Jundishapur J Microbiol. 2018 December; 11(12):e69136. doi: 10.5812/jjm.69136.
Published online 2018 November 11. **Research Article**

Distribution of Hepatitis C Virus Genotypes and Related Risk Factors Among Iranian Blood Donors: A Penalized Logistic Regression

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Received 2018 April 07; **Revised** 2018 October 14; **Accepted** 2018 October 18.

Abstract

Background: The hepatitis C virus (HCV) is a blood born virus and the major cause of liver diseases worldwide. Distribution of HCV genotypes varies depending on geographical regions and routes of infection. Knowledge regarding the distribution of HCV genotypes and related risk factors plays an essential role in the control of HCV infection in the community.
Objectives: The current study aimed at determining the current distribution of HCV genotypes and related risk factors among Iranian blood donors.
Methods: In the current analytical, cross sectional study, 106 HCV-infected blood donors with detectable HCV RNA over the country were interviewed by trained physicians through a post-donation questionnaire on demographic, medical, and risk history from November 2015 to May 2017. The hepatitis C virus genotype was determined by sequencing of a segment of non-structural 5B region in HCV genome. Penalized logistic regression model was used for statistical analysis through STATA software.
Results: Hepatitis C virus genotype was determined in all subjects, and the genotype 3a was the most frequent (65, 61.32%), followed by 1a (31, 29.25%), and 1b (10, 8.49%). Based on the multivariable analysis results, tattooing (adjusted odds ratio: 2.76; 95% confidence interval: 1.03 - 7.37) was associated with HCV genotype 3a.
Conclusions: According to the results, it seems that changes in molecular epidemiology of HCV infection and replacement of HCV genotype 1a with 3a, characterized by an increase in genotype 3a and decrease in genotype 1a have occurred over the last decade among Iranian blood donors. Tattooing was an independent risk factor for HCV infection by genotype 3a.

Keywords: Hepatitis C Virus, Genotype, Blood Donors, Risk Factor

1. Background

Hepatitis C virus (HCV), as a blood borne virus, is the leading cause of liver diseases, which predisposes patients to cirrhosis and hepatocellular carcinoma worldwide. Approximately 71 million people are chronically infected with HCV all over the world with the annual mortality rate of 3% (1, 2). In Iran, the overall prevalence of HCV is 0.6% in the general population (3, 4). Hepatitis C virus seropositivity decreased from 0.05% in 2014 (4) to 0.03% in 2016 among Iranian blood donors (according to the quality assurance/quality control deputy of Iranian Blood Transfusion Organization (IBTO).

Hepatitis C virus is an enveloped, positive-stranded linear RNA virus with approximately 9.5 kb genome, which belongs to the family of *Flaviviridae*. The non-structural 5B (NS5B) region of HCV genome coded for an error prone RNA-dependent RNA polymerase enzyme causes high heterogeneity in HCV genome. As a consensus, 7 major genotypes with multiple subtypes in each genotype are distinguished (5). Each genotype has 30% - 35% diversity with the others and there is a 15% difference in the subtypes of each genotype (6). Distribution and prevalence of HCV genotypes vary in different geographical regions. The most prevalent HCV genotypes, with global distribution are one and three. In the neighboring countries, 1b in Turkey, 3a in Pakistan and Afghanistan, and 4 in Saudi Arabia are predominant (7-10). Hepatitis C virus genotypes 1a and 3a are the most dominant subtypes among Iranian blood donors and patients infected with HCV (10-14). Globally, the most common route of HCV transmission is intravenous drug abuse (IVDA) in the developed countries and blood transfusion in the developing countries without

screening donated blood. In some developing countries with the screening donated blood, nosocomial transmission and transmission through high-risk procedures are prevalent (1, 15-17). Previous molecular epidemiologic studies showed association between HCV genotypes 1a and 3a with IVDA and HCV genotypes 1b and 2 with blood transfusion (18-22).

Hepatitis C virus genotyping is a powerful epidemiological tool to identify new subtypes and trace transmission routes (23). Sequence analysis of proposed coding region in virus genome with enough diversity, such as NS5B, is introduced as a reference and obligatory method for HCV genotyping in epidemiological studies (24-26). Increasing knowledge regarding the distribution of HCV genotypes and related risk factors play an essential role in the control of HCV infection in the community. Recently, the risk factors of HCV infection among Iranian blood donors were determined (27, 28). Distribution and prevalence of HCV genotypes may change according to the mode of transfusion over time. There is a lack of data on the current distribution of HCV genotypes and related risk factors among Iranian blood donors.

2. Objectives

In the current study, distribution of HCV genotypes was determined by sequence analysis of NS5 region of HCV genome among Iranian blood donors. In addition, the relationship between HCV genotype and known and putative routes of HCV risk factors were evaluated.

3. Methods

3.1. Ethics Statement

The current study was approved by the Ethics Committee of High Institute for Research and Education in Transfusion Medicine, Tehran, Iran (code No: IR.TMI.REC.1394.1800).

3.2. Epidemiological Data Collection

The current analytical, cross sectional study was conducted on 106 HCV-infected blood donors with detectable HCV RNA countrywide from November 2015 to December 2016. All subjects were interviewed by trained physicians using the post-donation questionnaire containing; (1) demographic characteristics including gender, age, educational level, and marital status, (2) high risk behaviors such as IVDA, inhalation drug abuse, history of imprisonment, sharing a razor, extramarital sexual activity, alcohol consumption, and contact sport, (3) medical interventions such as blood transfusion, tooth extraction, history of hospitalization, history of surgery, history of intramuscular injection, and history of intravenous injection, and (4) high risk procedures such as tattooing and wet cupping.

3.3. RNA Extraction and Amplification

Viral RNA was extracted by TriPure Isolation Reagent (Roche, Germany), according to the manufacturer's instruction. The extracted RNA was eluted in 20 μL elusion buffer and used as the template for further testing. Hepatitis C virus genotyping was performed via amplifying and sequencing of a segment of NS5B region in the HCV genome. SuperScript III Reverse Transcriptase (200 U/μL, Invitrogen, USA) and primer PR3 were used to synthetize the complementary DNA (cDNA). Semi-nested polymerase chain reaction was performed to amplify a 380-bp segment of NS5B region in HCV genome as described elsewhere (29). Briefly, outer primers PR3 and PR4 were used in the first-round followed by inner primers PR3 and PR5 for the second-round.

3.4. Hepatitis C Virus Sequencing and Genotyping

Purified PCR products were sequenced bi-directionally using primers PR3 and PR5. CEQ™ 8000 Genetic Analysis System (Beckman Coulter) was used to edit and assemble the sequences. To identify the possible similarity of sequences, the Basic Local Alignment Search Tool (BLAST) was used. Using the Clustal W software, the consensus sequences were aligned with the HCV reference sequence of NS5B genotype/subtype obtained from GenBank. Mega7 software was used for the phylogenetic analysis. The HCV sequences longer than 200 bp were set down in GenBank under the accession numbers MG704694 to MG704794.

3.5. Statistical Analysis

Demographic characteristics, as well as known or hypothesized risk factors, were considered as independent variables and HCV genotype was considered as the dependent variable. Descriptive results were expressed as mean $\pm$ standard deviation (SD) or percentages. Analysis of Variance (ANOVA) was used to compare mean age in different genotype groups. Logistic Regression was performed to evaluate the association between HCV genotypes and risk

factors. To deal with sparse data bias, penalized logistic regression model via data augmentation using the prior log-F (2, 2) was performed (30-32). All variables with P value $\leq$ 0.2 in the univariate analysis were considered independent and subjected for multivariable analysis. Multivariable analysis of risk factors by the penalized logistic regression model was performed to adjust for confounding with alpha level of 0.05. Results were summarized as crude odds ratio (OR) and adjusted OR (AOR) with 95% confidence interval (CI). Statistical analysis was performed with STATA software (Stata 13 Corp., College Station, Texas).

4. Results

All 106 samples with detectable HCV RNA were investigated using amplifying and sequencing techniques. Hepatitis C virus genotype 3a was the most frequent with 65 (61.32%) subjects, followed by 1a with 31 (29.25) and 1b with 10 (9.43) cases. The questionnaires of two subjects were not informative. Table 1 shows demographic features of 104 subjects.

No significant difference was observed regarding the age among different genotypes, 38.3 $\pm$ 1.77 years in the subjects infected with genotype 1a, 37.6 $\pm$ 3.95 years in the ones infected with genotype 1b, and 37.52 $\pm$ 1.00 years in those infected with genotype 3a (P = 0.26). The risk factors data of two subjects were unavailable. Frequency of genotypes in the risk factors of the questionnaire is shown in Table 2.

Table 3 shows distribution of risk factors of HCV infection in Iranian blood donors related to HCV genotype.

Table 1. General Characteristics of HCVRNA Positive Iranian Blood Donors, 2015 - 2017[a]

Characteristic	Value
Age, y, mean $\pm$ SD	37.76 $\pm$ 8.89
Gender	
Male	102 (98.08)
Female	2 (1.92)
Marital status	
Single (unmarried, divorced, or widow)	32 (30.76)
Married	72 (69.33)
Educational level	
Under diploma	60 (57.69)
Diploma	30 (28.85)
Associate degree	5 (4.80)
Bachelor and higher	9 (8.65)

[a]Values are expressed as No. (%) unless otherwise indicated.

Since the scarcity of HCV genotype 1b did not allow a statistical analysis of differences in the prevalence of risk factors in donors infected with this genotype compared with those of blood donors infected with other genotypes, subjects infected with genotype 1b were excluded from further statistical analyses and genotype 1a was considered as the reference. In univariate analysis, sharing a razor, history of surgery, and tattooing had a significant impact; there was no association between distinct HCV genotype and other risk factors such as IVDA and blood transfusion (Table 3).

The results of univariate and multivariable analysis of risk factors by the penalized logistic regression model are summarized as crude and adjusted OR with 95% CI for the significant HCV genotype risk factors. Tattooing was independently associated with genotype 3a (Table 4).

5. Discussion

In the current study, based on the sequencing of a segment of NS5B in HCV genome, subtype 3a (61.32%) was the predominant subtype followed by 1a (29.25%) and 1b (9.43%). The results were in contrast with those of a study conducted on 96 Iranian blood donors in 2010, which showed that HCV subtype 1a was the most frequent subtype followed by 3a and 1b (14). In the current study, compared with the above mentioned study, a significant decrease in the prevalence of HCV genotypes 1a from 51.5% to 29.25% (P = 0.05) were observed. In addition, there is a weak evidence of an increase in the prevalence of HCV genotype 3a from 37.9% to 61.32% (P = 0.08). Hepatitis C virus genotype 1a was replaced with HCV genotype 3a among Iranian blood donors. Generally, a shift in genotype distribution in a given community might be reflected as a change in HCV epidemiology in the same community due to an increase in blood safety and improvement in medical exposure conditions; IVDA is the main risk factor for HCV transmission in many countries (15, 27, 28) that highlights the role of IVDA in a vast majority of acquired HCV infection in the country.

One explanation of the change in genotype distribution is the result of change in epidemiological parameters such as HCV risk factors assumed in previous studies from European countries, United States, East Asian countries, and neighboring countries (20, 33-43). Recent studies suggested an increase in HCV genotype 3a prevalence and decrease in the HCV genotypes 1a and 1b prevalence, and as a consensus shift from genotype 1a dominancy to genotype 3a in the Iranian patients (11, 12, 44-46). According to the current study, it seems that such a shift toward genotype 3a

Table 2. HCV Genotype Distributions According to the Risk Factors Among Iranian Blood Donors, 2015 - 2017

Risk Factor	HCV Genotype, No. (%)			Total[a], N = 104
	1a (30)	1b (10)	3a (64)	
Age ≥ 40, y	11 (32.35)	3 (8.82)	20 (58.82)	34
Male gender	29 (31.18)	9 (68.82)	64 (100)	102
Injecting drug abuse	15 (28.30)	4 (7.55)	34 (64.15)	53
Inhalation drug abuse	10 (27.03)	3 (8.11)	24 (64.86)	37
History of imprisonment	16 (27.59)	5 (8.62)	37 (63.79)	58
Sharing a razor	6 (40.00)	3 (20.00)	6 (40.00)	15
Extramarital sexual activity	10 (27.78)	3 (8.33)	23 (63.89)	36
Alcohol consumption	10 (24.39)	5 (12.20)	26 (63.41)	41
Contact sport	7 (35)	1 (5)	12 (60)	20
Blood transfusion	1 (16.67)	2 (33.33)	3 (50)	6
Tooth extraction	21 (26.92)	7 (8.97)	50 (64.10)	78
History of hospitalization	5 (27.78)	1 (5.56)	12 (66.67)	18
History of surgery	10 (20.83)	5 (10.42)	33 (68.75)	48
History of intramuscular injection	10 (22.73	5(11.36)	29 (65.91)	44
History of intravenous injection	6 (20.69)	5 (17.24)	18 (62.07)	29
Tattooing	7 (18.92)	3 (8.11)	27 (72.97)	37
Cupping	9 (33.33)	3 (11.11)	15 (55.56)	27

[a]Total number is sum of row values.

Table 3. Distribution of Risk Factors According to HCV Genotype in Iranian Blood Donors, 2015 - 2017

Risk Factor	HCV Genotype, No. (%)		Total, N = 94	P Value
	1a, 30 (100)	3a, 64 (100)		
Age ≥ 40, y	11 (36.67)	20 (31.75)	31 (32.98)	0.64
Male gender	29 (96.67)	64 (100)	93 (98.94)	0.44
Injecting drug abuse	15 (50.00)	34 (53.13)	49 (52.13)	0.79
Inhalation drug abuse	10 (33.33)	24 (37.50)	34 (36.17)	0.71
History of imprisonment	16 (53.33)	37 (57.81)	53 (56.38)	0.6
Sharing a razor	6 (20.00)	6 (9.38)	12 (12.77)	019
Extramarital sexual activity	10 (33.33)	23 (35.94)	33 (35.11)	0.82
Alcohol consumption	10 (33.33)	26 (40.63)	36 (38.30)	0.52
Contact sport	7 (23.33)	12 (18.75)	19 (20.21)	0.63
Blood transfusion	1 (3.33)	3 (4.69)	4 (4.26)	0.81
Tooth extraction	21 (70.00)	50 (78.13)	71 (75.53)	0.42
History of hospitalization	5 (16.67)	12 (18.75)	17 (16.09)	0.82
History of surgery	10 (33.33)	33 (51.56)	43 (45.74)	0.12
History of intramuscular injection	10 (33.33)	29 (45.31)	39 (41.49)	0.29
History of intravenous injection	6 (20.00)	18 (28.13)	24 (25.53)	0.42
Tattooing	7 (23.33)	27 (42.19)|	34 (36.17)	0.09
Cupping	6 (20.00)	8 (12.50)	14 (14.89)	0.34

Table 4. Odds Ratios, Adjusted Odds Ratios, and 95% Confidence Intervals of Significant Risk Factors Associated with HCV Subtypes Among Iranian Blood Donors, 2015 - 2017

Risk Factor	OR (95% CI)	P Value	AOR (95% CI)	P Value
Sharing of personal razor	0.48 (0.15 - 1.48)	0.19	0.42 (0.13 - 1.40)	0.16
History of surgery	1.99 (0.84 - 4.69)	0.12	2.08 (0.85 - 5.08)	011
Tattooing	2.19 (0.87 - 5.49)	009	2.76 (1.03 - 7.37)	0.04

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

occurred among Iranian blood donors over the last decade. It seems that the shift from HCV genotype 1a to 3a is linked to the role of IVDA in the distribution of HCV genotype 3a due to transmission of HCV genotype 3a from IVD abusers to the community with no history of IVDA through high-risk behaviors, other related modes of HCV transmission resulted in no difference in the prevalence of HCV genotype 3a in blood donors with a history of IVDA and blood donors with no history of IVDA. Further studies such as phylogenetic analyses should be conducted to shed light on the issue.

The considerable finding of the current study was no association between the corroborated IVDA and HCV genotype. However, there are controversies over the association between HCV genotype and IVDA in Iranian patients. Similar to the current study, some recent studies reported no association between HCV genotype and IVDA (47, 48). In contrast, another recent study on 142 Iranian viremic patients referred to the Taleghani Hospital in Tehran from 2007 to 2012 revealed an association between genotype 3a and IVDA (22). Nevertheless, no previous studies on the association between HCV genotype and risk factors among Iranian blood donors were available to compare the results. Moreover, former studies showed that IVDA was the main risk factor for infection with HCV genotypes 3a and 1a and blood transfusion was the main risk factor for infection with HCV genotypes 1b and 2 (18-21).

This epidemiological situation was concluded from analyzing the age data in different genotype groups. Patients infected with genotypes 3a and 1a were younger than those infected with genotypes 1b and 2. However, in the current study, no association was observed between distinct HCV genotype and age, or HCV risk factors such as IVDA or blood transfusion. On the other hand, recent studies showed that HCV genotype 3a in IVD abusers and HCV genotype 1a in patients receiving blood or blood products were the most common genotypes (14, 49-52). As shown in Table 2, genotype 3a was not the most frequent genotype just in IVDA donors.

In the current study, multivariable analysis of risk factors by the penalized logistic regression model showed that tattooing was independently associated with HCV genotype 3a with AOR: 2.76 (95% CI: 1.03 - 7.37, P = 0.04). This result was similar to that of the study conducted on patients in France (20), however, it was in contrast to that of a study conducted on patients from some central parts of Iran, which showed the association between tattooing and infection with HCV subtype 1a (48).

In the current study, no other HCV genotypes than 3a, 1a, and 1b were similar to those of the previous studies conducted on Iranian blood donors (14). The current preliminary study evaluated the association between HCV genotype and risk factors in Iranian blood donors. With regards to the same study, the population of study on distribution of HCV genotype among blood donors was included in the evaluation of association between HCV genotypes and risk factors; a subsequent limitation of low sample size was faced in the latter study, which may produce statistical bias to find other independent variables with small effect size correlated with HCV genotype.

5.1. Conclusions

The current study revealed that the predominant subtype was 3a. The results suggested changes in HCV genotype distribution among Iranian blood donors over the last decade. It seems that HCV genotype 1a was replaced with genotype 3a, characterized by an increase in genotype 3a, and decrease in genotype 1a among Iranian blood donors that may be due to indirect role of IVD abusers in the prevalence of HCV subtype 3a infection in the country. Further studies with larger sample size need to support the findings. In addition, tattooing was found to be an independent risk factor for HCV genotype 3a.

Acknowledgments

This article is part of the result of a PhD by research thesis in Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine. We wish to thank the Institute for the financial and moral support. The authors thank their colleagues in blood transfusion centers across the country for data and sample col-

lection. In addition, the authors thank all colleagues for their technical help at the Diagnostic Laboratory of Iranian Blood Transfusion Organization, Tehran, Iran.

Footnotes

Authors' Contribution: Fahimeh Ranjbar Kermani, performing a bulk of the study and manuscript preparation; Sedigheh Amini-Kafiabad and Kamran Mousavi Hosseini, supervising the study; Zohreh Sharifi and Mahtab Maghsudlu, contribution to the study design, interpretation of the data, and drafting of the manuscript; Mohammad Ali Mansournia, contribution to the statistical analyses. All authors revised and approved the final manuscript.

Conflict of Interests: Authors had no conflict of interest.

Funding/Support: The study was supported by High Institute for Research and education in Transfusion Medicine, Research Center of Blood Transfusion, Tehran, Iran.

References

1. Lanini S, Easterbrook PJ, Zumla A, Ippolito G. Hepatitis C: Global epidemiology and strategies for control. *Clin Microbiol Infect*. 2016;**22**(10):833–8. doi: 10.1016/j.cmi.2016.07.035. [PubMed: 27521803].
2. World Health Organization. *Hepatitis C [fact sheet], 2016*. Geneva: World Health Organization; 2016.
3. 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). e42291. doi: 10.5812/hepatmon.42291.
4. Word Healh Organization. *Global status report on blood safety and availability 2016*. Geneva: World Health Organization; 2017.
5. Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from central Africa. *J Clin Microbiol*. 2015;**53**(3):967–72. doi: 10.1128/JCM.02831-14. [PubMed: 25520447]. [PubMed Central: PMC4390628].
6. Smith DB, Pathirana S, Davidson F, Lawlor E, Power J, Yap PL, et al. The origin of hepatitis C virus genotypes. *J Gen Virol*. 1997;**78 (Pt 2)**:321–8. doi: 10.1099/0022-1317-78-2-321. [PubMed: 9018053].
7. 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]. [PubMed Central: PMC5016383].
8. Afridi SQ, Khan N, Akmal M, Ali S, S A, Bahadar S, et al. Distribution of HCV genotypes and RNA viral load along with hemato-biochemical analysis of HCV patients in Rahim Yar Khan, Okara and Toba Tek Singh districts of Punjab, Pakistan. *Hepat Mon*. 2017;**17**(7). e58442. doi: 10.5812/hepatmon.58442.
9. Oral Zeytinli Ü, Muhterem Yücel F, Daldaban Dinçer Ş, Yanilmaz Ö, Aksaray S, Özdil K. Distribution of hepatitis C virus genotypes in the region of İstanbul northern anatolian association of public hospitals. *Viral Hepatit Dergisis*. 2017;**23**(2):64. doi: 10.4274/vhd.27146.
10. 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]. [PubMed Central: PMC5097177].
11. Mahmud S, Al-Kanaani Z, Chemaitelly H, Chaabna K, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C virus genotypes in the Middle East and North Africa: Distribution, diversity, and patterns. *J Med Virol*. 2018;**90**(1):131–41. doi: 10.1002/jmv.24921. [PubMed: 28842995]. [PubMed Central: PMC5724492].
12. Khodabandehloo M, Roshani D. Prevalence of hepatitis C virus genotypes in Iranian patients: A systematic review and meta-analysis. *Hepat Mon*. 2014;**14**(11). e22915. doi: 10.5812/hepatmon.22915. [PubMed: 25685164]. [PubMed Central: PMC4310018].
13. 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]. [PubMed Central: PMC4894080].
14. Sharifi Z, Shooshtari MM, Kermani FR. Identification of HCV genotypes in HCV infected blood donors. *Indian J Microbiol*. 2010;**50**(3):275–9. doi: 10.1007/s12088-010-0059-0. [PubMed: 23100841]. [PubMed Central: PMC3450055].
15. World Health Organization. *Hepatitis C fact sheet*. Geneva: World Health Organization; 2018, [updated April 2017]. Available from: www.who.int/mediacentre/factsheets/fs204/en/.
16. 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].
17. Liakina V, Hamid S, Tanaka J, Olafsson S, Sharara AI, Alavian SM, et al. Historical epidemiology of hepatitis C virus (HCV) in select countries - volume 3. *J Viral Hepat*. 2015;**22**(S4):4–20. doi: 10.1111/jvh.12475.
18. Roman F, Hawotte K, Struck D, Ternes AM, Servais JY, Arendt V, et al. Hepatitis C virus genotypes distribution and transmission risk factors in Luxembourg from 1991 to 2006. *World J Gastroenterol*. 2008;**14**(8):1237–43. doi: 10.3748/wjg.14.1237. [PubMed: 18300350]. [PubMed Central: PMC2690672].
19. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, et al. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J Infect Dis*. 1995;**171**(6):1607–10. doi: 10.1093/infdis/171.6.1607. [PubMed: 7769300].
20. Bourliere M, Barberin JM, Rotily M, Guagliardo V, Portal I, Lecomte L, et al. Epidemiological changes in hepatitis C virus genotypes in France: Evidence in intravenous drug users. *J Viral Hepat*. 2002;**9**(1):62–70. [PubMed: 11851904].
21. Elghouzzi MH, Bouchardeau F, Pillonel J, Boiret E, Tirtaine C, Barlet V, et al. Hepatitis C virus: Routes of infection and genotypes in a cohort of anti-HCV-positive French blood donors. *Vox Sang*. 2000;**79**(3):138–44. doi: 10.1159/000031231. [PubMed: 11111231].
22. Salehi Moghadam F, Mohebbi SR, Hosseini SM, Romani S, Mirtalebi H, Azimzadeh P, et al. Phylogenetic analysis of hepatitis C virus strains and risk factors associated with infection and viral subtypes among Iranian patients. *J Med Virol*. 2014;**86**(8):1342–9. doi: 10.1002/jmv.23947. [PubMed: 24838700].
23. Nakano T, Lu L, Liu P, Pybus OG. Viral gene sequences reveal the variable history of hepatitis C virus infection among countries. *J Infect Dis*. 2004;**190**(6):1098–108. doi: 10.1086/422606. [PubMed: 15319860].
24. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology*. 2005;**42**(4):962–73. doi: 10.1002/hep.20819. [PubMed: 16149085].

25. Cantaloube JF, Laperche S, Gallian P, Bouchardeau F, de Lamballerie X, de Micco P. Analysis of the 5' noncoding region versus the NS5b region in genotyping hepatitis C virus isolates from blood donors in France. *J Clin Microbiol*. 2006;**44**(6):2051–6. doi: 10.1128/JCM.02463-05. [PubMed: 16757597]. [PubMed Central: PMC1489428].
26. Laperche S, Lunel F, Izopet J, Alain S, Deny P, Duverlie G, et al. Comparison of hepatitis C virus NS5b and 5' noncoding gene sequencing methods in a multicenter study. *J Clin Microbiol*. 2005;**43**(2):733–9. doi: 10.1128/JCM.43.2.733-739.2005. [PubMed: 15695672]. [PubMed Central: PMC548094].
27. Rezaei N, Amini-Kafiabad S, Maghsudlu M, Abolghasemi H. Risk factor analysis of hepatitis C virus seropositivity in Iranian blood donors: A case-control study. *Transfusion*. 2016;**56**(7):1891–8. doi: 10.1111/trf.13660. [PubMed: 27273657].
28. Kermani FR, Hosseini KM, Kafi-Abad SA, Maghsudlu M, Sharifi Z, Mansournia MA, et al. Update on transmission modes of hepatitis C virus among volunteer Iranian blood donors: Analysis of a matched case-control study by penalized conditional logistic regression. *Hepatitis Monthly*. 2018;**18**(10). doi: 10.5812/hepatmon.69395.
29. Sandres-Saune K, Deny P, Pasquier C, Thibaut V, Duverlie G, Izopet J. Determining hepatitis C genotype by analyzing the sequence of the NS5b region. *J Virol Methods*. 2003;**109**(2):187–93. doi: 10.1016/S0166-0934(03)00070-3. [PubMed: 12711062].
30. Mansournia MA, Geroldinger A, Greenland S, Heinze G. Separation in logistic regression: Causes, consequences, and control. *Am J Epidemiol*. 2018;**187**(4):864–70. doi: 10.1093/aje/kwx299. [PubMed: 29020135].
31. Greenland S, Mansournia MA, Altman DG. Sparse data bias: A problem hiding in plain sight. *BMJ*. 2016;**352**. i1981. doi: 10.1136/bmj.i1981. [PubMed: 27121591].
32. Greenland S, Mansournia MA. Penalization, bias reduction, and default priors in logistic and related categorical and survival regressions. *Stat Med*. 2015;**34**(23):3133–43. doi: 10.1002/sim.6537. [PubMed: 26011599].
33. van de Laar TJ, Koppelman MH, van der Bij AK, Zaaijer HL, Cuijpers HT, van der Poel CL, et al. Diversity and origin of hepatitis C virus infection among unpaid blood donors in the Netherlands. *Transfusion*. 2006;**46**(10):1719–28. doi: 10.1111/j.1537-2995.2006.00961.x. [PubMed: 17002628].
34. Cantaloube JF, Gallian P, Attoui H, Biagini P, De Micco P, de Lamballerie X. Genotype distribution and molecular epidemiology of hepatitis C virus in blood donors from southeast France. *J Clin Microbiol*. 2005;**43**(8):3624–9. doi: 10.1128/JCM.43.8.3624-3629.2005. [PubMed: 16081888]. [PubMed Central: PMC1233931].
35. 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].
36. Chlabicz S, Flisiak R, Kowalczuk O, Grzeszczuk A, Pytel-Krolczuk B, Prokopowicz D, et al. Changing HCV genotypes distribution in Poland–relation to source and time of infection. *J Clin Virol*. 2008;**42**(2):156–9. doi: 10.1016/j.jcv.2008.02.001. [PubMed: 18353714].
37. Zhou Y, Wang X, Mao Q, Fan Y, Zhu Y, Zhang X, et al. Changes in modes of hepatitis C infection acquisition and genotypes in Southwest China. *J Clin Virol*. 2009;**46**(3):230–3. doi: 10.1016/j.jcv.2009.08.003. [PubMed: 19729342].
38. Butt S, Idrees M, Akbar H, ur Rehman I, Awan Z, Afzal S, et al. The changing epidemiology pattern and frequency distribution of hepatitis C virus in Pakistan. *Infect Genet Evol*. 2010;**10**(5):595–600. doi: 10.1016/j.meegid.2010.04.012. [PubMed: 20438863].
39. Dias PT, Hahn JA, Delwart E, Edlin B, Martin J, Lum P, et al. Temporal changes in HCV genotype distribution in three different high risk populations in San Francisco, California. *BMC Infect Dis*. 2011;**11**:208. doi: 10.1186/1471-2334-11-208. [PubMed: 21810243]. [PubMed Central: PMC3199778].
40. Toyoda H, Kumada T, Takaguchi K, Shimada N, Tanaka J. Changes in hepatitis C virus genotype distribution in Japan. *Epidemiol Infect*. 2014;**142**(12):2624–8. doi: 10.1017/S0950268814000478. [PubMed: 24598252].
41. Chen W, Liao B, Hu F, Nie J, Lan Y, Li H, et al. Changing epidemiology of hepatitis C virus genotype among patients with human immunodeficiency virus/hepatitis C virus co-infection in China. *PLoS One*. 2016;**11**(9). e0161844. doi: 10.1371/journal.pone.0161844. [PubMed: 27603929]. [PubMed Central: PMC5014309].
42. Acero Fernandez D, Ferri Iglesias MJ, Buxo Pujolras M, Lopez Nunez C, Serra Matamala I, Queralt Moles X, et al. Changes in the epidemiology and distribution of the hepatitis C virus genotypes in North-Eastern Spain over the last 35 years. *Gastroenterol Hepatol*. 2018;**41**(1):2–11. doi: 10.1016/j.gastrohep.2017.09.004. [PubMed: 29150360].
43. Li W, Xi L, Cai Q, Kang Y, Zeng Q, Ding R, et al. Changes in the distribution of hepatitis C virus genotypes and their association with shifts in transmission routes in Henan, China. *J Public Health*. 2017;**26**(2):157–62. doi: 10.1007/s10389-017-0845-9.
44. Hajia M, Amirzargar A, Khedmat H, Shahrokhi N, Farzanehkhah M, Ghorishi S, et al. Genotyping pattern among Iranian HCV positive patients. *Iran J Public Health*. 2010;**39**(2):39–44. [PubMed: 23113005]. [PubMed Central: PMC3481755].
45. Jahanbakhsh Sefidi F, 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). doi: 10.5812/hepatmon.7991. [PubMed: 23550108]. [PubMed Central: PMC3582303].
46. Taherkhani R, Farshadpour F. Epidemiology of hepatitis C virus in Iran. *World J Gastroenterol*. 2015;**21**(38):10790–810. doi: 10.3748/wjg.v21.i38.10790. [PubMed: 26478671]. [PubMed Central: PMC4600581].
47. Amini S, Mahmoodi Farahani Majd Abadi M, Joulaie M, Ahmadipour M. Distribution of hepatitis C virus genotypes in Iran: A population-based study. *Hepat Mon*. 2009;**9**(2):95–102.
48. Mokhtari M, Basirkazeruni H, Rostami M. The correlation between different risk factors of hepatitis C and different genotypes. *Adv Biomed Res*. 2017;**6**:45. doi: 10.4103/2277-9175.204588. [PubMed: 28503500]. [PubMed Central: PMC5414405].
49. Farshadpour F, Makvandi M, Samarbafzadeh AR, Jalalifar MA. Determination of hepatitis C virus genotypes among blood donors in Ahvaz, Iran. *Indian J Med Microbiol*. 2010;**28**(1):54–6. doi: 10.4103/0255-0857.58731. [PubMed: 20061766].
50. Ranjbar Kermani F, Sharifi Z, Ferdowsian F, Paz Z, Zamanian M. Distribution of hepatitis C virus genotypes among chronic infected injecting drug users in Tehran, Iran. *Jundishapur J Microbiol*. 2013;**6**(3):265–8. doi: 10.5812/jjm.5191.
51. Ederth J, Jern C, Norder H, Magnius L, Alm E, Rognsvag BK, et al. Molecular characterization of HCV in a Swedish county over 8 years (2002-2009) reveals distinct transmission patterns. *Infect Ecol Epidemiol*. 2016;**6**:30670. doi: 10.3402/iee.v6.30670. [PubMed: 26854010]. [PubMed Central: PMC4744866].
52. Samimi-Rad K, Rahimnia R, Sadeghi M, Malekpour SA, Marzban M, Keshvari M, et al. Epidemic history of hepatitis C virus among patients with inherited bleeding disorders in Iran. *PLoS One*. 2016;**11**(9). e0162492. doi: 10.1371/journal.pone.0162492. [PubMed: 27611688]. [PubMed Central: PMC5017697].