Hepatitis Monthly 2009; 9(2): 122-127

ORIGINAL ARTICLE

Hepatitis C Virus Genotype Distribution in Shiraz, Southern Iran

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Background and Aims: **Approximately 2-3% of the world population are infected with hepatitis C virus (HCV), which is the single most common indication for liver transplantation. This study aimed to determine the hepatitis C viral genotypes in HCV monoinfected and human immunodeficiency virus (HIV)/HCV co-infected patients in southern Iran.**

Methods: **During 2004-2005, 273 patients who were infected with HCV were enrolled. Out of them, 250 subjects accepted blood sampling, and RNA extraction was undertaken for 238 subjects. Specific and nested polymerase chain reaction (PCR) - restriction fragment length polymorphisms (RFLPs) were performed to determine viral infection and genotype analysis. Liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the correlated risk factors were also determined.**

Results: **HCV-RNA was detected in 238 subjects. 50 had HIV/HCV co-infection, among whom 88%, 92% and 56% had positive a history of intravenous drug use, being in prison, and tattooing, respectively. 188 subjects showed HCV mono-infection, among whom 14.36% had major thalassemia and 37.76%, 16.48%, 3.72%, 2.65% and 29.25% had a history of being in prison, dental visits, hemophilia, hemodialysis and tattooing, respectively. Of 50 subjects with HIV/HCV co-infection, 40% were genotype la, 34% genotype 3a, and 26% genotype 1b, and one of the patients had mixed infection with 1b/3a. Among 188 subjects with HCV mono-infection, 44.1% were genotype la, 42.0% genotype 3a, and 13.8% genotype 1b. ALT and AST levels in all genotypes were compared.**

Conclusions: **This study showed that Genotype 1a, followed by 3a and 1b, were the most prevalent types in both groups. No other genotypes were detected, but mixed infection with 3a/1b was observed in one of the HIV/HCV co-infected patients. HCV genotype had no correlation with mode of transmission or liver enzyme abnormalities.**

Keywords: **HCV, Genotype, HIV, Iran**

Introduction

Hepatitis C is a worldwide infection affecting more than 170 million people around the world. Most of these patients are asymptomatic carriers, but in chronic cases, infection is established in 85% of the infected individuals. The chronic stage evolves to severe liver disease in almost 20% of the infected individuals [(1)]. Consequently, hepatitis C is frequently diagnosed in advanced clinical stages or when asymptomatic carriers present themselves as blood-donor candidates [(1)]. Approximately 2-3% of the world populations are infected with Hepatitis C virus (HCV) and it is one of the most common causes of liver failure and cancer, and the single most common indication for liver transplantation [(2, 3)]. In

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Received: *20 Jan 2008* ***Revised:*** *11 Mar 2008*
Accepted: *10 Aug 2008*

Hepat Mon 2009; 9 (2): 122-127

Iran, the prevalence of HCV infection in blood donors is about 0.12% [4]. It seems that the prevalence of HCV infection is less than 1% in our general population, but is increasing mostly because of problems such as intravenous drug use and needle sharing among drug addicts. In Iran, HCV infection is the most prevalent cause of chronic hepatitis and cirrhosis in special groups such as hemophiliac [5], thalassemic [6], and renal failure patients [7]. Different HCV isolates showed substantial nucleotide sequence variability throughout the viral genome [8] and six major genotypes [1-6] and over 50 proposed subtypes (e.g., a, b, c) have been identified worldwide [9]. These viral types and subtypes differ in their geographical distribution and antigenicity [8]. Types 1, 2 and 3 have worldwide distribution [10, 11], while types 4, 5 and 6 were found in distinct geographical areas [11, 12]. Interestingly, not only do the HCV genotypes seem to differ in nucleotide sequence and geographical distribution, but there is also evidence of biological differences between the three HCV genotypes. Patients with HCV subtype 1b have a poorer response to interferon alpha treatment [13, 14]. Patients infected with genotypes 1 and 4 with viral loads higher than 800,000 IU/ml must be treated for one year, while those infected with other genotypes may be treated for only 6 months [15, 16]. Therefore, genotyping is an important tool for prognosis and follow-up of infected patients. Viral genotype determination before treatment is currently advised as a routine assay. Mode of transmission may also affect distribution of HCV genotypes [17, 18]. Genotyping of HCV-RNA positive serum samples from 155 hemodialysis patients in Tehran, Iran demonstrated that subtypes 3a and 1a were predominant accounting for 30.3% and 28.8%, respectively. The distribution of other HCV genotypes showed 18.2% for genotype 1b; 16.7% for genotype 4, 3% for mixed genotypes 1a and 1b, and 3% for genotype 3b. Genotype 2 was not detected in this study [19]. The effect of HCV in the course of Human Immunodeficiency Virus (HIV) infection and vice versa has been extensively studied, but still remains highly controversial. This may be due to HCV genetic heterogeneity. During one study in Puerto Rico using Inno-LiPa II technique, they analyzed samples from 171 HCV-HIV1-coinfected intravenous drug users and 375 subjects from a general HCV population of unknown HIV or source of infection status. Similar HCV genotype distribution was detected in this population. HCV genotype 1a was the most frequent in intravenous drug users co-infected with HIV-1, followed by 1b and 3a. Twenty mixed infections and 5 undetermined genotypes were reported [20].

Since prevalence of HIV is high in Shiraz and many HCV positive patients from neighboring cities and provinces are referred to clinics in Shiraz, knowledge of HCV genotype distribution in HCV monoinfected and HCV-HIV coinfected individuals in this area is important in planning the future therapeutic measures. Therefore, this study was designed with the aim of contributing to this knowledge.

Patients and Methods

During 2004-2005, 223 HCV-monoinfected patients who referred to Nemazee Hospital affiliated to Shiraz University of Medical Sciences in southern Iran entered our study. Also, 50 HIV-HCV coinfected patients who voluntarily referred to Fars Counseling and Behavioral Modification Center for counseling and health care were enrolled in the study. All HCV infections were documented by enzyme-linked immunosorbent assay ELISA (Ortho Kit, Chiron Company) and polymerase chain reaction (PCR) while HIV infection was confirmed by serial ELISA (Bio-Rad, France) and western blot (Gene Lab, Singapore) tests. Patients were informed about the study and a written consent was obtained from each patient. The study protocol was approved in the ethics committee of our department. Among patients, 250 subjects accepted to have blood sampling. Serum samples were provided and kept at -70°C for further study on liver function tests (LFTs) and genotype analysis. RNA extraction was undertaken for 238 subjects, including 50 HIV positive and 188 HIV negative individuals, using the standard method. RNA extraction was impossible in 12 serum samples. The risk factors and liver enzymes were determined.

PCR genotyping primers

For specific and nested PCRs, four oligonucleotide primers from 5′ noncoding regions were provided from Seq Laboratory (Germany). In the first PCR cycle, the primers corresponding to HCV-1 sense were hybridized as nucleotide 268-251 F (AGCGTCTAGCCATGGCGT). Numbered and antisense nucleotides were arranged as 22 R (GCGGTCTACGAGACCT).

In the second cycle, the primers corresponding to sense-oriented nucleotides were 199-183 FN (GTGGTCTGCGGAASSGG) and antisense nucleotides were 26-43 RN

(GGGCACTCGCAAGCACCC).

PCR reagent I, which was composed of 50 mM of KCl, 1.5 mM MgCl2, 10 mM Tris-HCl (pH=9.0) and 1 x Taq DNA polymerase buffer (Fermentas), 200 µM of each of four dNTPs, 0.4 µM of each of the first cycle primers, and 1 U of Taq DNA polymerase (Fermentas) were prepared for the first cycle of amplification. The amplification procedure involved denaturation at 94°C for 3 minutes, followed by 35 cycles of amplification for 45 sec each at 94°C, 58°C and 72°C, respectively. PCR reagent II, which was composed of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH=9.0), and l x Taq DNA polymerase buffer (Fermentas), 200 M of each of the four dNTPs, 0.4 µM of each of the second cycleprimers, and 1 U of Taq DNApolymerase (Fermentas Co.) were prepared for the second cycle of amplification. The amplification procedure consisted of predenaturation at 94 °C for 3 min, 25 cycles for 1 min and then at 94°C, 65°C, and 72°C for 4 min. 15 µl out of 25 µl of specific PCR and nested PCR products were analyzed by horizontal gel electrophoresis on 2% agarose gel at 100 V/50 min. A single, 264 bp and 174 hp bands were visible in the gel under ultraviolet light, respectively.

The total volumes of nested-PCR products (25 µl) were divided into three tubes containing appropriate buffers. The restriction enzymes (Fermentas Co.) consisted of Apa I/Hinf I, EcoRII/ Hinf I, bash 1236 I. The other enzymes were similar to Mcomish *et al.*, 1993 method. The tubes were incubated with l U of the enzymes for 3 h and the digestion temperature was 37°C. If the samples could not be analyzed immediately after digestion, they were stored at -20°C before the analysis by vertical 12% polyacrilamide gel electerophoresis and the digested products were heated for 5 min. Ethidium bromide was used for staining of DNA fragments and identification under ultraviolet light.

Marker of 100 bp plus fermentase and undigested PCR products were included in every analysis and the genotype was deduced from the fragmentation pattern of the amplified DNA.

Results were expressed as absolute and relative frequencies. Frequencies and variable association were compared by the Chi-Square test. The statistical tests were performed using the SPSS software (version 15, Chicago, IL, USA) and a p value less than 0.05 was considered significant.

Results

HCV-RNA was extracted in 238 subjects, among whom 50 had HIV/HCV co-infection with an age range of 21-51 years old (median=39 years) while 64% were male and 36% were female. Among the HIV/HCV co-infected subjects, 88% (44/50) were intravenous drug users, 92% (46/50) had a history of being in prison, and 56% (28/50) had experienced tattooing. 188 cases, who were 7 to 72 years old (median= 35 years), showed HCV mono-infection while 78.7% were male and 21.3% were female. Among the HCV mono-infected subjects, 14.36% (27/188) were major thalassemic patients with recurrent blood transfusion, 37.76% (71/188) had a history of being in prison, 16.48% (31/188) had a history of dental visits, 3.72% (7/188) were hemophiliac, and 2.65% (5/188) and 29.25% (55/188) had a history of hemodialysis and tattooing, respectively.

On genotype analysis, out of 50 subjects with HIV/HCV co-infection, 40% were type la, 34% type 3a, and 26% type 1b, and one of the patients had mixed infection with 1b/3a (Table 1). Genotype 1a was the most common type in subjects with a history of drug injection, being in prison and tattooing. There was no statistically significant association between mode of transmission and HCV genotype (P>0.05). Table 1 also shows that out of 188 subjects with HCV mono-infection, 44.1% were type la, 42.0% type 3a, and 13.8% type1b. Genotype 3a was the most common type in patients with a history of prison, tattooing, and hemodialysis, while genotype 1a was the most common type in other high risk groups. No statistically significant difference was seen in relation to the prevalence of HCV genotypes between HIV/HCV co-infected and HCV mono-infected subjects (P>0.05).

Table 1. Genotype distribution in HCV positive patients according to their HIV infection status.

			HIV		Total
			Negative	Positive	
Genotype	3a	Count	79 (42.0%)	17 (34.0%)	96 (40.3%)
	1a	Count	83 (44.1%)	20 (40.0%)	103 (43.3%)
	1b	Count	26 (13.8%)	13 (26.0%)	39 (16.4%)
Total		Count	188 (100.0%)	50 (100.0%)	238 (100.0%)

χ^2=4.35, df=2, P value=0.133

Tables 2 and 4 demonstrate AST levels according to HCV genotype in HIV/HCV co-infected and HCV mono-infected individuals. There was no significant association between AST level and HCV genotype in either group (P>0.05). Similarly, no significant correlation was found between ALT level and HCV

Table 2. AST levels in HIV/HCV co-infected individuals according to HCV genotype.

			AST			Total
			<42	43-62	>63	
Genotype	3a	Count	5 (29.4%)	5 (29.4%)	7 (41.2%)	17 (100.0%)
	1a	Count	10 (50.0%)	4 (20.0%)	6 (30.0%)	20 (100.0%)
	1b	Count	9 (69.2%)	1 (7.7%)	3 (23.1%)	13 (100.0%)
Total		Count	24 (48.0%)	10 (20.0%)	16 (32.0%)	50 (100.0%)

χ^2=4.995, df=4, P value=0.288

Table 3. ALT levels in HIV/HCV co-infected individuals according to HCV genotype.

			ALT			Total
			<42	43-62	>63	
Genotype	3a	Count	9 (52.9%)	3 (17.6%)	5 (29.4%)	17 (100.0%)
	1a	Count	17 (85.0%)	3 (15.0%)	0 (0.0%)	20 (100.0%)
	1b	Count	9 (69.2%)	2 (15.4%)	2 (15.4%)	13 (100.0%)
Total		Count	35 (70.0%)	8 (16.0%)	7 (14.0%)	50 (100.0%)

χ^2=7.097, df=4, P value=0.131

Table 4. AST levels in HCV mono-infected subjects according to HCV genotype.

			AST			Total
			<42	43-62	>63	
Genotype	3a	Count	36 (52.9%)	13 (19.1%)	19 (27.9%)	68 (100.0%)
	1a	Count	53 (45.2%)	12 (16.4%)	28 (38.4%)	72 (100.0%)
	1b	Count	9 (42.9%)	3 (14.3%)	9 (42.9%)	21 (100.0%)
Total		Count	78 (48.1%)	28 (17.3%)	56 (34.6%)	162 (100.0%)

χ^2=2.84, df=4, P value=0.584

Table 5. ALT levels in HCV mono-infected subjects according to HCV genotype.

			ALT			Total
			<42	43-62	>63	
Genotype	3a	Count	32 (47.1%)	12 (17.6%)	24 (35.3%)	68 (100.0%)
	1a	Count	25 (34.7%)	8 (11.1%)	39 (54.2%)	72 (100.0%)
	1b	Count	8 (38.1%)	6 (28.6)	7 (33.3%)	21 (100.0%)
Total		Count	65 (40.4%)	26 (16.1%)	70 (43.5%)	162 (100.0%)

χ^2=8.019, df=4, P value=0.091

genotype in either group (P>0.05) (Table 3 & 5).

Discussion

The study of viral diversity provides a better understanding of the origin and dynamics of viral infections. Genetic variants of HCV are known to be widely spread around the world. Genotypes 1, 2 and 3 are found on all continents, but in some geographical areas, such as Africa and Southeast Asia, viral isolates are highly divergent and particular genotypes or subtypes are predominant [21, 22]. These data suggest the existence of a long term endemic infection in these areas and some investigators have hypothesized that HCV might have originated in such places [23].

Our study showed that Genotype 1a followed by 3a and 1b were the most prevalent types in both groups (HCV mono-infected and HIV/HCV co-infected patients). No other genotypes were seen, but mixed infection with 3a/1b was observed in one of the HIV/HCV co-infected patients. HCV genotype had no correlation with mode of transmission or liver enzyme abnormalities. During one study, a significant difference in the epidemic behavior of different HCV genotypes was found [24]. Genotypes 1 and 3 spread rapidly before blood donor screening methods were adopted, while infections with genotypes 4 and 6 followed a pattern of community-acquired diseases by an undefined social and domestic route. In addition, genotyping provides information about strain variation and potential association with disease severity. In our study, genotypes 1a (44.1%), 3a (42%), and 1b (13.8%) were the most prevalent types in HCV mono-infected individuals. Mixed infection and other genotypes were not seen in these patients. There was no significant association between the mode of transmission and HCV genotype. One study in Iran on 156 patients showed a major prevalence of HCV genotype 1 in 87 (55.8%) cases followed by genotype 3 (28.8%), and genotype 4 (1.3%). One patient (0.6%) had mixed infection with genotypes 1 and 3 and genotyping was impossible in 21 patients. There was no statistically significant association between habitat and genotype. However, genotype 4 was found only in north and west of country and mixed infection with genotype 1a and 1b only in central parts. In this study, the dominant genotypes in different regions of Iran were 1a, 1b and 3a in central and western areas, 1a and 3a in northern parts and 1a in southern and eastern regions [25]. In another study in Iranian patients with HCV infection from Tehran

and 5 other cities showed that genotype 1a was predominant (47%), followed by 3a (36%), 1b (8%) and 4 (7%) [26]. Our results were similar to these two studies from Iran. Also the patterns of our genotypes are similar to those of England [27], but different from other Middle East countries such as Republic of Yemen, Kuwait, Iraq, and Saudi Arabia, where genotype 4 is the most prevalent one [28]. HCV genotype 4 was detected in 50% and genotype 1b was found in nearly 40% of Saudi patients [29, 30].

In our study, genotypes 1a (40%), 3a (34%), 1b (26%) were the most prevalent types in HIV/HCV co-infected patients. Also mixed infection by genotypes 3a/1b was seen in one of these patients but other genotypes were not observed. In this group, there was no significant association between the mode of transmission and HCV genotype. Also no statistically significant difference was seen in relation to prevalent genotypes between HIV/HCV co-infected and HCV mono-infected individuals. To the best of our knowledge, no investigation was available on HIV/HCV co-infected individuals in Iran. In one study, HCV was evaluated in 134 consecutive patients with evidence of HIV infection, living in Campania, Italy and data were compared with those obtained from 252 HCV infected patients without evidence of HIV infection. The prevalence of HCV infection in HIV patients was 19.40% and the largest group of HIV/HCV co-infected patients (84.62%) was present in the intravenous drug users (IVDU). The distribution of HCV genotypes in HIV/HCV patients was different, compared to that observed in HCV control group. HCV genotypes 1a (50%) and 3a (23.08%) were more frequently detected in HIV/HCV patients, compared to HCV control group (5.16 and 5.56% for 1a and 3a, respectively). Conversely, HCV genotypes 1b (55.70%) and 2a/2c (30.26%) were more frequently present in HCV control group, compared to HIV/HCV patients (15.38 and 0% for 1b and 2a/2c, respectively) [31].

In our cross-sectional survey, due to limited duration of the study, the sample size was small. We conclude that similar studies as Keivani *et al.* article [32] are needed in different parts of Iran to determine the most common genotypes in various parts of the country. We demonstrated the predominance of 1a, 3a and 1b genotypes in our area which helps the health authorities for future planning.

Acknowledgements

The authors would like to thank the Office of Vice-Chancellor for Research of Shiraz University of Medical Sciences and Shiraz University for financial support and the Center for Development of Clinical Studies for typing assistance.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med.* 2001;**345**(1):41-52.
2. Rosen HR, Martin P. Viral hepatitis in the liver transplant recipient. *Infect Dis Clin North Am.* 2000;**14**(3):761-84.
3. Alavian SM, Gholami B, Masarrat S. Hepatitis C risk factors in Iranian volunteer blood donors: a case-control study. *J Gastroenterol Hepatol.* 2002;**17**(10):1092-7.
4. Alavian SM, Ardeshir A, Hajarizadeh B. Seroprevalence of anti-HCV among Iranian Hemophilia Patients. *Transfusion Today-Groningen.* 2000;**49**:4-5.
5. Alavian SM, Kafaee J, Yektaprast B, Hajarizadeh B, Doroudi T. The efficacy of blood donor screening in reducing the incidence of hepatitis C virus infection among thalassemic patients in Iran. *Transfusion Today-Groningen.* 2002:3-4.
6. Alavian SM, Einollahi B, Hajarizadeh B, Bakhtiari S, Nafar M, Ahrabi S. Prevalence of hepatitis C virus infection and related risk factors among Iranian haemodialysis patients. *Nephrology (Carlton).* 2003;**8**(5):256-60.
7. Okamoto H, Okada S, Sugiyama Y, *et al.* Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. *J Gen Virol.* 1991;**72**(Pt 11):2697-704.
8. Okamoto H, Kurai K, Okada S, *et al.* Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. *Virology.* 1992;**188**(1):331-41.
9. Takada N, Takase S, Takada A, Date T. HCV genotypes in different countries. *Lancet.* 1992;**339**(8796):808.
10. Bukh J, Purcell RH, Miller RH. At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. *Proc Natl Acad Sci U S A.* 1993;**90**(17):8234-8.
11. Cha TA, Beall E, Irvine B, *et al.* At least five related, but distinct, hepatitis C viral genotypes exist. *Proc Natl Acad Sci U S A.* 1992;**89**(15):7144-8.
12. Takada N, Takase S, Enomoto N, Takada A, Date T. Clinical backgrounds of the patients having different types of hepatitis C virus genomes. *J Hepatol.* 1992;**14**(1):35-40.
13. Kanai K, Kako M, Okamoto H. HCV genotypes in chronic hepatitis C and response to interferon. *Lancet.* 1992;**339**(8808):1543.
14. EASL International Consensus Conference on hepatitis C. Paris, 26-27 February 1999. Consensus statement. *J Hepatol.* 1999;**31 Suppl 1**:3-8.
15. Zylberberg H, Chaix ML, Brechot C. Infection with hepatitis C virus genotype 4 is associated with a poor response to interferon-alpha. *Ann Intern Med.* 2000;**132**(10):845-6.
16. McOmish F, Chan SW, Dow BC, *et al.* Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. *Transfusion.* 1993;**33**(1):7-13.
17. Qu D, Li JS, Vitvitski L, *et al.* Hepatitis C virus genotypes in France: comparison of clinical features of patients infected with HCV type I and type II. *J Hepatol.*

1994;**21**(1):70-5.
18. Driesel G, Wirth D, Stark K, Baumgarten R, Sucker U, Schreier E. Hepatitis C virus (HCV) genotype distribution in German isolates: studies on the sequence variability in the E2 and NS5 region. *Arch Virol.* 1994;**139**(3-4):379-88.
19. Hosseini-Moghaddam SM, Keyvani H, Kasiri H, *et al*. Distribution of hepatitis C virus genotypes among hemodialysis patients in Tehran--a multicenter study. *J Med Virol.* 2006;**78**(5):569-73.
20. Rios-Olivares E, Yamamura Y, Gomez MA, *et al*. HCV genotype analysis in HCV-HIV-co-infected Puerto Ricans who are injecting drug users: undetermined and mixed infections. *Cell Mol Biol (Noisy-le-grand).* 2001;**47**(6):1017-24.
21. Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. *Semin Liver Dis.* 1995;**15**(1):41-63.
22. Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group. *J Gen Virol.* 1995;**76** (Pt 10):2493-507.
23. Simmonds P. The origin and evolution of hepatitis viruses in humans. *J Gen Virol.* 2001;**82**(Pt 4):693-712.
24. Pybus OG, Charleston MA, Gupta S, Rambaut A, Holmes EC, Harvey PH. The epidemic behavior of the hepatitis C virus. *Science.* 2001;**292**(5525):2323-5.
25. Kabir A, Alavian SM, Keyvani H. Distribution of hepatitis C virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters: a preliminary study. *Comp Hepatol.* 2006;**5**:4.
26. Samimi-Rad K, Nategh R, Malekzadeh R, Norder H, Magnius L. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. *J Med Virol.* 2004;**74**(2):246-52.
27. Harris KA, Gilham C, Mortimer PP, Teo CG. The most prevalent hepatitis C virus genotypes in England and Wales are 3a and 1a. *J Med Virol.* 1999;**58**(2):127-31.
28. Ohno T, Mizokami M, Saleh MG, *et al*. Usefulness and limitation of phylogenetic analysis for hepatitis C virus core region: application to isolates from Egyptian and Yemeni patients. *Arch Virol.* 1996;**141**(6):1101-13.
29. Al-Knawy B, Okamoto H, Ahmed El-Mekki A, *et al*. Distribution of hepatitis C genotype and co-infection rate with hepatitis G in Saudi Arabia. *Hepatol Res.* 2002;**24**(2):95.
30. Osoba AO. Hepatitis C virus genotypes in Saudi Arabia. *Saudi Med J.* 2002;**23**(1):7-12.
31. Rendina D, Vigorita E, Bonavolta R, *et al*. HCV and GBV-c/HGV infection in HIV positive patients in southern Italy. *Eur J Epidemiol.* 2001;**17**(9):801-7.
32. Keyvani H, Alizadeh AH, Alavian SM, Ranjbar M, Hatami S. Distribution frequency of hepatitis C virus genotypes in 2231 patients in Iran. *Hepatol Res.* 2007;**37**(2):101-3.