Journal of Gastrointestinal Cancer (2021) 52:145–149
https://doi.org/10.1007/s12029-020-00367-2

ORIGINAL RESEARCH

Frequency of Hepatitis C Virus Genotypes in Patients Who Had Hepatocellular Carcinoma in Gastroenterological Hospitals of Tabriz

Saeed Tarzi[1] · Mahmoud Mansouri[2] · Sahar Pashaei Sarand[3] · Navid Shomali[4,5] · Rozita Tamjidifar[1] · Changiz Ahmadizadeh[1]

Abstract

Background Generally, hepatitis C has been identified as one of the major health issues that about 3% of the world's population have been threatened and affected by it (about 170 million people), and also, it can be considered a factor in acute and chronic hepatitis.
Methods The aim of this study is to determine the prevalence of HCV genotypes in Azerbaijan patients. In this study, sampling was done on the referred patients to the hospitals (Mahallati and Behbud Hospital). RNA was extracted after isolation of plasma, and then, after the synthesizing of cDNA, the sample was carried out to the laboratory for performing the real-time PCR in order to determine the genotypes.
Results The evaluation of HCV genotypes in positive plasma samples showed that dominant subsets were remarkable and the mean age of the patients was 37/3 ± 11/8 (in the age range of 2–63). Among the 235 patients,139 of them (59%) were male. Statistically, the average number of women was more than men (*T* test, $P < 0/05$). 1b genotype was reported 70% in the patients above 40 years old, and also, it was reported as 71/6% in the patients under 40 years old that was not statistically significant. The incidence of serotype 3a was higher among the patients younger than 40 years old (3a was 18.1% vs. 15%), and this serotype was prevalent among men (3a was 18.7% vs. 14.6%), which was statistically significant.
Conclusion The findings indicate that among Azerbaijan's patients with chronic hepatitis C, genotypes 1b (71.1%) and 3a (17%) were dominant.

Keywords Hepatitis C · Hepatocellular carcinoma · Genotype

Background

Hepatitis C virus is an infectious disease that is a kind of a single-strand positive RNA virus and the main cause of liver cirrhosis [1]. It is one of the known agents that affect the normal functioning of the liver and causes acute and chronic infection [2, 3]. Hepatitis C is one of the issues posed by the World Health Organization and threatens about 3% of the world's total population (approximately 170 million people) [4]. Genotype plays an important role in pathogenesis and hepatitis infection [5]. According to the latest studies, the hepatitis C virus had seven genotypes and at least 67 subsets [6]. In many studies, there is a close relationship between the hepatitis C genotype and the response to interferon (IFN) and pegylated interferon (Peg-INF), which can be effective in combination with ribavirin [7]. Hepatitis C genotypes are very important because they can provide valuable information and accordingly, can determine the duration of antiviral treatment, tolerance, or resistance to the hepatitis C virus [7, 8]. It should be noted that the prevalence of hepatitis C genotypes can be different and may show different

✉ Changiz Ahmadizadeh
changiz.ah67@gmail.com

[1] Department of Biology Ahar Branch, Islamic Azad University, Ahar, Iran

[2] The University of Tehran, Department of Applied Chemistry, Tehran, Iran

[3] Amirkabir University of Technology (Polytechnic of Tehran), Department of Applied Chemistry, Tehran, Iran

[4] Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

sensitivities to viral treatment (4). Based on previous studies, it had been showed that genotypes 2 and 3 have a higher sensitivity to antiviral therapy, while the success rate of these genotypes was higher than other genotype 1 [9]. The prevalence of the hepatitis C genotype is different between countries and geographical areas [10, 11]. Worldwide, the prevalence of genotypes 1, 2, and 3 is higher than the other genotypes [12]. In Iran, the prevalence of hepatitis C infection is significantly lower than in neighboring countries. Distribution and abundance of genotypes 4 to 6 varied geographically, so genotype 4 was more distributed in North Africa and the Middle East, genotype 5 in South Africa, and genotype 6 in Southeast Asia [13]. In Europe and America, predominant subtypes 1a and 1b have been observed and have caused disease (10, 11). According to reports in Iran, there are 1a subscales (47%) and 3a (36%) [14]. Genotype 1 was more prevalent in other countries such as Russia and Belarus [15–19].

Material and Method

Study Population

This is a cross-sectional study in which studied the patients with confirmed hepatitis C and the selected patients simultaneously had hepatocellular carcinoma. These 235 patients were selected among the patients who were admitted at Tabriz hospitals between June 2013 and June 2019. The clinicopathological features of patients are summarized in Table 1. To prove that patients with chronic hepatitis C also have hepatocellular carcinoma, a pathologist with specific diagnostic tests and/or biopsy confirmed it. An informed consent was obtained from each patient, and the ethical approaches were approved by the ethics committee of Tabriz University of Medical Sciences.

Collection and RNA Extraction of Samples and Genotyping

Blood samples were collected from each patient and 5 ml of them were poured into the EDTA-containing tubes, and then, the plasma was kept at 80 °C. RNA extraction was done according to the manufacturer protocol. cDNA synthesis and HCV genotyping were done according to manufacturer protocol too (Qiagen, Netherland).

Statistical Analysis

Statistical analyses were performed using SPSS version 17 software and analyzed by *T* test and square tests. The mean ($P < 0.05$) was statistically significant.

Table 1 Clinicopathological features of patients

Characteristics	Classification	No. of patients (%)
Age	55 <	93 (39)
	55 ≥	142 (61)
Sex	Male	139 (59)
	Female	96 (41)
Tumor size (cm)	0.1–2.0	68 (29)
	2.1–5.0	119 (50)
	5.1–10.0	42 (18)
	10.1–20.0	4 (2)
	0 or ≥ 20.1	2 (1)
Histologic differentiation	Well	28 (11)
	Moderate	86 (37)
	Poor	20 (9)
	Undifferentiated	3 (1)
	Unknown	98 (42)
Surgical intervention	No surgery or blank	176 (75)
	Ablation/destruction	22 (9)
	Resection	19 (8)
	Transplantation	4 (2)
	Other	14 (6)
Hepatic vein invasion	Positive	2 (1)
	Negative	233 (99)
Lymph Node Metastasis	Positive	17 (7)
	Negative	218 (93)
Clinical stage	I	89 (38)
	II	76 (32)
	III	43 (19)
	IV	27 (11)
Alcohol	Yes	143 (61)
	No	92 (39)
Complication of cirrhosis	Yes	42 (18)
	No	193 (82)
HBs Ag	Positive	164 (70)
	Negative	71 (30)
Anti-HCV	Positive	135
	Negative	0

Results

In this study, 235 patients with chronic hepatitis C who had hepatocellular carcinoma were selected prior to the use of antiviral drug treatment. The genotypes were as follows: genotype 1b was found in 71.1% of patients (165), genotype 3a in 17% of patients (40), genotype 2 as the most common genotype by affecting 6.8% of patients (16 subjects), genotype 1a in 1.7% of patients (4 people), and genotypes 1b and 3a which were infectious in 62.5%. It is noteworthy that in 6 cases, the combination of HCV infection in blood compounds was detected

Table 2 Demographic data and prevalence of HCV genotype in Azerbaijani patients in health centers of Iran-Tabriz

Genotype	1a	1b	3a	1a + 3a	1b + 3a	1a + 1b	*P* value
Frequency; *N* (%)	4 (1.7%)	165 (71.1%)	40 (17%)	69 (29.5%)	147 (62.5%)	19 (8%)	-
Age; mean ± SD	41.25 ± 12.7	43.14 ± 12.5	41.39 ± 10.10	41.2 ± 11.64	42.64 ± 12.21	41.11 ± 12.49	0.024
ALT	101.25 ± 51.25	65.16 ± 40.66	145.2 ± 195.98	98.27 ± 48.64	78.50 ± 88.45	73 ± 45.80	0.112
AST	61.25 ± 59.26	56.20 ± 37.64	78.2 ± 97.89	51.25 ± 38.66	61.56 ± 31.21	41.33 ± 19.28	0.215
HCV load	225,641.32 ± 211,289.23	195,720.76 ± 676,922.17	300,383.60 ± 470,344.66	215,366.21 ± 345,120.36	321,544.65 ± 233,554.55	290,227.77 ± 490,213.36	0.041
HCV RNA	*P* = 4 *N* = 0	*P* = 165 *N* = 0	*P* = 40 *N* = 0	*P* = 69 *N* = 0	*P* = 147 *N* = 0	*P* = 19 *N* = 0	-
HCV Ab	*P* = 4 *N* = 0	*P* = 165 *N* = 0	*P* = 40 *N* = 0	*P* = 69 *N* = 0	*P* = 147 *N* = 0	*P* = 19 *N* = 0	-
Gender	*M* = 1 *F* = 3	*M* = 72 *F* = 93	*M* = 12 *F* = 28	*M* = 21 *F* = 48	*M* = 52 *F* = 95	*M* = 6 *F* = 13	0.012

statistically significant (P value = 0.031). The HCV genotypes of these patients were determined based on nucleotide sequencing (Tables 2 and 3).

The mean age of the patients was 38/3 ± 11.8 (range 2–63). Among 235 Azerbaijani patients, 139 (59.1%) were male. Statistically, the mean number of women was more than men (*T* test, $P < 0/05$).

In patients over 40 years old, genotype 1b was 70%, whereas in patients less than 40 years, it was 71.6%, which was not statistically significant. Also, the incidence of serotype 3a was higher among the patients younger than 40 years old (18.1% vs. 15%), and this serotype was prevalent among men (18.7% vs. 14.6%), which was statistically significant (*T* test, $P < 0.05$).

Discussion

The HCV genotyping is one of the epidemiological parameters. Among 235 patients with hepatic carcinoma who were confirmed to have chronic hepatitis C, the most frequent genotypes of HCV in these patients were as follows: genotype 1b was the highest one (71.1%) followed by genotype 3a with 17%, genotype 2 with 6.8%, genotype 1a with 1.7%, and HCV hybrid genotype was 3.4%.

It is little known about the prevalence of HCV genotypes in different regions such as the Soviet Union, where hepatitis C is an endemic disease (19). Although this research was carried out for the first time in Eastern Azerbaijani, it was impossible to compare the results of this study with other cases. According to related articles, there were reports of the prevalence of hepatitis C genotype in the Soviet Union, which was consistent with the results of this study, so that this genotype is prevalent among the Soviet population, such as genotype 1b

Table 3 Demographic data and the prevalence of HCV genotypes among male and female Azerbaijani patients of health centers of Iran-Tabriz

Parameters	Female	Male	Total
No. (%)	96(40.9)	139(59)	235(100)
Mean age	39.6 ± 13.3	35.7 ± 10.1	37.3 ± 11.8
Type of HCV genotype and subtypes			
1a	2 (2.1)	2 (1.7)	4 (1.7)
1b	70 (72.9)	97 (69.8)	165 (71.1)
2	7 (7.2)	9 (6.5)	16 (6.8)
3a	14 (14.6)	26 (18.7)	40 (17.0)
Mixed infection	3 (3.1)	5 (3.6)	8 (3.4)
HCV mixed infection			
1a and 3a	-	1 (0.7)	1 (0.4)
1b and 2	-	1 (0.7)	1 (0.4)
1b and 3a	3 (3.1)	2 (1.4)	5 (2.1)
2 and 3a	-	1 (0.7)	1 (0.4)

Table 4 Distribution of hepatitis C virus genotypes and subtypes in the former Soviet Union and the Middle East

Region/ countries	Most common	Less common	References
Russia	1b (76.0)	3a (13.0), 2a (7.0)	[19]
Estonia	1b (71.0)	3a (24.0)	[33]
Uzbekistan	1b (64.2)	3a (25.0)	[22]
Belarus	1b (53.8)	3a (38.5), 1a (5.1)	[20]
Moldova	1b (89.0)	2 (4.0)	[19]
Latvia	1b (85.0)	3a (10.0)	[34]
Lithuania	1b (54.0)	3a (23.9), 2a (10.9), 2b (4.4)	[35]
Georgia	1b (59)	3a (27), 2a/2c (11), 1a (3)	[26]
Tajikistan	1b (84.6)	3a (7.6), 2a (5.7), 2c (1.9)	[23]
Non-Arab countries			
Iran	1a (39.7)	3a (27.5), 1b (12.1)	[28]
Turkey	1b (87.2)	1a (9.9)	[16]
Pakistan	3a (54.4)	3b (8.2), 1a (6.8), 1b (4.6), 4 (1.3)	[27]
Arab countries			
Saudi Arabia	4 (62.0)	1 (24.0), 2 (7.4)	[32]
Jordan	1a (40.0)	1b (33.3), 4 (26.7)	[24]
Kuwait	4 (38.0)	1 (27.0)	[31]
Iraq	4	1b, 1a, 3a	[36]
UAE	4 (45.4)	3a (23.8), 1a (15.0)	[29]
Oman	4	Not available	[36]

in this study. The dominant genotypes in the Soviet Union are as follows: Russia 76% (19), Belarus 53.8% [20], Estonia 71% [21], Moldova 89% (19), Uzbekistan 64.2% [22], Tajikistan 84% [23], Lithuania 54% [24], Latvia 85% [25], and Georgia 59% [26]. Table 4 shows the comparison between the results of our study with other countries. Also, the prevalence of HCV genotypes, and their serotype in Arab countries [24, 27–31], Middle East, and non-Arab countries [28, 32], is presented in Table 4.

It is thought that the differentiation of various serotypes including 1b which occurred about 70 to 80 years ago [37] is due to the Soviet separation and then the Bolshevik revolution in 1917 which makes it be more than 80 years old. Serotype 1b is mainly from blood and blood products have been transmitted (19), while in one of the centers in Russia, the incidence of serotype 3a among drug users and the general population was very high (18, 20).

The prevalence of HCV genotypes varied over the years. For example, in 1997 (19), strains 1b (90%) and 3a (10%), and in 2003 (22), strains 1b (64.2%) and 3a (25%) have been reported in Uzbekistan (22). This distribution of HCV genotypes in these countries indicates changes that need further study. On the other hand, genotype 1b was found to be 71.1%; 3a (17%) and 2 (6.8%) were the dominant genotypes. Therefore, most studies focusing on this issue should be used to determine the prevalence of HCV genotypes among the Azerbaijani population.

Also, in patients younger than 40 years, the incidence of serotype 3a was 18.1% vs. 15.0% ($P < 0.05$). These results are consistent with the recent findings, indicating a high prevalence of serotype 3a among young people in Germany [38], Iran [39], and Slovenia [40].

As a final remark of this research, dominant genotypes of HCV among the Azerbaijani patients with chronic hepatitis C were as follows: serotype 1b approximately 71.1%, serotype 3a about 17% and serotype 2 approximately 6.8%. This study was performed to determine the frequency of HCV genotypes in patients who had hepatocellular carcinoma; however, it should be done using larger sample size and a large population to determine the HCV genotype in different populations of the Republic of Azerbaijan.

Acknowledgments The authors thank the Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, for supporting this study and all the volunteers who played a key role in carrying out this research.

Compliance with Ethical Standards

Ethics Approval and Consent to Participate Informed consent was obtained from each patient, and the ethical approaches were approved by the ethics committee of Tabriz University of Medical Sciences.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Rho J, et al. Hepatitis C virus (HCV) genotyping by annealing reverse transcription-PCR products with genotype-specific capture probes. J Microbiol. 2008;46(1):81–7.
2. Ly KN, et al. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann Intern Med. 2012;156(4):271–8.
3. Supram HS, Gokhale S, Sathian B, Bhatta DR. Hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection among HIV infected individuals at tertiary care hospital in Western Nepal. Nepal J Epidemiol. 2015;5(2):488–93.
4. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77–87.
5. Young AM, Crosby RA, Oser CB, Leukefeld CG, Stephens DB, Havens JR. Hepatitis C viremia and genotype distribution among a sample of nonmedical prescription drug users exposed to HCV in rural Appalachia. J Med Virol. 2012;84(9):1376–87.
6. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318–27.
7. Zarkesh-Esfahani SH, Kardi MT, Edalati M. Hepatitis C virus genotype frequency in Isfahan province of Iran: a descriptive cross-sectional study. Virol J. 2010;7:69.
8. Umar M, Bushra HT, Ahmad M, Data A, Ahmad M, Khurram M, et al. Hepatitis C in Pakistan: a review of available data. Hepat Mon. 2010;10(3):205–14.
9. Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology. 1994;19(1):13–8.
10. Chlabicz S, Flisiak R, Lapinski TW, Kowalczuk O, Wiercinska-Drapalo A, Pytel-Krolczuk B, et al. Epidemiological features of patients infected with HCV genotype 4 in Poland: epidemiology of HCV genotype 4 in Poland. Hepat Mon. 2011;11(3):191–4.
11. Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Ann Intern Med. 1996;125(8):634–9.
12. Fallahian F, Najafi A. Epidemiology of hepatitis C in the Middle East. Saudi J Kidney Dis Transpl. 2011;22(1):1–9.
13. Bokharaei-Salim F, et al. Distribution of hepatitis C virus genotypes among azerbaijani patients in capital city of Iran-Tehran. Hepat Mon. 2013;13(9):e13699.
14. Samimi-Rad K, et al. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. J Med Virol. 2004;74(2):246–52.
15. Abdel-Moneim AS, Bamaga MS, Shehab GM, Abu-Elsaad AA, Farahat FM. HCV infection among Saudi population: high prevalence of genotype 4 and increased viral clearance rate. PLoS One. 2012;7(1):e29781.
16. Altuglu I, Soyler I, Ozacar T, Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. Int J Infect Dis. 2008;12(3):239–44.
17. Cornberg M, et al. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. Liver Int. 2011;31(Suppl 2):30–60.
18. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. Int J Infect Dis. 2006;10(4):272–7.
19. Viazov S, Kuzin S, Paladi N, Tchernovetsky M, Isaeva E, Mazhul L, et al. Hepatitis C virus genotypes in different regions of the former Soviet Union (Russia, Belarus, Moldova, and Uzbekistan). J Med Virol. 1997;53(1):36–40.
20. Olinger CM, Lazouskaya NV, Eremin VF, Muller CP. Multiple genotypes and subtypes of hepatitis B and C viruses in Belarus: similarities with Russia and western European influences. Clin Microbiol Infect. 2008;14(6):575–81.
21. Tarantola A, Abiteboul D, Rachline A. Infection risks following accidental exposure to blood or body fluids in health care workers: a review of pathogens transmitted in published cases. Am J Infect Control. 2006;34(6):367–75.
22. Kurbanov F, Tanaka Y, Sugauchi F, Kato H, Ruzibakiev R, Zalyalieva M, et al. Hepatitis C virus molecular epidemiology in Uzbekistan. J Med Virol. 2003;69(3):367–75.
23. Khan A, et al. Epidemiological and clinical evaluation of hepatitis B, hepatitis C, and delta hepatitis viruses in Tajikistan. J Med Virol. 2008;80(2):268–76.
24. Bdour S. Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. J Med Microbiol. 2002;51(8):700–4.
25. Ray SC, et al. Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis. 2000;182(3):698–707.
26. Sharvadze L, et al. Prevalence of HCV and genotypes distribution in general population of Georgia. Georgian Med News. 2008;165:71–7.
27. Alfaresi MS. Prevalence of hepatitis C virus (HCV) genotypes among positive UAE patients. Mol Biol Rep. 2011;38(4):2719–22.
28. Keyvani H, Alizadeh AH, Alavian SM, Ranjbar M, Hatami S. Distribution frequency of hepatitis C virus genotypes in 2231 patients in Iran. Hepatol Res. 2007;37(2):101–3.
29. Daw MA, Dau AA. Hepatitis C virus in Arab world: a state of concern. ScientificWorldJournal. 2012;2012:719494.
30. S. Pacsa, A., et al., *Genotypes of hepatitis C virus in Kuwait*. Vol. 10. 2001. 55–57.
31. Shobokshi OA, Serebour FE, Skakni LI. Hepatitis C genotypes/subtypes among chronic hepatitis patients in Saudi Arabia. Saudi Med J. 2003;24(Suppl 2):S87–91.
32. Ali A, et al. Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan. Virol J. 2011;8:466.
33. Tallo T, Norder H, Tefanova V, Krispin T, Schmidt J, Ilmoja M, et al. Genetic characterization of hepatitis C virus strains in Estonia: fluctuations in the predominating subtype with time. J Med Virol. 2007;79(4):374–82.
34. Jansons, J., et al., *Hepatitis C virus molecular epidemiology in Latvia*. Vol. 676. 2004. 65–70.
35. Ambrozaitis A, Z Agminas KS, Balc Iūnaite G, Widell A. Hepatitis C in Lithuania: incidence, prevalence, risk factors and viral genotypes. Clin Diagn Virol. 1995;4(4):273–84.
36. Gasim GI. Comment on: “hepatitis C virus in Arab world: a state of concern”. ScientificWorldJournal. 2012;2012:534173.
37. Kalinina O, Norder H, Vetrov T, Zhdanov K, Barzunova M, Plotnikova V, et al. Shift in predominating subtype of HCV from 1b to 3a in St. Petersburg mediated by increase in injecting drug use. J Med Virol. 2001;65(3):517–24.
38. Ross RS, Viazov S, Renzing-Köhler K, Roggendorf M. Changes in the epidemiology of hepatitis C infection in Germany: shift in the predominance of hepatitis C subtypes. J Med Virol. 2000;60(2):122–5.
39. Payan C, Roudot-Thoraval F, Marcellin P, Bled N, Duverlie G, Fouchard-Hubert I, et al. Changing of hepatitis C virus genotype patterns in France at the beginning of the third millenium: the GEMHEP GenoCII study. J Viral Hepat. 2005;12(4):405–13.
40. Seme K, Vrhovac M, Mocilnik T, Maticic M, Lesnicar G, Baklan Z, et al. Hepatitis C virus genotypes in 1,504 patients in Slovenia, 1993-2007. J Med Virol. 2009;81(4):634–9.