

Frequency Distribution of Hepatitis C Virus (HCV) Genotypes and its Association with Viral Loads in Chronic HCV Infected Patients of Isfahan, Iran

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(Received: 21 March 2014; accepted: 29 April 2014)

The level of disease proceeding are related to different factors, for example, genotype/subtype and viral load. Six important genotypes of HCV and multiplex subtypes have been determined worldwide, HCV Genotyping is considerable clinical option before planning therapy. The proof is that various genotypes of HCV will need diverse term and of course the anti-viral treatment. The purpose of the current study was to assign the dispensation sample of different genotypes of HCV showed in the research reign and their relationship with the viral load in patients suffering suffer from HCV. Patients having positive serology for HCV were chosen for current research (N=105). After that, viral load and genotyping anticipation were carried out utilizing Real-time PCR 59.05 percent (62/105) of samples were contaminated with genotype 3a and consequently genotype 1a and genotype 1b were detected in 13.33percent (14/105) and 8.57percent (9/105) of samples respectively. However, genotype 3 didn't deal with a prominently higher viral load in comparison with the other genotypes ($P=0.113$). Nevertheless, the prominently higher viral load was detectable among male HCV patients ($P=0.012$) in contrast to women infected with HCV The current research shows that genotype of HCV 3 computed mostly for almost 70.47 percent of the HCV septicity in Isfahan city. Moreover, genotype 3 was not relevance with more extremity of liver disease in contrast to other genotypes as estimated by viral load.

Key words: HCV, Genotyping, Viral load, Real time PCR.

Hepatitis C virus, an important proof of chronic liver disease can progress to cirrhosis with the developing peril of hepatocellular carcinoma^{1,2}. Nearly more than 170 million individuals are infected globally, but in Iran, it seems that its outbreak is less than 1%, in fact, it is lower than in most of the zonal countries^{3,4}. The level of disease succession relies on different features as important

prophesier of the succession result like genotype/subtype and viral load¹. All six essential genotypes of HCV and multiplex subtypes have been specified all over the world⁵. Genotyping for HCV is essential, clinically before programming therapy because different types of genotypes of HCV need varied continuity and the amount of anti-viral therapy. Different samples for HCV dispensation were discovered tilldate⁶. In some zones, only a solitary subtype appeared meanwhile in other zones, high genetic heterogeneity dispensed^{7,8}. On the other hand, viral load has been recorded as a prognostic feature as a response

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to antiviral treatment in a reverse relevance^{9,10}. Therapeutic impression is related entirely to lower pre-therapy viral load (< 800'000) in contrast to higher levels (> 800'000)^{11,12}. Also, chronic hepatitis C and viral load in persons with increasing serum alanine transaminase (ALT) levels perhaps have clinical correlation¹³. In this way, genotype of HCV, viral load and viral load reduction in early treatments plays pivotally in adapting and removing antiviral therapy¹⁴. In Iran, a limited research has been carried out as far as the correlation between pre-therapy viral load and genotypes of HCV is concerned. Thus, the current research was carried out considering the dispensation sample of HCV genotypes in Isfahan patients suffering from chronic hepatitis and their correlation with viral load.

METHOD

Patients

Patients with positive HCV serology (N=105) were chosen for HCV genotyping. The samples were obtained from patients of various health centers located in Isfahan, Iran, from 2011 to 2013. Each patient provided information in written form about his or her demographic feature, age, zone and infection evaluation time. The risk factors in the patients were divided sequentially as follows: drug injection abusers, thalassemia and hemophiliac sufferers, hemodialysis patients, individuals with unsafe sexual contacts and finally individuals with unknown risk factors. A written consent form was signed by the patients. The ethical committee and internal review board of the Zist Partake institution approved the protocol. The existence of anti-HCV antibodies in the sample was detected by an enzyme immunoassay according to the manufacturer's instructions (DIA. PRO. Diagnostic Bioprobes Srl, Italy)

RNA extraction and cDNA synthesis

Viral RNA was extracted from HCV positive serum samples in the protocol prepared by the commercial High Pure Viral RNA Kit (Roche Diagnostic GmbH, Mannheim, Germany). In all the above samples, using elution buffer, RNA was separated from the column. Then, 10 μ l (about 50 ng) of the extracted RNA was utilized for cDNA synthesis using 100 U of M-MuLV Reverse Transcriptase (Ferment as GmbH) and random

hexamer primers at 37°C for 50 minutes. The RTEs were modified at 96°C for 5 minutes, and 5 μ l of this cDNA were suggested for the Real Time PCR response.

HCV genotyping and quantitation of HCV-RNA

Quantitation of HCV-RNA in serum was performed on all patients' serum samples utilizing Real Time PCR (primers and RT-PCR reagents from Strata gene, Qiagen, USA according to producer's directions)¹⁵ Low viral load was signified as lower than 100 $\times 10^3$ IU/L, average signified as equal to 100-1000 $\times 10^3$ IU/L, and high viral load was signified as higher than 1000 $\times 10^3$ IU/L¹⁶. For all the modules genotyping of HCV was performed utilizing molecular genotyping of HCV ways¹⁷. Two μ l of cDNA was utilized for the development of 470-bp region from the HCV 5'NCR+Core zone in first round PCR. Each first round PCR module was related to two second-rounds nested PCR developments. It was primarily carried out with mix-A primers and secondarily with mix-B primers in a response content of 20 μ l. Mix-A had genotype-specific primers for 1a, 1b, 1c, 3a, 3c and 4 genotypes and mix-B included genotype-specific primers for 2a, 2c, 3b, 5a, and 6a genotypes. The second round PCR proceeds were electrophoresed on a 2% agarose gel, then, stained with ethidium bromide and was observed under UV Tran illuminator. A 100-bp DNA ladder (Invitrogen, Corp., California, and USA) carried out in pergel as a DNA size marker.

Statistical analysis

The obtained information was resolute utilizing SPSS win integrates a statistical package sample (SPSS Inc., Chicago, IL). For scale data, resemblance between two groups was done utilizing Mann-Whitney test. A P value < 0.05 was considered significant.

RESULTS

Out of total 105 HCV positive patients, 87 (82.9 %) were males and 18 (17.1%) were females (Table 1). There were 41 (39.04%) drug-injection patients, 12 (11.43%) patients with multiple sexual partners, 7 (6.66%) thalassemia sufferers, 4 (3.81%) hemophiliacs and 3 (2.86%) and hemodialysis patients 38 (36.19%). The patients responded in a form to the queries related to other minor risk factors like injuries, surgeries, Frequency breakup

of the HCV positive patients were 14(13.33%), 9(8.57%), 3(2.86%), 62 (59.05%), 4(3.81%) and 5(4.76%) genotypes of 1a, 1b, 2a, 3a, 3b, and unknown respectively. 8(7.62%) patients were

infected with mixed HCV genotypes. 5 (4.76%) patients were infected with the genotypes 1a₊3a; and 3(2.86%) by 1a₊ 1b. The highest frequent HCV genotype belonged to 54.28% of patients who were

Table 1. Pattern of HCV genotype among the infected patients (N=105)

Genotype	Subtype	Male (%) (N=87)	Female (%) (N=18)	Total (%) (N=105)
Genotype 1	1 a	9(10.34%)	5(27.77%)	14(13.33%)
	1b	8(9.20%)	1(5.55%)	9(8.57%)
Genotype 2	2a	2(2.30%)	1(5.55%)	3(2.86%)
Genotype 3	3a	53(60.92%)	9(50%)	62(59.05%)
	3b	3(3.44%)	1(5.55%)	4(3.81%)
Unknown Genotype		4(4.60%)	1(5.55%)	5(4.76%)
Mixed Genotype	5 (4.76%) 1a ₊ 3a	8(9.20%)	0	8(9.20%)
	3(2.86%) 1a ₊ 1b			

Table 2. Pattern of HCV genotypes/subtypes in different age groups (N=105)

Genotype/ subtype	Age groups (Yr)					
	10-20	21-30	31-40	41-50	51-60	>60
1a	0	0	11 (10.47%)	3 (2.86%)	0	0
1b	0	0	4 (3.81%)	0	0	0
2a	0	1 (0.95%)	2 (1.90%)	0	0	0
3a	0	19 (18.09%)	29 (27.62%)	6 (5.71%)	6 (5.73%)	2 (1.90%)
3b	0	7 (6.67%)	2 (1.90%)	0	0	0
Unknown	0	0	3 (2.85%)	1 (0.95%)	0	1 (0.95%)
Mixed genotypes	0	1 (0.95%)	6 (5.73%)	1 (0.95%)	0	0
Total	0	28 (26.66%)	57 (54.28%)	11 (10.48%)	6 (5.73%)	3 (2.85%)

Table 3. HCV RNA viral load categories and their distribution in sex and genotype in Isfahan city

Genotype/subtype	Viral load			P value
	< 600'000	600'000-800'000	> 800'000	
Genotype 3(n=71)	19 (26.76%)	43 (60.56%)	15 (21.12%)	0.113
Other genotypes (n=34)	19 (55.88%)	10 (29.41%)	5 (14.70%)	
Male(n=87)	23 (26.44%)	46 (52.87%)	18 (20.69%)	0.012
Female(n=18)	11 (61.11%)	5 (27.78%)	2 (11.11%)	

in the range of 31-40 years of age. The lowest frequency was seen for > 60 years old (Table 2). Viral load distribution was classified into three categories according to its viral load levels, such as low (< 600'000 IU/mL), intermediate (600'000-800'000 IU/mL) and high (> 800'000 IU/mL). The HCV RNA viral load in HCV genotype 3 infected patients were 19 (25.67%), 41 (55.41%) and 14 (18.92%) for low, intermediate and high categories, respectively (Table 3). For all other genotypes except 3, the values for low, intermediate and high viral load categories were 17 (54.84%), 10 (32.26%), and 4 (12.9%), respectively. Viral load distribution was also categorized sex wise; for males, it was 23/87 (26.44%), 46/87 (52.87%) and 18/87 (20.69%) whereas for females it was 11/18 (61.11%), 5/18 (27.78%) and 2/18 (11.11%) for low, intermediate and high viral load, respectively. Fifty nine percent (62/105) of cases was infected with genotype 3a followed by genotype 3b in 8.57percent (9/105) and unknown in 4.76percent (5/105) of cases. Genotype 3 was not associated significantly ($P=0.113$) with a higher viral load. Also, the significant higher viral load was observed among male HCV patients ($P=0.012$) as compared to HCV female patients (Table 3).

DISCUSSION

In this research, we investigated the dispensation of HCV genotypes and the correlation of these genotypes with sex and viral load. The results clearly showed that there is a modulation among the HCV genotypes and sex as different HCV genotypes were broadcasted with important various ratios between males and females ($P<0.05$). In spite of the findings in our study, an insignificantly important difference was observed by Idrees and Riazuddin¹⁸ in sex and modulation between genotypes in Pakistan. Also, in Libya, the circulation genotype of HCV 1 was found to be totally related with males, whereas genotype 4 was optimally found in females¹⁹. In our research, genotype 3 was apparent to be more in males in relation to females. The present research clearly indicated that high circulation amount of HCV infection was apparent between the age groups 31-40. Our findings were similar to the Ahmad et al.'s research¹⁴. However, The maximum circulation amount was seen between the groups of ≤ 40

years²⁰. These findings clearly depict that early distinction of HCV might be due to public awareness about HCV infection, especially in this part of the globe. Information analysis of this study showed that genotype 3a (59.05%) has a high circulation amount which is followed by 1a and 1b in chronic HCV infected patients. The similar findings were as well reported in the studies from Pakistan, Turkey and India^{18,21,22,23}. Nevertheless, the geographical distribution of HCV genotypes varies globally, where 1a genotype is more frequent in the US and Europe, 1b in the US, Europe and Japan, genotype 3 in southeastern Asia and India, type 4 most prevalent in North Africa and the Middle East, and finally the types five and six are most frequent in South Africa and the Middle East. Undoubtedly, Iran, located in the Middle East, serves as a connection between Far East and Near East and there have been regional reports on the hepatitis genotyping among special high risk groups within the country. However, very few reports are available about its prevalence in Isfahan (Iran). In a study carried out by Zarkesh *et al.* on 97 patients in Isfahan, it was revealed that the prevalence of genotypes were as follows: 3a (61.2%), 1a (29.5%) and 1b (5.1%)^{21,22,24,25}. This finding effectually suggests that there is a switch correlation between viral load and response amount to standard antiviral treatment⁹. Our research was carried out integrating some important conception for the therapeutic hindrance. Genotype 3 is the most prevalent genotype in Isfahan because detection and treatment are important to get a high level lookout for the response of virological (SVR)²⁶ Wagner *et al.* (2005) and Dalgard *et al.* (2004) showed that genotype 3 HCV infected patients with low baseline viral load (HCV-RNA, < 600'000-800'000 IU/mL) had more chance to get stabilized with the response of virological (SVR) as compared with those indicating a high viral load (HCV-RNA > 600'000-800'000 IU/mL)^{11,12}. In the present study, 43 (60.56%) patients infected with genotype 3 exhibited an average viral load (600'000-800'000) after the quantification of HCV RNA. These findings are quite helpful to program antiviral treatment personally for HCV patients infected with genotype 3 which in turn will decrease the economic load, influence antiviral treatment, and perhaps advance optimum response amounts. It is worth mentioning that 39.04% of patients suffered

from drug-injection out of the total 60.95% cases who maintained other risk factors and the frequency of genotype 3a in this group was found to be significantly higher than that in the other groups (47.6% Vs 28.2%), $P=0.001$. The present findings are in concurrence with other studies²⁷ but in the patients with *blood transfusion* demonstrated that the genotype 1a was predominant (10.4%) followed by 1b and 3a. In the present study, the patients who didn't respond to the questions or claimed other minor risk factors, was 38(36.19%) HCV-RNA positive where the popularity of the, our findings genotype 3a was strangely more than other groups ($P>0.05$). Besides our findings indicated that few drug-injection cases due to some reasons, concealed the injection. Likewise, previous research studies in Iran have reported a greater prevalence rate of type 3a among the drug injection groups, as compared to other groups²⁸. To sum up, this research shows that more than one genotype of HCV circulates in Isfahan, Iran. Predominant genotype was 3a followed by 1a and 1b.

CONCLUSION

In the present study, we concluded that HCV genotype 3a is the most prevalent genotype circulating in this region of the world. Moreover, the majority of the infected patients were aged 31-40 years. Baseline viral load was significantly higher in patients infected with HCV genotype 3 (subtypes a & b) as compared to other genotypes such as 1 (subtypes a, b, c), 2 (subtypes a), mixed and unknown genotypes.

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