

Frequency Distribution of Hepatitis C Virus Genotypes in District Karachi, Pakistan

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ABSTRACT

AIM: Hepatitis C viral infection is a major health issue in the globe especially in developing countries like in Pakistan with prevalence rate of about 6% of the total population. True epidemiological data in developing countries about this infection is almost inadequate. The main objective of this study was determined the existence of different HCV genotypes in study area, in gender and in different age groups.

METHODS: It was a prospective, observational study carried for HCV genotyping from Jan, 2011 to March 2011 at Genome Center for Molecular Based Diagnostics and Research (GCMBDR) Lahore. A total of 375 HCV positive samples were received by GCMBDR Lahore from various areas of Karachi. These HCV Positive samples

were used for genotyping by using nested and reverse transcriptase polymerase chain reaction.

RESULTS: Molecular genotyping assay was carried out for 375 HCV positive serum samples. Out of 375 HCV positive serum samples 153(40.8%) were male and 222 (59.2 %) were female. Prevalence breakup of the circulating genotypes was: genotype 3a was detected in 220 (58.67%) patients followed by untypable 52 (13.87%), 1a 44 (11.73%), 2a 11 (2.93%), 3b 11 (2.93%), 1b 8 (2.13%) and 29 (7.73%) patients showed mixed infection of two different genotypes.

CONCLUSION: It is concluded that most prevalent genotype was 3a in district Karachi. Most predominant prevalence was seen in age group of 20-40.

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Key words: HCV; Genotype; Gender disparity; Karachi; GC-MBDR

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INTRODUCTION

Hepatitis C Virus (HCV) was isolated in 1989 as the causal agent of most cases of post-transfusional and sporadic acute and chronic viral hepatitis^[1,2]. While a member of the Flaviviridae family, HCV is an enveloped positive single stranded RNA virus having genome size of 9.6 kb encode a polyprotein precursor of 3000 amino acids and non-coding regions (3'NCR and 5'NCR) which use as internal ribosomal entry site (IRES) and is necessary for viral RNA cap independent translation^[3,4].

HCV infected about 3% of world population mostly in developing countries including Pakistan where HCV infected population is 6%^[5]. The primary hosts of HCV are B lymphocytes and hepatocytes^[6,7] producing 1,013 viral particles each day, although in the late stage of

infection^[8]. HCV transmission may occur through intravenous drug users, contaminated needles, sharing of tooth brushes, razors and also through sexual contact^[9]. In saliva and milk, HCV have been found, but transmission via breast milk has not been documented^[10]. HCV transmission from infected mother to neonate might be 3-5% and transmission through needle stick injury is about 5%^[11,12].

Around the world six major genotypes and more than hundred of subtypes are classified^[13]. The different forms of HCV genotypes are generally studied for clinical management of the infection, epidemiology, and vaccine development^[14]. Distribution pattern of HCV genotypes is different in different geographical regions of the world^[15-20]. HCV genotypes 1, 2, and 3 are present globally while subtypes different in different geographic area^[21], 1a and 1b Subtypes are most well-known in Europe and United States of America^[21]. The most common subtype 1b is present in Japan^[22] and in China as well^[23]. HCV subtype 3a frequently is circulating in Pakistan^[24]. However there is no such available data reporting the prevalence of HCV genotypes in district Karachi, thus aim of this study was to determine prevalence of HCV genotypes in district Karachi, in gender and in different age groups.

METHODS

Sample collection

Samples were collected from different regions of Karachi, all of the HCV RNA positive sera samples were received, for HCV Genotyping till Jan, 2011 to March, 2011. All of the 375 HCV RNA positive sera genotyping were performed at (Genome Center for Molecular Based Diagnosis and Research) GCMBDR Lahore, Pakistan. Separate written informed consent was not taken from these chronic HCV patients specifically for the current study as this investigation was a part of the reliable procedure in a regular workup of Molecular detections. This study was designed to estimate the frequency distribution of HCV genotypes among both genders as well as different age groups of HCV positive individuals

Qualitative and Quantitative detection of Hepatitis C RNA

The viral nucleic acid was isolated from each 100 µL patient's sera sample through Genra RNA extraction kit (Puregene, Minneapolis, MN 55441 USA) according to manufacturer's instruction. The HCV Complimentary DNA (cDNA) containing 5'NCR was synthesized using 100 units of Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and outer antisense primer at 5pM concentration.

For qualitative detection of HCV amplicons, HCV cDNA was subjected to two rounds of PCR-amplification (first round of PCR and nested PCR) using 2 units of Taq DNA Polymerase in a 20 µL reaction. PCR products were resolved on 2% agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

HCV viral RNA was quantified in all PCR qualitative positive samples using Smart Cycler II Real-time PCR (Cepheid, Sunnyvale, Calif. USA) utilizing HCV RNA quantification kits (Sacace Biotechnologies, Italy). The SmartCycler II system is a PCR system by which amplification and detection were accomplished concurrently with TaqMan technology (Applied Biosystems, Foster City, Calif) using fluorescent probes to detect amplification after each replicating cycle.

HCV Genotyping

AnaGen (USA) kit was used for HCV genotyping. Complimentary DNA (cDNA) was synthesized from about 10 µL of purified totalRNA was used to synthesize Complimentary DNA (cDNA) using MMLV-

RT for 50 min at 37°C. HCV cDNA was further subjected to two rounds of PCR amplification using HCV specific primers. In the first round, 2 µL of cDNA was used to amplify 470-bp product spanning HCV 5'NCR+Core region. PCR product amplified in the first round were subjected to nested PCR using primer mix-A, containing genotype 3a, 3c, 1a, 1b, 1c and 4 specific primers or primer mix-B containing genotype 3b, 2a, 2c, 5a and 6a specific primers. PCR products were resolved on 2% agarose gel, stained with ethidium bromide and visualized under UV transilluminator. The genotype-specific cDNA bands identified the HCV genotype in each sample.

Statistical Analysis

All results were presented in percentage form and were analyzed through Statistix 9 version software by One-Way ANOVA analysis and p less than 0.05 were considered significant.

RESULTS

A total of 375 serum sample (153 males and 222 females) were collected HCV-positive patients from district Karachi. The mean age of patients was 40 years. The frequency of Hepatitis C virus genotypes for the patients analyzed is shown in table 1 and 2.

Prevalence of different HCV genotypes in district Karachi

Table 1 shows that 3a 220 (58.67%) is the most common genotype circulating in studied population followed by untypable 52 (13.87%), 1a 44 (11.73%), 2a 11 (2.93%), 3b 11 (2.93%), 1b 08 (2.13%) while the remaining 29 (7.73%) patients showed mixed infection of two different genotypes. All the HCV genotypes were distributed significantly ($p < 0.05$) in infected population of district Karachi.

Table 1 Frequency distribution of HCV genotypes in various areas of district Karachi.

HCV genotypes	Subtypes	No. of isolates (n=375)	Percentage
1	1a	44	11.73%
	1b	08	2.13%
2	2a	11	2.93%
	3a	220	58.67%
3	3b	11	2.93%
	Untypable	52	13.87
Mixed genotypes		29	7.73
Total		375	100%

Gender wise incidence of HCV genotypes

All the studied HCV genotypes were distributed with same fashion in male and female patients (Table 2). Male patients were less susceptible 153 (40.8) to HCV infection as compared to female patients 222 (59.2). HCV genotypes were statistically ($p < 0.05$) distributed both in male and female patients.

Table 2 Gender wise distribution of HCV genotypes in district Karachi.

Gender	Distribution of HCV genotypes							Total
	1a	1b	2a	3a	3b	Untypable	Mixed genotypes	
Male	19	02	05	91	06	20	10	153 (40.8%)
Female	25	06	06	129	32	05	19	222 (59.2)

Distribution pattern of HCV genotypes in different age groups

Prevalence rates of HCV genotypes in various ages groups of district Karachi is shown in table 3. Of the total 375 patients samples successfully genotyped in age group of ≤ 20 genotype 3a was observed in 12 patients, untypable genotypes were seen in 2 patients and 2 patients were infected by two different mixed genotypes. In

age group of 21-40 the most common genotype was 3a observed in 122 patients followed by 1a in 28 patients, 1b in 6 patients, 2a in 4 patients, 3b in 9 patients. 21 patients showed untypable genotype and the remaining 15 patients showed mixed genotypes. In age group of 41-60 showed 3a the predominant genotype seen in 84 patients, 1a, 1b, 2a and 3b were observed in 15, 2, 7 and 2 patients respectively and the remaining 26 patients showed untypable genotype and 12 patients showed mixed genotypes. The patients of age group ≥ 60 showed 3a genotype in 2 patients, 1a in 1 patient and 3 patients showed untypable genotype. From the results it was noted that 3a was the most common genotype observed in all age groups. Most frequent prevalence 54.66% of all HCV genotypes was seen in age group of 21-40 as compared to other age groups. All the HCV genotypes was significantly ($p \leq 0.05$) circulating in all age groups of the studied population.

Table 3 Frequency distribution of HCV genotypes in various ages groups of district Karachi.

Age groups	Different HCV genotypes							Percentage
	1a	1b	2a	3a	3b	Untypable	Mixed genotypes	
≤ 20	0	0	0	12	0	02	02	4.26%
21-40	28	06	04	122	09	21	15	54.66%
41-60	15	02	07	84	02	26	12	39.46%
≥ 60	01	0	0	02	0	03	0	1.6%
Total	44	08	11	220	11	52	29	100%

DISCUSSION

Hepatitis C virus infection is one of the leading causes of chronic liver disease and most common indication for liver transplantation in US and other developed countries^[25]. Hepatitis C is one of the major disease burdens on the world. Nearly 3% population's of world has been infected by HCV^[26]. There are about one hundred and seventy million individual were infected by Hepatitis C virus worldwide and about 3-4 million peoples are newly infected each year^[27,28]. HCV prevalence is alarmingly high in the developing countries including Pakistan and HCV infection is rapidly spreading in the general population in rural areas due to poor health infrastructure and has a devastating impact on the economy of Pakistan as well^[29]. Prevalence pattern of HCV genotypes might be different in various areas and in different groups of the same community^[30]. In the entire study a total of 375 samples of district Karachi were examined for genotyping Hepatitis C virus. It is the largest and mostly educated city and main financial center of Pakistan. Its population is estimated about 13 to 20 million^[31,32]. Karachi is the main center of higher education in South Asia and in the Muslim World^[33]. In this setup predominant genotype was 3a 220 (58.67%), followed by untypable 52 (13.87%), 1a was 44 (11.73%), mixed genotypes were 29 (7.73%), 2a was 11 (2.93%), 3b was 11 (2.93), 1b was 08 (2.13%), and 2b 0 (0%). Frequent prevalence of untypable genotypes has earlier been reported from different parts of Pakistan^[24]. The sequencing of these untypable genotypes is necessary not only for future treatment but also important for research purposes.

Such results were in concurrence with the data reported in district Karachi, Pakistan^[34] as the most prevalent genotypes was 3a 392(85%) followed by genotype 1 (11.2%) and only two patients was seen with mixed infection of genotype 3a+1a, although some patients were also the carriers of genotype 2, 4 and 5. According to large scale study conducted in Pakistan and observed genotypic distribution were 1a (8.35%), 1b (3.01%), 1c (0.15%), 2b (0.80%), 2a (7.52%), 2c (0.09%), 3a (49.05%), 3b (17.66%), 3c (0.75%), 4 (1.49%), 5a (0.18%), 6a (0.12%), untypable (6%) and (4.80%) were mixed infection^[24]. Other studies has also been confirmed that the predominant genotype

in Pakistan is 3a with prevalence rate of about 50% followed by genotype 3b and 1a, respectively^[35-39]. In present study we are unable to isolate 4, 5 and 6 HCV genotypes these are appears to be common in North Africa, South Africa and in Hong Kong respectively^[40-41]. The study revealed that patients of age groups 0-20 years of district Karachi have less or no infection of HCV. The reason might be due to their least exposure to some of the high risk environmental factors causing the infection. Age group 21-40 have high rate of HCV infection. This infected population had variable history of exposure to the HCV risk factors like blood transfusion, minor/major surgeries, dental procedures and reuses of contaminated needles/syringes.

Finally in our data analysis female population 222 (59.2) was more affected as compared to male infected population 153 (40.8%) of district Karachi. This frequent prevalence of HCV infection in female might be due the fact that female of this area is socially more active than male and highly expose to various risk factors and thus get infection more easily.

In our country due to financial problems, physicians generally do not suggest the genotype testing, as they assume HCV- 3a genotype more prevalent and responder to interferon therapy, but due to frequent prevalence of untypable and 1a genotypes and for improved dose management, time course of interferon treatment and sustainable response thus significance of genotyping cannot be ignored.

CONCLUSION

It is concluded from the present study that most common HCV genotype is 3a in district Karachi. A frequent incidence rate of untypable genotypes was seen in patients that necessitate further analysis for the successful genotyping by improving the sensitivity and specificity of used genotyping assay or utilizing viral sequencing procedure.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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