

Research Article

Frequency distribution of hepatitis C virus genotypes circulating in Hyderabad, Sindh

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Abstract

Hepatitis C virus (HCV) is the second most common cause of viral hepatitis worldwide. HCV genotype is the strongest predictive factor for sustained virological response (SVR). The patients with different HCV genotypes act differently in response to alpha interferon therapy, thus, the patient genotype plays vital role in treatment outcome. The aim of the present study was to assess molecular epidemiology of HCV genotypes and subtypes circulating in Hyderabad, Sindh. A cross sectional study was conducted for a period of six months from July 2015 to December 2015. A total of 577 samples of HCV positive patients were collected for analysis of HCV genotypes and subtypes using Real-time PCR Assay. Out of 577 cases 57.54% (n=332) were male and 42.46% (n=245) were females. Age groups between 21 and 60 years comprised 90.47% (n=522) of the patients. HCV 3 genotype was most frequent genotype infecting 489 (84.75%) of the patients. The second most common variety was untypeable 9.36% (n=54). A little number of patients yielded mixed variety of genotypes. The prevalence of other genotypes as follows: Genotype 1 (0.52%), 1, 1a (0.87%), 1, 1b (2.43%), 1b (0.17%), 2 (0.69%), 3, 4 (0.87%), 4 (0.35%). Genotypes 5 and 6 were not identified in any patient enrolled in this study. In summary, HCV genotype 3 was the most prevalent at Hyderabad, Sindh. Despite the availability of antiviral therapy, apparent increase in HCV 3 genotype burden seems alarming and warrants urgent measures to prevent the spread of HCV infection.

Keywords: Genotyping; HCV; Hepatitis C; Hyderabad; SVR

Introduction

Viral hepatitis is major public health concern across the world. Out of six main viruses, HCV has been reported to cause a prolonged chronic type of hepatitis infection in susceptible patients [1]. HCV is an enveloped virus which was first reported in 1989 [2]. HCV possesses single stranded non-

segmented, linear, ribonucleic acid (RNA) enclosed in nucleocapsid, surrounded by envelope and appears icosahedral in shape [3]. HCV infection is the second commonest cause of viral hepatitis with significant clinical problems worldwide. Eleven different genotypes of HCV have been reported, out of the six genotypes are

recognized as major types of HCV. Determining the genotype of HCV is very important as the duration of HCV treatment has been shown to be genotype associated, where some genotypes of HCV are treated in shorter duration while others require more time to obtain SVR and eradication of virus successfully [4, 5]. Thus, identification of genotype is strongly recommended before onset of treatment in order to get promising results in treatment and for clearing virus. The recommended duration for treatment of genotypes 2 and 3 is 24 weeks whereas for genotype 1 infected patients the duration is longer such as 48 weeks [6, 7]. HCV infection is a large health care burden worldwide, but because of asymptomatic and often latent nature of the disease, it's difficult to calculate the incidence rate until the clinical manifestations. Worldwide prevalence rates of HCV infection has been changed due to awareness in many countries about the leading risk factors of spread of the virus such as blood transfusion related transmission of HCV and intravenous drug use [8]. It has generally been known that the spread and transmission of HCV primarily occurs through blood transfusion and parenteral exposures with contaminated syringes [9-11]. Different HCV genotypes have been used for epidemiological investigations, development of vaccine, and clinical management and response to anti-viral therapy [12]. Out of major six genotypes, three genotypes including genotype 1, genotype 2 and genotype 3 are distributed across the world and their prevalence has been reported to vary from place to place according to geographic locations. Subtypes 1a and 1b are widely distributed in Europe and United States of America [13], while 1b is most prevalent Europe and Asia. Subtype 2a is common in China and Japan and 2b is most common in north Europe and North America, where as genotype 2c is widely circulating in

northern Italy and other regions of western northern Europe. Subtype 3a is most prevalent in south Asia and Australia (40%). Genotype 4 is widely distributed in the Middle East, while subtype 4a is commonly circulating in Egypt and 4c in central Africa. The subtype 5a has been reported to be more common in South Africa and the subtype 6a is only circulating in Macau, Vietnam and Hong Kong [14]. Other genotypes and subtypes although not common but found in some regions include 7a and 7b circulating in Thailand, 8a, 8b and 9a are found in Vietnam, and in Indonesia 10a and 11a are common genotypes [15, 16]. Genotype 3 and untypable genotypes are highly prevalent in the Pakistan [17, 18]. In view of increasing prevalence of HCV and changing patterns of genotypes involved in infection, the present study was aimed to assess prevalence of HCV genotypes and subtypes infecting people in Hyderabad, Sindh.

Materials and methods

Sampling and study duration

A cross sectional prospective study was undertaken at institute of Microbiology, University of Sindh, Jamshoro and Asian Institute of Medical Sciences (AIMS) Hyderabad from six months from July 2015 to June 2015. HCV RNA positive patients of more than 16 years in age and chronically infected with HCV, were enrolled in the study. A total of 577 blood samples were collected from HCV patients for determining HCV genotypes.

Collection of blood sample and serum separation

Using sterile syringe, 5 ml of blood was collected from each patient by veinpuncture. Blood samples were immediately added to BD vacutainer serum separation tubes and processed for serum separation as per manufacturer's guidelines. Briefly, the gel tubes were inverted five times, and allowed to stand 30 minutes for clotting, and then centrifuged at 3000 rpm for 10 minutes. The

gel in tubes forms a physical barrier between serum and blood cells during centrifugation. After centrifugation separated serum obtained and the sample was ready to be further processed. Samples were either used immediately or stored at -20°C for future use

HCV genotyping assay

Genotyping analysis was carried out using reverse transcription-polymerase chain reaction (RT-PCR) using Abbott Real Time HCV Genotype assay kit as per manufacturer's guidelines (Abbot, USA). The genotyping kit uses genotype-specific fluorescent-labeled oligonucleotide probes to detect six major genotypes including genotype 1, genotype 2, genotype 3, genotype 4, genotype 5, and genotype 6, as well as two subtypes such as 1a and 1b. The primers target the 5'UTR region of HCV genome for the classification of genotypes 1 through 6, and the NS5B region to determine the subtype 1a and 1b of HCV (Abbott Real Time HCV Genotype II).

Results and discussion

The current study has investigated the types and frequency of HCV genotypes in patients from Hyderabad, Sindh. The data revealed that out of 577 HCV positive samples subjected for genotyping, 332 (57.54%) were male and 245 (42.46%) were females. Females were slightly less in number than the male patients studied in the present study.

Moreover, the genotyping data demonstrated that HCV genotype 3 was the most frequent genotype infecting 84.75% (n=489) of the total HCV patients. The second most common variety was un-typeable with 9.36% (n=54) samples. A little number of patients yielded mixed variety of genotypes. The prevalence of other genotypes included Genotype 1 (0.52%), 1a (0.87%), 1b (2.43%), 2 (0.69%), 3, 4 (0.87%) and 4 (0.35%). Genotypes 5 and 6 were not identified in any patient enrolled this study.

Genotyping of samples yielded 8 different genotypes and subtypes including (1,1a),

(1,1b), (1b), (2), (3), and (3, 4) (Table 1). However, HCV genotypes 1a, 5, and 6 were not detected among patients studied. Genotype 3 was the most prevalent one detected in 84.75% of patients, followed by untypeable genotype (9.36%) and then genotype 1b, 1a and mixed genotypes (3 & 4). Genotype 1 was the least prevalent and was detected in only in 0.52% patients. The studied patients were categorized in three different age groups and then prevalence of age associated HCV genotypes among male and female patients was determined (Table 2 & 3). A slight difference in gender-wise distribution was found in genotypes. Genotype 3 was the most prevalent in both male and female patients. Seven genotypes were common in both genders; however, genotype 4 was only detected in males but not in females in the present study.

HCV 3 was most prevalent genotype followed by (1, 1b), (1 1a), (3, 4), (2), (1), and (4). The present study has also demonstrated mixed HCV genotypes; however, only one pattern of mixed genotypes (3, 4) with a very low prevalence was noticed. The second most common type was unidentified/untypeable genotype. The pattern of genotype distributions in male and females revealed that although HCV genotype 3 was the most common genotype in both genders, no substantial difference in the distribution of genotypes between two genders was observed.

The findings of present study are in agreement with previous studies from other parts of Pakistan and especially Karachi. Other genotypes and subtypes, although were less frequent, included 1, 1a 1b, 2, 3, and 4. Genotype 4 was only detected in males not in females (Table 3). Despite of availability of antiviral therapy, apparent increasing pattern of HCV 3 patient's burden especially Hyderabad is alarming and needs special measures to prevent the transmission of

infection. Periodical investigations of HCV genotypes are needed to monitor distribution

patterns of genotypes to facilitate treatment options and preventive strategies.

Table 1. Distribution of various HCV genotypes circulating in Hyderabad

Genotypes	(n=)	(%)
1	3	0.52
1a	0	0
1,1a	5	0.87
1,1b	14	2.43
1b	1	0.17
2	4	0.69
3	489	84.75
3,4	5	0.87
4	2	0.35
5	0	0
6	0	0
Untypeable	54	9.36
Total	577	100

Table 2. Age and gender wise distribution of HCV genotypes among male patients

MALE													
Age groups in years	1	1a	1,1a	1,1b	1b	2	3	3,4	4	5	6	Untypeable	Total
1 to 20	--	--	--	--	--	--	11	--	--	--	--	--	11
21 to 40	2	--	1	2	--	--	87	2	--	--	--	18	112
41 to 60	--	--	2	7	--	1	162	--	2	--	--	13	187
61-Above	--	--	--	--	--	1	18	1	--	--	--	2	22
Total	2	0	3	9	0	2	278	3	2	0	0	33	332
%	0.35	0.00	0.52	1.56	0.00	0.35	48.18	0.52	0.35	0.00	0.00	5.72	57.54

Table 3. Age and gender wise distribution of HCV genotypes among female patients

FEMALE													
Age groups in years	1	1a	1,1a	1,1b	1b	2	3	3,4	4	5	6	Untypeable	Total
1 to 20	--	--	--	--	--	1	5	1	--	--	--	--	7
21 to 40	1	--	--	1	1	1	90	--	--	--	--	9	103
41 to 60	--	--	2	3	--	--	103	1	--	--	--	11	120
61-Above	--	--	--	1	--	--	13	--	--	--	--	1	15
Total	1	0	2	5	1	2	211	2	0	0	0	21	245
%	0.17	0.00	0.35	0.87	0.17	0.35	36.57	0.35	0.00	0.00	0.00	3.64	42.46

Conclusions

The present study demonstrated that the HCV genotypes distribution was variable based on age and gender. Most commonly affected age was 40-60 years and almost equally common in both genders. Eight different genotypes and subtypes were found circulating in Hyderabad. Genotype 3 was found most common genotype in Hyderabad.

Authors' contributions

Conceived and designed the experiments: SA Tunio & S Bano, Performed the experiments: NL Pathan, Analyzed the data: SA Tunio & AN Jatt, Contributed materials/ analysis/ tools: AN Jatt, NL Pathan & S Bano, Wrote the paper: SA Tunio & S Bano.

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