Research Article

Distribution of Hepatitis C Virus Genotypes in Rasht, Capital City of Guilan Province, Northern Part of Iran

Dariush Naghipour-Khalkhaliani,[1] Daryoush Behzadpour,[2,*] and Seyed Dawood Mousavi Nasab[3]

[1]Department of Environmental Health Engineering, School of Health, Gilan University of Medical Sciences, Rasht, IR Iran
[2]Department of Virology, Rasht Branch, Islamic Azad University, Rasht, IR Iran
[3]Department of Research and Development, Pasteur Institute of Iran, Tehran, IR Iran

[*]*Corresponding author*: Daryoush Behzadpour, Department of Virology, Rasht Branch, Islamic Azad University, Rasht, IR Iran. Tel: +98-9113482716, Fax: +98-791341509, E-mail: dbehzadpour@gmail.com

Abstract

Background: Hepatitis C infection is a major health problem around the world. It exhibits high genetic diversity, characterized by regional variations in genotype distribution. It seems that the hepatitis C virus genotypes are differently distributed between distinct geographical regions and also associated with different clinical outcomes and response to antiviral therapy.

Objectives: The current study was performed to identify the distribution of HCV genotypes among chronic patients, who were HCV positive in Rasht, Capital City of Guilan Province, and Northern Part of Iran.

Patients and Methods: The cross sectional study was carried out from June 2014 to February 2015. A total of 83 patients with anti-HCV-positive specimens were enrolled. Detection of HCV-RNA by Real Time-Polymerase Chain Reaction (RT-PCR) was performed from anti-HCV positive specimens, and HCV genotypes among plasma samples from RNA-HCV positive patients were then investigated using type-specific primers.

Results: The mean age of patients was 49.6 ± 11.2 years (range: 19 to 62). Hepatitis C virus RNA was detected in 81 out of 83 patients with positive HCV antibodies. The HCV G1a was identified as the most abundant HCV subtype 42 (51.8 %). Furthermore, HCV G3a was observed in 23 (28.4%) patients followed by G2a, in 6 (7.4%), and G1b in 10 (12.4%). The frequency of HCV genotypes in males was higher than females. G1a was the most frequent genotype in patients over 40 years of age and also G3a was the most frequent in patients under 40 years old, yet this difference was not significant.

Conclusions: The current results indicate that G1a is the most prevalent genotype in in the study region. Also, the study gives greater evidence of the role of gender between different HCV genotypes in Rasht. Therefore, it is essential to determine the predominant subtypes of HCV in each region of Iran.

Keywords: Hepatitis C Virus, Genotype, Rasht, Distribution

1. Background

Hepatitis C Virus (HCV) is an infectious pathogen belonging to genus Hepacivirus and the Flaviviridae family that has infected more than 180 million people worldwide (1). Also, it was estimated that 1.75 million new HCV infections occurred worldwide in 2015; based on estimation, the number of individuals infected annually by HCV infection is estimated to be 3 to 4 million (2-4).

The most frequently affected regions are Mediterranean and European regions, with a prevalence of 2.3% and 1.5%, respectively (3, 5). Depending on the country, hepatitis C virus infection can be involved in certain populations and/or in general populations. There are multiple genotypes of HCV and their distribution vary based on region. Currently, the virus is classified to 7 genotypes and 67 subtypes with genotypes varying substantially in their geo-

graphical distribution (6). The most prevalent genotype is genotype 1 (subtypes 1a and 1b in the USA and Europe), followed by genotypes 3 (South Asia and Australasia), 2 (Sub-Saharan Africa), and 4 (Central Africa to the Middle East), respectively (7). Genotype 1 is predominant in non-Arab countries, including Turkey, Iran, and Cyprus (8).

In Iran, subtypes 1a, 3a, and 1b are the most prevalent subtypes (9). Hepatitis C prevalence in the general population of Iran is less than 0.5% (0.1% in females and 1.0% in males) (10). This distribution of HCV genotypes varies by region, as indicated by previous reports. These reports differed in 4 geographical regions of Iran, such that G1a and G3a were predominant genotypes in northern regions (11). Also, there are controversies regarding the correlation of HCV genotypes with any clinical outcome. A combination of different factors, such as the genotype, have been sug-

gested to play a role in liver disease progression. Although the new therapy, Direct-Acting Antivirals (DAAs), is the current standard treatment for hepatitis C, this therapy type differs from one genotype to another, so that pre-therapy genotyping and prediction of liver damage severity is necessary (12).

2. Objectives

Considering that the data of HCV genotypes distribution can improve the current understanding of HCV epidemics in a region and that genotypes play a crucial role in assessing therapeutic decisions and approaches, therefore the present study aimed at determining the distribution of HCV genotypes in Rasht, Capital City of Guilan Province, Northern Part of Iran.

3. Patients and Methods

3.1. Study Population

All patients were registered at the Medical Clinical Centre of Rasht, a central part of Gilan province, between June 2014 and February 2015. The protocol of the present study was approved by the Ethics Committee of Gilan University of Medical Science. Consent forms were signed by all subjects. Also, a questionnaire consisting of demographic information, was completed for each patient, including clinical data, such as age, gender, Alanine Aminotransferase (ALT), HBsAg, and anti-HCV antibody status.

This study included 83 subjects with HCV infection. The inclusion criteria of this study were as follows: (a) age of more than 18 years old with the presence of anti-HCV antibodies (positive anti-HCV), (b) serum Alanine Aminotransferase (ALT) higher than the reference range, and (c) lack of treatment history for this illness. Also serum ALT levels in HCV-infected patients were higher than the reference range. All received samples were screened for HCV-RNA by RT-PCR. Negative HCV PCR and genotyping were excluded. Therefore, 81 RNA HCV positive samples for HCV genotyping were entered in the study.

3.2. Hepatitis C Virus RNA Isolation

Six milliliters of whole blood sample were collected from each individual and all plasma samples were isolated from each 0.5 M EDTA-treated blood samples by centrifuging at 3000 rpm for 15 minutes, and then were preserved at -80°C. According to the manufacturer's instructions, RNA was extracted from 200 μL of plasma, using the QIAamp viral RNA kit (Qiagen, Hilden, Germany).

3.3. Complementary DNA Synthesis

After extraction, viral RNA was resuspended in a total volume of 20 μL and reverse transcription was carried out with 200 U of Moloney murine reverse transcriptase, (Fermentas GmbH, Germany), random hexamer (0.5 μM), (Fermentas GmbH, Germany), 0.5 mM each of dideoxynucleotides (dNTPs), (Fermentase GmbH, Germany), and reverse transcriptase reaction buffer 5x for 1 hour at 37°C, followed by an enzyme inactivation step at 95°C for 15 minutes in a thermocycler machine (MJ Research, Watertown, MA, USA).

3.4. RNA Hepatitis C Virus-Polymerase Chain Reaction

The sequences of PCR primers, specific for 5'-UTR region of HCV genome, have already been published (13). The sequence was as follows: ExF; 5'-AGCGTCTAGCCATGGCGT-3', ExR; 5'-GCACGGTCTACGAGACCT-3', InF; 5'-GTGTCTGCGGAACCGG-3', InR; 5'-GGGCACTCGCAAGCACCC-3'. The first round of PCR reaction mixture contained 5 μL of cDNA as template, 5 μL of 10x PCR buffer (Roche, Germany), 1 μL of 10mM dNTPs, 1 μL of each external primer and 0.3 μL of Taq DNA polymerase. The final volume was adjusted to 50 μL. The thermocycler machine (Eppendorf, Germany) was programmed at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 55 seconds, annealing temperature at 55°C for 40 seconds, and extension at 72°C for 40 second, and 10 minutes at 72°C for final extension. In the second round (for nested PCR) 3 μL of the first round PCR product was used as template and 1 μL of each internal primer was added to the reaction. Other PCR components were similar to the first round except annealing temperature, which was 64°C for 40 seconds, and the final volume was adjusted to 50 μL. Finally, the PCR product was loaded onto 2% agarose gel, electrophoresed, and then visualized under a UV transilluminator. The PCR fragment was 174 bp.

3.5. Hepatitis C Virus Genotyping

In this study, the PCR protocol was modified, and has previously been reported (14). Genotypes were determined by performing PCR, using specific primers for the target core region of the HCV genome (15). After cDNA synthesis, the PCR were performed. Briefly, cDNA synthesis and PCR amplification were performed in a 50-μL reaction volume, containing 20 μL of RNA, 50 pmol of each of primer, 200 μM of each deoxynucleotides (dNTPs), 10 U of Avian Myeloblastosis Virus Reverse Transcriptase (AMV RT) (Promega Madison, WI, USA), 2.5 U of Taq DNA polymerase (Promega Madison, WI, USA), 20 mM Tris-HCl (pH 8.4), and 50 mM KCl. Reactions were performed in a thermal cycler (Eppendorf, Germany), programmed as follows: 42°C

for 30 minutes, 95°C for 5 minutes, 40 cycles at 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, and 72°C for 5 minutes. Amplicons were then resolved by agarose gel electrophoresis (2%) with genotype-specific band sizes, which were compared with a 50-bp DNA ladder (Figure 1 A-D). Genotype 1a generates a 208-bp PCR product, genotype 1b, 2, and 3a generate 234, 337, and 285 bp PCR products, respectively. The plasma with different genotypes of HCV was used as a positive control for detection of different genotypes of HCV. Genotyping was confirmed by sequencing with gold standard methods, therefore, several samples were sent for sequencing.

3.6. Statistical Analysis

Analysis was performed by the SPSS software version 17.0, using descriptive values, such as mean, standard deviation, and Fisher's exact probability test. T-test analysis was used for quantitative variables. P values of < 0.05 were considered statistically significant.

4. Result

Eighty-two patients with positive anti-HCV were recruited in this cross sectional study, out of which 81 were RNA HCV positive by PCR (Figure 2). The picture of the HCV genotyping products based on amplicon size is shown in Figure 1 A-D.

Demographic and HCV genotype distribution of all patients of this study are presented in Table 1. The mean age of patients was 49.6 ± 11.2 years (range: 19 to 62). The mean age of female participants was statistically higher than males (54.2 ± 3.8 versus 50.6 ± 4.6, respectively) ($P < 0.05$). The majority of the patients were aged 31 to 40 years, followed by 21 to 30 years.

Hepatitis C Virus G1a was identified as the most abundant HCV subtype (42; 51.8 %). Furthermore, HCV G3a was observed in 23 participants (28.4%) followed by G2a, in 6 (7.4%), and G1b in 10 (12.4%). The HCV genotypes 5a and 6a were not detected in any of the HCV RNA positive patients. G1a was more frequent (85.7%) in male patients. The distribution of HCV genotypes in males was higher than females (68.3% versus 31.7%). G1a was the most frequent genotype in patients over 40 years of age (56.7% versus 41.2%) and G3a was the most frequent in patients under 40 years old (45.8% versus 38.3%), yet this difference was not significant. Also, the distribution of HCV genotypes based on other clinical data is shown in Table 2.

5. Discussion

Epidemiological studies in different regions and patients of the world show differences in distribution of HCV

Figure 1. Identification of Hepatitis C Virus Genotypes by Polymerase Chain Reaction Amplification
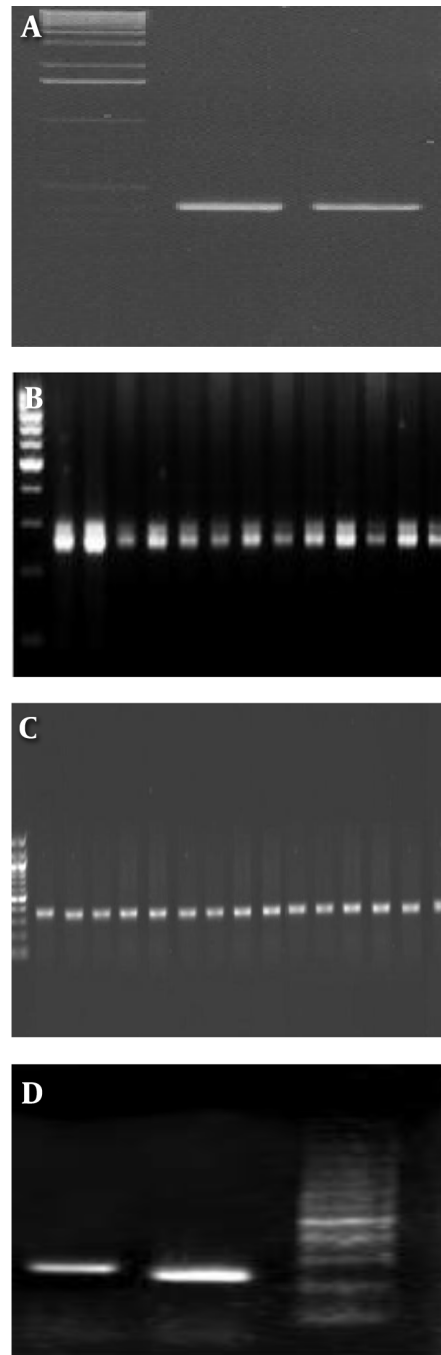
From left to right: 100 bp DNA ladder (Fermentas, Germany) and positive samples and C+ = positive control. A, G1a amplicon size is 208bp; B, G1b amplicon size is 234 bp; C, G2a amplicon size is 337bp; D, G3a amplicon size is 285bp.

genotypes, therefore, the distribution of hepatitis C genotypes can contribute to health and treatment programs.

Figure 2. Identification of RNA Hepatitis C Virus by Polymerase Chain Reaction amplification.
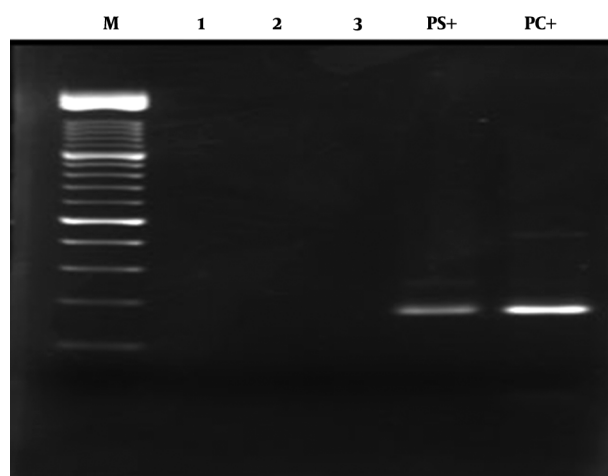
From left to right: M = 100 bp DNA Ladder (Fermentas, Germany), 1 = Negative sample, C- = Negative control, 2, 3, 4 = Positive Samples (PS+), C+ = Positive Control (PC+) respectively. The PCR products size was 174 bp.

Table 1. Demographic and Clinical Data for the 81 Hepatitis C Virus Patients

Variables	N	%	Mean ± SD
Age groups			49.6 ± 11.2
21 - 30	34	42	
31 - 40	18	22.2	
41 - 50	17	21	
≥ 50	12	14.8	
Gender			
Male	32	39.5	
Female	49	61.5	
ALT (IU/L)			41.5 ± 12.1
AST (IU/L)			27.3 ± 12.9

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Also, HCV genotype determination is an important need for clinicians in order to decide about the duration of antiviral treatment, because it is associated with the severity of liver damage and response to antiretroviral therapy (16). The geographical distribution of HCV genotypes is important for epidemiological studies in terms of distribution and possible risk groups. Therefore, it is necessary to determine the predominant genotype of the virus in each region, however, the aim of this study was to identify the prevalence of HCV genotypes in Rasht, Iran.

In the current study, HCV G1a was identified as the most abundant HCV genotype, which is the most prevalent worldwide and is the most frequently found in Northern Europe and North American (17). Previous reports showed that the most abundant HCV genotype was G1a (18), yet, in other studies conducted in Shiraz, Isfahan, and Mazandaran, G3a was the most common HCV genotype (19-21). The current results confirmed that the dominant genotype in Guilan province was 1a, which is in accordance with other reports from the same area of Iran. Based on previous studies in Guilan on HCV-infected patients and high risk individuals, such as hemodialysis and Beta-thalassemia, the most common genotype has been G1a (11, 22). Also, recent studies indicate that G1a 3a and 1b are the most prevalent genotypes in Iran (17). As shown previously, the distribution of HCV genotypes in Gilan in different studies has not changed, especially in high risk individuals; as these individuals have frequent blood transfusion and are at risk of exposure to infectious agents, such as HCV, this pattern may be due to low migration of different populations and the type of circulated HCV genotype. According to recent reports, a change in the distribution of HCV genotypes has been reported in a number of countries around the world (23-25). Therefore, the shift in the genotype circulation, especially in high risk individuals, may have a significant impact on clinical management of the patients as well as on the public health, therefore, this recent pattern can be concluding accurate clinical management of the patients.

In Iran, located in the Middle East, there has been regional reports on hepatitis genotyping among infected patients. Compared to the studies of Iran's neighboring countries, it can be understood that the most common genotype is G1, found in approximately 50% to 75% of samples from Turkey and Bahrain (26-28). No study is available on HCV genotypic variation from Iran, Bahrain, and Pakistan, which could give cohesive circulation of HCV genotypes. Classification by viral genotype at the national and regional level, and a better understanding of viral diversity within different populations, will also critically inform the rational design and testing of HCV vaccines. Genotype diversity is particularly high in many Southeast Asian countries, and also in Western Europe, perhaps as a result of population immigration from Africa and/or Asia, therefore, the current results confirmed that it is better to investigate the distribution of HCV genotypes in each region of Iran based on periodic programs (29).

Male gender was reported as an independent predictive factor for HCV infection in previous studies (30). In this study, it was indicated that the difference of distribution of HCV genotypes between males and females was statistically significant. This may be because most of the HCV infected patients were male. Thus, it was not possible to analyze the distribution of HCV genotypes, according to gen-

Table 2. Distribution of Hepatitis C Virus Genotypes Based on Demographic and Clinical Data

Variables	GT3 (n = 23)	GT1a (n = 42)	GT2 (n = 6)	GT1b (n = 10)	P Value
Gender					0.001
Male	10 (43.5%)	36 (85.7%)	3 (50%)	6 (60%)	
Female	13 (56.5%)	6 (14.3%)	3 (50%)	4 (40%)	
Age, mean ± SD	43.1 ± 11.3	48.5 ± 1.8	46.1 ± 1.7	43.5 ± 4.7	0.32
ALT	23.3 ± 12.7	34 ± 11.7	29.4 ± 6.4	29.4 ± 3.4	0.56
AST	21.7 ± 11.8	28.6 ± 11.5	19.7 ± 11.8	18.6 ± 11.5	0.35

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

der, because of the low sample size. Similar studies have shown that the prevalence of hepatitis C in the general population of Iran is less than 0.5% (0.1% in females and 1.0% in males) (10); also, the difference in the distribution of HCV genotypes between males and females in HCV patients was statistically significant (31-33). In another report, the distribution of HCV G1 was found to be significantly associated with male gender, while G4 has frequently found in females (34). Also, in other studies, it was shown that there is no difference between both genders (35).

Epidemiological differences in age distribution of the HCV genotype have been previously investigated (17). The current results showed that distribution of HCV genotypes did not vary with age. European studies show that G1a and G3a are relatively more common in young individuals. In some studies, G1b-infected patients were significantly older than those infected by other genotypes and a significant proportion of younger patients were infected with G3 (21). In some studies, it was determined that G1a and G1b were more common in people older than 50 years of age, whereas G1b was the most common subtype among people younger than 20 years old (36). Hepatitis C virus increased in older patients compared with younger individuals, whereas there was no significant difference between males and females, which is incompatible with the current findings. In addition, according to a previous study from Iran, the prevalence of HCV infection in males was higher than females.

In conclusion, in the present study, G1a was the most prevalent genotype in Rasht, Iran. A high rate of HCV infection was reported in male patients. Further information is required on the epidemiology of HCV genotypes, as response to treatment is reported to vary in different types of HCV. This study had some limitations, such as subtype, with a relatively small sample size, and clinical data, such as therapy, yet the results may be helpful for understanding the genotype distribution of HCV in Rasht and can provide important information about HCV prevalence among different ages and gender.

Footnotes

References

1. Mohd Hanafiah K, 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**(4):1333–42. doi: 10.1002/hep.26141. [PubMed: 23172780].

2. Degenhardt L, Peacock A, Colledge S, Leung J, Grebely J, Vickerman P, et al. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. *Lancet Global Health*. 2017;**5**(12):e1192–207.

3. Organization WH. *Global hepatitis report 2017: executive summary.* 2017.

4. Mirminachi B, Mohammadi Z, Merat S, Neishabouri A, Sharifi AH, Alavian SH, et al. Update on the prevalence of hepatitis c virus infection among iranian general population: A systematic review and meta-analysis. *Hepat Mon*. 2017;**17**(2).

5. Sadeghi F, Salehi-Vaziri M, Almasi-Hashiani A, Gholami-Fesharaki M, Pakzad R, Alavian SM. Prevalence of Hepatitis C Virus Genotypes Among Patients in Countries of the Eastern Mediterranean Regional Office of WHO (EMRO): A Systematic Review and Meta-Analysis. *Hepat Mon*. 2016;**16**(4). e35558. doi: 10.5812/hepatmon.35558. [PubMed: 27274353].

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. doi: 10.1002/hep.26744. [PubMed: 24115039].

7. Thrift AP, El-Serag HB, Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. *Nat Rev Gastroenterol Hepatol*. 2017;**14**(2):122–32. doi: 10.1038/nrgastro.2016.176. [PubMed: 27924080].

8. Ghaderi-Zefrehi H, Gholami-Fesharaki M, Sharafi H, Sadeghi F, Alavian SM. The Distribution of Hepatitis C Virus Genotypes in Middle Eastern Countries: A Systematic Review and Meta-Analysis. *Hepat*

Mon. 2016;**16**(9). e40357. doi: 10.5812/hepatmon.40357. [PubMed: 27826320].

9. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol.* 2016;**22**(34):7824–40. doi: 10.3748/wjg.v22.i34.7824. [PubMed: 27678366].

10. 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. doi: 10.1016/j.ijid.2009.11.032. [PubMed: 20362479].

11. Ashourizadeh B, Bashizadeh-Fakhar H, Fallahchai SS, Goleijani-Moghaddam R. *Distribution of Genotypes of B and C Hepatitis Viruses in Guilan Province.* 2012.

12. Baharlou R, Romani B, Kiani SJ, Sadeghi K, Shadmand E, Fazel H. Genotype-related variations in proinflammatory and regulatory cytokine levels in treated and treatment-naive HCV-infected patients. *Med Microbiol Immunol.* 2017:1–10.

13. Pohjanpelto P, Lappalainen M, Widell A, Asikainen K, Paunio M. Hepatitis C genotypes in Finland determined by RFLP. *Clinic Diagnos Virol.* 1996;**7**(1):7–16.

14. Mohamed NA, Rashid ZZ, Wong KK. Hepatitis C virus genotyping methods: evaluation of AmpliSens((R)) HCV-1/2/3-FRT compared to sequencing method. *J Clin Lab Anal.* 2014;**28**(3):224–8. doi: 10.1002/jcla.21670. [PubMed: 24478138].

15. Gushchin AE, Hoskova OM, Shipulin GA. [Development of "Amplisens-HCV-genotype" reagent set for identification of hepatitis C virus genotypes 1a, 1b, 2a and 3a]. *Vopr Virusol.* 2003;**48**(3):45–8. [PubMed: 12894481].

16. Dal Molin G, Ansaldi F, Biagi C, D'Agaro P, Comar M, Croce L, et al. Changing molecular epidemiology of hepatitis C virus infection in Northeast Italy. *J Med Virol.* 2002;**68**(3):352–6. doi: 10.1002/jmv.10210. [PubMed: 12226821].

17. Khodabandehloo M, Roshani D. Prevalence of hepatitis C virus genotypes in Iranian patients: a systematic review and meta-analysis. *Hepat Mon.* 2014;**14**(12). e22915. doi: 10.5812/hepatmon.22915. [PubMed: 25685164].

18. Sefidi FJ, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Bokharaei-Salim F. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. *Hepat Mon.* 2013;**13**(1).

19. MohammadAli D, MehdiSaberi F, KamranBagheri L, Davood M, Abbas-Behzad B, Alireza S. Hepatitis C virus genotype distribution in Shiraz, southern Iran. *Hepat Mon.* 2009;**2009**(2, Spring):122–7.

20. 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. doi: 10.1186/1743-422X-7-69. [PubMed: 20331907].

21. Ebrahimzadeh P, Haghshenas M, Babamahmoodi F. The Prevalence of Hepatitis C Virus Genotypes in Mazandaran Province, Iran. *Jundishapur J Microbiol.* 2017;**10**(4). doi: 10.5812/jjm.40750.

22. Joukar F, Khalesi AK, Jafarshad R, Rahimabadi MS, Mansour-Ghanaei F. Distribution of hepatitis C virus genotypes in haemodialysis patients of Guilan, northern Islamic Republic of Iran. *East Mediterr Health J.* 2012;**18**(3):236–40. [PubMed: 22574476].

23. Ross R, Viazov S, Renzing-Kohler 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.

24. 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. doi: 10.1002/jmv.21427. [PubMed: 19235845].

25. Svirtlih N, Delic D, Simonovic J, Jevtovic D, Dokic L, Gvozdenovic E, et al. Hepatitis C virus genotypes in Serbia and Montenegro: the prevalence and clinical significance. *World J Gastroenterol.* 2007;**13**(3):355–60. [PubMed: 17230602].

26. Kabakci Alagoz G, Karatayli SC, Karatayli E, Celik E, Keskin O, Dinc B, et al. Hepatitis C virus genotype distribution in Turkey remains unchanged after a decade: performance of phylogenetic analysis of the NS5B, E1, and 5'UTR regions in genotyping efficiency. *Turk J Gastroenterol.* 2014;**25**(4):405–10. doi: 10.5152/tjg.2014.7083. [PubMed: 25254523].

27. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol.* 2014;**61**(1 Suppl):S45–57. doi: 10.1016/j.jhep.2014.07.027. [PubMed: 25086286].

28. Janahi EM, Al-Mannai M, Singh H, Jahromi MM. Distribution of Hepatitis C Virus Genotypes in Bahrain. *Hepat Mon.* 2015;**15**(12). e30300. doi: 10.5812/hepatmon.30300. [PubMed: 26977163].

29. 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. doi: 10.1002/hep.27259. [PubMed: 25069599].

30. Zamani F, Sohrabi M, Poustchi H, Keyvani H, Saeedian FS, Ajdarkosh H, et al. Prevalence and risk factors of hepatitis C virus infection in amol city, north of iran: a population-based study (2008-2011). *Hepat Mon.* 2013;**13**(12). e13313. doi: 10.5812/hepatmon.13313. [PubMed: 24358039].

31. Keshvari M, Alavian S, Behnava B, Miri S, Tabatabaei S, Abolghasemi H. Distribution of hepatitis C virus genotypes in iranian patients with congenital bleeding disorders. *Iran Red Crescent Med J.* 2010;**2010**(6):608–14.

32. Samimi-Rad K, Shahbaz B. Hepatitis C virus genotypes among patients with thalassemia and inherited bleeding disorders in Markazi province, Iran. *Haemophilia.* 2007;**13**(2):156–63. doi: 10.1111/j.1365-2516.2006.01415.x. [PubMed: 17286768].

33. Hafez AA, Baharlou R, Nasab SDM, Vasmehjani AA, Shayestehpour M, Joharinia N. Molecular epidemiology of different hepatitis C genotypes in serum and peripheral blood mononuclear cells in jahrom city of iran. *Hepat Mon.* 2014;**14**(5).

34. Brahim I, Akil A, Mtairag el M, Pouillot R, Malki AE, Nadir S, et al. Morocco underwent a drift of circulating hepatitis C virus subtypes in recent decades. *Arch Virol.* 2012;**157**(3):515–20. doi: 10.1007/s00705-011-1193-7. [PubMed: 22160625].

35. Rouabhia S, Sadelaoud M, Chaabna-Mokrane K, Toumi W, Abenavoli L. Hepatitis C virus genotypes in north eastern Algeria: A retrospective study. *World J Hepatol.* 2013;**5**(7):393–7. doi: 10.4254/wjh.v5.i7.393. [PubMed: 23898373].

36. Niu Z, Zhang P, Tong Y. Age and gender distribution of Hepatitis C virus prevalence and genotypes of individuals of physical examination in WuHan, Central China. *Springerplus.* 2016;**5**(1):1557. doi: 10.1186/s40064-016-3224-z. [PubMed: 27652130].