# Why Is Gallbladder Cancer Common in the Gangetic Belt?

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Ruhi Dixit and V.K. Shukla

**Abstract**

Carcinoma of the gallbladder (CaGB) is the commonest malignancy of the biliary tract. There is wide variation in the incidence of carcinoma of the gallbladder in India ranging from 1.2 per 100,000 for males and 0.9 per 100,000 for females in southern India to 4.5 per 100,000 males and 10.6 per 100,000 females in northern India. The incidence is highest in eastern Uttar Pradesh and western Bihar regions of India. This wide geographical variation in the incidence of carcinoma of the gallbladder suggests that environmental factors might be playing an important role in its causation. Both these regions lie downstream of the river Ganges which is the main source of water for all uses such as drinking water and for irrigation. The river Ganges receives an extremely high load of pollutants in the form of untreated domestic sewage, industrial and agricultural effluents containing aromatic hydrocarbons, nitrosamines and chemicals such as nitrates and nitrites which are by-products from domestic sewage. Pesticides which are frequently used in agricultural industry can also play a role in CaGB. Typhoid infection is prevalent in this region which may also be associated with the gallbladder carcinogenesis. Lifestyle and smoking have also been correlated with the CaGB. Adulteration in our cooking oil (mustard) by sanguinarine and diethylnitrosamine has also been found to be linked with CaGB. It is possible that carcinoma of the gallbladder is the disease of multifactorial etiology.

**Keywords**

Gallbladder cancer • Gangetic belt • Cholelithiasis • Heavy metals

## 12.1 Introduction

Gallbladder cancer (GBC) is the third most common gastrointestinal neoplasm and it is mostly common in northern India (Shukla et al. 1985).

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A survey by the Indian Council of Medical Research (ICMR) described that the incidence of GBC in women in northern India is one of the highest in the world (National Cancer Registry Programme 2002). We can find a wide geographical variation in the incidence of GBC in India. It actually varies from 1.2 per 10,000 for males and 0.9 per 10,000 for females in northern India. Its occurrence is higher in the eastern UP and western Bihar regions of India. These regions are situated near the river Ganges. This river water might be an important factor for the GBC incidence in such areas only. River Ganges is the prime source of water in these areas for drinking water and irrigation. The river Ganges is highly polluted with industrial waste, domestic sewage and agriculture effluents. Such environmental factors also play a role in the gallbladder carcinogenesis in these regions. The Ganges water usually consists of pollutants like pesticides; heavy metals such as cadmium, chromium and lead; and industrial wastes having aromatic hydrocarbon and nitrosamines and chemicals (nitrates and nitrites) which are by-products from domestic sewage (Gupta et al. 2005a, b). Various factors play a role in GBC as it is mentioned in a study that there is no single agent for gallbladder carcinogenesis, but it is known to be a disease of multifactorial aetiology (Kowalewski and Todd 1971). Following are the factors which might be playing a role in gallbladder carcinogenesis.

### 12.1.1 Metallothionein

Metallothionein (MT) is the low molecular weight metal-binding protein mostly present in the liver, kidneys, heart, testes and brain. MT usually binds to heavy metals by making clusters of thiolate bonds. They store these MT ions in the liver and play a protective role against heavy metal toxicity (Kägi and Kojima 1987; Freedman and Peisach 1989; Frazier and Din 1987; Liu et al. 1990). It has been reported in experimental model that normal tissues which are deficient of MT are more prone to cadmium (Cd) carcinogens (Frazier and Din 1987; Liu et al. 1990). Therefore, we look for the expression of MT in GBC in comparison to cholelithiasis and normal gallbladder. The results of this study showed an increased expression of MT in GBC. It's overexpression has also been reported in breast, gastric and oesophageal cancers (Schmid et al. 1993; Monden et al. 1997; Hishikawa et al. 1997). Heavy metals like cadmium are excreted and concentrated by the hepatobiliary system. Those tissues which show low or no expression of MT were more susceptible to Cd carcinogenesis as MT prevents such tissues from heavy metal toxicity. Thus, we concluded from this study that high-level expression of MT in GBC cases may indirectly reflect the exposure to Cd and other heavy metal toxicity and their higher concentration in the gallbladder. Content of heavy metals like Cd found to be at a higher concentration in the potable water samples studied in Varanasi region (Krishnamurti and Vishwanathan 1990) has been reported, where GBC is so common, and, therefore, this may also be an aetiological factor of GBC in this part of India (Shukla et al. 1998a, b).

### 12.1.2 Heavy Metals and Gallbladder Cancer

Ganges receives domestic sewage and industrial agriculture effluent. High concentrations of certain heavy metals are found in sewage, irrigation and potable water. It has been reported that high concentration of cadmium and other heavy metals is found to be present in water from such region. These heavy metals have also been found to be implicated in cancer progression (Leonard 1983). We also tried to see the association of GBC with exposure to heavy metals. Cadmium, chromium and lead concentrations were estimated in GBC, cholelithiasis and healthy control. Their concentrations were significantly higher in carcinoma of the gallbladder in comparison to gallstones. These metals were also found in drinking water of such region which confirms the hypothesis. They are known as the chemical carcinogens; therefore, we reported that their high biliary concentration in GBC patients might be a causing factor of gallbladder cancer (Shukla et al. 1998a, b).

### 12.1.3 Micronutrients

Micronutrients can also be a factor modifying the multistage process of carcinogenesis. Various studies have shown that deficiency of micronutrients is found to be associated with cancer. Cases of gallbladder cancer (I), cholelithiasis (II) and healthy controls (III) were included in a study. Selenium (Se), zinc (Zn), copper (Cu), manganese (Mn), ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) concentrations were estimated in serum, bile and gallbladder tissue in groups I and II and only serum in group III. We have found low serum, biliary and tissue concentrations of Se, Zn and vitamin E in patients having GBC in comparison to cholelithiasis. Earlier study on heavy metals from one group has shown higher biliary concentrations of cadmium (Cd), chromium (Cr) and lead (Pb) in carcinoma of the gallbladder. They are known chemical carcinogen. This, when associated with the reduced levels of protective micronutrients like Se, Zn and vitamin E, is involved in preventing damage to membrane lipids, protein and nucleic acids by acting as free radical scavenger; this could further promote the multistage process of carcinogenesis (Shukla et al. 2003).

Other than micronutrients, there exist some other trace elements such as copper (Cu) and zinc (Zn) which can play a role in carcinogenesis. An elevated serum copper and low zinc levels have been found in various cancers like in breast cancer (Garofalo et al. 1980; Gupta et al. 1991), gastrointestinal tract cancer (Inutsuka and Araki 1978; Gupta et al. 1993) and lymphomas (Margerison and Mann 1985). In a similar way, zinc serum levels have been found to be decreased in different malignancies (Garofalo et al. 1980; Gupta et al. 1991, 1993, 2005a, b; Inutsuka and Araki 1978; Margerison and Mann 1985; Gray et al. 1982). Therefore, we have demonstrated alteration in the serum, biliary and tissue levels of Cu and Zn in patients with GBC and cholelithiasis. We concluded that patients with GBC show alterations in the Cu and Zn levels of serum and tissue. Thus, Cu/Zn ratio could also be a factor for the prevalence of this gallbladder disease in northern part of India.

### 12.1.4 Nitrate and Nitrite

India is an agriculture-based country. In recent years, use of nitrate-based fertilizers has been increased. Thus, it leads to high intake of nitrate through drinking water and vegetables. We found a higher biliary concentration of nitrate in GBC patients in comparison to cholelithiasis in our study. Similar results have also been shown in gastric cancer, brain tumours and hepatocellular carcinoma (Fraser et al. 1980). The high nitrate content in bile may be converted to nitrite and in turn nitrosamine which is known to be carcinogenic and could initiate gallbladder carcinogenesis (Shukla et al. 2004).

### 12.1.5 Pesticides

Pesticides in crop protection are used in excessive quantity, and it all ultimately mixes with the Ganges water, and water is frequently used in the daily requirements like for drinking and irrigation. It has already been demonstrated by various studies that pesticides play a crucial role in carcinogenesis (Nayak et al. 1995; Rehana et al. 1996); it can even cause liver cell carcinogenesis in experimental model (Ito et al. 1995; Kolaja et al. 1996). Another research was conducted by our earlier work was done to look at the pesticides presence in the causation of gallbladder cancer. A total of sixty patients of gallbladder diseases have been collected, of which 30 have GBC and 30 have cholelithiasis. Bile and blood samples were collected to estimate the pesticides' concentration. Organochlorine pesticides have shown earlier an association with the oesophageal and stomach cancers (Ditraglia et al. 1981). We also evaluated the concentration of some pesticides in gallbladder cancer patients. The estimated results have shown significantly higher concentration of BHC and DDT in gallbladder cancer patients when compared with cholelithiasis. Thus, it can

also be an important factor in gallbladder carcinogenesis in our region (Shukla et al. 2001).

### 12.1.6 Bile and Bacteria

Bacterial presence can also be a causative factor for the gallbladder carcinogenesis. Primary bile acids change to secondary bile acids, due to the chronic bacterial infection which leads to the development of tumour (Hill 1986). In our earlier study in 2007, we tried to see the association of gallbladder cancer with chronic bacterial infection. We observed higher bile culture positivity in GBC in comparison to cholelithiasis and control (Sharma et al. 2007). Another study concluded that concentration of biliary deoxycholate was higher in GBC as compared to cholelithiasis (Shukla et al. 1993). In a previous study, we demonstrated the role of bacterial degradation of primary bile acids in GBC, and the study reported that the bacterial degradation of primary bile acids could also be a factor for gallbladder cancer (Pandey et al. 1995). Typhoid and paratyphoid can also correlate with the incidence of GBC in North India as these two are very common in such parts (Shukla et al. 1985). It is also concluded that approximately 2.5 % of total number of enteric fever cases is known to associate with the biliary system which leads to a chronic carrier state (Old 1990). Gallbladder diseases, including carcinoma, are common in the northern part of India and so are *Salmonella typhi* infection and typhoid carrier state. The typhoid carrier state may be one of the possible mechanisms of gallbladder carcinogenesis. There is 8.47 times more risk of developing carcinoma of the gallbladder in culture-positive typhoid carriers than the noncarriers (Welton et al. 1979; Nath et al. 1997, 2008; Shukla et al. 2000).

### 12.1.7 Dietary Factors

Other factors which may also correlate with the gallbladder carcinogenesis are dietary factors. Various studies concluded from their observation that intake of animal proteins, fats and oily food increases the risk of GBC, whereas intake of vegetables and fruits decreases the risk in patients for developing GBC (Kato et al. 1989). Another work also stated that ingestion of vegetables, fibre, vitamin C and vitamin E showed a lower risk of GBC but a higher risk is associated with the addition of sugar in desserts with the causation of biliary tract cancer (Moerman et al. 1995). We also (2002) evaluated the role of diet in gallbladder carcinogenesis. Significant odds ratios were seen with the consumption of radish (OR 0.4, 95 % CI 0.17–0.94), green chilies (OR 0.45, 95 % CI 0.21–0.94) and sweet potato (OR 0.33, 95 % CI 0.13–0.83) among vegetables and mango (OR 0.4, 95 % CI 0.16–0.93), melon (OR 0.3, 95 % CI 0.14–0.64) and papaya (OR 0.44, 95 % CI 0.2–0.64) among fruits. We concluded that vegetables and fruits showed a protective effect on gallbladder carcinogenesis but red meat (beef and muffin) was found to be associated with increased risk of gallbladder cancer (Pandey and Shukla 2002).

### 12.1.8 Mustard Oil

Mustard oil is frequently used as cooking medium in this part of country. It is already reported that mustard oil has an inflammatory response (Shukla and Arora 2003; Shukla et al. 2003). They were reported to have a tumorigenic response on consumption of mustard oil. Edible mustard oil is usually adulterated with argemone oil because argemone seeds closely resemble with the mustard seeds and colour of both the oils are the same (Ghosh et al. 2005). Sanguinarine and diethylnitrosamine are found to be present in the adulterated and fried mustard oil. Sanguinarine is an alkaloid of argemone oil. Recent studies have shown that argemone oil and isolated sanguinarine possess genotoxic and carcinogenic potential which is implicated in DNA damage in the blood of dropsy patients (Das et al. 2005). Similarly, diethylnitrosamine has also been reported to induce hepatocarcinogenesis (Bhosale et al. 2002). We also investigated the association of mustard oil as cooking medium with gallbladder cancer in northern

India. Concentration of sanguinarine and diethylnitrosamine were estimated in gallbladder cancer and cholelithiasis patients, and it was reported that sanguinarine and diethylnitrosamine concentrations in the gallbladder tissue increased in patients with gallbladder cancer compared to patients with cholelithiasis. We reported that there is an association between mustard oil and GBC (Dixit et al. 2013).

## 12.2 Lifestyle, Reproduction Factors and Risk of GBC

We also evaluated the risk of gallbladder cancer association with the lifestyle, parity, menstrual and reproductive factors. Sixty-four newly diagnosed GBC cases were included in the study and the detailed information regarding smoking, chewing habits, alcohol drinking and parity, menstrual and reproductive factors were collected in a pro forma. We stated that tobacco and chewing were associated with increased odds of gallbladder cancer. Similarly early menarche, late menopause, multiple pregnancies and childbirth increased the risk of gallbladder cancer (Pandey and Shukla 2003). A recent study also described a higher risk of developing gallbladder diseases in older, multiparous women and men with diabetes. They also showed an increased risk of gallbladder diseases by the intake of chickpeas and unsafe water and in villages having heavy water pollution (Unisa et al. 2011). Cigarette smoking is an important health hazard and nicotine is one of the main carcinogens known. Nicotine is found to be involved in DNA damage, genetic instability and inhibition of apoptosis (Campain et al. 2004). Our recent study was aimed to assess the role of nicotine in gallbladder carcinoma. A significant higher value of tissue nicotine concentration was observed in the gallbladder carcinoma group. These elevated nicotine concentration in tissue samples from gallbladder cancer is indicative of its strong association with the disease (Basu et al. 2012).

From the above discussion, we can conclude that multiple factors are playing a role in gallbladder carcinogenesis. So, it might be possible that such environmental factors are associated with the development of gallbladder cancer in the Gangetic plane.

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