

Occurrence of HCV genotypes in different age groups of patients from Lahore, Pakistan

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Abstract

Background: Hepatitis C virus is a small, enveloped single stranded, positive sense RNA virus. Different genotypes are distributed in different geographical areas of the world. Determination of HCV genotype is a powerful tool for the treatment of chronic and acute liver disease.

Method: The present study was carried out to find the occurrence of different HCV genotypes in the city of Lahore, a populous city of Pakistan from January 2010 to December 2010. Blood sample of patients positive for anti HCV by ELISA as well as HCV by PCR were collected and plasma was separated. HCV viral RNA load was analyzed in these samples using Real Time PCR. Qiagen HCV mini kit for RNA extraction and Qiagen HCV amplification kit for PCR amplification were used. Amplicons were subjected to HCV genotyping using Third Wave Technology.

Results: Among 489 patients, 211 (43.1%) patients were males and 278 (56.9%) were females. Occurrence of HCV in the age group of 36-45 years was 32.5 %. Occurrence of HCV genotype 1 was 9.6% (47), genotype 3a was 80.77% (395), genotype 3h was 1.0% (5), genotype 4 was 4.9% (24), co-infection of genotypes 1 & 2 was 0.2% (01), co-infection of genotypes 1 & 3 was 0.6% (03) and co-infection genotypes 1 & 4 was 0.4% (02).

Conclusion: HCV genotype 3a is most prevalent HCV genotype in subjected population during said duration with most infected people from 26 to 35 years of age. Female population is having more of HCV infection as compared to males.

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Introduction

Infection with Hepatitis C Virus (HCV) is estimated at more than 200 million people worldwide, representing more than 3% of the world's population [1]. HCV consists of an envelope derived from host membranes into which are inserted the virally encoded glycoproteins E1 and E2, surrounding a nucleocapsid and a positive sense single stranded RNA genome which has been characterized and sequenced [2]. Phylogenetic analysis of HCV sequence has revealed 6 major genotypes and more than 50 subtypes [3,4]. HCV genotypes differ from each other in their nucleotide sequence by 31-34% and in their amino acid sequence by 30% [5]. The genotype determination is a relevant clinical practice. It helps to predict the probability of sustained virological response i.e. 40%-45% for genotype 1 as compared with 70%-80% for genotypes 2 and 3. It is also used routinely to determine duration of treatment i.e. 48 weeks for genotypes 1 & 4, and 24 weeks for genotypes 2 and 3 [6].

Genotypes 1, 2 and 3 are distributed almost worldwide [7,8]. Half of the hepatitis C patients from South of Brazil were infected by genotypes 2 and 3 [9]. Types 4, 5 and 6 have been found in distinct geographical areas [3,8,10]. Genotype 5 is mainly dominant in South Africa and also present in less percentage in other countries [11]. Genotype 6 is common in South East Asia, Asian countries and Asian Australians [12]. In Romania, HCV subtype 1a was 5.4 %, subtype 1b was 92.6%, subtype 3a was 0.8% and subtype 4a was 1.2% [13]. In the Swat district of Pakistan, HCV genotype 3a

had been reported to be predominant followed by mixed genotype infection and then 3b [14]. Genotype 7, 8 and 9 had been reported from Vietnam [15]. Genotype 10 and 11 were identified in patients from Indonesia but there had been a conflict for the classification of HCV isolate and scientists proposed that genotypes 7 through 11 must be considered as variants of the same group and must be classified as a single genotype, type 6 [15,16]. From Pakistan, few studies are available on the distribution of various HCV genotypes and its route of transmission based on small sample sizes [17-19].

Methods

All the work was done in PCR section of Pathology Department, Jinnah Hospital, Lahore from January 2010 to December 2010. Three milliliter blood sample was collected from each of 489 HCV positive patients along with their age, gender and place of living. Plasma was separated from each sample using the recommended standard procedure given by Qiagen extraction kit. Extraction of HCV RNA from each sample was done using QIAamp Viral mini kit by Qiagen cat no. 52906. Amplification was achieved by using Atrus HCV RT-PCR kit by Qiagen from Germany. HCV genotyping of amplified samples was detected using Invader HCV Reagents on Palm Cycler for amplification and Cytoflour for detection of genotype. The Invader® chemistry is composed of two simultaneous isothermal reactions. A primary reaction specifically and accurately detects single-base changes, insertions, deletions and changes in gene and

chromosome number for genetic, pharmacogenetic and infectious diseases. A second reaction is used for signal amplification and generic Present study was carried out to find the occurrence of different HCV Genotypes in the city of Lahore, Punjab of Pakistan. In the present study a total number of 489 HCV positive cases (which were also anti HCV positive by ELISA) were selected. HCV load was analyzed in these samples using Real Time PCR. Genotypes of all samples were determined using Third Wave Technology.

Results

Among 489 patients 211 (43.1%) patients were male and 278 (56.9%) were female. Out of 211 (43.1%) anti HCV positive males, 24 (4.9%) had HCV genotype 1, 168 (35.4%) had HCV genotype 3a, 03 (0.6%) had HCV genotype 3h, 10 (2.0%) had HCV genotype 4, 01 (0.2%) had co-infection of genotypes 1 & 3 and untypable were 5 (1.02%). Among 278 (56.9%) anti HCV positive females, 23 (4.7%) had HCV genotype 1, 227 (46.4%) had HCV genotype 3a, 02 (0.4%) had HCV genotype 3h, 14(2.9%) had HCV genotype 4, 01 (0.2%) had co-infection of genotypes 1 & 2, 02 (0.4%) had co-infection of genotypes 1 & 3 and 02 (0.4%) had co infection of genotypes 1 & 4 and untypable were only 7 (1.43%) [Table I].

Out of 489 patients, 15.5% (76) were from 15-25 years age group. Out of which 2.0% (10) had HCV genotype 1, 12.3% (60) had HCV genotype 3a, 0.8% (04) had HCV genotype 4, 0.2% (01) was co infected with HCV genotypes 1&3 and 0.2% (01) was co

infected with HCV genotypes 1 & 4. Out of 489 patients, 33.9% (166) were from 26-35 years age group.

HCV Genotype	Male	Female	Total
Type 1	24 (4.9%)	23 (4.7%)	47(9.6%)
Type 3a	168 (34.4%)	227 (46.4%)	395 (80.8%)
Type 3h	03 (0.6%)	02 (0.4%)	05 (1.0%)
Type 4	10 (2.0%)	14 (2.9%)	24 (4.9%)
Type 1 & 2 Co-infection	-	01(0.2%)	01 (0.2%)
Type 1& 3 Co-infection	01 (0.2%)	02 (0.4%)	03 (0.6%)
Type 1 & 4 Co-infection	-	02 (0.4%)	02 (0.4%)
Untypable	5 (1.02%)	7 (1.43%)	12 (2.45%)

Table I: Distribution of HCV genotypes with genders

Out of 489 patients, 15.5% (76) were from 15-25 years age group. Out of which 2.0% (10) had HCV genotype 1, 12.3% (60) had HCV genotype 3a, 0.8% (04) had HCV genotype 4, 0.2% (01) was co infected with HCV genotypes 1&3 and 0.2% (01) was co infected with HCV genotypes 1 & 4. Out of 489 patients, 33.9% (166) were from 26-35 years age group. Out of which 2.9% (14) had HCV genotype 1, 27.4% (134) had HCV genotype 3a, 0.6% (03) patients had HCV genotype 3h, 2.0% (10) had HCV genotype 4 and 0.2% (01) was co infected with genotypes 1 & 3. Out of 489 patients, 32.5% (159) were from 36-45 years age group. Out of which 2.5% (12) had HCV genotype 1, 27.4% (134) had HCV genotype 3a, 0.2% (01) had HCV genotype 3h, 1.2%

Age Groups	Type 1	Type 3a	Type 3h	Type 4	Type 1 & 2 co-infection	Type 1 & 3 co-infection	Type 1 & 4 co-infection	Untypable	Total
15-25 Years	10 (2.0%)	60 (12.3%)	-	04 (0.8%)	-	01 (0.2%)	01 (0.2%)	-	76 (15.5%)
26-35 Years	14 (2.9%)	130 (26.5%)	03 (0.6%)	10 (2.0%)	-	01 (0.2%)	-	08 (1.7%)	166 (33.9%)
36-45 Years	12 (2.5%)	134 (27.4%)	01 (0.2%)	06 (1.2%)	01 (0.2%)	-	01 (0.2%)	04 (0.8%)	159 (32.5%)
46-55 Years	08 (1.7%)	52 (10.6%)	01 (0.2%)	04 (0.8%)	-	01 (0.2%)	-	-	66 (13.5%)
56-65 Years	02 (0.4%)	16 (3.3%)	-	-	-	-	-	-	18 (3.7%)
> 65 Years	01 (0.2%)	03 (0.6%)	-	-	-	-	-	-	04 (0.8%)
Total	47 (9.6%)	395 (80.77%)	05 (1.0%)	24 (4.9%)	01 (0.2%)	03 (0.6%)	02 (0.4%)	12 (2.4%)	489 (99.9%)

Table II: Distribution of HCV genotypes with age groups

(06) had HCV genotype 4, 0.2% (01) was co-infected with HCV genotypes 1 & 2 and 0.2% (01) was co-infected with HCV genotypes 1 & 4. Out of 489 patients, 13.5% (66) were from 46-55 years age

(16) had HCV genotype 3a. 489 0.8% (04) patients were from > 65 years age group. Out of which 0.2% (01) had HCV genotype 1 and 0.6% (03) had HCV genotype 3a [Table II].

HCV Genotype	Frequency	Percent
Type 1	47	9.6
Type 3a	395	80.77
Type 3h	05	1.0
Type 4	24	4.9
Type 1 & 2 Co infection	01	0.2
Type 1 & 3 Co infection	03	0.6
Type 1 & 4 Co infection	02	0.4
Untypable	12	2.45
Total	489	99.9

Table III: Occurrence of HCV genotypes

group, out of which 1.7% (08) had HCV genotype 1, 10.6% (52) had HCV genotype 3a, 0.2% (01) had HCV genotype 3h, 0.8% (04) had HCV genotype 4 and 0.2% (01) was co-infected with HCV genotypes 1 & 3. Out of 489 patients, 3.7% (18) were from 56-65 years age group. Out of which only 0.4% (02) had HCV genotype 1 and 3.3%

Among 489 anti HCV positive cases on ELISA, occurrence of HCV genotype 1 was 9.6% (47), genotype 3a was 80.77% (395), genotype 3h was 1% (05), genotype 4 was 4.9% (24), co infection of genotypes 1 & 2 was 0.2% (01), co infection of genotypes 1 & 3 was 0.6% (03) and co infection genotypes 1 & 4 was 0.4% (02) and untypable were 2.45% (12) [Table III].

Discussion

Interest of HCV genotyping by group screening is increased many fold as it is useful for the solution of epidemiological questions and development of vaccines against HCV. It has also been shown to be valuable to facilitate therapeutic decisions and strategies [20,21]. Severity, progression and response to the treatment of disease

caused by HCV vary according to the genotype of HCV [22,23].

Knowledge of occurrence of different HCV genotypes in different areas of Pakistan will help in therapeutic implications. In the present study, occurrence of various genotypes of HCV, in Lahore city, were observed. All plasma samples were anti HCV positive on ELISA and thus were further tested for HCV genotyping. The data shows that HCV infection is more common in females residing in Lahore as compared to males [20]. Results were analyzed by keeping the age and gender categorical variable and patients were divided in six different age groups. It is found that patients belonging to age group 26-35 years are more infected with HCV followed by the age group of 36-45 years. A similar reported same kind of results that people ≤ 40 years of age were more affected with HCV in comparison to those > 40 years of age in Lahore [24]. A study reported that HCV prevalence observed was highest among an age group of 13-50 years [25]. In present study prevalence of HCV genotypes is also recorded with reference to gender and it is found that highest prevalence of type 3 exists among both genders. Genotype 3a predominates among the patients residing in Lahore, genotype 1 and genotype 4 follows it. This data favors the results of Ahmad who reported that the most commonly detected genotype in their study was genotype 3 (59.1%), with predominant subtype 3a (55.9%) and 3b (3.2%) [24]. Genotype 1a was (23.6%) while genotype 4 (13.7%) comprised of the subtypes a (12.5%) and b (1.2%). It is also reported the

occurrence of HCV genotype 3a in 40.96%, 3b in 15.66%, 1a in 9.63%, and 1b in 2.40% of hepatocellular carcinoma tissue (HCT) samples [26]. They also found 24 (28.91%) mixed types. Another study by Butt et al., in 2010 showed prevalence of 3a (62%), 3b (9%), 1a (3%), 2a (2.144%), mixed (4.718%) and un type able (17.16%) [27]. Another study reported that genotype 1 was the most frequently genotype found in all regions of Brazil. In present study prevalence of mixed infection is quite negligible [28].

This is dire need to carry out a detailed study on patterns of HCV genotype amongst the population of Lahore for the proper and better control on spread of disease.

Conclusions

- Anti HCV positivity was relatively more predominant in females than male population.
- HCV RNA was common in the age group of 26-35 followed by the age group of 36-45 years.
- Genotype 3a was the common genotype found in the population of the city of Lahore, Pakistan and it was also the common genotype in both males and females.

Competing interests

All the authors have no financial or non-financial competing interests.

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