeting c-Met in Liver Cancer

c-Met is a valuable biomarker in predicting cancer prognosis and treatment outcome; its prognostic potential has been studied in many cancers including the liver. Aforementioned, high c-Met expression is common in HCC and is related to decreased survival of HCC patients [93–97]. Its prognostic value in patients who underwent surgery or received prior sorafenib treatment is discussed in many studies; one study has shown that HCC patients who received surgical treatment have high c-Met expression in larger tumors, also in patients with higher AFP levels, portal vein invasion, and higher tumor-node-metastasis stage [99]. Other studies also have shown that HCC patients who have high tumoral c-Met levels benefit more from systematic treatment [100].

Therefore several approaches are developed against c-Met targeting its constitutive signaling. One approach is to target its binding with HGF by using decoy c-Met or domains of c-Met

Table 2 HGF/c-Met and Gas6/Axl expression levels in HCC cell lines from CCLE database

	HGF/c-Met		AXL/Gas6	
HCC cell lines	c-Met	HGF	AXL	Gas6
HEP3B217	High	Low	Low	Low
HEPG2	Moderate	Low	Moderate	Low
HLF	High	Low	High	High
HUH1	High	Low	Moderate	Low
HUH6	Moderate	Low	Low	High
HUH7	Moderate	Low	Low	Low
JHH1	High	Low	Low	Moderate
JHH2	High	Low	Moderate	High
JHH4	High	Moderate	High	High
JHH5	High	Moderate	Moderate	Moderate
JHH6	High	Low	High	High
JHH7	High	Low	Low	Low
LI7	High	Low	High	Moderate
NCIH684	High	Low	Low	Moderate
PLC/PRF/5	Moderate	Low	High	Low
SK-HEP-1	High	Low	High	High
SNU-182	Moderate	Low	High	High
SNU-387	High	Low	High	High
SNU-398	Low	High	Moderate	Moderate
SNU-423	High	Low	High	High
SNU-449	High	Low	High	Moderate
SNU-475	High	Moderate	High	Moderate
SNU-761	High	Low	High	Low
SNU-878	High	Low	High	Moderate
SNU-886	High	Moderate	High	High

as antagonists, or through the use of antibody-based therapeutics that bind directly to HGF or c-Met, and compete or neutralize to prevent ligand-receptor interaction [85, 101]. Regarding HGF-based targeting, NK2, NK4, and uncleavable HGF are commonly used. Among them, NK4 (intramolecular fragment of HGF which contains the four kringle domains) is a more promising analog compared to NK2, and is used as a competitive antagonist targeting both c-Met activation and angiogenesis [102].

Another approach is developing selective or multi-targeting small-molecule tyrosine kinase inhibitors against c-Met kinase activity. Crizotinib and cabozantinib are well-known agents that belong to the family of ATP binding competitors that suppress the activatory domain of c-Met [85]. Tivantinib is developed as an oral drug targeting tyrosine kinases independent of adenosine triphosphate (ATP). Tivantinib was previously developed as a selective c-Met inhibitor; however, further studies uncovered its off-target effects [103, 104]. Crizotinib is developed as an effective c-Met inhibitor, and initial findings record its selective effects on both c-Met and anaplastic lymphoma kinase (ALK), but further studies identified its role also on Recepteur d'Origine Nantais (RON) and ROS1 [105]. Cabozantinib is also a multi-target drug showing activity against several RTKs including c-Met, RET, AXL, VEGFR-2, c-Kit, and FLT3 [106]. Together with cabozantinib, tivantinib is used in a second-line treatment after sorafenib in HCC. In a phase II clinical study, HCC patients were recorded to benefit better from tivantinib as a second-line treatment when c-Met expression is higher in HCC tumors, compared to the placebo group [100]. Median OS, progression-free survival, and median time to progression were found to be increased in the same study. Also they have shown that independent of the treatment regimen, patients in the placebo group with higher c-Met tumoral expression had significantly lower survival when compared to placebo patients with low c-Met tumoral expression. Following this phase II study with tivantinib, METIV-HCC phase III trial started. Biopsy samples before and after sorafenib treatment of HCC patients were positive for c-Met, and sorafenib treatment again increased c-Met expression from 35% to 69% [107]. Lorenzato et al. and Renzo et al. have detected somatic c-Met mutations not in primary tumors but in metastatic cells; together these data stress the pivotal role of c-Met in metastatic clone selection and drug resistance [108, 109].

Although c-Met is a promising target for cancer treatment, clinical trials like METIV-HCC (with c-Met inhibitor tivantinib) failed to show significant improvement [107]. It is arguable that appropriate patient selection criteria might be one reason. Hughes and Siemann suggested that criteria should not be limited just to c-Met overexpression, tumor type, and *MET* amplification but rather c-Met activation [110]. Since non-canonical activation and sustained signaling of c-Met have a significant role in carcinogenesis even in the absence of overexpression, taking c-Met activation into consideration might allow better patient selection and better targeted therapy. Also it should be noted that the expression and activity of c-Met have a very fine balance during the development of HCC. Knockdown and total inactivation of c-Met was also related to tumorigenesis, keeping this balance is critical and might have a role in the outcome of treatments [111].

2.1.4 The Role of c-Met in Treatment Response in Liver Cancer

Overexpression and activation of c-Met have been extensively associated with acquired resistance to cancer therapeutics. Resistance against targeted monotherapies and systemic therapies against multikinases or radiotherapy was found to be acquired in association with elevated c-Met expression and activity [112–114].

As a first-line treatment, sorafenib has been the most extensively studied drug by means of treatment response in HCC. Mechanisms overcoming sorafenib response include alternative targeting of c-Met. Akt inhibitors, when used solo, were found to activate c-Met in sorafenib-resistant HCC cells, but dual inhibition of both Akt and c-Met using respective inhibitors MK2206 and capmatinib was suggested as a second-line treatment alternative and shown to

suppress sorafenib-resistant HCC cells both in vitro and in vivo [115]. Supportingly, HGF induction of sorafenib-treated HCC cells was shown to induce Akt-Erk-EGR1 expressions through c-Met activation to gain resistance against apoptosis and become more invasive [116]. This effect was reversed by inhibiting c-Met activation through the use of PHA-665752 and EGR1 silencing with targeted siRNA. Studies also show that in the tumor microenvironment, HGF secretion is directly related to drug resistance. Co-culture of hepatic stellate LX2 cells with HuH-7 cells led to secretion of HGF from LX2, and blockage of Akt was shown to overcome sorafenib resistance generated by HGF induction [117]. In addition, the role of tumor-associated M2 macrophages in sustaining Akt-Erk signaling downstream of c-Met through HGF secretion was shown to contribute to sorafenib resistance in HCC [118]. As a positive feedback loop, M2 macrophages accumulated in sorafenib-resistant tumors where HGF acts as a chemoattractant to the macrophages [118]. Recent cell culture data has also supported the role of autocrine HGF secretion in c-Met activation in sorafenib-resistant cells, rendering them more invasive and metastatic, which might be reversed with the use of specific c-Met inhibitor and HGF-neutralizing antibody [119]. The studies of exosomes also enlightened another mechanism of c-Met activation in tumor niche to induce sorafenib resistance; exosomes isolated from more invasive cells were found to be more efficient in developing resistance [120].

Receptor crosstalk and heterodimerization are usually attributed as a key factor in gaining resistance to RTK-targeted therapies. Drugs targeting multiple RTKs were also efficient in overcoming sorafenib resistance. One successful strategy is to target both c-Met and VEGFR2 by using cabozantinib, which also targets AXL and RET [121]. The role of cabozantinib was more evident in c-Met overactive HCC xenografts. Lenvatinib is the only other first-line drug for advanced HCC therapy, and resistance mechanisms are recently identified. Not surprisingly, c-Met was found to have a role. HGF stimulation was shown to reverse lenvatinib's anti-proliferative and anti-invasive effects on HCC cells, and c-Met inhibitor PHA-665752 rescues the effect of HGF [122].

2.2 AXL and its Role in Liver Cancer Progression and Treatment Response

2.2.1 AXL Signaling Pathway

The receptor tyrosine kinase AXL was first isolated as a transforming gene from two different chronic myelogenous leukemia (CML) patients that progressed to blast crisis in 1988 [123]. Three years later, it was cloned from a CML patient, characterized and named as AXL derived from the Greek word "anexelekto" which means uncontrolled [124]. Concurrently nearly at the same time, it was cloned from a chronic myeloproliferative disorder patient by an independent group and named UFO addressing the unknown function [125]. Surprisingly, another group also cloned murine AXL and called it adhesion-related kinase (Ark) again in the same year [126].

Gene AXL is located on human chromosome 19q13.1 and encodes 20 exons. 104 kDa full-length protein is firstly translated and consequently glycosylated at six N-link glycosylation sites and forms approximately 140 kDa [127]. AXL protein consists of two immunoglobulin-like (Ig-like) domains and two fibronectin type III (FNIII) domains in the extracellular space of cells followed by a single pass transmembrane domain and conserved intracellular kinase domain [128] (Fig. 2a). Those domain structures are also found in receptor tyrosine kinases Tyro3 and Mer. They, altogether, form a subgroup of RTK named TAM family [129]. Gas6 (growth arrest-specific protein 6) is a common ligand for the TAM family [130]. However, it has the highest affinity for AXL [131]. Gas6 is a 75 kDa vitamin K-dependent protein consisting of a Gla domain, four EGF-like domains, and two LG-like domains, respectively [128]. It is mainly expressed by leukocytes, fibroblasts, endothelial cells, smooth muscle, and bone marrow cells [132]. Under normal conditions, Gas6 is

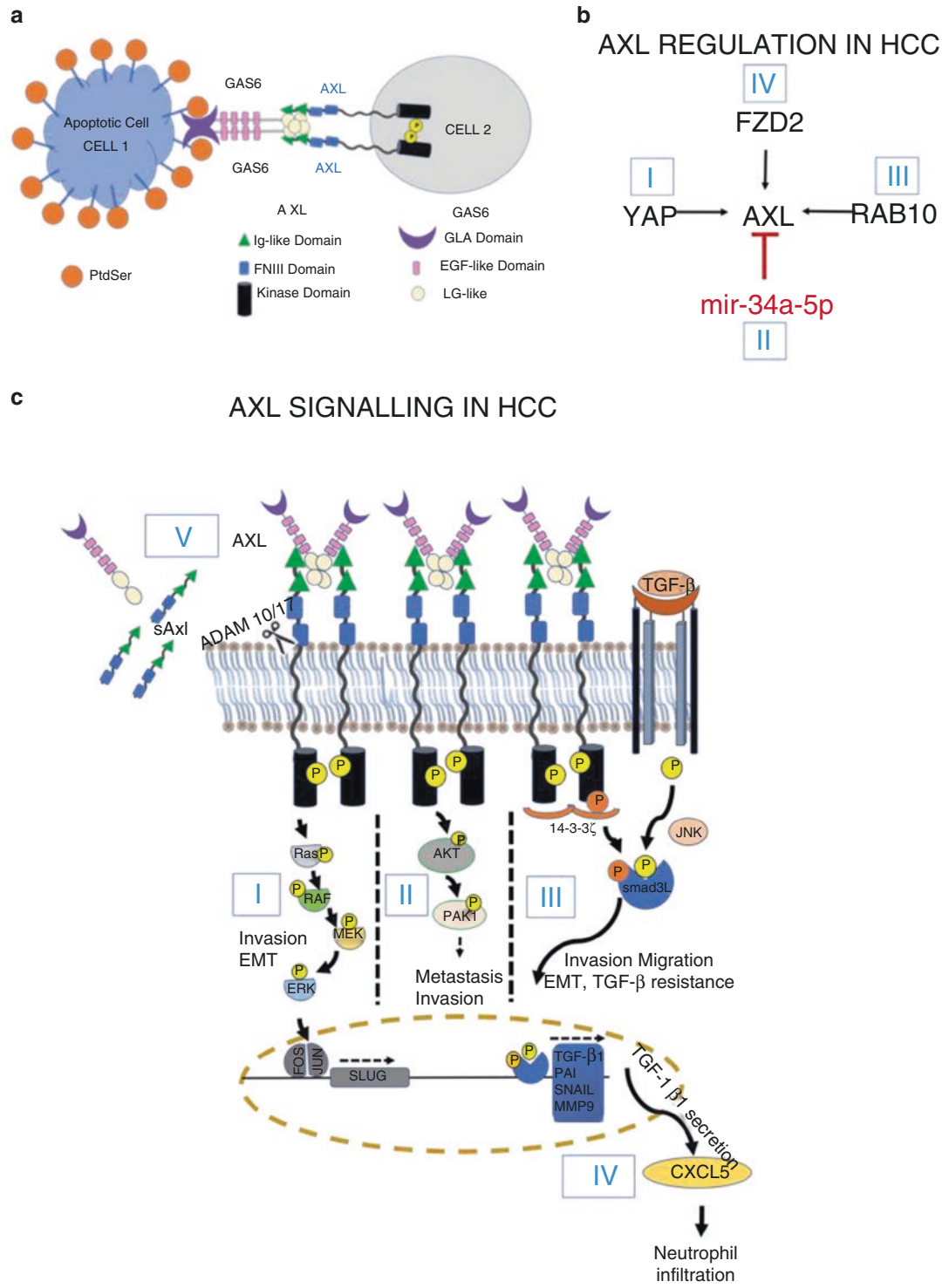

Fig. 2 (**a**) Canonical AXL-GAS6 binding adapted from [190]. (**b**) Elucidated regulator molecules of AXL in HCC adapted from [176, 178, 181, 182, 184, 191]. (**c**) AXL signaling pathways involved in tumor progression (I-II-III-IV) [167, 170, 187, 189]. AXL is proteolytically processed by ADAM10/17 as a sAXL (V) [192]

expressed only by Kupffer cells (macrophages) in the liver [133]. During regeneration or in development, Gas6 expression is observed in oval cells, biliary cells, and sinusoidal cells. Hepatic stellate cells express Gas6 during myofibroblastic change. AXL expresses in hepatic stellate cells and oval cells as well. Whether the interaction between Gas6 and AXL is in the paracrine or autocrine manner is unknown. However, they contribute to hepatic regeneration [134].

In canonical ligand-dependent activation of AXL, two Gas6 molecules are required to bind two AXL receptors for signal transduction [128]. Gas6 can only bind to AXL from its LG-like domain if the Gla domain of Gas6 binds to PtdSer of apoptotic cells [131, 135] (Fig. 2a). PtdSer normally confines the inner leaflet of the cell membrane. When cells undergo apoptosis, PtdSer "eat-me" signal flips out and is exposed on the outer leaflet. PtdSer becomes accessible for the binding of Gas6 [136]. Therefore, AXL and Gas6 interaction would then provide a bridge between apoptotic cells and macrophages for phagocytosis [137].

Receptor dimerization of AXL upon Gas6 binding firstly serves Y698, Y702, and Y703 phosphorylation and then Y779, Y821, and Y866 [138]. As a result of the phosphorylation, multiple pathways such as PI3K/Akt, RAS/MAPK, STAT3, NF-κB, and PLCγ/PKC are activated. AXL/Gas6 signaling controls many pivotal cellular processes from cell survival/proliferation to cell motility [139, 140].

Nevertheless, several publications have appeared in recent years documenting ligand-independent activation of AXL. AXL signaling may be initiated by either homodimeric or heterodimeric activation. When AXL was highly overexpressed on the surface of the cell, spontaneous AXL-AXL homodimeric activation and autophosphorylation may occur. For instance, AXL ligand-independent homodimeric activation has been observed in vascular smooth muscle cells under hypoxia or during H_2O_2 treatment of metastatic human lung adenocarcinoma cells [141, 142]. AXL heterodimerizes with both TAM family and non-TAM family members such as FMS-like tyrosine kinase 3 (FLT3), EFGR, c-Met, and platelet-derived growth factor receptor (PDGFR) [143, 144]. Although the demonstration of Mer and AXL heterodimerization is still lacking, Tyro-3 and AXL form heterodimer in gonadotropin-releasing hormone (GnRH)-secreting neuronal cells in the absence of ligand [145]. AXL crosstalk with FLT3 in progenitor cell of NK cells [143]; EGFR in NSCLC and breast cancer [144, 146]; and c-Met, PDGFR [144], and Her2 [147] in breast cancer has been previously reported in the literature. Ligand-independent activation of AXL has also been observed in vascular endothelial cells through crosstalk with αvβ3 integrin as a result of shear stress [148].

Once AXL signaling starts, it has been revealed that AXL regulates many pivotal pathways related to cell survival, proliferation, invasion, migration, and dedifferentiation, cytokine production, apoptotic protection, EMT, and matrix adhesion [149].

2.2.2 The Role of Aberrant AXL Signaling in Cancer and HCC

AXL is found to be aberrantly expressed in many cancers including brain [150], esophageal [151], thyroid [152], head and neck [153], prostate [154], ovarian [155], gastric [156], colorectal [157], and breast cancer [158], lung adenocarcinoma [159], non-small cell lung carcinoma (NSCLC) [160], glioblastoma [161], mesothelioma [162], B-cell chronic lymphocytic leukemia [163], acute myeloid leukemia (AML) [164], CML [124], melanoma [165], and liver cancer [166]. Multiple studies demonstrated the aberrant expression of AXL in HCC tissues when compared to adjacent non-tumor tissues by both mRNA and immunohistochemistry analysis [167–169]. Supportively, AXL is also overexpressed in many HCC cell lines (Table 2). However, not all of the HCC cell lines (Table 2) and tissues exhibit AXL overexpression. Some HCC patients not at all have AXL positivity [168–170]. Nonetheless, it has been discovered that AXL positivity in the tumor is strongly correlated with a high recurrence rate and poor overall survival. Besides, the expression of AXL is also associated with vascular invasion, metasta-

sis, and advanced tumor stage [168, 171]. Overexpression of AXL in human HCC cell lines shows enhanced cell growth, morphology change, migration, and invasiveness. AXL knockdown HCC cells via RNAi exhibit reduced cell growth [168], invasion capacity, and tumor size [167]. A study on mouse hepatocellular carcinoma cell lines has been demonstrated that AXL expression is also associated with fast proliferation rate, migration, invasion, anchorage- independent growth in vitro and peripheral lymph node metastasis transendothelial invasion in vivo experiments [171].

Although the exact overexpression mechanisms of AXL are unknown, (1) YAP (Yes-associated protein) through Hippo pathway, (2) microRNA mir34a, (3) cellular trafficking regulator RAB10, and (4) FDZ2 could be major regulators of aberrant AXL signaling in HCC (Fig. 2b).

Hippo is an important signaling pathway required for organ homeostasis and development regulated by extracellular matrix elasticity and cell shape [172]. In normal condition, cell-to-cell interaction generates actin polymerization that activates mammalian sterile 20-like kinase 1/2 (MST 1/2) and subsequently large tumor suppressor homolog 1/2 (LATS1/2). Activated LATS1/2 phosphorylates YAP resulting in its proteasomal degradation. However, the Hippo pathway is inhibited in cancer cells or in regeneration. Therefore, YAP molecule translocates to the nucleus, and complex with transcription factor TEAD. This complex turns on anti-apoptotic and cell proliferative genes [173] (Fig. 2b-I). Hippo pathway is also a significant regulative and tumor suppressor pathway in the liver. It controls liver tissue growth, regeneration, and carcinogenesis [173]. The downstream target YAP transgenic mouse displays a huge liver size. It harbors dysplastic hepatocytes [174]. Both transcript and protein levels of YAP increase in HCC tumor tissues. Its expression is correlated with tumor recurrence and poor survival rate in HCC [175]. Direct associations of AXL and YAP was analyzed since non-tumorigenic cell line MIHA forms AXL expressing tumors in nude mice after Yap1 overexpression. Chromatin immunoprecipitation (ChIP) assay and luciferase reporter activity proved that AXL is under control of the TEAD-YAP complex [176] (Fig. 2b-I). A short while ago, α2β1 integrin was added upstream of the Hippo pathway in HCC. When extracellular collagen and laminin bind to α2β1 integrin of cells, MST1 activity is suppressed and YAP becomes free to translocate to the nucleus leading to transcription of YAP-targeted genes including AXL [177] (Fig. 2b-I).

Another upstream regulator of AXL has been discovered recently [178]. Ras-related GTP binding protein RAB10 that controls intracellular vesicle trafficking from endoplasmic reticulum to Golgi [179] is upregulated in HCC. Knockdown of RAB10 increases the expression level of genes related to the RTK signaling pathway including AXL. RAB10 expression is also associated with tumor invasion, migration, and metastasis [178]. However, further substantiates are demanded to test whether those oncogenic potential depends on AXL or other pathways (Fig. 2b-II).

Direct binding of mir-34a-5p (a strand of mir-34a) to 3'UTR of AXL was proved by both luciferase reporter assay and sequencing [180, 181]. Regulation of AXL is confirmed by both miRNA-34a-5p overexpression and miRNA-34a-5p inhibitor treatment [181] (Fig. 2b-III). miR-34a is activated by p53 and has a significant role in HCC development. miR-34 overexpression resulted in G1 arrest and a decrease in cell proliferation, invasion, and migration [182]. Its expression also has diminished in the hepatocarcinogenesis of rats induced by methyl deficiency [183]. miRNA-34a-5p expression negatively correlated with cancerous tissues [181].

Very recently, multiple kinobead/LC-MS to find EMT-responsible genes has revealed that FZD2 regulates AXL expression [184] (Fig. 2b-IV). FZD1 downstream molecule STAT3 silencing in HCC cells via RNAi leads to a decrease in AXL expression suggesting STAT3 may be found upstream of AXL as well. However, the same study also discovered that silencing of AXL impairs Y705 phosphorylation of STAT3 [184].

AXL is involved in various signaling pathways such as PI3K, MAPK, and TGF-β pathways

due to its overexpression in HCC. In the PI3K/Akt pathway, AXL activation causes signal transduction followed by PI3K, Akt, and PAK1 activation (Fig. 2c-I). In this context, the silencing of AXL by shRNA decreases both PI3K and its downstream molecules' (Akt and PAK1) expressions. The invasive and metastatic ability of cells also reduced with the downregulation of AXL [167]. Additionally, AXL signaling through MAPK pathways including RAS, RAF, MEK, and ERK subsequent phosphorylation, respectively, turns on gene expression of Slug. Therefore HCC cells undergo EMT and gain invasive property (Fig. 2c-II) [170]. Finally, it has been discovered that AXL is a critical mediator of the transition of the tumor suppressor role of the TGF-β pathway to the pro-oncogenic role. Under normal physiological conditions, TGF-β regulates the growth of hepatocytes by induction of apoptosis [185]. However, TGF-β has elevated expression in HCC. TGF-β1 levels are correlated with HCC progression and poor prognosis [186]. Consistently, microarray data of human HCC collection revealed two clusters of TGF-β-dependent antitumorigenic and tumorigenic genes are upregulated [187]. Under normal physiological conditions, ligand binding to TGF-β receptors leads to receptor activation, and phosphorylation of Smad3 causes its nuclear translocation to open cell cycle arrest and apoptosis-inducing genes in hepatocytes. However, in HCC, AXL takes a role. Reichl et al. have shown that collaboration of AXL with the TGF-β pathway is implicated in tumor progression, TGF-β resistance, invasion, EMT, and migration [171]. Activated AXL physically interacts with 14–3-3z and non-canonically phosphorylates Smad3 at linker site resulting in nuclear translocation of Smad3L to turn on pro-oncogenic responsive genes including TGF-β1, PAI1, MMP9, and Snail [171, 187] (Fig. 2c-III). The secretion of TGFβ1 suppresses immune surveillance in the tumor microenvironment and supports tumor progression [188]. Related to the contribution of AXL-TGF-β signaling axis on tumor microenvironment, it has been recently discovered that CXCL5 expression is controlled by the crosstalk of AXL with TGF- β signaling. Long-term treatment of TGF-β increases CXCL5 expression via AXL. AXL knockout cells by CRISPR-Cas9 impair CXCL5 activation as well. CXCL5 is known for its role in neutrophil migration (Fig. 2c-IV). Nevertheless, overall patient survival is short if the patient had CXCL5 expression. Neutrophil migration may be used to promote tumor microenvironment by AXL-TGF-β signaling as a conclusion [189].

2.2.3 AXL in Diagnosis and Treatment Response of HCC

As previously described, new diagnostic biomarkers are needed for diagnosis and prognosis of HCC [193]. Soluble AXL (sAXL) is proposed as a potential biomarker for early diagnosis and prognosis of HCC [194–196]. AXL can proteolytically be cleaved from its extracellular region by ADAM10/17. It is shed from cells and can be detected in serum as sAXL [Fig. 2C-V] [192]. Reichl et al. discovered that total protein levels of AXL in HCC cell lines are correlated with sAXL release [196]. Supportively, several cohort studies with HCC and cirrhosis and control samples from both China and Europe showed that the sAXL levels are elevated in both AFP-positive and AFP-negative HCC patients. sAXL was able to detect early stage of HCCs when AFP levels were low. The sensitivity of sAXL was higher than AFP for differentiating HCC patients from healthy controls. Moreover, a combination of AFP and sAXL could provide an increase in the accuracy, sensitivity, and specificity of HCC detection [194–196]. sAXL appears to be a highly specific marker for HCC since patients with breast, ovarian, and colorectal cancers displayed unchanged levels of sAXL [192]. Cholangiocarcinoma and liver adenoma patients also release low levels of sAXL, but patients with nonmalignant chronic liver disease did not show high sAXL level [195]. Moreover, sAXL levels increase in correlation with the stage and progression of HCC [192, 196]. For instance, more metastatic or invasive HCC tumors display higher amounts of sAXL compared to those with noninvasive or non-metastatic HCCs [196].

AXL expression is associated with resistance against different drugs including DNA-damaging

agents such as cisplatin in AML [164] and esophageal [142] and ovarian cancers [155], doxorubicin in NSCLC [197] and AML [164], docetaxel in prostate cancer [198] and RTK inhibitors such as lapatinib in breast cancer [199], imatinib in CML [200] and gastrointestinal stromal tumors (GIST) [201], nilotinib in CML [202], sunitinib in renal cell carcinoma [203], afatinib in gastric cancer [156], osimertinib [204], erlotinib and gefitinib in NSCLC [205], as well as sorafenib in HCC [169]. Elevated phosphorylation of AXL has been observed in sorafenib-resistant HCC cells that exhibit more invasive and migratory phenotype. AXL knockdown in HCC cells increased their sensitivity to sorafenib. Such cells displayed impaired motility and invasive characters as well [169].

Several studies have ascertained that chemoresistance of tumors via AXL overexpression is regulated through long non-coding RNAs or microRNAs. For instance, long non-coding RNA DANCR is a mediator of cisplatin resistance by activating AXL/NF-κB axis in glioma cells [206]. miR-34a-5p was shown to interact physically with the 3'UTR of AXL, and its expression decreases AXL-mediated cisplatin resistance in HCC cells [181]. Similarly, a decrease in AXL expression and drug transporter protein MDR1 by ectopic expression of miR-34a reverses chemoresistance against doxorubicin [207].

2.2.4 Targeting AXL Signaling in Liver Cancer

Since its identification, AXL has become an increasingly attractive target for anti-cancer therapies. Multiple AXL inhibitors have been under development [208]. Small-molecule inhibitors [209], anti-AXL mAbs [210, 211], nucleotide aptamers [212–214], and decoy receptors [215] have been developed as inhibitors of AXL signaling.

Concerning HCC, only small-molecule R428 (bemcentinib, BGB324) [216] and bosutinib [217] have been explored. R428 is a highly selective inhibitor of AXL. The preclinical efficacy of R428 was first shown in breast cancer, decreasing metastasis and angiogenesis in vivo and delaying invasion and migration in vitro. R428 also gives a synergistic response with cisplatin to inhibit liver micrometastasis [216]. R428 also blocks cell viability, leads to G1 cell cycle arrest, and disrupts colony formation in AXL-expressing HCC cells. Following R428 treatment, HCC cells exhibited diminished fluorodeoxyglucose 18 (18F-FDG) uptake in a time-dependent manner, as an indication of decreased tumor cell metabolism [169]. Bosutinib (SKI-606) which is known as an inhibitor of Src and Abl kinases in CML also inhibited AXL autophosphorylation in HCC cells [217]. Also, bosutinib decreased AXL-dependent HCC invasion [170].

Metformin, an antidiabetic drug, interferes with hepatic gluconeogenesis [218]. Recently, it was shown to display anti-proliferative activity in solid tumors [219] including HCC [220] and cholangiocarcinoma (CCA) [221]. CCA originates from the bile duct in the liver and is the second most common primary liver cancer after HCC [5]. Metformin incrementally decreased proliferation of human CCA cell lines, and this effect was associated with inhibition of AXL phosphorylation [221]. Elevated AXL expression has been observed in metformin-resistant prostate cancer cell line [222].

As previously stated, cabozantinib is a non-specific multikinase inhibitor that also targets AXL. It was approved by the European Medicines Agency (EMA) and FDA as a second-line treatment for advanced stage and sorafenib-resistant HCC patients [223].

3 Synergism of c-Met and AXL Pathways, Crosstalk, and Inhibition in HCC

As clearly outlined earlier in this chapter, HCC is a devastating malignancy for which current therapies do not offer satisfactory results. Although the multikinase inhibitor sorafenib has opened a window of hope to combat HCC, the overall outcomes are far from being satisfactory. One reason for extraordinary resistance of HCC to therapy is the high complexity of the disease.

The development of technologies that enable next-generation sequencing of DNA and RNA and

large-scale tumor molecular profiling has revolutionized the field of cancer precision medicine which is defined as "the use of therapeutics that are expected to confer benefit to a subset of patients whose cancer displays specific molecular or cellular features" [224]. The success of a personalized approach to HCC management is dependent on the availability of reliable biomarkers. Clearly, development of "multiple-biomarkers panels" to predict tumor response to drug or drug combinations while avoiding excess toxicity has brought predictive biomarkers to the center of cancer therapy. During the past few years, there has been great progress in the generation of big omic data across multiple modalities in HCC from primary to metastatic tumors, from bulk tissues to single cells, and from preclinical models to patients. These new developments will empower the identification of new clinically relevant biomarkers (reviewed in [209]). In particular, among these big datasets, gene expression data consistently provided the best predictive power compared to other genetic or epigenetic datasets [225]. In addition, computational findings obtained from independent datasets including tumor-derived cell lines and tumors showed that signaling pathways serve as robust and powerful biomarkers [226]. A management plan aims to target a list of HCC molecular features, such as gene expression signature and signaling networks, that can be used to complement conventional target-based approaches. Recalling the fact that HGF/c-Met and Gas6/AXL pathways are highly active in HCC and related to the resistance to sorafenib, co-targeting HGF/c-Met and Gas6/AXL signaling pathways may hold promise for the treatment of HCC.

4 Biological Significance of the Potential Bidirectional Crosstalk between HGF/c-Met and Gas6/AXL Signaling Pathways

As discussed earlier in the chapter, HCC is driven by cumulative changes in the hepatocyte genome and epigenome affecting cell signaling and behaviors, allowing cells to escape from cytotoxic or targeted therapies. In addition, the crosstalk between tumor cells and their surrounding microenvironment including cellular (such as fibroblasts, invading inflammatory cells, endothelial cells, hepatic stellate cells, pericytes, adipocytes) and non-cellular stroma components (such as extracellular matrix components and growth factors) modulates HCC biology by effects on cancer signaling pathways in tumor cells. In such a rich microenvironment, the crosstalks between pathways and feedback inhibition constitute a complex network of signal transduction that drives dynamic and adaptive cellular responses. HGF/c-Met and Gas6/AXL pathways are examples for such interactions as they enable bidirectional information exchange between microenvironment, regulating tumor growth, metastasis, and drug resistance.

Although the c-Met and AXL receptors are frequently upregulated and co-expressed during hepatocarcinogenesis, their crosstalk is yet to be established in HCC. However, success of the cabozantinib, an inhibitor of both c-Met and AXL, in previously treated patients and the co-expression of c-Met and AXL suggest that they interact with each other during hepatocarcinogenesis.

In general, the crosstalk between receptor tyrosine kinases and non-receptor kinases occurs in a ligand-dependent or ligand-independent manner. Particularly, growth factor receptors appear to be in relatively close proximity, so that they can form complexes upon stimulation by their cognate ligands [227] (Fig. 3). The c-Met receptor is often complexed with AXL on the plasma membrane of various cancer cells such as glioblastoma, melanoma, and breast cancer cells [116]. In these cancer models, HGF treatment induces activation of both c-Met and AXL and formation of highly polarized c-Met-AXL clusters on the plasma membrane [228]. HGF also induces both the expression and phosphorylation of AXL in a ligand-independent manner [228, 229]. HGF increases motility and invasion through stimulation of a rapid and dynamic cytoskeleton reorganization by activat-

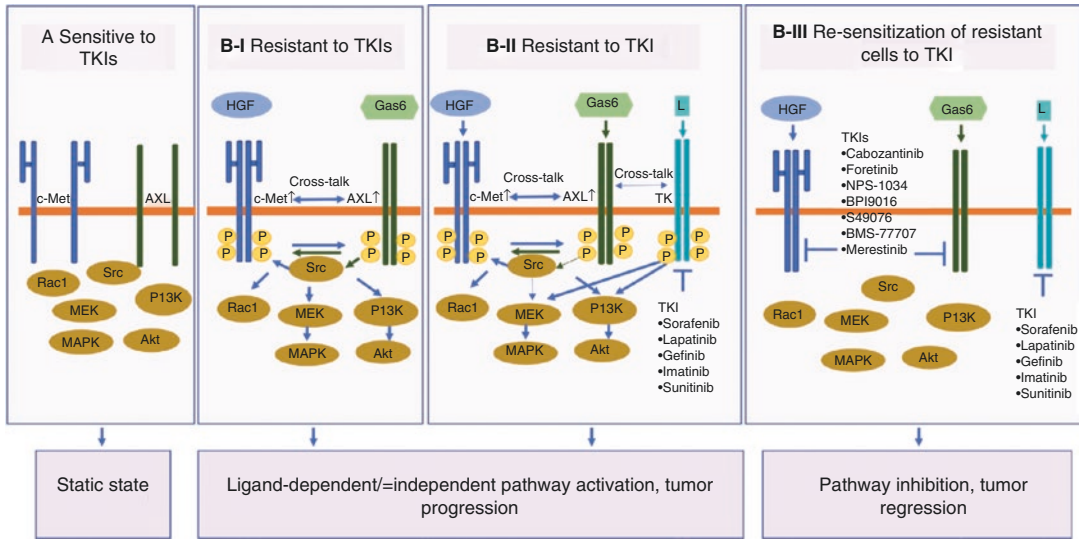

Fig. 3 Scheme summarizing the potential mechanisms by which c-Met and AXL crosstalk drives resistance to tyrosine kinase inhibitors (TKI)

ing GTPase RAC1, a process requiring both c-Met and AXL kinase activities [228]. A positive association between c-Met and AXL in bladder cancer was also detected [229]. In this model, similar to the mechanism described above, c-Met activation increased the expression of AXL and transactivated AXL signaling through the MEK/ERK pathway [229]. In clear cell renal cell carcinoma (ccRCC) and HCC cells, Gas6/AXL signaling transactivates c-Met through Src kinase to maximize cellular invasion [230]. Similar interactions occur in NSCLC as HGF-dependent c-Met activation enhances the AXL activity, and AXL positively regulates c-Met activity without affecting c-Met expression [231]. Moreover, studies show that miR-34a that is downregulated in cancers, including breast and gastrointestinal cancers, suppresses both c-Met and AXL expressions, causing a reduction in cellular migration and invasion ability [191, 232–234].

Since c-Met and AXL share many common elements in their signaling pathways including Akt, MAPK, Src, and Rac1, it is clear that there are many opportunities for c-Met to transactivate AXL and vice versa to modulate signals in HCC. However, such studies are lacking, and it will be extremely useful to explore mechanisms of co-activation of c-Met and AXL in HCC.

5 Scientific Rationale for Targeting the Crosstalk between c-Met and AXL

It has been also well documented that aberrant regulation of crosstalk has been associated with intrinsic and acquired drug resistance. The crosstalk between c-Met and AXL has been proved to allow the interplay with each other in some pathological processes including drug resistance. For instance, crosstalk involving c-Met and Akt pathways [115] and PI3K/Akt and JAK-STAT pathways [235] and the activation of hypoxia-inducible pathways [236, 237] are involved in the acquired resistance to sorafenib, the current first-line therapy for HCC. More recently, AXL and EMT pathways have been implicated in the development of acquired resistance to sorafenib [169]. Particularly, in tumors with high levels of EGFR, such as lung and triple-negative breast cancer (TNBC), c-Met and AXL dimerize with this receptor and initiate signaling that interferes

with the antitumor effects of anti-EGFR therapies. Promisingly, the treatment foretinib (a c-Met, AXL, Ron, Kdr, Flt-3, and Flt-4 inhibitor) in combination with lapatinib (a dual EGFR and HER2 inhibitor) effectively restored lapatinib sensitivity [199] and reduced Akt phosphorylation that led to cell cycle arrest and reduction of migration and invasion in TNBC cells [236]. In addition to the association with EGFR inhibitor resistance, the crosstalks of AXL and c-Met are essential for lung cancer metastasis in NSCLC [231, 238]. Moreover, targeting c-Met/AXL/FGFR crosstalk using S49076, an oral ATP-competitive inhibitor of c-Met, AXL, and FGFR1–3, improves the antitumor efficacy of radiotherapy in NSCLC [239]. In the first-in-human phase I study, S49076 demonstrated limited single-agent activity with a tolerable safety profile in patients with advanced solid tumors [240]. In this phase I study, S49076 was encouraged for combination therapies. Later, it was combined with gefitinib (EGFR inhibitor) in phase I clinical trials, in c-Met/AXL dysregulated NSCLC patients who have progressed on EGFR-targeted treatment [241]. Promisingly, this combination therapy was found to be well tolerated [241]. In addition, in TNBC, the crosstalks between EGFR/c-Met/AXL have been associated with a lack of response to ErbB family-targeted inhibitors [144]. In this model, EGFR signaling transactivates AXL which is colocalized with c-Met on the plasma membrane leading to transactivation of c-Met too [144]. A similar drug resistance mechanism was detected with imatinib therapy against c-kit/alpha-PDGFR in gastrointestinal stromal tumors (GIST) which was reversed by targeting AXL/c-Met [242]. In a renal carcinoma (RCC) preclinical model, c-Met- and AXL-mediated resistance against sunitinib, anti-angiogenic therapy, induces EMT-associated gene expression changes that includes Snail and beta-catenin [243]. The use of Cabozantinib, a potent inhibitor of VEGFR, AXL and c-Met, was shown to inhibit both c-Met and AXL activations and promote pro-metastatic behaviour and angiogenesis [243]. The efficacy of cabozantinib was also tested in a phase II randomized controlled trial, ECOG-ACRIN 1512, in NSCLC [244]. The design of the trial was based on the feasibility of using cabozantinib alone or combined with erlotinib in patients with EGFR wild-type NSCLC [244]. The trial identified signals of clinically meaningful efficacy superior to that of erlotinib alone [244]. Later, cabozantinib has been approved for the use in the first- and second-line settings in patients with advanced RCC [245]. As mentioned earlier in this chapter, in a double-blinded, randomized phase III trial that compared cabozantinib with placebo in previously treated patients with HCC, cabozantinib resulted in longer overall survival and progression-free survival than placebo [238]. Overall, cabozantinib inhibits multiple receptor tyrosine kinases that play roles in HCC pathogenesis and treatment resistance involving c-Met and AXL. However, resistance to cabozantinib is also common [246, 247]. Notably, in RCC preclinical model, sunitinib treatment linked to acquired cross-resistance to cabozantinib losing inhibitory effect on c-Met signaling and driving survival mechanism [247].

Targeting c-Met and AXL cooperativity in HCC is a rational approach given the known efficacy of cabozantinib that inhibits both c-Met and AXL activities. Co-targeting can be achieved through drug combinations or through the design and development of a single compound that is able to inhibit multiple oncoproteins, in this case c-Met and AXL. There are some non-selective tyrosine kinase inhibitors that inhibit both c-Met and AXL simultaneously. BMS-777607, a small molecular inhibitor of c-Met and AXL, significantly blocks the HGF-stimulated activation of c-Met signaling, including MEK-MAPK and PI3K-Akt pathways [248, 249]. In prostate cancer in vitro models, BMS-777607 treatment inhibited cell migration and invasion activated by HGF [249]. In a sarcoma rodent tumor model, BMS-777607 impaired angiogenesis and metastasis through blocking c-Met signaling [250]. Complete tumor stasis was achieved in the human gastric carcinoma xenograft model following oral administration without any obvi-

ous toxicity [248]. In a similar tumor model, BMS-777607 reduced AXL phosphorylation in the hypercellular tumor regions, in the migratory front of tumor cells, and in the vascular proliferative region within glioblastoma xenograft model [251]. Currently (2020) the maximum tolerated dose of BMS-777607 (ASLAN002) in subjects with advanced or metastatic solid tumors is under investigation (ClinicalTrials.gov Identifier: NCT01721148 and NCT00605618). Another dual-targeting inhibitor, NPS-1034, was used to prevent the crosstalk between AXL and c-Met under EGFR inhibition [238]. This is a newly developed drug (NPS-1034) that targets both c-Met and AXL and was tested in combination with EGFR inhibitors, gefitinib or erlotinib using gefitinib- or erlotinib-resistant cells [238]. This combinatorial treatment synergistically inhibited cell proliferation and induced apoptosis in resistant cells and also was found to be effective in xenograft mouse models of resistant cells [238]. Promisingly, phase I study with BPI-9016, a novel small-molecule inhibitor that simultaneously targets both c-Met and AXL, with NSCLC patients showed antitumor activity and favorable safety and pharmacokinetic profiles [252]. Additionally, merestinib (LY2801653) that inhibits c-Met, AXL, RON, MKNK1/2, and NRTK1/2/3 was shown to inhibit migration, invasion, and concomitant in vivo tumor growth through downregulation of c-Met signaling in preclinical models of tumors including AML, gastric cancer, and NCSLC [253, 254]. This compound is currently being investigated in patients with advanced or metastatic cancer (ClinsicalTrials.gov Identifier: NCT03027284, NCT01285037, NCT03125239, NCT03292536, and NCT02711553).

Overall, preclinical and clinical studies have revealed that the downregulation of c-Met and AXL signaling disrupts key steps in metastatic cascade and tumor growth (Table 3). This indicates that targeting c-Met and AXL may hold promise for the treatment of HCC, owning active c-Met and AXL signaling. Since pharmacodynamic readout of dual inhibitors such as NPS-1034, BPI9016, and BMS-777607 is encouraging, further investigation of them in combination therapies can be used with sorafenib to combat c-Met and AXL resistance and sensitize cells to sorafenib. Cautiously, the probability of efficacy would have to be balanced against likely toxicity. Thus new clinical studies with well-designed patient selection criteria, dose selection, and adverse event profile will potentially demonstrate benefit.

6 Future Perspective

As briefly reviewed earlier, HCC is one of the most lethal cancer types. Although the poor outcome is largely secondary to a high proportion of patients who are diagnosed with advanced disease, the prognosis of HCC is also influenced by the inherent biological aggressiveness and the high metastatic potential of this malignancy. Treatment options remain limited with little progress over the last decades. From 2007 to 2017, sorafenib remained the only systemic agent with proven clinical efficacy for advanced HCC patients. Since 2017, with the approval of several new agents, there have been improvements in the treatment landscape of advanced HCC. Among these agents, regorafenib, lenvatinib, and cabozantinib as the targeted therapies, nivolumab and pembrolizumab as the immune checkpoint inhibitors, and ramucirumab as the anti-angiogenic have been shown to be effective in patients with HCC. In addition to these agents, multiple new agents have been under investigation in the clinical trials. However, persistence of drug resistance remains a major challenge leading to decreased drug response and the recurrence of disease. The overall cause of drug resistance is complex due to high plasticity and adaptability of HCC and the dynamic interactions between tumor cells and the microenvironment. Thus a comprehensive understanding of tumor biology will lead to identification of new targets and relevant pathways serving as diagnostic, predictive, and prognostic biomarkers. Thanks to novel technologies and "-omics"-based characterization efforts, the molecular profiling of HCC is evolving rapidly that can offer not only identification of multiple new targets but

Table 3 Examples of inhibitors targeting c-Met and AXL

Inhibitors	Targets	Cancer type studied	Biological activities	Phase	Toxicological profile	Reference
Foretinib	c-Met, AXL, Ron, Kdr, Flt-3, and Flt-4	TNBC, NSCLC	Inhibition of migration, invasion, growth, and metastasis	Preclinical	–	[198]
NPS-1034	c-Met, AXL	NSCLC	Inhibition of proliferation, induction of apoptosis	Preclinical	–	[238]
BPI9016	c-Met, AXL	NSCLC	Inhibition of tumor growth	Phase I	Well tolerated	[252]
S49076	c-Met, AXL, FGFR1–3	NSCLC	Improvement of the antitumor activity of radiotherapy	Phase I	Well tolerated	[239]
		NSCLC	Improvement of the antitumor activity of gefitinib	Phase I	Well tolerated	[240]
Cabozantinib	c-Met, AXL, Kdr, TIE2, Flt3, c-kit	NSCLC, RCC	Longer overall survival	Phase II	Well tolerated	[246, 247]
		HCC	Longer overall survival and progression-free survival	Phase III	Well tolerated	[21]
BMS-777607	c-Met, AXL	Prostate cancer	Inhibition of migration and invasion	Preclinical	–	[251]
		Sarcoma	Inhibition of angiogenesis and metastasis	Preclinical	–	[251]
		Gastric cancer	Tumor stasis	Preclinical	–	[251]
		Advanced or metastatic cancer		Phase I (under investigation)		[251]
Merestinib	c-Met, AXL, RON, MKNK1/2, and NRTK1/2/3	AML, gastric cancer, NCSLC	Inhibit migration, invasion, and tumor growth	Preclinical		[253, 254]

also identification of the subset of patients who might respond to particular combinatorial therapies. Since HCC is a systemic disease already at a time of diagnosis, management of HCC requires a moving and dynamic planning of therapy. Considering heterogeneity and multifocal nature of HCC, therapy planning can be started by multiregional sampling of a patient's tumors and combination of agents, with each agent targeted to the features of different sub-clones and microenvironmental compartments. Recalling the fact that acquired resistance to an initially effective therapy is a greater challenge in the management of HCC, leading to relapse of the disease and poor prognosis, addressing multifactorial drug resistance mechanisms is imperative to achieve better outcome for this disease. Especially, bypassing certain drug target signaling pathways through molecular crosstalks and feedback loops that gives cancer cells survival advantage is one of the common drug resistance mechanisms in the development of resistance. Regarding the fact that c-Met plays a central role in resistance to targeted therapies and the high degree of crosstalk between c-Met and other signaling molecules suggest that c-Met inhibition is unlikely to be effective as a monotherapy. Thus, identification of crosstalk partners of c-Met such as AXL involved in the

tumorigenesis may be clinically relevant to HCC and provide important biomarkers for combinatorial therapies. Therefore, targeting the crosstalk between c-Met and AXL might be an important approach to enhance effectiveness of therapy.

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Hepatocellular Carcinoma in Morocco

Younes Cherradi

HCC in Morocco: What's the Current Situation?

1 General Features.
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1 General Features

Management of HCC is of great interest in Morocco. The Declaration of Rabat for African fight against viral hepatitis and HCC, first issued in 2008, has been updated in 2011 and 2015 [1]. The last update in February 2015—in the context of the fifth African Middle East Congress on Digestive Oncology—focused on strategies to be implemented in order to reduce the incidence of HCC in African countries [1]. In this chapter, we present the epidemiological profile of HCC in Morocco and the specific features of this tumor in Moroccan patients.

1.1 Incidence

Morocco is a country with low endemicity of HCC [2]. According to Global Cancer Observatory, Morocco and Nepal have the lowest estimated age-standardized incidence rates (ASIR) per 100,000 people for HCC (equally about 1.1) [2]. Also, the age-standardized mortality rates (ASMR) from liver cancer in both genders have been established as low in Moroccan population [2]. However, if it seems evident that Morocco is a country with low incidence of HCC, it's important to mention the increasing trends of this cancer's incidence and mortality rates in Morocco from 1990 to 2017 moving, respectively, from 1.6 to 1.74 and from 1.73 to 1.83 [3]. The low HCC incidence in Morocco is explained by the multifocal actions and strategies adopted by Moroccan health authorities in order to reduce risk factor incidence and prevent HCC development. Genetic hypotheses are also implicated.

1.2 Causes

Regarding etiological factors of HCC occurrence in Morocco, chronic hepatitis is the first incriminated pathogen. Chronic hepatitis C is the major causal agent of HCC in the country. Yapali et al.

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reported chronic hepatitis C as a primary etiological factor for HCC occurrence in 36% of patients followed by chronic hepatitis B in 31% and alcohol in 14% [4]. Among 440 Moroccan patients with HCC in Ibn Sina Hospital during the period from 2001 to 2015, Essaid et al. confirmed the prominence of hepatitis C virus (HCV) and hepatitis B virus (HBV) as the leading causes of HCC in Morocco with, respectively, 69.7% and 15.2% [5]. Chronic alcoholic cirrhosis was reported as an etiology in only 0.9% of all patients' series. A study conducted in Marrakech University Hospital on 76 cases of HCC confirmed those findings with chronic hepatitis C as a causal agent in 18.5% of patients and chronic hepatitis B in 12.5% [6]. The same findings were confirmed in the Global Burden of Disease study in 1990 and 2017.

Chronic hepatitis B is the second cause of HCC in Morocco. The profile of HBV is characterized by a predominance of genotype D and negative HBe antigen [7]. Occult hepatitis B infection (OBI) is a frequent but underdiagnosed condition; it corresponds to the presence of HBV DNA in the liver of patients with negative HBs antigen (with HBV DNA detectable or not in the serum) [8]. In Moroccan patients with negative HBs antigen and cryptogenic cirrhosis, OBI should not be missed out. It was demonstrated that OBI was strongly associated with the severity of the underlying hepatopathy with HCC more frequent in case of association of chronic hepatitis C and OBI compared to OBI-positive cryptogenic patients [8]. The presence of OBI has been advanced as an independent risk factor for hepatocellular carcinoma development in Hepatitis C virus carriers suggesting a possible synergistic mechanism [8].

If aflatoxin B1 (AFB1) is incriminated in many sub-Saharan African countries as a potential causal agent of HCC, AFB1 levels in cereals are, apparently, two to three times lower in Morocco, and TP53 mutation seems exceptional [9]. In a study including 42 patients with HCC from Western North African (WNA) countries-with 35 patients from Morocco-, only one TP53 R249S mutation has been reported [9].

1.3 HCC Risk Factors in Moroccan Population

- Hepatic Condition.

HCC is evidently related to liver hepatopathy with cirrhosis as a pre-cancerous condition. In a retrospective study conducted at Hassan II[d] University Hospital among 148 cases of HCC, advanced Child-Pugh, elevated alpha-fetoprotein level (>400 ng/ml), the presence of metastases, and the invasion of the portal vein were recognized as bad prognosis factors in Moroccan population with liver cirrhosis[10].

- Risk Factors of HCC Development After Antiviral Therapy.

In the study published by Cherradi et al. and including 369 Moroccan patients previously treated for chronic hepatitis C, HCC occurred in 5% of all treated patients ($n = 20$). Advanced age (>50 year old) when HCV infection is diagnosed, severe fibrosis (F >2 according to the Metavir score), and the absence of sustained virological response (SVR) were strongly associated with the development of HCC in treated patients [11, 12]. HCC incidence was significantly less important in SVR patients' group with only 2.3% of patients who developed HCC versus 12.5% in nonresponder population [12] which confirms the evidence that achieving SVR have a mainly protective role against HCC development.

According to the same study, severe fibrosis was the unique significant predictive factor of HCC occurrence in the group of patients who achieved SVR. This is highlighting the continuous need for regular screening for HCC in patients treated for chronic hepatitis C even after achieving SVR, especially in case of advanced fibrosis. It's important to mention that patients considered for this study were all treated by pegylated interferon. No local data are available regarding direct-acting antiviral agents (DAAs).

- Emerging Risk Factors: Obesity, Diabetes, and Lifestyle.

Between 1960—when Mediterranean food model was still present—and 2021, Moroccan food and lifestyle have considerably changed leading to a significant elevation of obesity and diabetes prevalence over the last decades. The prevalence of obesity among the Moroccan adult population has significantly rose from 4.1% in 1984/1985 to 10.3% in 1998/1999 and 13.3% in 2000. Similarly, the trends of persons with overweight condition or obesity are increasing [13, 14].

In patients with HCC from Morocco and North African countries, diabetes has been clearly established as a risk factor for development of cirrhosis and primary liver cancer [15]. When present, diabetes increases eightfold the risk of HCC occurrence [15]. This is extremely important, especially when we know that 2.5 million (M) Moroccans are diabetic, 2.4 M are in prediabetes condition, and 10 M are overweight (including 63% of women and 16% of children) [14]. The contribution of nonalcoholic steatohepatitis in liver cancer incidence in Morocco has increased from 9.3 to 11.8% from 1990 to 2017 [3].

2 Commonest Manifestations, Diagnosis, and Treatments

2.1 Commonest Presentation of HCC:

In the Moroccan population, since maternal fetal transmission mode is rare, HCC is occurring in older patients, mainly on cirrhotic liver. According to the Firwana et al. series including 440 HCC, mean age at diagnosis was 63 years old [5]. It is similar in the Pratic et al. series where mean age was 59 years old [6]. Development of HCC seems to be prominent in male gender with a sex ratio of 1.7 in both Firwana et al. and Pratic et al. series [5, 6]. However, a study among 148 patients in Hassan IId University Hospital showed female predominance [10].

HCC is mainly occurring in patients with cirrhosis. In Morocco, diagnosis is usually made in the context of a screening strategy in patients with chronic hepatopathy. Clinical presentation at diagnosis is not specific. Main clinical complaint in Moroccan patients is abdominal pain as reported by Firwana et al. and Pratic et al. with, respectively, 25% and 75% [5, 6]. Among 440 considered patients, Sixty-one percent were asymptomatic at diagnosis, and HCC was diagnosed during regular ultrasonography screening in 38.5% of cases [5].

2.2 Screening: A Key Strategy in HCC Management:

In Morocco, with a great consciousness of the impact of screening on early tumor detection, eligibility for curative treatment, and patient overall survival, regular screening was adopted early as a mandatory part of the follow-up of patients with chronic liver condition. Screening strategy consists of periodical ultrasonography exam in the hand of expert every 6 months. Alpha-fetoprotein may be considered as prognostic factor but is not necessary in Moroccan gastroenterologists practice.

Khannoussi et al. compared screening findings and HCC prognosis during two consecutive periods: the first period between 1994 and 1998 with 225 diagnosed HCC and the second period between 2001 and 2007 including 270 cases of HCC [16]. In the first period, the screening consisted of both serum alpha-fetoprotein dosage and ultrasonography (US) examination every 3 to 6 months. In the second period, only ultrasonographic examination was regularly performed every 6 months. The implementation of US screening impacted evidently the HCC early diagnosis which moved from 18% between 1994 and 1998 to 54% between 2001 and 2007 [17]. Screening allowed the initiation of HCC treatment with curative intention in 41% of patients. Mean nodule size at diagnosis in regularly screened patients was 31 mm compared to 63 mm in the others [16]. US screening in cirrhotic patients allowed an early diagnosis of HCC with reduced size of tumors and better hepatic function (Child-Pugh score) offering more therapeutic options for patients. Thus, the place of screening as a major tool in the improvement of prognosis and management of HCC was

stated; biannual abdominal ultrasound examination was sufficient and most efficient with no need of alpha-fetoprotein.

2.3 A Preventive Approach

Many actions have been taken by Moroccan health authorities to reduce the incidence of HCC in Moroccan population and may explain the local low incidence of HCC:

- Screening for both hepatitis B and C is mandatory in blood transfusion centers in Morocco since 1995.
- HVB vaccination is systematic for all newborns since 1999.
- Hepatic ultrasound screening is mandatory in patients with chronic liver condition and is recommended by national scientific societies of gastroenterology.
- To decrease the risk of hepatitis B maternal fetal transmission mode, HBs antigen positivity screening is mandatory during pregnancy. Human hepatitis B immunoglobulin antibodies are now available in Morocco for the prevention of mother-to-child transmission in newborns in the case of a hepatitis B virus carrier-mother.
- Access to antiviral therapies has been improved, and DAAs are now easily available for patients with chronic hepatitis B and C. However, efforts are still needed for universal health coverage; Moroccan Government started in 2021 working on social security reform in order to allow equal access to health services and benefit from a basic social security.
- In 2019 and in order to reduce the impact of obesity and diabetes, as emerging risk factors of HCC development, Morocco was the first country in North Africa and the Middle East region to adopt taxation policies of beverages and sweetened products [14].
- The role of preventive approach regarding viral hepatitis-related HCC is present in Moroccan national plan 2020–2029 against cancer.

2.4 Treatment of HCC in Morocco

Except for hepatic transplantation, which is undoubtedly a cornerstone in the management of HCC, all other therapeutic options (alcoholization, radiofrequency ablation, chemoembolization, surgical resection, biotherapies) are available in Morocco. However, few data are published making difficult to establish a cartography of therapeutic strategies in Moroccan patients with HCC.

According to the experience of M. Benazzouz and R. Afifi at Ibn Sina University Hospital in Rabat with a large series including 440 HCC, treatment was curative in 41.5% of patients. As a result of screening, percutaneous approach was mainly possible (30%) with alcohol injection, radiofrequency ablation, and acetisation, respectively, in 17.9%, 9.7%, and 2.5% of patients [5]. Surgical resection was an option in 13% of cases, and only one patient benefited from liver transplantation [5]. Palliative and symptomatic treatments were considered, respectively, in 19.3% and 33.8% of patients [5]. Unpublished data from both experts reported total necrosis rate of 87%, 89% and 94%, respectively, after alcoholization of 164 HCC, acetisation of 45 HCC and radiofrequency of 27 HCC. Survival rate was, respectively, 47% at 5 years in patients who benefited from alcoholization, 66% at 3years after acetisation and 87% at 2 years in patients treated by radiofrequency ablation.

Data from Mohammed Vth University Hospital in Marrakech are slightly different. Among 76 cases of HCC, curative treatment was only possible in 14.4% of patients (with radiofrequency ablation in seven patients, surgical resection for three patients, and liver transplantation in one case). Therapeutic approach was mainly palliative and symptomatic with, respectively, 26.3% and 52.6% of all patients [6].

For patients with advanced unresectable HCC in Morocco, trans-arterial chemoembolization (TACE) and sorafenib are available therapeutic options as palliative approach.

Regarding response to TACE, a study was conducted in Hassan II^{d} University Hospital in

Fez, Morocco, among 162 patients diagnosed with 225 unresectable HCC. Continuous complete response (CR) was obtained in 14 patients (8%). It was significantly associated with male gender, chronic hepatitis C as a causal agent, location in the segments VI and VII of the liver, and complete blush extinction on digital subtraction angiography [18]. Hepatic condition was also a major determinant in CR with Child-Pugh A, MELD score ≤ 19, and BCLC B and C stages strongly correlated to CR [18].

Liver transplantation (LT) is a major issue in Morocco. Despite many legislative and technical improvements, too much efforts are still needed. Less than 30 liver transplants have been performed in Morocco with the first successful liver transplantation totally performed by a Moroccan team (Benkabbou et al.) in 2016 [19]. It is certain that the development of LT programs in Morocco will bring vital opportunities for Moroccan patients with HCC and meeting indications and will improve significantly quality of healthcare and survival rate for this category.

3 A General Overview of HCC-Related Research

Pineau et al. stated that genomic stability prevails in North African HCC and demonstrated that genetic presentation of HCC in WNA countries is mainly different from Egypt and other African countries hypothesizing that the low endemicity may be explained by the low prevalence of mutagenic (exceptional TP53 mutations) or cytotoxic (low consumption of alcohol) agents [9]. Many other researches investigated the genetic aspects of HCC in Morocco especially the p53 pathway recognized as the most important target for hepatic carcinogenesis aberrations. Akil et al. proposed that variants of the transcriptional co-activator genes (EP300 and PCAF) may influence HCC risk with an interesting association between the Val/Val genotype of the EP300 at codon 997 and Ser/Ser genotype of the PCAF at codon 386 and the risk to develop HCC in Moroccan population [20]. Rebbani et al. explored the potential implication of a single nucleotide polymorphism (SNP) in the MDM2 (the murine double minute 2) in development of HCC. The GG genotype of SNP309 showed a significant association with a higher risk of HCC occurrence [21].

In a case-control study enrolling 74 HCC, Jadid et al. suggested the implication of the SOCs3 polymorphism in the modulation of HCC development by affecting mRNA expression in chronic HCV-infected patients [22]. SOCs3, the suppressor of cytokine signaling 3, is a potent regulator of cytokine signal transduction which is involved in negative control on JAK/STAT pathway [23]. Those findings are highlighting the specific molecular presentation of HCC in Morocco. Large pooled studies with multicentric recruitment are needed to explore more aspects of hepatocarcinogenesis pathways in the local population.

4 Conclusion

HCC in Morocco shows specific features. Due to genetic profile and a multifocal preventive approach, incidence is considerably low. Chronic hepatitis C is the major causal agent. A national register dedicated to HCC will help to collect all resources about this cancer and make all unpublished data available for researchers. More multicentric studies are needed to understand better all clinical, therapeutic, and molecular aspects of HCC in Moroccan patients. Also, there is a serious need to implement a national program for liver transplantation to optimize survival rate and act on cirrhosis as it's the precancerous condition.

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Hepatocellular Carcinoma in Lebanon and Its Association with Thalassemia

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1 HCC in Lebanon

Hepatocellular carcinoma (HCC) is a relatively rare cancer in Lebanon. According to the national cancer registry data for the years 2005–2015, the crude incidence rate of liver cancer in Lebanon ranges from 2.2 to 3.7 (males) and 1.9 to 3.4 (females) per 100,000. Unpublished data from a major tertiary care center in Lebanon, the American University of Beirut Medical Center (AUBMC), reveal that 37 liver cancers were reported in 2018, making it the 18th most common type of cancer. The mean age was 62.6 years, predominantly males (62.2%). On histology, 11 out of 37 tumors (39.7%) were HCC. The reported number of HCC cases from AUBMC was 9, 6, and 12 in 2015, 2016, and 2017, respectively. Liver cancer rates have been rising in Lebanon. A study by Shamseddine et al. over a 5-year period from 2003 to 2008 showed the highest annual percent change of all cancers (13.6% in males and 18.3% in females) [1].

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Yaghi et al. studied 92 patients in Lebanon diagnosed with HCC and collected from 3 large university medical centers over a 5-year period between 1998 and 2003. The average age was 60.5 ± 22.3 years, and the male/female ratio was 5.6:1. Viral hepatitis was the most common cause (83.7%) of the underlying liver disease, and hepatitis B infection was more common than hepatitis C infection (67.4% versus 19.6%). Other etiologies included alcoholic liver disease in 8.2% and other miscellaneous rare causes such as primary biliary cirrhosis, hemochromatosis, autoimmune hepatitis, and tyrosinemia [2]. HCC occurred in the background of liver cirrhosis in the majority of patients (91 of 92 patients). Child-Pugh class at time of HCC diagnosis was A (34.8%), B (39.3%), and C (25.8%), respectively. Model of end-stage liver disease (MELD) score was available in 79 patients, and the mean MELD score was 9.4 ± 7.6 [2]. HCC was diagnosed in 36.1% of patients within the first 2 years after the diagnosis of cirrhosis and in 72.4% within 5 years. In 42.4% of cases, the diagnosis was established during follow-up of cirrhosis with a mean follow-up period of 40 months. The average size of tumor nodules was 5.6 cm, and the number of nodules varied from single (46.1%) to two (15.7%) to three (5.6%), while 32.6% had diffuse HCC. Forty patients had less than three nodules <3 cm of diameter or a single tumor of less than 5 cm [2]. Portal vein thrombosis was present in 38.2% of the patients. Sixty-five percent of the patients were ineligible for curative resection based on the

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presence of diffuse disease or tumor associated with portal vein thrombosis [2]. Overall survival was 44.8%, 32.8%, and 17.6% at 1, 2, and 3 years, respectively. The reported prognostic factors that were identified using univariate analysis included "age >55, bilirubin <3.2 mg/dL, HCC as the first manifestation of liver disease, eligibility for a curative treatment, International Normalized Ratio (INR) <2, MELD score >18, and the presence of portal vein thrombosis" [2]. The reported 6-month mortality was 38.8%, and the factors that were associated with survival beyond 6 months included "INR< 2, bilirubin <3.2 mg/dL, tumor <5 cm of diameter or 3 nodules of <3 cm of diameter without portal vein thrombosis, MELD score <16, lower Child-Pugh class, and the absence of portal vein thrombosis" [2].

2 HCC and Thalassemia

Thalassemia is a genetic disorder based on underlying molecular defects in the α-globin or β-globin gene clusters, which form the basis of the various inherited forms of α-thalassemias or β-thalassemias and the defective hemoglobin synthesis leading to ineffective erythropoiesis and anemia [3, 4]. Based on the transfusion requirements, patients are commonly classified into transfusion-dependent (TDT) for those who are not to survive without lifelong blood transfusions by producing sufficient hemoglobin and non-transfusion-dependent thalassemias (NTDT) [4]. Both populations are at increased risk of iron overload as the liver is the primary site of storage of excess iron [5]. Iron overload development in TDT patients is secondary to blood transfusion. In patients with NTDT, however, it is mainly due to increased intestinal absorption. Hepcidin levels are low; thus, the excess iron is absorbed into the system and released into the circulation causing preferential portal and hepatocyte iron loading. This ultimately results in an increase in free iron into the blood, which can lead to end-organ damage, such as endocrinopathies, cirrhosis, and malignancies [6].

Thalassemia patients used to die at an early age secondary to anemia and heart failure [7]. With the advent of wider and more correct use of iron-chelating agents as well as advancements in MRI technology for the assessment of iron overload in the liver and heart, the lifespan of thalassemia patients has increased [8]. Since the year 2000, the improved survival of thalassemia patients has increased the incidence of disease complications, which were less likely to develop [9, 10]. Among these complications, one of the most worrying in recent years is hepatocellular carcinoma (HCC). The incidence of HCC in patients with thalassemia has been increasing with time [6]. Epidemiologic data based on an Italian registry reported a steady increase in the number of HCC cases in thalassemia patients in Italy from 8 in 1993–1997 to 31 in 2008–2012 [11]. Compared to the general population, most patients with thalassemia develop HCC at age <50 years, and there is no discernible difference in incidence between men and women in this group of patients [6].

Worldwide, the major risk factors for liver cancer are infection with the hepatitis B and C viruses [12], while the reported risk factors for HCC in thalassemia patients include iron overload and chronic hepatitis B and C [13]. Chronic viral hepatitis is a risk factor for HCC in patients with thalassemia [6]. Due to blood transfusions, many patients are or have been infected with HCV or HBV, namely, those born before 1990 or those born in countries where universal HBV vaccination and safe blood transfusion programs are still not completely applied [10]. Worldwide, 0.3–5.7% of thalassemia patients are HBsAg-positive [10]. The reported prevalence of anti-HCV antibodies in thalassemia patients ranges from 4.4% to 85.4% [11]. However, HCC has also been reported in non-transfused patients and in those who are HCV- and HBV-negative. Therefore, other risk factors, primarily iron overload, are involved in hepatocarcinogenesis in patients with thalassemia (Fig. 1) [10].

Hepcidin is a key regulator responsible for iron balance and functions by decreasing iron absorption from the gut and its release from the reticuloendothelial system [6]. Ineffective erythropoiesis along with the subsequent hemolytic anemia leads

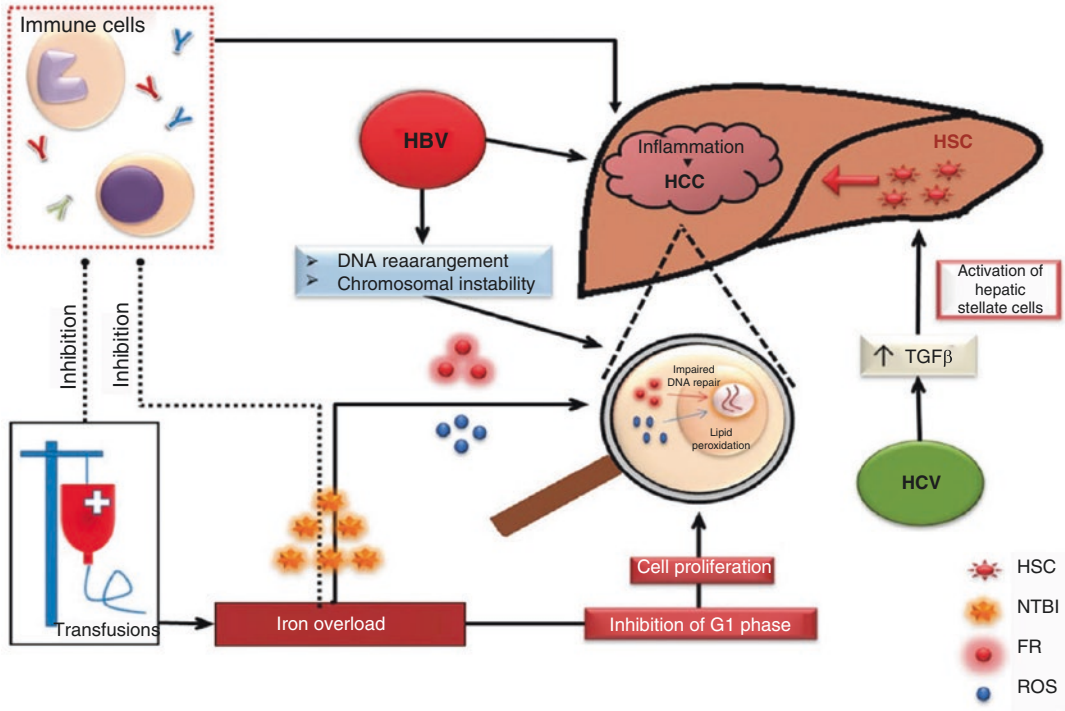

Fig. 1 Different mechanisms related to HCC development in thalassemia patients. (FR free radicals, HBV hepatitis B virus, HCV hepatitis C virus, HCC hepatocellular carcinoma, HSC hepatic stellate cells, NTBI non-transferrin-bound iron, ROS reactive oxygen species, TGFβ transforming growth factor β). (Used with permission from Finianos et al. [13])

to an increase in the level of hepcidin and thereby results in an increase in iron absorption from the gut. In addition to the need for frequent transfusions to ameliorate the resulting anemia, this results in an iron overload state [13]. Free iron is believed to generate reactive oxygen species, which can cause peroxidation of membrane fatty acids and consequent formation of toxic byproducts that disrupt DNA and impair protein synthesis, thereby triggering malignant transformation through mutations in tumor suppressor genes (such as p53) and DNA repair genes [14]. Iron overload can also promote malignant transformation of the liver by accelerating the pathway from fibrosis to cirrhosis by activating stellate cells and the profibrogenic effects of lipid peroxidation [15]. It has also been suggested that iron overload may contribute to cancer formation by inducing immunologic aberrancies [6].

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An Overview of Hepatocellular Carcinoma (HCC) in Lebanon: A Focus on Hepatitis B- and Thalassemia-Related HCC

Sally Temraz and Ali T. Taher

1 Epidemiology of HCC in Lebanon

Lebanon is a 10,452 sq.km. country on the Mediterranean Sea with an estimated population of four million inhabitants and a life expectancy of 80 years. Hepatocellular carcinoma (HCC) is a relatively scarce cancer in Lebanon, ranking 18th among males and females with an age-adjusted incidence rate of 4.2 and 2.3 per 100,000 males and females, respectively. According to the latest WHO data published in 2018, there were 227 newly diagnosed liver cancer cases and 211 liver cancer-related deaths in Lebanon. The age-adjusted death rate is 2.29 per 100,000 of population making Lebanon #174 in the world. According to the Lebanese Ministry of Public Health, liver cancer cases increased from 84 cases for a population of 3.987 million in 2005 to 150 cases for a population of 4.597 million in 2016 for both genders [1].

2 Risk Factors of HCC in Lebanon

Although many culprits are found to cause this aggressive cancer, most cases arise from chronic hepatitis B (HBV) and hepatitis C virus (HCV) infection; of these two viruses, HBV accounts for 80% of causes of viral hepatitis [2]. The remaining cases of HCC can be attributed to a profusion of other reasons such as aflatoxins in the diet, alcoholic and non-alcoholic fatty liver diseases, obesity, and some metabolic diseases such as hemochromatosis [3].

2.1 HBV and HCV Infections

Most HCC cases in Lebanon have hepatitis B virus (HBV)-related HCC, mainly through liver cirrhosis, accounting for nearly two thirds of patients [4, 5]. The prevalence of HBV in Lebanon is 1.74%. Figure 1 shows the distribution of HBV by region. The majority of cases (63%) are between 20 and 60 years [6]. HBV infection was recorded among 2.4% of prisoners, a relatively higher seroprevalence compared to the general Lebanese population [7]. On the other hand, among 204 men who have sex with men and female sex workers aged 18 years and above, the rate is much lower than the general population with only one testing positive for HBV (0.99%) [8]. HBV infections are characterized by HBV genotype D. In this regard, a study of 61 HBV carrier blood donors from Lebanon was performed. Genotype D was the only type detected, with the majority of the strains related to subgenotype D1 and few strains related to subgenotype D2 [9].

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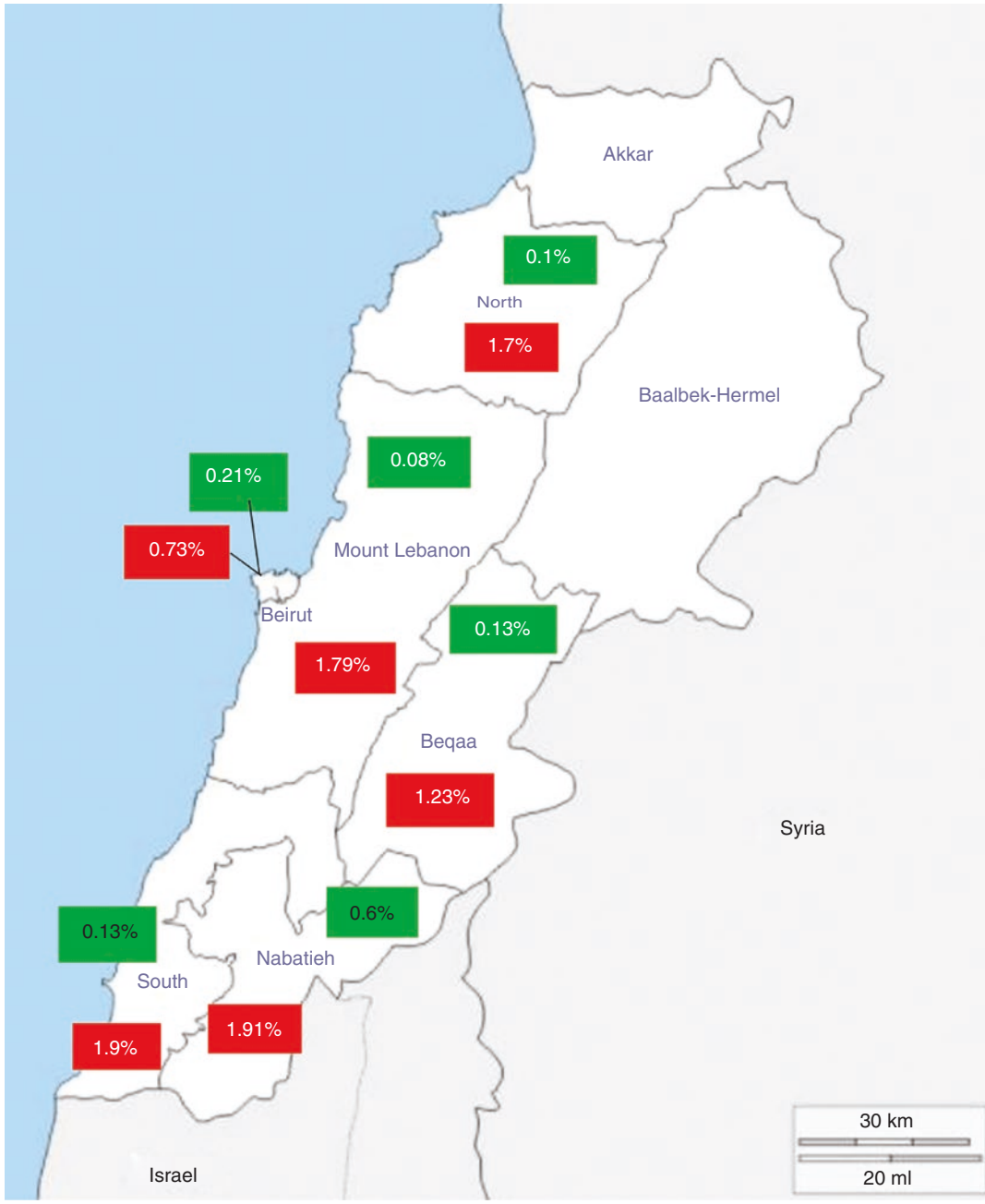

Fig. 1 The distribution of HBV cases (shown in red) and HCV cases (shown in green) among regions in Lebanon

Another risk factor includes HCV infection in 20% of patients [4, 5]. The prevalence of HCV in Lebanon is 0.21%. Figure 1 shows the distribution of HCV by region. HCV genotype 1 is most common (50%) followed by genotype 4 (33%) and genotype 3 (17%). Most cases of HCV infection (71%) occur in those older than 40 years [6].

Beirut, despite the overpopulation, has the least prevalence of hepatitis B (0.73%), mainly because of a higher socioeconomic status. On the

other hand, higher rates are found in Nabatieh and the South mainly due to vaccination program failure because of lack of education and inefficient awareness campaigns [10, 11]. The modes of transmission of HBV in Lebanon are predominantly horizontal, resulting from the exposure of abraded skin, cuts, minor open wounds, or mucosal surfaces to blood or body fluids containing HBV from the afflicted subjects [12]. Of note, the HBV and HCV carrier state in Lebanese blood donors is 1–2% and 0.1–0.6%, respectively [13–15].

2.2 Alcoholic and Non-alcoholic Liver Disease

Alcohol abuse is responsible for 8% of HCC cases in Lebanon. According to the Lebanese Epidemiologic Survey on Alcohol (LESA), 11.2% of Lebanese adults experienced alcohol use disorders in the prior 12 months (abuse, 6.2%; dependence, 5%) in 2011 [16]. Non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of metabolic syndrome which is based on obesity and insulin resistance. Epidemiologic data clearly demonstrates that NAFLD and obesity-related disorders are significant risk factors for tumor development in HCC [17]. A recent study evaluating the nutritional profile and the dietary patterns of Lebanese NAFLD patients demonstrated that 40% of cases belonged to the high fruit group as compared to 30% following a high meat and fast food dietary pattern. Both groups increased the odds of NAFLD by fourfold [18]. National studies from Lebanon report increasing rates of overweight and obesity that is more evident in females and consistent with the growing epidemic of obesity worldwide [19, 20].

2.3 Aflatoxin Contamination

Aflatoxins are food-borne secondary fungal metabolites that are hepatotoxic, hepatocarcinogenic, and mutagenic. Aflatoxin M1 (AFM1) has been recently classified by the International Agency for Research on Cancer (IARC) as a class 1 carcinogen after several studies demonstrated the increased risks for HCC in individuals exposed to it. AFM1 presents a threat to public health since it tends to linger in milk and dairy, and many studies proved its capacity to remain intact and relatively stable after heat treatment such as pasteurization or ultra-high temperature treatment [21]. In Lebanon, milk and dairy are recognized for their high nutritional value, and they are consumed by the majority of people from different regions and ages. Milk is also used as a major ingredient in the manufacturing of a wide array of Lebanese food especially dairy, pastries, and sweets. A recent study from Lebanon reported on the occurrence of AFM1 from 868 collected samples of raw cows' milk, pasteurized and UHT cows' milk, and dairy products. Results showed contamination in raw milk, pasteurized and UHT milk, and dairy products at a range of 0.011–0.440 μg/L, 0.013–0.219 μg/L, and 0.015–7.350 μg/L, respectively, with 28%, 54.5%, and 45.5%, respectively, of samples with AFM1 above maximum tolerable limit set by the European Commission [22]. AFM1 consumption was shown to be associated with 0.0041 additional cancer cases per 100,000 persons per year [22].

3 Surveillance, Prevention, and Management of HCC in Lebanon

Government strategies to address the burden of HCC include surveillance, prevention, and management of the disease. As part of surveillance, the government has initiated the National Cancer Registry since 2004, the behavioral risk factors for non-communicable diseases in Lebanon, and the National Hepatitis Epidemiological Program for Hepatitis B and Hepatitis C. As part of prevention, several public health interventions have been implemented since 1994 which have led to the decrease in the prevalence of HBV cases in Lebanon. These include HBV vaccination of newborns since 1998, the mandatory pre-

marital screening implemented by the Lebanese government since 1994, educational and awareness campaigns regarding the disease, and screening and vaccinating high-risk groups. The decrease in prevalence of HCV from 0.7% to 0.21% in Lebanon is mainly due to efficient awareness campaigns and most importantly to the instauration of national guidelines for the treatment of HCV. Moreover, accurate blood testing for HCV in blood banks using ELISA technique as well as systematic screening of drug abusers in rehabilitation centers may have also played a role. As part of managing the disease, the government restructured cost sharing and insurance design in all institutions; these included fast-tracked, higher reimbursement rate and risk-sharing agreements for oncology and immunology products by the National Social Security Fund (NSSF), the creation of a national health technology assessment (HTA) unit by the Ministry of Public Health (MOPH), yearly tender BIDs by MOPH, cost-effectiveness local studies for oncology and immunology, and cost-sharing agreements by MOPH. Lately, National Management Guidelines of the disease were set by a national experts group established by the MOPH and the WHO.

4 Diagnosis and Treatment of HCC

The diagnosis of HCC can be difficult and often requires the use of one or more imaging modalities. Ideally, tumors should be detected when they are $\leq$2 cm in size so that all treatment options can be offered. However, HCC is frequently diagnosed at a late stage because it is usually asymptomatic. Nonspecific symptoms associated with advanced stage of HCC may include jaundice, anorexia, weight loss, fatigue, and upper abdominal pain. Most commonly implemented diagnostic strategies in Lebanon involve the use of multiphasic liver protocol CT with IV contrast or multiphasic contrast-enhanced MRI in conjunction with the serum biomarker alpha-fetoprotein (AFP) to determine the perfusion characteristics, extent and the number of lesions, vascular anatomy, and extrahepatic disease.

Surgical resection, liver transplantation, and ablation offer a potential for cure for HCC patients; however, only 20% of patients are suitable for primary surgical management at the time of diagnosis [23]. Liver transplant rates remain low in Lebanon mainly because of the sensitivity of the Arab culture towards the issue of possible loss of dignity of the dying process through organ procurement. This obstacle continues to hinder the progress in transplants in the region and has led to living-related liver transplants as an alternative source of organs for transplant. Patient survival in Lebanon following liver transplant was 76% at 1, 5, and 10 years [24]. The remaining 80% cases of HCC are diagnosed at advanced stages when curative treatments become nonfeasible [25]. The prognosis of HCC patients is dismal with a 5-year survival rate less than 5%, a median overall survival of 1 year [26], and a 5-year recurrence rate of nearly 80% [27]. Thus, patients with advanced HCC are offered nonsurgical approaches such as chemotherapy, targeted therapy, immunotherapy, transhepatic chemoembolization (TACE), RT, or percutaneous ethanol injection (PEI) [28–31].

5 HBV-Related Hepatocarcinogenesis

Both indirect and direct mechanisms are involved in HCC oncogenesis by HBV. HCC-promoting HBV factors include chronic inflammation, HBV integration, HBV mutations, epigenetic mechanisms, and HBV-encoded oncoproteins (e.g., HBx) (Fig. 2).

5.1 Chronic Inflammation

Following HBV infection, a robust T-cell immune response is stimulated to combat the infection. This results in hepatocyte necrosis, inflammation, and consequently regeneration, to compensate for lost hepatocytes [32]. Thus, failure by the immune system to clear HBV results in sustained

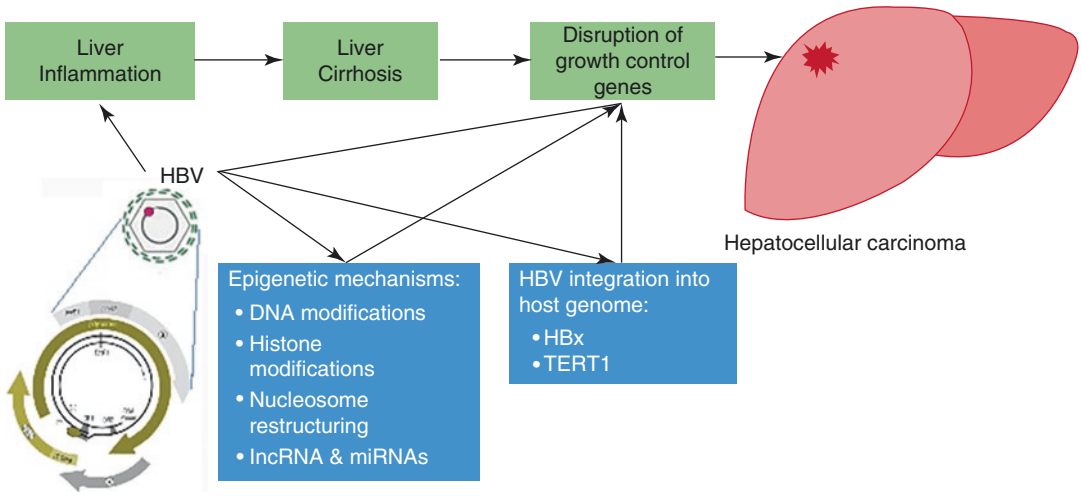

Fig. 2 HBV infection may develop to chronic hepatitis and progress to HCC, or to liver cirrhosis and subsequently HCC. Molecular mechanisms of HBV-related HCC involve (1) chronic inflammation; (2) epigenetic factors that confer cell growth advantage; (3) integration of HBV DNA into the host genome and activation of host genes controlling cell proliferation; and (4) direct promotion of cell proliferation by viral proteins (mainly HBx)

cycles of necrosis-inflammation-regeneration. Immune-mediated liver injury is often associated with elevated ALT levels, and elevations in tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β levels are often observed in the sera of HBV-infected patients [33]. Additionally, long-lasting hepatic inflammation caused by host immune responses during chronic HBV infection can promote liver fibrosis, cirrhosis, and HCC progression due to accelerated hepatocyte turnover rates and the accumulation of mutations [34].

5.2 HBV Integration

The HBV genome is found integrated in the host genome in nearly 80–90% of HBV-associated liver tumors and in around 30% of non-HCC liver tissue adjacent to HCC [35], and this integration appears prior to the occurrence of HCC [36]. Mechanisms by which the integration of HBV DNA could contribute to hepatocarcinogenesis include host DNA alterations at several cancer-relevant genes including cyclin A, telomerase reverse transcriptase (TERT), platelet-derived growth factor receptor-beta (PDGFRB), mitogen-activated protein kinase 1 (MAPK1), and others [37, 38].

TERT is located on chromosome 5p and is directly associated with HBV genome integration in the TERT locus [39]. HBx functions as a transcriptional activator and suppressor and has effects on hepatocellular apoptosis [40]. HBx proteins may upregulate the transcriptional activation of human telomerase transcriptase [41]. Cis-activation of human TERT mRNA by HBx gene may also play a role in hepatocarcinogenesis [42].

5.3 HBV Mutations

Recent studies observed that single nucleotide polymorphisms (SNPs) of genes including GSTM1 (Glutathione S-Transferase Mu1), GSTT1 (Glutathione S-Transferase Theta1), STAT4 (Signal Transducer and Activator of Transcription 4), TPTE2 (Transmembrane Phosphoinositide 3-Phosphatase and Tensin Homolog 2), DCL1 (CD302 Molecule), KIF1B (Kinesin Family Member 1B), and PGD (Phosphogluconate Dehydrogenase) are associated with increased risk of HBV-mediated HCC [43]. Moreover, mutations in the tumor suppressor p53 (TP53), WNT pathway (APC, AXIN1, CTNNB1), and epigenetic enzymes (ARID1A,

ARID2, MLL4) have also been reported in HBV-mediated HCC [43, 44].

5.4 Epigenetic Mechanisms

Epigenetic modifications are heritable changes in gene expression that do not result from changes in the genomic sequence. These could be attributed to DNA modifications (methylation of cytosine residues generating 5-methylcytosine, oxidation of 5-methylcytosine to 5-hydroxymethylcytosine), histone modifications (methylation, acetylation, phosphorylation, and ubiquitination of N-terminal of histone tails), nucleosome restructuring by ATP-dependent chromatin remodeling complexes, and altered expression of long non-coding RNAs (lncRNA) and microRNAs (miRNAs) [45].

Several miRNAs involved in the Toll-like receptor (TLR) signaling pathway play a critical role in innate immunity against HBV infection [46]. For instance, miR-145 and miR-148a target TLR3; miR-200b, miR-200c, miR148a, miR-455, and let-7-family members target TLR4; let-7b and miR-155 target TLR7; and miR-148a targets TLR9, and all of which are involved in HBV infection [46, 47].

5.5 HBV-Encoded Oncoproteins

The HBV genome encodes at least four proteins [HBsAg, a core protein (splice variant: HBeAg), a DNA polymerase, and the HBx protein] that are translated from mRNAs transcribed from HBV covalently closed circular DNA and/or from HBV genome sequences integrated into the host genome [48].

A truncated mutant of HBsAg increases HBV-related tumorigenesis through the downregulated expression of transforming growth factor (TGF) BI associated with the TGFβ-SMAD pathway [49]. Furthermore, HBsAg enhances the IL-6–STAT3 pathway, thereby increasing the HBsAg-mediated malignant potential of HBV-associated HCC [50]. HBeAg is associated with the host immune response and cytokine production, both of which play roles in HBV-associated HCC [51].

Overexpression of the HBV polymerase due to core gene deletion enhances HCC cell growth by inhibiting miR-100 [52]. Results of a recent study revealed that transgenic mice expressing the reverse transcriptase domain of HBV polymerase in their livers developed early cirrhosis with steatosis by 18 months of age, with 10% subsequently developing HCC [53].

The viral regulatory protein HBx contributes critically to HBV replication and is thought to be closely related to HBV oncogenicity [54]. HBx transactivates binding sites for the transcription factors AP-1 and NF-κB [55], activates the p53-RB and β-catenin pathways [56, 57], and is involved in chromatin remodeling [58] and transcriptional modulation in hepatocarcinogenesis [59].

6 Thalassemia-Related Hepatocarcinogenesis

Thalassemia is a genetically transmitted, quantitative disorder of hemoglobin. It is divided into two main categories, transfusion-dependent thalassemia (TDT)—patients who are not capable of producing sufficient hemoglobin to survive without blood transfusions—or non-transfusion-dependent thalassemia (NTDT). Patients with β-thalassemia intermedia, hemoglobin H disease, and mild-to-moderate forms of hemoglobin E/β--thalassemia often fall under the classification of NTDT, whereas patients with β-thalassemia major and severe forms of hemoglobin E/β--thalassemia are classified as having TDT [60].

HCC is a major life-threatening cancer that is becoming more frequently identified in thalassemia patients mainly because of the increased life span of these patients which previously had limited survival [61, 62]. The incidence of HCC among thalassemia major patients was 2% during a 1-year observation period, which is almost the same as the risk of HCC in the general population [63]. Most patients with thalassemia develop HCC at age <50 years, and there is no noticeable difference in incidence between men and women in this

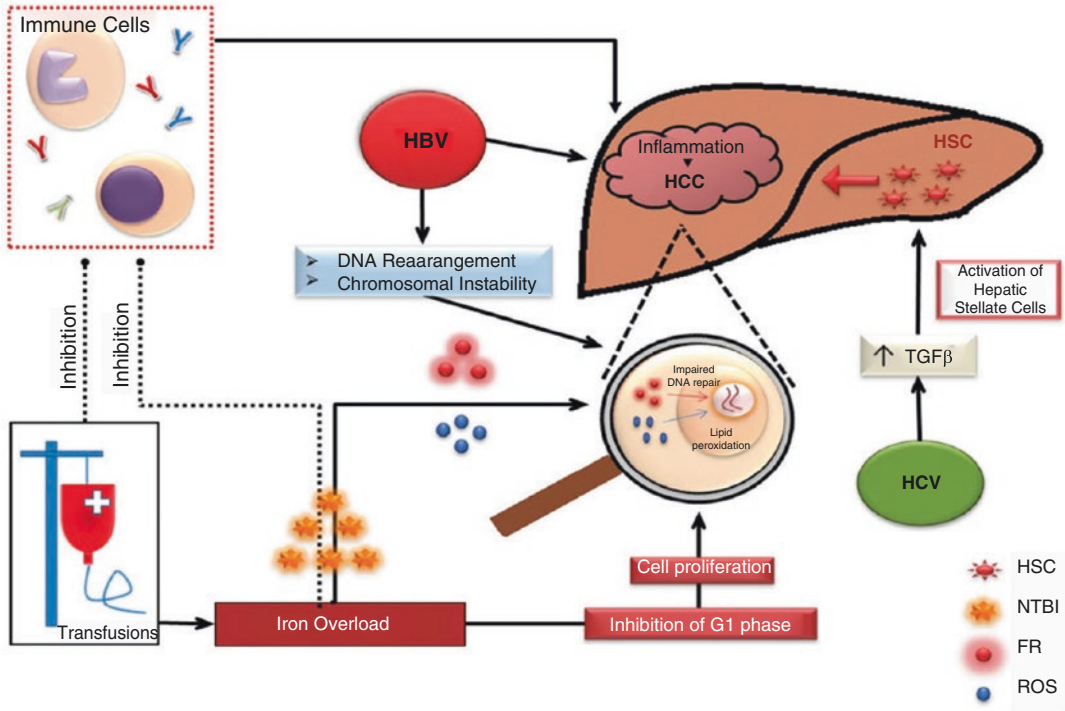

Fig. 3 Different mechanism related to HCC development in thalassemia patients. FR free radicals; HBV hepatitis B virus, HCV hepatitis C virus, HCC hepatocellular carcinoma, HSC hepatic stellate cells, NTBI non-transferrin-bound iron, ROS reactive oxygen species, TGFβ transforming growth factor β. (Adapted from Taher A, 2018)

group of patients [63, 64]. The two mainly established risk factors for the development of HCC in thalassemia include iron overload and viral hepatitis with or without cirrhosis [61, 62] (Fig. 3).

6.1 Iron Overload

In TDT patients, iron overload is secondary to regular transfusions, while in NTDT patients, it develops from increased intestinal absorption and increased release of recycled iron from the reticuloendothelial system (due to the suppression of hepcidin synthesis in the liver) [65]. One mechanism by which free iron is believed to trigger malignant transformation is through the generation of reactive oxygen species (ROS), which causes peroxidation of membrane fatty acids and subsequent formation of toxic byproducts that impair protein synthesis and disrupt DNA, leading to mutations in tumor suppressor genes (such as p53) and DNA repair genes [66]. Iron overload may also promote malignant transformation in the liver through the acceleration of fibrosis to cirrhosis by activation of stellate cells and through the profibrogenic effects of lipid peroxidation [67]. Cases of HCC in NTDT, hepatitis C-negative patients who have significant iron overload have been reported, which further highlight the role of iron overload as a definite risk factor for HCC [68, 69]. Thus, screening thalassemia patients using magnetic resonance imaging (MRI)-based liver iron concentration (LIC) measurement and liver ultrasound is strongly recommended for early detection of iron overload and HCC, respectively [68].

6.2 HCV/Cirrhosis

Chronic viral infection related to blood transfusions is another risk factor for HCC in thalassemia patients. Of the HCC in thalassemia reported in the literature, 88% are infected with HCV (either as HCV Ab or HCV RNA) [64]. HCV-mediated carcinogenesis is either directly related to the virus or to complications of HCV hepatic infection like fibrosis and cirrhosis. HCV genes seem to induce the production of transforming growth factor β and consequently activating hepatic stellate cells that are responsible for hepatic fibrosis [70]. It is important to mention that chronic hepatitis C and liver iron overload have been proposed to work in synergy to increase the HCC risk, which is reflected by the fact that hepatic iron is often increased in patients who have chronic HCV infection secondary to an HCV-induced decrease in serum hepcidin levels [71, 72].

7 Secondary and Tertiary Prevention of HBV- or HCV-Related HCC

In chronic HBV carriers, the risk of HCC is higher in those with certain host factors such as older age, male gender, African origin, the presence of cirrhosis, chronic hepatic necroinflammation, alcohol use, coinfection with chronic HCV or HIV, metabolic syndrome, and genetic polymorphisms or viral factors such as HBV DNA levels and presence of specific viral mutations. HBV DNA level can be reduced by antiviral drugs, thus rendering them the main treatment target for chronic HBV [73]. Two main classes of drugs control viral replication: interferon (IFN) and nucleos(t)ide analogs (NA). The use of NA specifically entecavir and tenofovir disoproxil decreases the HCC incidence rate [74] and is shown to be associated with regression of fibrosis and, to a lesser extent, reversal of cirrhosis [75, 76]. The use of IFN in chronic HBV was also shown to reduce the risk of HCC in a selected subgroup of patients with early cirrhosis who responded to IFN compared with controls; however, the response rate in chronic HBV is disappointing and incurs numerous adverse effects [77, 78]. Specific to chronic HBV, in those who already developed HCC, tertiary prevention mainly involves initiation (if not given before the HCC) or continuation of antiviral therapy. The beneficial effects of NA are observed in patients who received curative liver resection for HCC [79] and also in patients receiving radiofrequency ablation [80]. However, there is not yet enough evidence showing the beneficial effect of NA in prevention of HCC recurrence. Also, the effect of NA on HCC in patients with inoperable HCC undergoing locoregional therapy is not well defined.

With the use of direct-acting antiviral (DAA) agents as anti-HCV therapy, HCV infection can be cured with a minimum of side effects. Antiviral therapy against HCV has a positive impact on the risk of development of HCC [81, 82] and has shown excellent rates of sustained virologic response (SVR). A few recent studies have raised concerns on the increased risk of HCC occurrence or recurrence after DAA therapy [83–86]. However, other studies support that treatment with DAAs is not associated with increased HCC risk compared to treatment with IFN or in DAA-unexposed patients [87–90]. Although prospective trial is needed to clarify this controversy, surveillance for HCC occurrence or recurrence is required among patients with an SVR at risk of liver disease progression, irrespective of the antiviral agent used [91]. In patients without cancer, recent data highlight the potential consequences of delaying antiviral treatment on subsequent risk of HCC and support treatment of all patients with HCV before their progression to advanced fibrosis and cirrhosis, because progression to cirrhosis might be associated with substantial downstream costs related to the need for lifelong HCC surveillance and/or cancer care for those who develop HCC [87].

8 Conclusions

Although HCC rates in Lebanon are relatively low compared to other regions of the world, they are on the rise. It is anticipated that the increased incidence in the future will be more related to nonviral causes such as obesity, aflatoxin contamination, and alcohol abuse and less to viral causes as HCV and HBV infection rates have been decreasing as a result of efficient governmental strategies undertaken to control the virus. Finally, thalassemia patients are also emerging to be at high risk of developing HCC which has not been previously an issue as the survival rates of thalassemia patients were low and HCC did not pose an issue beforehand. Now, the emergence of HCC as a pressing morbidity in thalassemia suggests the need for structured HCC screening programs. Moreover, the development of HCC can be avoided by preventing or treating the HCC risk factors, namely, chronic viral hepatitis and iron overload. Although little data has been published on HCC treatment in thalassemia, the following modalities have been proven both safe and effective in selected patients with thalassemia: (1) surgical resection, (2) chemoembolization, and (3) simultaneous percutaneous radiofrequency thermoablation and ethanol injection [62].

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Hepatocellular Carcinoma in Pakistan: An Update

Abu Bakar Hafeez Bhatti

1 Introduction

Cancer remains a leading cause of death worldwide. There were 18.1 million new cases and 9.5 million cancer-related deaths worldwide in the year 2018. By 2040, the number of cancer-related deaths is expected to rise to 16.4 million [1]. Liver cancer is the fifth most common cancer in men and the ninth most common cancer in women. In 2018, there were over 840,000 new cases of liver cancer in the world [2]. Pakistan is one of the eight countries in South Asia (Fig. 1).

Based on the United Nations Children's Fund (UNICEF) data, the estimated population of Pakistan is 212, 228, 286 and the under-five mortality rate is 67.2 per 1000 live births [3]. It is the fifth largest country in the world population wise, but lags behind in various important determinants of health care when compared with neighboring countries [4, 5].

There is a steady increase in the incidence of liver cancer in Pakistan [6, 7]. Between 85% and 90% of the liver cancer originates from the hepatocytes. Hepatocellular carcinoma (HCC) in Pakistan represents a unique challenge in a number of ways. In the absence of a functional national cancer registry, our inferences are based on results from hospital-based registries. Hepatitis C virus infection is the most common risk factor for HCC, which is in contrast with some of the other countries in the Asia Pacific region, where hepatitis B infection is endemic. Moreover, there is very limited data on natural history of non-hepatitis C and B HCC [8]. Consequentially, applicability of established guidelines remains questionable, yet we need to reach a national consensus on HCC management. Well-known risk factors for HCC include viral infections, alcohol consumption, autoimmune and hereditary disorders, diabetes, and obesity [9–11].

Based on available data, age-standardized rate for HCC in Pakistan is 7.6 per 100,000 persons per year for males and 2.8 for females [12, 13]. In this chapter, we have attempted to review the epidemiology of HCC in Pakistan, with special focus on surveillance and diagnostic and treatment strategies more relevant to developing countries.

2 Risk Factors for HCC

2.1 Hepatitis C and B Virus Infection

To date, hepatitis C virus (HCV) and hepatitis B virus (HBV) infection is the most common risk factor for progression to liver cirrhosis and HCC in Pakistan. Most patients present in the fifth decade of life [14–16]. Pakistan is one of the few

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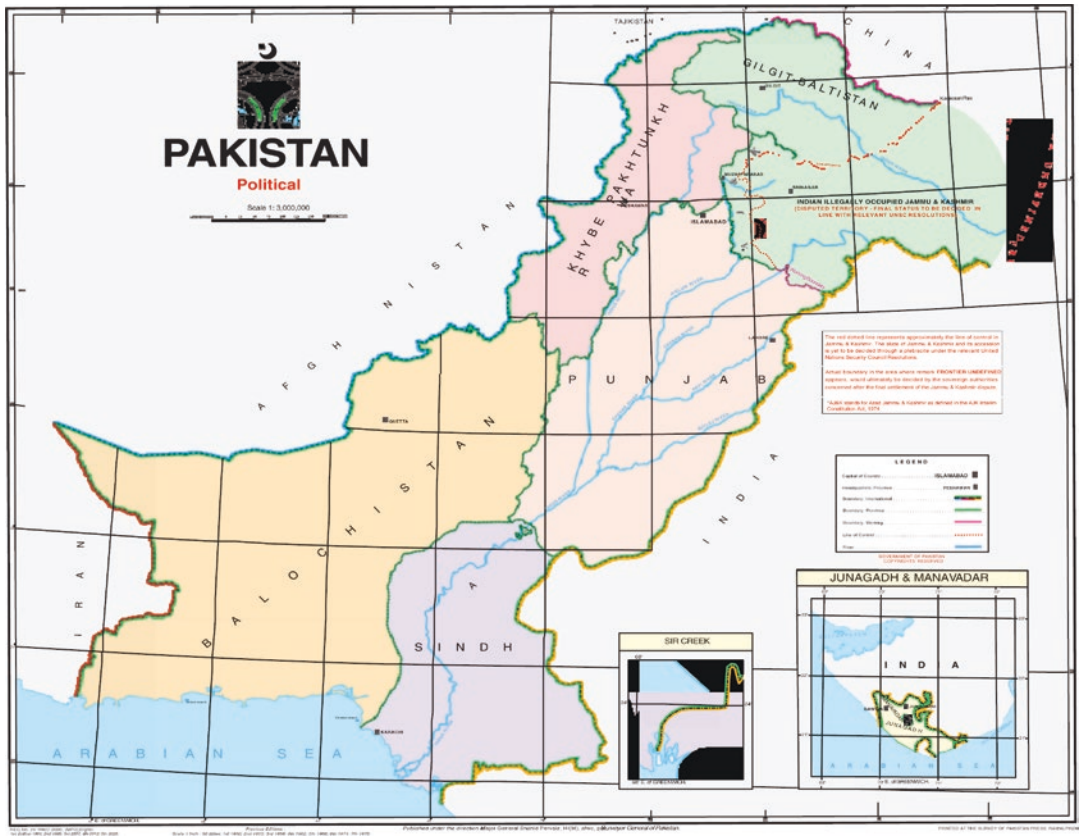

Fig. 1 Political map of Pakistan (www.surveyofpakistan.gov.pk)

countries in the world with >3% anti-HCV antibody positivity [17–19]. Approximately 58% patients have HCV, while 25% have HBV infection [20]. However, HCV antibody positivity can vary from 24% to 72.5%, while hepatitis B surface antigen positivity ranges between 13.1% and 51.2% [21–23]. This reported variability stems from patchy nature of available information, high prevalence of HCV and HBV in certain pockets of the country, and lack of national cancer surveillance programs [8].

Factors implicated in the spread of HCV infection include rural population, illiteracy, unscreened blood products, use of unsterilized instruments for shaving, misuse of injectables, and minor surgical procedures particularly circumcision [24]. In fact it has been shown that the risk of HCV transmission is increased when circumcision is performed by barbers and not in the hospital setting [25, 26]. Up to 48% barbers might use unsterilized blades for shaving. This behavior might be replicated when performing circumcision [27]. In addition, distinct phytogenetic clustering of HCV in Pakistan has also been implicated in exponential spread of HCV [28]. All these factors might account for high prevalence of HCV and not HBV in our country.

2.2 HIV Coinfection

In HIV-positive patients, HCV or HBV coinfection leads to accelerated progression to cirrhosis and HCC. A significant number of HIV-positive patients might have HCV or HBV coinfection. Sexual promiscuity and blood transfusions are the major risk factors [29]. HCV and HBV coinfection in HIV patients is a growing problem in Pakistan but remains underreported. The available data are predominantly based on high-risk

groups like prisoners. These studies have shown an alarming rate of >50% coinfection in HIV-positive patients [30, 31]. Reasons for the high coinfection rate include illegal drug use, homosexuality, etc. The impact of HCV or HBV infection in HIV-positive Pakistani patients largely remains unknown.

2.3 Non-alcoholic Fatty Liver Disease

The risk of HCC is increased two to four times in patients with diabetes mellitus (DM). Studies from the West and Asia have confirmed these findings [32, 33]. DM not only accelerates liver fibrosis and development of HCC, but contributes to poor outcomes. Moreover, after curative treatment, risk of recurrence is also increased in the presence of DM [34]. Non-alcoholic fatty liver disease (NAFLD) is a common problem in DM. NAFLD is associated with presence of fat in the liver after exclusion of other contributory factors like alcohol and certain drugs. It ranges from simple steatosis to fibrosis, cirrhosis, and HCC [35]. NAFLD is a growing problem in the world including Asia and Pakistan. This is due to modernization and urbanization associated with increasing wealth, sedentary lifestyle, and dietary changes predisposing to obesity [36]. Chronic liver disease due to non-alcoholic steatohepatitis (NASH) may soon be the most common reason for liver transplantation in the world [37]. Fatty liver disease has reached epidemic proportion in Pakistan but still remains unnoticed [38–40].

2.4 Aflatoxin-Mediated HCC

Aflatoxins are difuranocoumarin derivatives of *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi contaminate crops in warm, humid climates both during the growth and the storage phase [41]. Aflatoxin B_1 (AFB1) is the most potent hepatocarcinogen and is found in contaminated human food stuff [42]. AFB1 is known to have a synergistic effect and increases the risk of HCC development many folds in the presence of HBV infection [43].

It is estimated that about 45 million of the world's population is exposed to aflatoxins. There are various types of aflatoxins, and AFB1 is the most important in terms of clinical impact. In high-income countries, due to strict governmental regulations, effective screening ensures that food stuff exposed to aflatoxin does not reach consumers and is safely discarded. Regulations to control dietary exposure to AFB1 are either non-existent or difficult to implement in low-income countries. Thus infected food stuff can gain entry into the consumer markets. Least, they are consumed by the family members, friends, and neighbors of the farmers growing these crops which leads to spread of infection [41]. In fact, it has been shown that more than 50% of corn-based products and 18.3% rice products in Pakistan might have AFB1 contamination [44].

3 Screening and Diagnosis for HCC

Since HCC has a median subclinical period of 3.2 years, screening with early detection has a substantial impact on outcomes [45, 46]. Although ultrasound is highly sensitive in detecting HCC, its ability is limited in obese and cirrhotic patients [47]. Significant differences in regional guidelines on HCC screening and diagnosis exist. The American Association for the Study of Liver Disease (AASLD) recommends 6 monthly ultrasound (US) with or without AFP in high-risk patients [48]. The Asian Pacific Association for the Study of the Liver (APASL) recommends US and AFP, while the European Association for the Study of the Liver (EASL) recommends US alone [49, 50].

For lesions >1 cm in size, diagnostic criteria for HCC rely on dynamic imaging of the liver with CT or MRI scan. In most cases, a lesion with arterial enhancement and venous washout is typical for HCC. In patients with atypical liver lesions, biopsy can be performed to establish diagnosis. There is considerable controversy with regard to diagnosis and management of lesions <1 cm in size [47, 51, 52].

Majority of high-risk patients do not undergo screening in Pakistan. There is a large variation in

terms of choice of investigations and time duration between them [53]. In the presence of cirrhosis, it is difficult to detect early HCC. Less than 10% patients are diagnosed with HCC on screening in Pakistan, and that perhaps explains the late presentation and poor prognosis in the majority [20, 21]. Less than 2% patients are diagnosed with HCC on CT findings alone in Pakistan. A combination of CT, AFP, and histopathology is used in most patients [21]. HCC is >5 cm in more than 40% patients, ≥50% patients have >1 tumor nodule, while majority have advanced liver failure at the time of presentation [22].

To summarize, patients with HCC generally have advanced disease at presentation and only are eligible for definitive treatment. In the absence of nationally accepted guidelines for diagnosis of HCC, majority of patients end up undergoing an array of expensive investigations for establishing a diagnosis.

4 HCC Treatments

HCC has a poor prognosis even in developed countries, and 5-year survival is only 10%. Survival is even worse in developing countries, and mortality is roughly equivalent to incidence rates [54, 55]. Tumor characteristics (size, multinodularity, and vascular invasion), underlying liver function (Child-Pugh score), and performance status (Eastern Cooperative Oncology Group Performance Status) play an important role in survival [56, 57].

Global trends in HCC treatment are not uniform and do not adhere to the most widely applied Barcelona Clinic Liver Cancer (BCLC) staging algorithm. These trends are dictated by availability of treatment facilities and technical skills. Treatment may vary for the same stage across different regions. Transarterial chemoembolization (TACE) is the most frequently used first treatment in North America, Europe, China, and South Korea, percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) in Japan, and resection in Taiwan (Fig. 2) [58].

Liver resection and liver transplantation Surgery remains the most effective treatment for patients with HCC. In carefully selected patients, liver resection and transplantation can achieve 5-year overall survival of 40% and 70%, respectively [59–61]. In majority of cases, HCC develops on a background of cirrhosis, and liver resection is not always feasible due to underlying liver failure. Liver transplantation (LT) not only removes the HCC but also removes the failing liver.

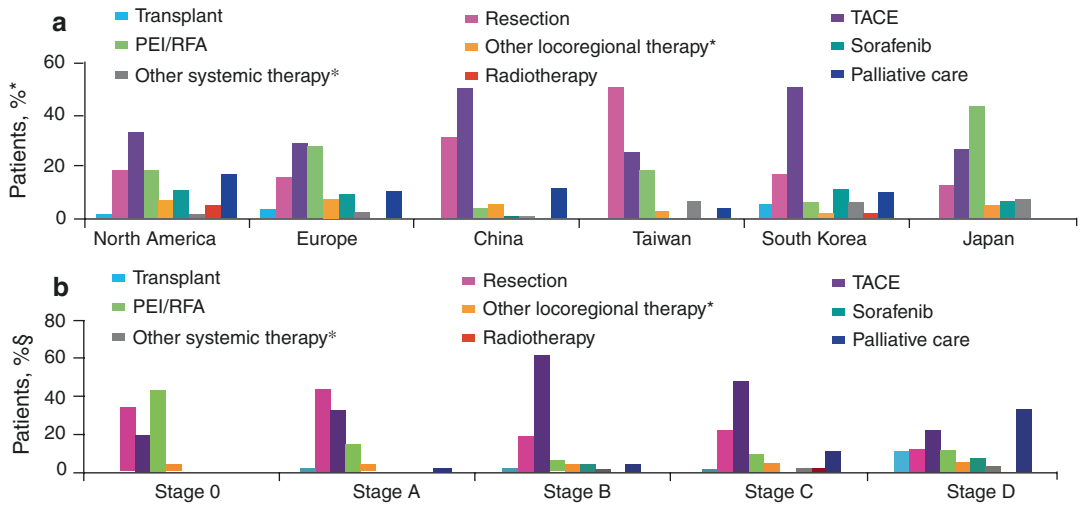

Fig. 2 (**a**) First recorded HCC treatment by country and region. (**b**) BCLC stage, *Liver Int*. 2015 Sep; 35(9): 2155–2166. https://doi.org/10.1111/liv.12818

Very few centers in Pakistan offer the full spectrum of liver resections and transplant services. The field of LT and liver surgery is rapidly evolving nationally. The last decade has proven to be much more promising in terms of development of HCC treatment facilities in the country. At present, there are two well-established transplant centers and at least five facilities where major liver resections are possible. The most experienced transplant program in Islamabad is nearing 1000 living donor liver transplants, while a government-funded transplant program in Sindh province is providing care to patients who cannot afford this treatment [62, 63]. Considering the burden of HCC and cirrhosis in Pakistan, there is an urgent need to develop more treatment facilities in the country. Unfortunately, since health care in Pakistan is predominantly out of pocket, it is imperative that government support for transplantation becomes more effective. In addition, a strong system of insurance coverage needs to be developed to bear the cost of expensive treatments [64].

Local ablative therapies Percutaneous ethanol ablation (PEA), RFA, and microwave ablation (MWA) are the various ablative treatment options. Access to these treatments is limited to few centers in the major cities. In order to improve waiting times and ease of access to these treatments, their availability should be ensured in smaller cities. In particular, relatively cheaper options like PEA deserve more attention. PEA was the first treatment option that became available, while RFA was introduced in the year 1999. RFA is believed to be superior in terms of clinical response and overall survival. However, this superiority has only been demonstrated in Asian studies. It is believed that there is substantial risk of bias in these studies [65]. PEA remains a cheaper yet effective treatment for HCC. In terms of cost-effectiveness, a single session of PEA costs 300 US dollars, while other ablative treatments can cost up to 1500 US dollars. PEA needs to be reintroduced into clinical practice, and its widespread availability should be made possible on national level.

Chemo and radio embolization Transarterial radioembolization is currently not available in Pakistan. Much like other HCC treatments, TACE and TAE are available only in major cities. Most patients do not have access to these facilities and, as a result, do not receive treatment for HCC. Moreover, a single TACE session can cost up to 1500 US dollars.

Systemic therapy In 2007, a tyrosine kinase inhibitor, sorafenib, became the first Food and Drug Administration (FDA)-approved systemic treatment for HCC. The drug confers only modest benefit in overall survival and comes with significant side effects. Other multikinase inhibitors like lenvatinib and regorafenib might have superior clinical efficacy and have recently been approved [66]. Sorafenib is the standard of care for patients with advanced HCC in Pakistan. Newer multikinase inhibitors are pending access to Pakistani markets. They remain very expensive with limited clinical benefit. At present, a month's course of sorafenib might cost around 1000 US dollars. There is a need to develop local or regional alternatives that are easily available and affordable. Moreover, these products need to be regularized so that ease of access is ensured in far-flung parts of the country. On the other hand, certain herbal compounds have shown moderate activity against HCC in the laboratory. There is a need to explore these cost-effective options for HCC treatment. A specific plant derivative *Berberis lycium* has shown moderate activity in G2 phase of HCC growth [67]. These alternatives might become useful options for treatment or palliation for HCC.

5 Prevention and Screening

The most effective method for HCC control is via prevention and screening. Various potential issues in HCC care and their solutions have been summarized in Table 1.

HBV and HCV The recent decline in HBV-related infection, cirrhosis, and HCC in Pakistan can be attributed to effective implementation of

Table 1 Major problems in HCC control in Pakistan and their potential solutions

Issues	Potential solutions
Incidence and prevalence of hepatitis C and hepatitis B are unknown	Implementation of national screening program
Lack of awareness regarding risk factors Low screening rate High prevalence of diabetes and obesity Aflatoxin control IV drug abuse Circumcision HIV coinfection	Public education via audiovisual dissemination regarding risk factor prevention Maintenance of healthy lifestyle and exercise Strict implementation of pre- and post-harvest control policies Mandatory disposal of used syringes Identification of high-risk behaviors Policies to prevent circumcision in community Identification of high-risk population
Insufficient HPB/liver transplant, palliative care, and cancer centers Enormous demand and supply gap	Development of infrastructure with collaboration of public and private sector Easy access to cheaper options like ethanol ablation for smaller HCC Legislation to provide easy access to local or regional alternatives to systemic therapies for HCC
Shortage of technical skills	Acquire technical skills via national and international exposure and collaboration
Controversial aspects of HCC management Knowledge gap	Collective decision-making Online tumor boards Personalized care for individual patients
Low remission rates for hepatitis C	Access to new treatments at affordable price Market access to local or regional brands with cost-effective options
Scattered cancer registriestrue cancer incidence and prevalence unknown	National cancer registry

Modified from: Hafeez Bhatti et al. [8]

HBV vaccination programs across the country. Nevertheless there are certain pockets with very high prevalence of HBV infection. This is due to complete lack of awareness and availability of HBV vaccination. Outreach programs need to be developed to ensure safe and effective delivery of vaccination in such high-risk areas and education of native population [68]. On the contrary, HCV infection continues to be a growing menace, and more than half of the HCC in Pakistan is secondary to HCV infection. With the availability of more effective anti-HCV medications, the future might appear promising as far as HCV-related HCC is concerned. However, HCV eradication at present remains a mere dream than reality. It has been shown that massive efforts and support are required to achieve this goal and HCV elimination does not appear likely until 2030 unless major incentives are given by the government [69]. There is a need to promote locally manufactured products for HCV treatment. Moreover, high-risk behaviors associated with spread of HCV and HBV infection need to be discouraged by media campaigns and strict legal actions. Use of contaminated syringes should be discouraged, while disposable or sterilized instrumentation for dental procedures, circumcision, shaving, etc. should be promoted.

5.1 NAFLD

Diet and lifestyle might play an important role in development of HCC. Recent evidence suggests that adhering to healthy diet including fruits, vegetables, and fibers might protect from HCC. Healthy lifestyle including exercise helps overcome detrimental effects of energy-rich diet consumption and reduces cancer-related mortality [70]. A large meta-analysis has suggested that coffee intake might be linked with reduced risk of HCC [71]. Similar effects might also been seen with fish and vitamin E intake. On the contrary, tobacco has been strongly associated with development of HCC, and its use needs to be discouraged.

5.2 Aflatoxin-Related HCC

Several methods have been implied to control spread of aflatoxins in the community. These include development of resistant cultivars, chemical and physical control, and biological control [72].

1. By altering the agricultural practices in areas with high dietary AFB1 intake. For example, a change to a rice-based diet led to a significant decrease in AFB1 ingestion in China [43]. However, changes in dietary practices in low-income countries might face criticism due to resource limitations and the inability of government to come up with alternative options supporting both the nutritional and economic needs of the community.
2. Pre-harvest prevention can be achieved with adequate irrigation and spraying of fungicides [43, 73, 74].
3. Post-harvest prevention can be achieved effectively by simple measures like sun drying on cloth rather than earth, hand sorting to remove crops with molds, and improved storage practices avoiding warm moist environments. However, such efforts require governmental involvement with provision of storage facilities to farmers in low-income countries [43, 72–74].
4. Perhaps the most effective method of AFB1control is biological. In biological control, non-toxigenic strains of aspergillus are developed, which can then replace the toxigenic types at the time of crop colonization [72].

HCC is a growing problem in Pakistan. Although treatment of HCC is challenging, its occurrence at large remains preventable with simple measures. Our population remains unaware of simple changes in behavior and practices that might significantly reduce its incidence. There is a need to develop policies and ensure their strict implementation at national level pertaining to prevention, surveillance, and access to various treatments in order to reduce HCC in Pakistan.

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Part IV

The Future

Future Directions

Brian I. Carr

The Middle East comprises a range of countries with huge differences in income per capita, national wealth and its distribution, and hygiene and its practices: the ultra-wealthy and the ultra-poor. The causes of HCC and especially the cofactors are hugely varied. Some countries, such as Egypt, have a very high percent of HCV-based HCC, while others, such as Turkey, have a high percentage HBV-based HCC. Many countries in addition are joining the western epidemic of obesity. Some suggestions for improving our services to HCC patients follow.

1. Tumor registries need to be taken seriously.

   We cannot properly allocate resources and improve the quality of our services, without accurately knowing the size of the problem and what help is needed and where. Many major hospitals do not seem to have systematic data collection and often individually – collected databases are like silos and not shared. Quite often, there is no salary allocation for the support staff that are needed to collect the HCC patient data and enter it into a hospital-based system.
2. National neonatal HBV vaccination programs.

   Some countries have been very forward-looking and diligent with this. Others, less so. Yet, HBV is a preventable major cause of HCC, when neonatal vaccination is used. The widespread national adoption of cheap neonatal HBV vaccination, to be included with other neonatal vaccines, will have major long-term benefits.
3. Obesity is an increasing problem in the modern Middle East, as it is in so much of the western world. Since it is a modern epidemic and the cause of so much chronic illness, an educational program in schools and the workplace, similar to that used for smoking cessation, is also likely to yield long-term societal benefits, especially since obesity-associated chronic liver disease (NAFLD, NASH) is a fast-rising cause of HCC, just as near-universal HBV vaccination is resulting in a decrease in HBV-based HCC.
4. Increase in the use of surveillance. A large number of our newly diagnosed HCC cases occur when the symptoms of advanced disease lead to a physician visit. The diagnosed HCC at this time is often too advanced for treatments with curative intent. Yet, many of the factors that lead to HCC development are well-recognized and include cirrhosis from any cause, chronic HBV with or without cirrhosis, HBV plus HCV, and the combinations of chronic hepatitis with chronic alcohol consumption. Such at-risk patients are often known to the medical system and need to have 6-monthly surveillance ultrasound examinations. This will lead to an increased proportion

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of patients being diagnosed with earlier and hence more treatable HCCs.

5. We need better biomarkers.

   AFP is a time-honored and useful HCC biomarker in the 50% of cases in which it is elevated. However, there is evidence that currently available biomarker panels are more sensitive for diagnosis. These include GALAD score, a combination of **g**ender, **a**ge, AFP-**L**3%, **A**FP, and **D**CP. AFP, AFP-L3, and DCP kits are sold with all three assays together. GALAD seems especially useful in detecting small obesity-associated HCCs. Although there are several new candidate serum biomarkers being evaluated, such as proteomic, glycomic, and genetic markers that have gone through early stages of biomarker validation for the early detection of HCC, these markers still need to be validated in well-curated cohorts. It is expected that glypican-3, osteopontin (a secreted phosphoprotein), C-reactive protein (CRP), Golgi protein-73, soluble Axl (sAxl, a transforming receptor tyrosine kinase member of the tumor-associated macrophage family), circulating microRNAs, and methylated DNA (epigenetic) markers in cell-free circulating DNA are likely to become clinically validated in the near future.

6. We need serum, plasma, and tissue biobanks.

   As discussed in #6 above, there is a huge amount of biodiscovery being conducted in worldwide laboratories for early diagnosis of HCC. However, once a suitable candidate biomarker is identified, it needs to be validated using blood or tissues from patients with confirmed HCC diagnosis and preferably with known etiology. Otherwise it can take years for basic research workers to evaluate marker candidates. These repositories are especially important now that new TKIs and ICIs get FDA approval, but only with use of companion molecular tests of the drug targets. Thus, HCC biorepositories are an investment in the future of our subject.

7. Inhibition of horizontal transmission of hepatitis B or C.

   Although the screening of blood transfusion donors has been a well-learned lesson, many areas for transmission exist in daily life that could be reduced by popular education. This is especially true in personal service industries, such as barber shops where razors and knives can be used for multiple customers, with associated micro-abrasions and transmission of blood-borne hepatitis (and other) viruses. Similar concerns involve village-level circumcisions by barbers or local practitioners of circumcision, as well as for female genital mutilation, and 25% of the worldwide cases occur in the Middle East and North Africa.

8. More liver transplant centers are needed.

   Since liver transplantation is the only curative treatment for both HCC and the underlying diseased liver, it will be important to develop more centers with liver transplantation in the region. Since the surgical skills are already available in a few specialist centers in the Middle East, this should be an achievable goal.

9. Middle East-focused clinical trials are needed.

   The SHARP phase III trial of sorafenib versus placebo gave results for OS of 10.7 months in the sorafenib arm and 7.9 months in the placebo arm. Most patients were from Europe. The Asia-Pacific trial with identical design gave OS as 6.6 months in the sorafenib arm versus 4.2 months in the placebo arm. Thus, Asian patient survival on sorafenib was worse than European survival on placebo. This incredible difference highlights the fact that patients with the same disease, but in different peoples, can have quite different results with the same drug. There is far too little information on cancer drug tolerance and tumor responses in the peoples of the Middle East. Given that the population of the Middle East of about 250 million people is equivalent to that of the USA, this sizeable potential market should deserve the attention of international pharmaceutical companies, with respect to clinical trials of new HCC drugs. Numbers of HCC cases may be similar, being 13/100,000 in Egypt and Qatar and 5/100,000 in MENA and Turkey, as against approximately 13.6 and 4.7/100,000 in the

USA for males and females, respectively. Having comparable numbers of HCC cases, the Middle East represents a sizeable market for new HCC drugs, which should be drawn to the attention of the manufacturers. Especially doing trials of the new immune checkpoint inhibitors (IHIs) should make these new agents available to many of our patients. Given the size of our populations, this same argument speaks to the merits of tasting biomarkers for earlier diagnosis in our peoples.

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The Need for Region-Wide HCC Collaborations

Brian I. Carr

Each medical center in each town of each country does its best to treat its cancer patients, including those with HCC. We all depend upon a certain flow of ideas and activity. This can be expressed as:

- Prevention: hepatitis, alcohol, dietary contaminants, obesity
- Surveillance for early diagnosis: 6-monthly ultrasounds, biomarkers
- Evaluation of diagnosed patients: multispecialty tumor board
- Treatment selection or sequencing of choices: definite or other therapies

Each of these has limitations and needs for improvement. Several suggestions follow, on what can be better achieved by collaboration than by individual practitioners.

1. Transnational HCC Patient Clinical Database

   In order to estimate the size of the HCC problem and to quantitate the resources that will be needed, some confidence is needed in an assessment of the number of annual new cases.

   Furthermore, most clinical analyses of HCC patient subsets or prognostic factors suffer from insufficient power due to small patient numbers. A big step forward will be the ability to analyze combined patient information on several thousand patients.

2. Shared National Databases to Identify Geographic Hotspots

   A key to identifying unusual environmental determinants of disease is to compare incidence rates in differing geographical locations. Some areas may be HCV or aflatoxin B_1 hotspots. Since many countries in the Middle East are amalgams of various tribes and ethnicities, some with different sensitivity to various HCC causes, the study of shared national database comparisons will be helpful in this regard.

3. Middle Eastern HCC Biobank

   The rapidly increasing identification of signatures in serum, circulating plasma, and HCC tissues that can identify both HCC subsets and prognostic features requires a prospectively gathered specimen biobank or repository, with an associated spreadsheet of the clinical features of the de-identified patients associated with each specimen, including demographics, underlying hepatic disease, and radiologically identified determinants of tumor biology: maximum tumor size, number of tumor nodules, presence and extent of portal vein thrombosis, as well as results of standard blood count, liver function tests, serum alpha-fetoprotein (AFP) levels, and hepatitis testing.

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4. Public Health Teaching on Public and Private Sanitary Practices

   It is clear that much but not all HBV transmission is vertical, via the maternal-fetal route. However, other HBV and all HCV transmission is through the horizontal round, via transfusion of contaminated blood or its products, nonmedical drug administration parenterally, or other methods of exposure to human blood or secretions, such as in the personal care professions, including hairdressers, tattoos, acupuncture, and medical or dental procedures. Public health education and perhaps inspection of the premises of these practitioners to ensure use of proper sterilization techniques or adoption of single-use and disposable needles and razors would be protective from horizontal viral transmission in daily life.
5. Transnational HCC Conferences to Foster Education, Collaboration, and Surveillance

   The support by governments, ministries of health, and local authorities of transnational HCC meetings to disseminate techniques on limiting virus transmission, surveillance, new treatments, and approaches and multi-institutional clinical trials would seem to be effective ways of improving professional knowledge and encouraging collaborations.
6. Transnational Clinical Trials: Access to New Drugs and Clinical Trial Experience

   As explained in the chapter on New Directions, we cannot assume that the results of a clinical trial with a new drug that are applicable to one ethnic group are necessarily either safe or effective in all other ethnic groups. Many drug activating and detoxifying enzyme levels can be different among different groups of people. It would seem reasonable the new classes of drugs that are approved elsewhere should first be subject to confirmatory clinical trials in our region, before being given approval for use in our region. By use of transnational protocols, such trials can rapidly accrue the required patient numbers.
7. Fabrication of Biosimilar Drugs for Expensive New Drugs

   Local fabrication will address the availability of otherwise expensive drugs for ordinary citizens. India provides an example of a country that has several pharmaceutical companies, some of them now major corporations, that have focused on the production or synthesis of biosimilar drugs that sell at a fraction of the cost of those produced by pharmaceutical giants in the USA and Europe. This would bring new business and income to our region and make new drugs available to both governments and individuals at a lower cost than if imported.
8. Identification of Barriers to Early Diagnosis

   Too many of our newly diagnosed HCC patients are diagnosed at an advanced stage when they cannot benefit from putatively curative therapies. The answer is to diagnose their tumors earlier. This requires knowing who is at risk and screening them. Screening ultrasonography is cheap and easy and does not need referral to major hospitals. Implementation of more aggressive screening, perhaps with the newer portable and handheld ultrasound machines, might be a simple and practical means of increasing the numbers of patients who get diagnosed at earlier and thus treatable HCC stages. Perhaps a decrease in platelet count in patients at risk might be a useful prompt for surveillance ultrasonography.
9. Quality of Life Issues

   How well are we serving our patients' needs?

   As physicians and surgeons, we tend to focus on objective measures of disease extent or response to our treatments. As patient families attest, an HCC patient is much more than the product of scans and blood tests. The whole patient needs to be considered, from symptoms (pain, nausea, weight change, ascites, mobility) to hope, fear, anxiety, depression, self-image, and interactions with family and caregivers. As medical professionals, we are not always sensitive or

pro-active with respect to the psychological and emotional needs of our patients and usually do not have the time in our busy clinics to deal with them. Yet they are often foremost in the minds of our patients. The quality of their lives is often intricately linked to their minds and emotions. A first step in helping them is for us to be aware of and be able to quantify these issues. In this respect, several validated quality of life questionnaires or tools have been made available and can be downloaded from the Web without cost. Examples are CDC Health related quality of life (HRQOL) questionnaire; WHO Quality of Life Instrument; Global Quality of Life Scale; EORTC QLQ-C30, and the FACT-G (Factual Assessment of Cancer Therapy, FACT) score, among others. Translations of any one of these into Arabic, Turkish, and Iranian languages should serve a large percentage of our patients.

A questionnaire needs to be given to each patient at various intervals during his or her disease course and then evaluated. The difference from all other testing in medicine is that the result is based on the opinion of the patient and not the service professional. We actually get the opinion of the patient this way. Too few such studies are done in our region, so it is difficult to discern what our patients are actually thinking and feeling about their disease course and the treatments that we offer.

10. Unmet Needs for HCC Therapy
    - Prevention of obesity; anti-inflammatory agents
    - Biomarkers for patients with low-AFP HCC
    - Effective PVT therapy
    - Post-resection adjuvant therapies: HBV treatments, anti-inflammatories
    - Downstaging for transplantation
    - Expansion of transplant centers

There are several identifiable unmet needs in the subject of HCC studies, most of which require collaborative multi-institutional studies to advance. As always, the most important is HCC prevention. While the uses and benefits of HBV vaccination are widely acknowledged, other preventative causes remain. In Sect. 4 above, the need for improvement in sanitary behavior is mentioned. In addition however, as HBV rates are falling, obesity rates are climbing and are a predisposing factor for HCC. This can only be approached at a national or local government level through education and counselling.

Furthermore, it is increasingly clear that inflammation is an important part of the hepatocarcinogenic process. As mentioned in chapter "Biological Aspects of HCC", epidemiologic studies have suggested that anti-inflammatory agents can decrease the incidence of several cancer types, including HCC. Perhaps a clinical trial in this region of aspirin or NSAIDs in people at risk for HCC might be useful and point the way to another preventive action.

One of our clinically common and frustrating experiences is that in the 50% or more of HCC patients who do not have elevated serum AFP levels, we do not have another clinical biomarker to guide response to therapy, only expensive scans. Both serum CRP or GGT levels have been suggested to be useful as disease activity monitors in this situation, but new and sensitive biomarkers are still needed. In this regard, it has recently been shown that HCCs evolve during the course of the disease process in individual patients. Thus, the baseline biopsy may not reflect the same level of tumor aggressiveness as 6–12 months later. Some form of noninvasive biopsy is therefore needed that can be repeated safely. An obvious candidate is the so-called liquid biopsy, based upon measurement in a sample of peripheral blood of circulating HCC cells or cell fragments or circulating HCC cell DNA. Some epigenetics test (DNA methylation) kits have already been approved for some other cancer types for this purpose. It would be ideal for following HCC during the course of a patient's disease process.

Although multiple new tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) have been recently approved for clinical use in advanced stage HCC, none of them have been shown to reverse portal vein thrombosis (PVT), which has been reported in 30–40% of HCC patients and is a major negative prognostic factor. Some form of new systemic agent or radiation or the combination is needed, and recent reports of combining TARE and SBRT look encouraging, but need to be prospectively validated.

Another major issue is the high HCC recurrence rates after technically resectable HCC. Only ^{131}I-lipiodol has so far been shown effective in reducing post-resection recurrences. Perhaps the new ICIs or anti-inflammatory therapies could be useful in the adjuvant setting, especially since the causative (virus) factor is still present. In this respect, some tertiary HBV therapy results look encouraging.

Finally, once HCC is present, the only therapy that removes both the tumor and the inflammatory background is liver transplantation. Greater effort at downstaging the tumor mass pre-transplant and expansion of liver transplant availability are still needed. Both aims are fully accomplishable in our region.

HCC in the Middle East

Brian I. Carr

The Middle East comprises a heterogeneous collection of mainly Muslim and Arab countries. In some discussions, nearby countries have also been included, such as those central Asian republics neighboring Iran and those north African countries to the west of Egypt and stretching to the Atlantic. A flexible and inclusive view is taken in this book. The differences in liver cancer or hepatocellular carcinoma (HCC) causes, incidence, and clinical characteristics among the countries of this region provide an amazing opportunity to examine the influences of geography, crops, and diet on the one hand, ethnicity on the other hand, and local behavior on the yet other hand. Most countries, to an outsider to the Middle East, might appear homogeneous. Yet they tend to be ethnically diverse, such as Arabs and Africans in Egypt, Berbers and Arabs in Morocco, Turks and Kurds in Turkey, and Persians, Azeris, and Kurds in Iran.

HCC incidence ranges 20-fold across countries, from 22.0 to 1.5 per 100,000 males (females usually have lower incidence) in Egypt versus Iran, respectively. In East Asia, where HCC is most frequent, the major causes are chronic hepatitis B or hepatitis C, Aflatoxin B_1 contamination of stored and unrefrigerated grains and nuts, alcoholism, and increasingly, obesity. The countries of the Middle East are predominantly Muslim, so alcoholism has not been a major factor; nor is Aflatoxin B_1 contamination of farm products. However, hepatitis B is a major issue, with differing incidence and genotypes in the various countries. Similarly, hepatitis C is also a major issue. Furthermore, some areas have populations with combination hepatitis B and C or hepatitis B and D. As in the West and Asia, increasing prosperity has been accompanied by increases in obesity, another HCC risk factor. Several countries also have high rates of inherited metabolic liver diseases, likely due to consanguineous marriages. The availability of expensive but effective new anti-hepatitis drugs is hugely variable, as is the availability of interventional radiology for administering chemoembolization, or the considerable infrastructure and support that is needed for potentially curative liver transplantation. There is also wide variation on the concept of brain death, to enable cadaveric organ donation, and on the feasibility of live organ donation for unrelated recipient patients.

There has been massive social change in the region during the past 20 years, both positive and negative. Thus, there has been large upward social mobility in some areas such as Saudi Arabia and the Gulf Emirates (peace and wealth) and in Oman and Morocco (peace) and downward mobility in other areas such as Yemen (war and starvation), Syria (war and population displacement), and Libya (war). The central Asian countries (Turkmenistan, Azerbaijan, Uzbekistan) have by and large prospered. These social changes have been associated with changes in medical needs and availability.

The book is divided into several sections. Firstly, a general description of HCC, its causes and incidence and frequent modes of presentation. The 5 largest countries by population are Egypt, Turkey, Iran, Iraq and Saudi Arabia. The incidence of HCC is highest in Egypt and Saudi

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Arabia and is low in Turkey and Iran. These countries are considered in separate chapters to evaluate their relative risks and country-specific HCC considerations. Since prevention is the key to long-term survival for most cancers, including HCC, separate chapters are written for hepatitis B, hepatitis C, and other HCC causes. Likewise, the best long-term survival seems to be for liver transplantation as a treatment modality (both cancer and diseased organ are removed), and this merited its own chapter, including the ethics, donor procurement, and technical considerations.

The reader of this book will gain insights into the societies and their mores in a politically very important part of the world, in so far as these considerations help us understand a critically important cancer. The understanding of the different causes of HCC in the most populous countries will inform the approaches needed to the all-important prevention and early diagnosis strategies. An appreciation of the different treatment modalities, their uses and limitations, availability, and costs will help inform rational treatment provision and selection. The possibilities of country-wide or region-wide centers of excellence in HCC management is also considered.

For governments needing to plan social and medical services for their peoples and for international aid agencies needing to prioritize donations and projects, a knowledge of the causes, epidemiology, and treatments of HCC, the second highest cause of death from cancer worldwide, is likely to prove useful. It is with these aims that the current book has been written.

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