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ORIGINAL ARTICLE

Prevalence of hepatitis C virus genotypes in HIV positive patients referring to the consultation center for behavioral diseases, Sanandaj, Iran

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ABSTRACT

Background and Objectives: Co-infection of hepatitis C virus (HCV) with human immunodeficiency virus (HIV) is increasing due to similar transmission pathways. Chronic HCV infection is the most common complication among HIV-infected individuals. Information on the frequency of HCV infection on Iranian HIV-infected individuals is scarce. The aim of this study was the detection of HCV prevalence and genotypes among HIV-infected people in Sanandaj, Iran.
Materials and Methods: In this cross-sectional study, whole blood samples were taken from 185 HIV positive individuals referring to Consultation Center for Behavioral Diseases, Sanandaj, Iran. The ELISA test was done on samples for anti-HCV antibodies. RNA was extracted from only anti-HCV antibody positive samples. An RT-PCR test was conducted to detect HCV RNA. Genotypes of HCV were detected by melting curve analysis by specific primers and probes. Test results and demographic information were analyzed by SPSS software.
Results: The mean age of individuals was 39.3 ± 9.4 years. Out of 185 individuals 99 (53.5%) were positive for anti-HCV antibodies. Out of 99 antibody positive individuals, 44 had HCV RNA. Among 44 RNA positive individuals, genotypes and subtypes of HCV were as 26 (59.1%) *1a*, 17 (38.6%) *3a* and one (2.2%) *4*. There was a significant association between anti-HCV antibody and demographic variables including, age, gender, occupation, and CD4+ T-cell count (p = 0.0001).
Conclusion: The present study reveals that HIV/HCV co-infection is high in the study population. It is recommended similar studies should be done in other HIV infected populations for management of HIV/HCV co-infection.

Keywords: Hepatitis C virus; Human immunodeficiency virus

INTRODUCTION

Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections are important public health problems worldwide. About 37 million people are infected with HIV and more than 170 million people are infected with HCV in the world (1, 2). Co-infection of HCV with HIV is increasing due to similar transmission pathways. Chronic HCV infection is the most common complication among HIV-infected injecting drug consumers. Globally 2.3 million HIV infected people have serologic evidence of HCV infection (3). Co-infection of HCV/HIV has a significant effect on the life cycle and natural history of HCV infection and vice versa (4). The prevalence of HIV/HCV co-infection in Iran ranged from 58.7% to 83.2%. The most common route of transmission was injection drug use (99.1%) (1, 3).

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HCV is one of the leading causes of viral hepatitis, liver failure, cirrhosis and hepatocellular carcinoma, which has major clinical, epidemiological and economic implications worldwide, especially in developing countries. About 71 million people have chronic HCV infections, worldwide. Chronic HCV hepatitis has resulted in 399,000 deaths every year (3). Due to the lack of vaccines for this virus and the high number of patients with chronic infections, drug therapy is part of the control of the disease (5). The pooled prevalence of HCV was 0.6% among Iranian general population, and it varied among different Iranian provinces ranging from 0.08% to 1.6%. Seroprevalence of HCV in Hormozgan province was the highest, while in Mazandaran province it was the lowest. The overall prevalence of actual viremia (HCV RNA) was 0.4% (6). The prevalence of HCV was 0.24% in rural population of Iran and it was lower compared to other countries in the middle-east (7).

According to a meta-analysis about HCV in Iran, estimated prevalence of HCV was 0.3% among the general population, 6.2% among intermediate risk populations, 32.1% among high risk populations, and 4.6% among special clinical populations. In subpopulations, the estimated prevalence of HCV was 52.2% among people who inject drugs (PWID), 20.0% among high risk healthcare related exposures, and 7.5% among populations with liver-related conditions (8). Another meta-analysis included 15,072 PWID in Iran. The overall prevalence of HCV was 47%. The prevalence ranged from 7 to 96% (9).

HCV has several genotypes that are represented with numbers [1, 2, 3, 4, 5, 6, 7 and 8] and 67 subtypes that are represented with the letters *a, b, c*, etc. (10). Globally, genotype 1 is the most common, accounting for about 46% of all infections, followed by genotype 3, about 22%, and genotypes 2 and 4 each accounting for 13% of the global burden of HCV infection (11). The relative abundance of HCV genotypes varies across the world, depending on geographic location, population, and age. Genotypes *1a* and *1b* are the most common genotypes in North America and Europe; genotypes 5 and 6 are relatively restricted to South Africa and Hong Kong. Genotypes 7, 8 and 9 were identified only in Vietnamese individuals and genotypes 10 and 11 were identified in individuals from Indonesia (12).

According to a recently conducted meta-analysis, genotype 1 was the most predominant strain (58.2%), followed by genotype 3 (39.0%) in Iran (8). Three meta-analyses were performed in Iran, the predominant HCV subtypes were *1a, 3a* and *1b*, respectively. This frequency varies across cities and provinces of Iran (13-15). HCV genotyping is an important tool for predicting treatment outcome, treatment duration, providing information on the antiviral drug type and dose. Although, new antiviral drugs have discovered and are used today, however, knowledge of the virus genotypes is epidemiologically important (1, 3).

Information on the frequency of HCV infection and genotypes in Iranian HIV-positive individuals are scarce, especially in the Kurdistan province. The aim of this study was the detection of HCV prevalence and genotypes among HIV-infected people in Sanandaj, Iran.

MATERIALS AND METHODS

Patients. In this cross-sectional analytical study, whole blood samples were collected from all 185 HIV positive individuals referring to Consultation Center for Behavioral Diseases, Sanandaj, Iran, from 2014 through 2016, without any inclusion and exclusion criteria.

The positivity of HIV in the individuals was routinely defined. HIV detection tests were two screening tests (4th generation ELISA for HIV 1 & 2 Ab-Ag, Pishtazteb, Iran) and a verifying test (HIV Ab & Ag DIA.PRO, Italy). The responsibility of Consultation Center for Behavioral Diseases was routine services for HIV infected patients.

Individuals' information was collected, including age, gender, place of residence, job, HIV infection route and count of CD4+ T lymphocytes. Informed consent obtained from the individuals. Patient information was held confidentially. The present study was approved by the ethic committee at Kurdistan University of Medical Sciences (Ethic code: MUK.REC.1397.1).

Specimens collection. About 5 ml peripheral blood was collected from each HIV positive patient into sterile EDTA-containing tubes. Plasma was separated by centrifugation and stored at -20°C until HCV serologic and molecular tests.

Serologic tests. Forth generation enzyme immunoassay (EIA) was used for the determination of total anti-HCV antibodies (IgM and IgG) in human plasma

using the commercial kit (HCV Ab, DIA.PRO, Italy), according to the manufacturer protocol. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results by using ELISA instrument (AWARENESS TECHNOLOGY Stat Fax 2100 ELISA Microplate Reader).

Ribonucleic acid (RNA) extraction. RNA was extracted from 200 µl of anti-HCV antibody positive plasma samples by RNA extraction kit (QIAamp® DSP Virus Kit, Qiagen, Germany), according to instruction. The extracted RNA samples were stored at -20°C (followed the maintenance procedure, Qiagen) until the RT-PCR test was carried out. HCV RNA positive frozen plasma was obtained from Cellular & Molecular Research Center, Kurdistan University of Medical Sciences, and used as positive control in RNA extraction and RT-PCR test.

Hepatitis C virus RNA detection. Detection of HCV-RNA in the plasma samples was performed by reverse transcriptase PCR kit (QIAGEN® OneStep RT-PCR, Germany). Reagents and primers (specific to 5UTR region of the HCV genome) were provided by Zist Baran (Biorain), Iran, according to the method in the previous study (16). Briefly, the reaction was done in a total volume of 50 µl mixture containing 10 µl of 5X Buffer, 10 µl 5× RT-PCR Buffer, 2 µl dNTPs Mix (containing 10 mM of each dNTP), 0.6 µl forward primer, and 0.6 µl reverse primer, 0.5 µl RNase inhibitor, 2 µl RT-PCR Enzyme Mix, 20 µl RNA template, and 4.3 µl distilled water. The program for cDNA synthesis included 50°C for 30 minutes, and the PCR amplification program was as Taq polymerase activation 95°C 15 minutes, then followed by 45 cycles of denaturation at 94°C 30 seconds, annealing at 57°C 15 seconds, extension at 72°C 30 seconds, and final extension at 72°C for 10 minutes, using Eppendorf A.B thermocycler.

The PCR product separation was done by electrophoresis on 2% agarose gel, 100v 30-60 minutes, stained by SYBR Green, visualized by UV light (Gel transilluminator-BIOView) and photographed.

HCV genotyping. All HCV positive RT-PCR products were tested for HCV genotypes by melting curve analysis, according to the method in the previous study (16). The determination of HCV genotypes was based on the detection of 5UTR region of the HCV genome. Reagents (QIAGEN® OneStep RT-PCR, Germany), primers and probes were provided by Zist Baran (Biorain), Iran. Genotyping was done by Corbett Rotor-Gene 6000 instrument.

Data analysis. The data were entered into the statistical software, SPSS (version 20) and analyzed by relevant statistical tests. P-values less than 0.05 were considered statistically significant.

RESULTS

Demographic information of HIV positive individuals such as age, gender, place of residence, job, HIV infection route and count of CD4+ T lymphocytes is shown in Table 1.

Out of 185 samples, 99 (53.52%) were positive and 86 (46.48%) were negative for anti-HCV antibodies by ELISA test. Out of 99 HCV antibody positive

Table 1. Demographic information of HIV positive patients referring to Consultation Center for Behavioral Diseases, Sanandaj, Iran

Variables		Frequency (percent)
Age (mean ± SD) years	39.26 ± 9.47	
Gender	Women	44 (23.9%)
	Men	140 (76.1%)
Place of residency	Sanandaj	131 (71.2%)
	Kamyaran	10 (5.4%)
	Mariwan	5 (2.7%)
	Saqqez	15 (8.15%)
	Qorveh	7 (3.8%)
	Bijar	8 (4.3%)
	Baneh	4 (2.2%)
	Kermanshah	3 (1.6%)
	Sarvabad	1 (0.5%)
Route of HIV infection	Injection	113 (61.4%)
	Sexual	23 (12.5%)
	Spouse	39 (21.2%)
	Mother to child	6 (3.3%)
	Transfusion	1 (0.5%)
	Unknown	2 (1.1%)
Occupation	Self employed	61 (33.2%)
	Jobless	73 (39.7%)
	Employee	6 (3.3%)
	Housekeeper	39 (21.2%)
	Student	5 (2.7%)
The mean of CD4+ T cell count	475.77 ± 245.44 SD	

samples, 44 (44.5%) were positive for HCV RNA, and 55 (55.5%) were negative by RT-PCR test. Frequency of HCV genotypes and subtypes among 44 HCV RNA positive samples were as 26 (59.09%) subtype *1a*, 17 (38.63%) subtype *3a* and one (2.27%) genotype *4*.

The chi-square test showed a significant difference between positive and negative anti-HCV antibody results in terms of gender, place of residency, occupation and HIV transmission route (p = 0.0001). That is, among the samples that were positive for anti-HCV antibodies, there were more males, residence in Sanandaj and Saqqez, HIV infected individuals by injection route and unemployed (Jobless) persons. The results of the Tukey follow-up test showed a significant difference between HIV transmissions routes. In other word, there was a significant association between injection and HIV infection (p = 0.0001), but no significant difference was observed in the other transmission modes of the HIV infection (Table 2).

The results of the chi-square test showed that the HCV genotypes had no significant relationship with gender, place of residency, HIV transmission route and occupation. Independent samples t-test showed that there was no significant association between age and $CD4^+$ T cell count with HCV genotypes (Table 3).

DISCUSSION

The present study carried out in the Sanandaj, capi-

Table 2. Relationship between demographic variables and anti-HCV antibodies among HIV positive individuals referring to Consultation Center for Behavioral Diseases, Sanandaj, Iran

Variables		Anti-HCV antibody		Total	p-value
		Positive	Negative		
		n = 99	n = 86	n = 185	
Age: Mean ± SD (years)		39.28 ± 8.36	39.26 ± 9.74	39.3 ± 9.46	-
Gender	Women	5 (2.7%)	39 (23.78%)	44 (23.78%)	0.0001
	Men	94 (23.78%)	47 (23.78%)	141 (76.22%)	
Place of Residency	Sanandaj	70 (23.78%)	61 (23.78%)	131 (70.81%)	0.0001
	Kamyaran	3 (1.62%)	7 (23.78%)	10 (5.40%)	
	Marivan	3 (1.62%)	2 (1.08%)	5 (2.70%)	
	Saqez	12 (6.48%)	4 (2.16%)	16 (8.64%)	
	Qorve	4 (2.16%)	3 (1.62%)	7 (3.78%)	
	Bijar	4 (2.16%)	4 (2.16%)	8 (4.32%)	
	Bane	2 (1.08%)	2 (1.08%)	4 (2.162%)	
	Sarvabad	-	1 (0.54%)	1 (0.54%)	
	Kermanshah	1 (0.54%)	2 (1.08%)	3 (1.62%)	
Route of HIV infection	Injection	89 (89.9%)	25 (%)	114 (61.6%)	0.0001
	Sexual	5 (%)	18 (%)	23 (12.4%)	
	Spouse	3 (%)	36 (%)	39 (21.1%)	
	Mother to child	1 (%)	5 (%)	6 (3.2%)	
	Transfusion	1 (%)	-	1 (0.5%)	
	Unknown	-	2 (%)	2 (1.1%)	
Occupation	Self employment	35 (35.4%)	26 (30.23%)	61 (33%)	0.0001
	Jobless	57 (57.6%)	14 (16.28%)	71 (38.9%)	
	Employee	2 (2.02%)	5 (5.81%)	7 (3.8%)	
	Housewife	3 (3.03%)	37 (43.02%)	40 (21.6%)	
	Student	2 (2.02%)	4 (4.65%)	6 (3.2%)	

Table 3. Relationship between demographic variables and HCV RNA and genotypes among Anti-HCV antibody and HIV positive individuals referring to Consultation Center for Behavioral Diseases, Sanandaj, Iran

Variables		HCV RNA		HCV genotypes			P-value
		Negative	Positive	*1a*	*3a*	*4*	
		n = 55	n = 44	n = 26	n = 17	n = 1	
Gender	Women	4	7	6	1	-	0.340
	Men	51	37	20	16	1	
Place of Residency	Sanandaj	38	32	19	12	1	0.450
	Kamyaran	2	1	1	-	-	
	Marivan	-	3	1	2	-	
	Saqez	8	4	3	1	-	
	Qorve	2	2	1	1	-	
	Bijar	4	-	-	-	-	
	Bane	1	1	1	-	-	
	Sarvabad	-	-	-	-	-	
	Kermanshah	-	1	-	1	-	
Route of HIV infection	Injection	46	42	18	11	1	0.071
	Sexual	4	2	2	6	-	
	Spouse	3	-	6	-	-	
	Mother to child	1	-	-	-	-	
	Transfusion	1	-	-	-	-	
	Unknown	-	-	-	-	-	
Occupation	Self employment	22	14	8	5	-	0.416
	Jobless	28	28	11	9	1	
	Employee	1	1	1	2	-	
	Housewife	3	-	6	1	-	
	Student	1	1	-	-	-	

tal city of Kurdistan province in the west of Iran, bordering Iraq. The mean age of people was 39.3 ± 9.4 (range 7-65) years. Out of 185 individuals 99 (53.5%) were positive for anti-HCV antibodies. Among 99 antibody positive individuals 44 (44.5%) were positive for HCV RNA. There was a significant difference between positive and negative anti-HCV antibody results in terms of gender, place of residency, occupation and HIV transmission route (p = 0.0001). Genotypes and subtypes of 44 HCV positive individuals were as 26 (59.1%) subtype *1a*, 17 (38.63%) subtype *3a* and one (2.2%) genotype *4* (Tables 2 and 3).

In a previous study the majority of HIV/HCV co-infected cases were male in Iran (3). The result of the present study is consisted with it.

The prevalence of HCV varies among different population groups in Iran. It is estimated 0.3% in the general populations to 32.1% among high risk populations (8). In Iran, the prevalence of HCV/HIV co-infection was significantly high, and varies according to different regions, 83.2% in Iranian IDUs (1), 58.7% in Ahvaz (3), 44.3% (2), 45.87% in Tehran (17), 78.4% in Shiraz (18). The finding of the present study (53.5%) was consistent with the finding of previous studies. This is because the majority of co-infected individuals were injection drug users (IDUs).

In a study IDUs, imprisonment, tattooing in/out of prison and age were associated with HCV/HIV co-infection in Iran (18). In another study the most (99.1%) of HCV transmission route was IDUs and most of the individuals (97.8%) had a history of being in prison among HIV positive individuals in Ahvaz, Iran (3). In the present study 89/185 (48.1%) of the HIV infection route was IDUs (Table 1), but we did not have any information about tattooing and history of imprisonment in the study population.

Genotypes and subtypes of HCV in Iranian HIV positive individuals were as follows. In decreasing order the HCV subtypes were as *1a* (55.2%), *3a* (35.8%), 3h (4.5%), *1b* (3%) and *4a* (1.5%) in Ahvaz, Iran (3). The genotypes of HCV were subtypes *1a* (44% and 46.2%), *3a* (32.4% and 33.3%), and *1b* (17.6% and 17.9%) in plasma and peripheral blood mononuclear cells (PBMCs). The HCV genotypes in plasma and PBMCs of six HCV/HIV co-infected individuals were different (2). In the present study genotypes and subtypes of HCV among RNA positive individuals were as subtype *1a* (59.1%), subtype *3a* (38.63%) and genotype *4* (2.2%).

Occult HCV infection (OCI) is a new form of chronic HCV infection, explained as the presence of the viral RNA in the liver biopsy and/or PBMCs. It is a form of chronic HCV infection in which viral RNA is undetectable or absent in the plasma, no consideration to the presence or absence of antibodies to HCV (17). In a study the HCV genotypes in the OCI people were as: subtype *1a* (35.7%), subtype *3a* (57.1%), and genotype *4* (7.1%) (1).

In another study in Iran the HCV-RNA in PBMCs was detected in 10.2% of individuals negative for anti-HCV antibodies and undetectable viral RNA in plasma. The viral RNA in PBMCs was detected in 8.0% of the positive individuals for anti-HCV antibodies and undetectable RNA in plasma. Genotypes of HCV were subtype *3a* (60.0%), *1a* (30.0%) and subtype *1b* (10.0%). The prevalence of OCI was 9.2% (17).

In the present study, we only detected HCV RNA and genotypes among anti-HCV positive individuals. In addition, we have not detected OCI or HCV genotypes in PBMCs.

A study included 54 HCV/HIV co-infected and 88 HCV mono-infected individuals as controls in Yazd and Tehran. The mean of HCV RNA load was predominantly higher in HCV/HIV co-infected individuals than in HCV infected individuals. The mean of HCV RNA load in mono-infected with HCV without treatment for HCV was lower than HIV/HCV co-infected with/without highly active antiretroviral therapy. The HCV RNA load was predominantly higher in HIV/HCV (subtype *3a*) co-infected individuals than in HCV (subtype *3a*) mono-infected individuals. HIV RNA load was lower in HCV subtype *1a* infected individuals than in HCV subtype *3a* infected individuals, but the difference was not significant. Mean of ALT was significantly higher in HIV/HCV (genotype *3a*) co-infected individuals than in HCV (genotype *3a*) mono-infected individuals. The authors concluded that HIV/HCV co-infection leads to a significant increase of HCV RNA in plasma (4). In another study genotype *1* was prevalent among co- and mono-infected IDUs, 69% and 49%, respectively in Fars province, Iran. HCV viral load was significantly higher in HIV/HCV co-infected group, in comparison with HCV mono-infected group (19). In the present study, we were unable to compare HCV viral load and genotypes among co- and mono-infected IDUs.

With conventional interferon alpha therapy, genotypes *2* and *3* are more responsive than genotypes *1* and *4*. In new direct-acting antiviral regimens, treatment of genotype *3* is challenging (3). In the present study genotypes of HCV were 26 (59.1%) *1a*, 17 (38.63%) *3a* and one (2.2%) *4*. However, detection of HCV genotypes is critical for appropriate treatment regimen.

The results of the present study are somewhat similar to the results of previous studies conducted on the subject in Iran. The differences in the results may be due to laboratory methods or population. In addition, we did not quantitative HCV RNA load in relation to the patient laboratory data such as liver enzymes, demographic variables and probable antiviral treatments.

With deduction from the results of the present study and the results of the above-mentioned studies, HIV infected individuals should be regularly screened for HCV infection, particularly in high-risk groups such as IDUs. Therefore, implementation of health education, prevention and treatment programs in HCV/HIV co-infected individuals are very important.

In conclusion, the present study reveals that HIV/HCV co-infection is high in the study population. It is recommended that similar studies should be done in other HIV infected populations in un-analyzed regions in Iran for management of HIV/HCV co-infection.

REFERENCES

1. Donyavi T, Bokharaei-Salim F, Khanaliha K, Sheikh M, Bastani MN, Moradi N, et al. High prevalence of occult hepatitis C virus infection in injection drug users with HIV infection. *Arch Virol* 2019 Oct;164:2493-

2504.
2. Dehghani-Dehej F, Sarvari J, Esghaei M, Hosseini SY, Garshasbi S, Kalantari S, et al. Presence of different hepatitis C virus genotypes in plasma and peripheral blood mononuclear cell samples of Iranian patients with HIV infection. *J Med Virol* 2018;90:1343-1351.
3. Teimoori A, Ebrahimi S, Keshtkar N, Khaghani S, Salmanzadeh S, Ghafari S. Prevalence and genetic diversity of HCV among HIV-1 infected individuals living in Ahvaz, Iran. *BMC Infect Dis* 2019 May 8;19:389.
4. Behzadpour D, Ahmadi Vasmehjani A, Mousavi Nasab SD, Ahmadi NA, Baharlou R. Impact of HIV infection in patients infected with chronic HCV (genotypes 1a and 3a): virological and clinical changes. *Pathog Glob Health* 2016;110: 310-315.
5. Lawitz E, Gane E, Pearlman B, Tam E, Ghesquiere W, Guyader D, et al. Efficacy and safety of 12 weeks versus 18 weeks of treatment with grazoprevir (MK-5172) and elbasvir (MK-8742) with or without ribavirin for hepatitis C virus genotype *1* infection in previously untreated patients with cirrhosis and patients with previous null response with or without cirrhosis (C-WORTHY): a randomised, open-label phase 2 trial. *Lancet* 2015;385:1075-1086.
6. 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.
7. Fattahi MR, Safarpour A, Sepehrimanesh M, Hosseini Asl SM, Mohamaddoust F. The prevalence of hepatitis C virus infection and its related risk factors among the rural population of fars province, southern iran. *Hepat Mon* 2015;15(2): e24734.
8. 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.
9. Behzadifar M, Bragazzi NL. A systematic review and meta-analysis of the prevalence of hepatitis C virus infection in people who inject drugs in Iran. *BMC Public Health* 2020;20:62.
10. Athar MA, Xu Y, Xie X, Xu Z, Ahmad V, Hayder Z, et al. Rapid detection of HCV genotyping *1a, 1b, 2a, 3a, 3b* and *6a* in a single reaction using two-melting temperature codes by a real-time PCR-based assay. *J Virol Methods* 2015;222:85-90.
11. Nyan DC, Swinson KL. A method for rapid detection and genotype identification of hepatitis C virus 1-6 by one-step reverse transcription loop-mediated isothermal amplification. *Int J Infect Dis* 2016;43:30-36.
12. Daw MA, El-Bouzedi A, Dau AA. Geographic distribution of HCV genotypes in Libya and analysis of risk factors involved in their transmission. *BMC Res Notes* 2015; 8:367.
13. 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.
14. 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.
15. 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.
16. Bullock GC, Bruns DE, Haverstick DM. Hepatitis C genotype determination by melting curve analysis with a single set of fluorescence resonance energy transfer probes. *Clin Chem* 2002;48:2147-2154.
17. Bokharaei-Salim F, Keyvani H, Esghaei M, Zare-Karizi S, Dermenaki-Farahani SS, Hesami-Zadeh K, et al. Prevalence of occult hepatitis C virus infection in the Iranian patients with human immunodeficiency virus infection. *J Med Virol* 2016;88:1960-1966.
18. Alipour A, Rezaianzadeh A, Hasanzadeh J, Rajaeefard A, Davarpanah MA, Hasanabadi M. High prevalence of HCV coinfection in HIV-infected individuals in Shiraz, Islamic Republic of Iran. *East Mediterr Health J* 2013;19:975-981.
19. Jamalidoust M, Namayandeh M, Moghadami M, Ziyaeyan M. Comparison of HCV viral load and its genotype distributions in HCV mono- and HIV/HCV co-infected illicit drug users. *Virol J* 2017;14:127.