ORIGINAL ARTICLE

Identifying genotype profile of chronic hepatitis C infection in Southwest Iran

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Background: Hepatitis C virus (HCV) infection is one of the most important risk factors for liver failure which can lead to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Approximately 170–200 million (almost 3% of the world's population) people have been reported to have HCV infection worldwide. HCV has six genotypes and multiple subtypes. HCV genotyping and identification of subtypes are critical steps for HCV vaccine development. **Materials and Methods:** In this community-based study, we aimed to investigate the HCV genotypes in infected patients referring to the laboratory of Hajar Hospital of Shahrekord city (the capital of Chaharmahal and Bakhtiari Province) in Iran from November 21, 2016, to October 21, 2017. During 2016–2017, the sera were obtained from 2377 individuals referring to the laboratory of Hajar Hospital of Shahrekord, Iran. The anti-HCV antibody was tested for all sera by enzyme-linked immunosorbent assay test. Following HCV RNA isolation and cDNA synthesis, HCV genotype detection was performed by quantitative reverse transcription-polymerase chain reaction. **Results:** Genotypes 3, 1a, and 1b were found in 28.6% (95% confidence interval [CI]: 17.0%–40.0%), 9.5% (95% CI: 2.1%–17.0%), and 3.2% (95% CI: 0.0%–7.6%) of the patients, respectively. In 5 patients (7.9%, 95% CI: 1.1%–14.8%), however, we did not observe any genotypes. We could not find any significant difference between the plasma viral load of infected patients and different genotypes. There was no significant difference either between age groups and genotypes ($P > 0.05$). **Conclusion:** The findings of the present study determined that HCV genotype 3 was the predominant genotype followed by the genotypes 1a and 1b in Chaharmahal and Bakhtiari Province.

Key words: Genotype, hepatitis C virus, Iran

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INTRODUCTION

Hepatitis C virus (HCV), a member of the Flaviviridae family, is an enveloped, single-stranded RNA virus, whose only host is human.[1] HCV has seven genotypes and multiple subtypes (for instance, 1a and 1b).[2] HCV infection is one of the most important risk factors for liver failure and can lead to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Approximately 170–200 million (almost 3% of the world's population) people have been reported to have HCV infection worldwide.[3,4] The prevalence of HCV serotype in the general population of Iran has been reported as 0.5%–1%[5] and 6.7%[6] and 20%[7] in hemodialyzed and thalassemia patients, respectively. In 1989, HCV was identified as the most important cause of hepatitis following blood transfusion.[8] More than 25 years after discovering HCV, the development of preventive and therapeutic vaccines for HCV is still one of the most important challenges for researchers. The high rate of mutation in the virus and its many strategies to

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evade the host immune system are the important reasons for the complexity of the vaccine design.[9,10] According to the documentations, rates of infection are generally categorized into three groups including high (>3.5%), intermediate (1.5%–3.5%), and low (<1.5%). Central and East Asia, the Middle East, and North Africa fall in the first group. South and Southeast Asia, sub-Saharan Africa, Central and Southern Latin America, Caribbean, Oceania, Australasia, and Central, Eastern, and Western Europe belong to the second group. The third group includes Asia-Pacific, Tropical Latin America, and North America.[11] In neighboring countries of Iran, the prevalence of HCV infection is very different which has been reported as 0.8%, 2.4%, 3%, and 8.7% in Kuwait, Turkey, Pakistan, and Azerbaijan, respectively.[12] In Iran, the prevalence of HCV infection has been reported as 0.3% among first-time blood donors.[13] On the other hand, the overall prevalence of HCV infection varies across different provinces ranging from 0.3% to 1.6%.[12,14,15] According to the literature, 7 genotypes (1–7) and more than 60 subtypes (e.g. subtype 1a and 1b) have been reported for HCV strains.[16] Genotypes 1–3 are widely distributed around the world.[17] The prevalence of HCV subtypes (1a and 3a), the most frequent subtypes of HCV in Iran, has been reported 47% and 36%, respectively.[18] Chaharmahal and Bakhtiari Province is an attractive city for passengers and tourists every year. A recent study on 3000 samples older than 15 years old in Chaharmahal and Bakhtiari Province (urban and rural areas) revealed that the prevalence of HCV was twice as large in men (1.2%) than in women.[19]

Since HCV is one of the causative agents of chronic liver disease and that is a global public health problem, putting a significant burden on the health-care and economical system, so molecular epidemiological studies on HCV genotypes can display significant differences in their global distribution and prevalence. Knowing the genotypes of HCV can bring new insights for health and economic outcomes of HCV cure through testing, managing, and treating adults infected with HCV. Therefore, this community-based study aimed to investigate HCV genotypes in infected patients referring to the laboratory of Hajar Hospital of Shahrekord University of Medical Sciences from November 21, 2016, to October 21, 2017.

MATERIALS AND METHODS

Serological assay

In this study, the sera were obtained from 2377 individuals referring to the laboratory of Hajar Hospital in Shahrekord, Iran, from November 21, 2016, to October 21, 2017. Initially, the blood samples (8 ml) from participants were collected in ethylenediaminetetraacetic acid tubes. Following plasma separation, the anti-HCV antibody was tested for all sera by enzyme-linked immunosorbent assay (ELISA) test (Dia.pro, Italy). The samples of positive sera which were detected for HCV antibody were aliquoted and stored immediately at −70°C further analysis.

Hepatitis C virus RNA extraction, cDNA synthesis, and quantitative reverse transcription-polymerase chain reaction

HCV RNA isolation was performed from the sera of patients using RNA extraction kit (RIBO-prep, Moscow, Russia). The RNA concentration was detected by calculating the absorbance ratio OD260 nm/280 nm using NanoDrop 2000 Spectrophotometer (Thermo Scientific, MA, USA). Then, purified RNA was directly subjected for cDNA synthesis. Detection of HCV genotypes was performed using the AmpliSens® HCV-Monitor-FRT real-time polymerase chain reaction (PCR) kit (InterLabService Ltd., Moscow, Russia), according to the manufacturer's instructions. The transcription level of glyceraldehyde 3-phosphate dehydrogenase was used as an endogenous control. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression of genes. Quantitative reverse transcription (qRT)-PCR reactions were run using the Rotor-Gene 6000 instrument (Corbett Research, Sydney, Australia).

Statistical analysis

Descriptive statistics are reported with the number (%) for qualitative variables, median (interquartile range) for abnormally distributed quantitative variable (viral load), and mean ± standard deviation for quantitative variable with normal distribution (age). Independent *t*-test (to compare difference between the age of males and females), Fisher's exact test (to assess associations between age categories and genotypes), Spearman correlation (to assess correlation between viral load and age), and Kruskal–Wallis test (to compare plasma viral load of different genotypes) were performed in IBM SPSS 19.0 (IBM SPSS Statistics, ARMONK, New York, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Sample description

Out of 2377 participants who were examined for hepatitis C, ELISA test was positive for only 63 patients. Among them, the real-time test was positive in 31 (49.2%, 95% confidence interval [CI]: 37%–62%) patients. The mean age of the participants was 39.8 ± 13.2 years (range, 8–80, 95% CI: 36.5–43.2) with a male predominance (82.5%, 95% CI: 73%–92%). There was no significant difference between the age of males and females ($P = 0.47$).

Information about hepatitis C virus genotypes

The median HCV viral load among infected patients was

665,710 (4,886,042). There was no correlation between viral load and age (P = 0.35). Genotype 3 which was detected in 18 (28.6%, 95% CI: 17.0%–40.0%) samples was the most frequent type, followed by 1a (9.5%, 95% CI: 2.1%–17.0%), others (7.9%, 95% CI: 1.1%–14.8%), and 1b (3.2%, 95% CI: 0.0%–7.6%). In this study, we did not find the genotypes 2 and 4 in our population study. Our results suggest that there was no difference between the plasma viral load of different genotypes [P = 0.39, Table 1]. The associations between age categories and genotypes are reported in Table 2. Although the main type among all of the age categories was genotype 3, no significant association was detected based on Fisher's exact test (P = 0.66). However, the association between gender and different genotypes was significant [P = 0.04, Figure 1].

DISCUSSION

One of the major public health concerns is HCV infection which causes fulminant hepatitis, hepatocellular carcinoma, and cirrhosis. The distribution of HCV genotypes is various across different areas and patients.[20]

Determining the dominant genotype of HCV in each region through epidemiological studies is essential for antiviral planning as genotype-based antiviral therapy increases the chance of success.[21]

In the current study, we measured anti-HCV antibodies in specimens obtained from patients susceptible for HCV infection. They were then confirmed using qRT-PCR method which is employed for diagnosing and monitoring patients infected with HCV according to the European and American Liver Society guideline.[22]

In the present study, the initial screening of patients was done by ELISA. We further used the qRT-PCR method to confirm the screening and to prevent false-positive and false-negative results.

Different prevalence of HCV infection has been reported in the general population in different provinces of Iran.[12-14] These discrepancies may be due to factors such as lifestyle (as most patients are not honest in describing their lifestyle, and more importantly sometimes curiosity in some lifestyle issues is inconsistent with ethical considerations), undesirable habits, inappropriate quality of public health services, and different influence of confirmatory techniques after screening by ELISA.[13,23]

The prevalence of HCV infection is higher in neighboring countries of Iran including Pakistan (4.7%), Afghanistan (1.1%), Iraq (7.1%), Turkey (1%–2.1%), and Qatar (6.3%).[24] Globally, Egypt has been reported as a country with the highest HCV prevalence (17.5%).[23] In our population, the prevalence of HCV infection was calculated 1.1%.

Our results are in line with previous results which reported that the prevalence of HCV infection in men is higher than in women. In a study performed in Kermanshah Province of Iran, across a 1721 urban population, during 2005–2006, the prevalence rate of HCV infection for the male was 5-fold higher than for the female.[15] In 2017, the same rate was achieved in another study conducted by Ghezeldasht *et al*.[13]

The findings of the present study revealed that the pattern of HCV genotypes was 3, 1a, and 1b, with the frequency rates of 58.1%, 19.4%, and 6.4%, respectively. These results are consistent with the findings of Zarkesh-Esfahani *et al*. from Isfahan[25] as well as the results provided by Hadinedoushan *et al*. from Yazd.[26] All of these findings suggest that the prominent genotypes were 3a, 1a, and 1b,

Table 1. Association between plasma viral load and different genotypes

Genotype	*n* (%)	Plasma viral load Median (IQR)	*P*
3	18 (28.6)	599694 (4766785)	
1a	6 (9.5)	2721262 (12538245)	0.39
1b	2 (3.2)	4542659 (2641494)	
5 or 6	5 (7.9)	37746 (3972764)	

Table 2. Association between different age categories and genotypes

Age group	3	1a	1b	5 or 6	Total	*P*
<31	2 (6.5)	1 (3.2)	0 (0)	0 (0)	3	
31-40	9 (29.0)	4 (12.9)	1 (3.2)	2 (6.5)	16	0.66
41-50	4 (12.9)	1 (3.2)	0 (0)	3 (9.7)	8	
>50	3 (9.7)	0 (0)	1 (3.2)	0 (0)	4	

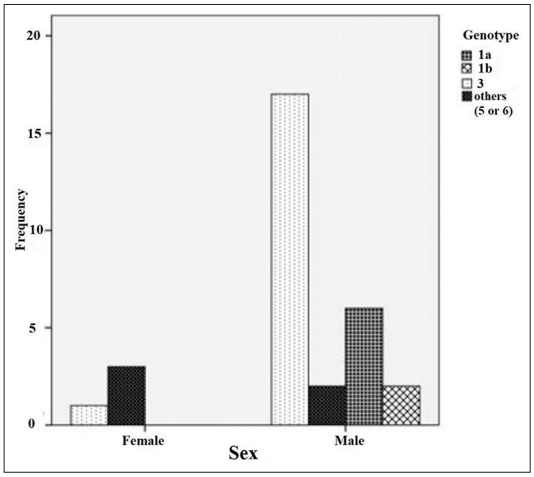

Figure 1: Association between gender and different genotypes. Results were reported as number (%) and the associations were examined using Fisher's exact test

consecutively. A similar pattern of HCV genotype in Iran has also been observed in multiple countries worldwide. HCV genotypes 1 and 3 are the dominant and predominant subtypes in Iran, respectively.[27,28] In line with our survey, several studies in India, Pakistan, and Malaysia have identified that HCV genotype 3 was the predominant type in these countries.[29]

The most important limitations of this study are the lack of study variables. Based on the evidence from the International Agency for Research on Cancer has classified as Group 1 carcinogens, six viruses associated with the origin of certain types of human cancers: Epstein–Barr virus, Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8, human papillomavirus, hepatitis B virus (HBV), HCV, and human T-lymphotropic virus type 1. It would be valuable to evaluate functional studies to characterize the biological properties of HCV in hepatocellular carcinoma development using *in vitro* and *in vivo* model systems.

Since HBV and HCV are associated with non-Hodgkin's lymphoma and liver cancer, it is worth mentioning to establish a causal role of HCV in human carcinogenesis. Identifying possible synergies between environmental factors and HCV with hepatocellular carcinoma can have potential effects in controlling the disease.

CONCLUSION

The critical step to predicting the response rate and duration of the treatment for HCV antiviral therapy is genotyping because the patients who need more aggressive management are helped for screening. Therefore, in view of the points mentioned above, the findings of the present study determined that HCV genotype 3 was the predominant genotype followed by the genotypes 1a and 1b in Chaharmahal and Bakhtiari Province.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Manns MP, Buti M, Gane E, Pawlotsky JM, Razavi H, Terrault N, *et al*. Hepatitis C virus infection. Nat Rev Dis Primers 2017;3:17006.
2. Kermani FR, Kafi-Abad SA, Hossein KM, Maghsudlu M, Sharifi Z, Mansournia MA. Association of HCV genotype with viral load among Iranian blood donors: A penalized logistic regression. Med J Islam Repub Iran 2019;33:149.
3. Farag MM, Sofy AR, Mousa AA, Ahmed MA, Alganzory MR. Molecular assay and genotyping of hepatitis c virus among infected Egyptian and Saudi Arabian Patients. Virology (Auckl) 2015;6:1-0.
4. Zidan A, Scheuerlein H, Schüle S, Settmacher U, Rauchfuss F. Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in Iran and worldwide. Hepat Mon 2012;12:e6894.
5. Bokharaei-Salim F, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Nasseri S. Distribution of hepatitis C virus genotypes among Azerbaijani patients in capital city of Iran-Tehran. Hepat Mon 2013;13:e13699.
6. Hamissi J, Hamissi H. Occurrence of hepatitis B and C infection among hemodialyzed patients with chronic renal failure in Qazvin, Iran: A preliminary study. Int J Collab Res Intern Med Public Health 2011;3:89.
7. Ebrahimzadeh P, Haghshenas M, Babamahmoodi F. The prevalence of hepatitis C virus genotypes in Mazandaran province, Iran. Jundishapur J Microbiol 2017;10(4):e40750.
8. Jafroodi M, Davoudi-Kiakalayeh A, Mohtasham-Amiri Z, Pourfathollah AA, Haghbin A. Trend in prevalence of hepatitis C virus infection among β-thalassemia major patients: 10 years of experience in Iran. Int J Prev Med 2015;6;89.
9. Li HC, Lo SY. Hepatitis C virus: Virology, diagnosis and treatment. World J Hepatol 2015;7:1377-89.
10. He L, Cheng Y, Kong L, Azadnia P, Giang E, Kim J, *et al*. Approaching rational epitope vaccine design for hepatitis C virus with meta-server and multivalent scaffolding. Sci Rep 2015;5:12501.
11. Hanafiah KM, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333-42.
12. Alavian SM, Ahmadzad-Asl M, Lankarani KB, Shahbabaie MA, Ahmadi AB, Kabir A. Hepatitis C infection in the general population of Iran: A systematic review. Hepat Mon 2009;9:211-23.
13. Ghezeldasht SA, Hedayati-Moghaddam MR, Shamsian K, Fathimoghadam F, Bidkhori HR, Rezaee SA. Prevalence of hepatitis c virus infection in general population of Mashhad, Northeastern Iran. Iran J Public Health 2017;46:408-13.
14. Merat S, Rezvan H, Nouraie M, Jafari E, Abolghasemi H, Radmard AR, *et al*. Seroprevalence of hepatitis C virus: The first population-based study from Iran. Int J Infect Dis 2010;14 Suppl 3:e113-6.
15. Sayad B, Shams AS, Keyvani H, Rezaei M, Asadi T, Vaziri S, *et al*. Seroepidemiology of hepatitis C in Kermanshah (West of Iran, 2006). Hepatitis Monthly 2008;8:141-6.
16. Mokhtari C, Ebel A, Reinhardt B, Merlin S, Proust S, Roque-Afonso AM. Characterization of samples identified as hepatitis C virus genotype 1 without subtype by Abbott real time HCV genotype II assay using the new Abbott HCV genotype plus RUO test. J Clin Microbiol 2016;54:296-9.
17. 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;11:191-4.

18. Hajiani E, Hashemi SJ, Masjedizadeh A, Shayesteh AA, Jalali F. Genotypic analysis of hepatitis C virus in Khuzestan province, Southwestern Iran. Middle East J Dig Dis 2011;3:126-30.
19. Moezzi M, Imani R, Karimi A, Pourheidar B. Hepatitis C Seroprevalence and risk factors in adult population of Chaharmahal and Bakhtiari province of Iran in 2013. J Clin Diagn Res 2015;9:LC13-7.
20. Keshvari M, Alavian S, Behnava B, Miri S, Elizee PK, Tabatabaei S, *et al*. Distribution of hepatitis C virus genotypes in Iranian patients with congenital bleeding disorders. Iran Red Crescent Med J 2010;12:608.
21. Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Hepatitis C virus (HCV) genotype 1 subtype identification in new HCV drug development and future clinical practice. PLoS One 2009;4:e8209.
22. Calvaruso V, Craxì A. 2011 European Association of the Study of the Liver hepatitis C virus clinical practice guidelines. Liver Int 2012;32:2-8.
23. Taherkhani R, Farshadpour F. Epidemiology of hepatitis C virus in Iran. World J Gastroenterol 2015;21:10790-810.
24. Khodabandehloo M, Roshani D. Prevalence of hepatitis C virus genotypes in Iranian patients: A systematic review and meta-analysis. Hepat Mon 2014;14:e22915.
25. 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.
26. Hadinedoushan H, Salmanroghani H, Amirbaigy MK, Akhondi-Meybodi M. Hepatitis C virus genotypes and association with viral load in Yazd, central province of Iran. Hepat Mon 2014;14:e11705.
27. 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;61:77-87.
28. Mahmud S, Akbarzadeh V, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Iran: Systematic review and meta-analyses. Sci Rep 2018;8:150.
29. Mohamed NA, Zainol Rashid Z, Wong KK, Abdullah SA, Rahman MM. Hepatitis C genotype and associated risks factors of patients at University Kebangsaan Malaysia Medical Centre. Pak J Med Sci 2013;29:1142-6.