

EPIDEMIOLOGICAL CHANGES IN HEPATITIS C VIRUS GENOTYPES AND THEIR ROUTES OF TRANSMISSION IN PAKISTAN

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ABSTRACT

Background: Characterization of Hepatitis C virus (HCV) into its genotypes plays a vital role in therapeutic recommendation, geographic characterization and to determine the sources of transmission of the virus

Objectives: The present study was arranged to examine the changing epidemiology of different HCV genotypes and their routes of transmission in Pakistan.

Methods: The present retrospective cohort study was conducted at the pathology department of Shalamar hospital Lahore from November 2007 to November 2015. HCV genotyping was performed. Geno typing was done by multiplex PCR using type specific primers.

Results: Genotype 3 was found the most common type in HCV RNA positive cases (68.10%) followed by genotype 1, 2, 4, 5 and 6 (14.66%, 5.76%, 2.31%, 0.06%, and 0.02% respectively). 5.06% cases remained unclassified and in 4.04% more than one genotype was detected. In last eight years a gradual increasing trend in HCV genotype 1 & 4 and decreasing trend in genotype 2 & 3 was examined. The most probable routs of HCV transmission were noted in dental surgery, general/gynaecological surgery and barbers (22.28%, 20.14% and 11.16%).

Conclusions: Downward trend was observed in the prevalence of HCV genotype 3. On contrary, the prevalence of genotype 1 and 4 was observed to be rising. Transmission of HCV was strongly associated with nonsterile razors, general/ gynaecological or dental surgery.

Key words: HCV; genotypes; changing epidemiology; Pakistan.

INTRODUCTION

Hepatitis C is a communicable liver disease caused by the infection of Hepatitis C virus (HCV) and may lead from a mild illness of a few weeks to the severe long lasting disease. More than 185 million individuals in the world are living with HCV chronic infection and more than 350,000 deaths occurs every year with this disease.^{1,2} The highest rate of Hepatitis C in the world is reported from Egypt, Pakistan and China (15%, 4.8% and 3.2%).^{1,2}

HCV is an enveloped spherical virus with approximately 9600 nucleotides long positive single stranded RNA genome and belongs to the *Flaviviridae* family.³⁻⁵ HCV genome is highly heterogeneous and the variation of the nucleotide sequence is not consistently distributed across the whole genome, some regions are highly variable and some are conserved.^{6,7}

HCV genotypes, subtypes and quasispecies are based on three levels of HCV genomic variations. The classification of Hepatitis C virus into its genotypes is

based on the first level of genetic variation where the nucleotide sequence of HCV genome shows more than 30% variability. At the second level each major genotype is subdivided into its subtypes when it exhibits more than 20% variation in its nucleotide sequence within the genotype.⁸⁻¹¹ These genotypes and subtypes play a key role to examine the geographical prevalence of different HCV genotypes, their source of transmission, their relation to the particular risk groups, to predict the response of HCV infected patients to Interferon therapy and to decide the therapy duration and management.¹²⁻¹⁵

The frequency of HCV genotypes and their subtypes varies region to region. In North and South America, Europe, Russia, China, Japan, Australia, New Zealand and India the contribution of HCV subtypes 1a, 1b, 2a, 2c, and 3a is considered more than 90%.^{16,17} In Egypt, North Africa, Central Africa, and the Middle East the most frequently reported HCV genotype is 4. Genotype 5 and 6 are commonly found in South Africa and South East Asia respectively.^{18,19} According to the

studies reported by different groups from Pakistan, Mostly the population of this country is infected with genotype 3 especially its subtype 3a.^{14,20-23}

In previous studies reported from different areas of the Pakistan changing epidemiology of different HCV genotypes in this country is not properly addressed. Additionally, except one study reported by Idrees and Riazudin in 2008 all other studies were based upon the small number of samples and were not able to explain the prevalence of all six HCV genotypes (1 to 6).

The following study was arranged to see the frequency of different HCV genotypes, their changing epidemiology and their probable routes of transmission in Pakistan. The present study was based upon a sufficient number of the samples (4981). The findings of this study may play an important role in epidemiological, pathological and virological characterization of the virus. It may also help in effective therapeutic management and future planning to eliminate the virus from our population and in vaccine development.

MATERIALS AND METHODS

Patients: Anti-HCV positive (by ELISA) samples were received at the Pathology department of Shalamar Hospital Lahore for further processing to detect HCV RNA and specific genotype of the virus. The age range of the patients was 2 – 70 years both in males and females, while the mean, median and mode was 36 ± 6 , 35.5 and 40 respectively. The patient participated in the present study were from different areas of the province Punjab of Pakistan. All the patient information including age, sex, address, previous HCV infection related investigations, foreign tour history and the history of any family member HCV infection was registered.

Statistical Analysis

To analysis the data SPSS version 11 software was used. The results for all variables were set in the form of rates (%). To evaluate the positive association among the categorical variables Fisher's exact and Chi Square tests were used and $p < 0.05$ was defined as significant.

RESULTS

Hepatitis C virus was genetically classified into its six major genotypes (1 to 6) and nine subtypes (1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a) in 4528 (90.90%) out of 4981 HCV RNA positive samples (Fig. 2). In 5.06% HCV RNA positive samples HCV remained unclassified and were declared as untypable. Mixed genotypes (More than one) were detected in 4.04% samples (Table-1).

Genotype 3 was the most prominent type among the HCV infected patients (68.10%). Second and third common genotypes were 1 and 2 (14.66% and 5.76%), while the percentage of genotype 4 was 2.31%. The percentages of genotype 5 and 6 were very low (0.06% and 0.02%). Among the HCV subtypes, the most common subtype was found 3a (47.80%) followed by 3b, 1a, 1b, 2a, and 2b (20.30%, 9.13%, 5.52%, 4.62% and 1.14%) (Table 1 and Fig. 3).

The changing epidemiology of different HCV genotypes was observed from November 2007 to November 2015. Decreasing trend was examined in genotype 2 and 3 in last five years. Decline was 6.80% to 5.63% in genotype 2 and 72.80% to 63.64% in genotype 3. Major instability was seen in genotype 1 (especially its subtype 1a) and genotype 4 where the trend was significantly on increasing side ($p = 0.001$). Because the presentation of genotype 5 and 6 was rare so to assess their epidemiological change during the said period was difficult. All the results regarding the changing epidemiology of different HCV genotypes are revealed in table 4.

The probable source of HCV transmission was observed in 71.22% HCV infected patients. Out of those 22.28% were due to dentists, 11.16% due to barbers, 20.14% due to general or gynaecological surgery, 11.46% due to infected family members, 4.28% due to blood or blood product transfusion and 1.89% were due to piercing or tattooing. In 28.78% cases the source of HCV transmission was unclear (Table 5).

A total of 19.17% HCV patients infected with different HCV genotypes had foreign tour history out of those 12.83% were males and 6.34% females. With 5.52% males and 3.53% females a total of 451 (9.05%) patients had a Middle East tour history, whereas 504 (10.12%) had Europe or USA tour history (7.31% males and 2.81% females) (Table 6).

Table 1: Presentation of different HCV genotypes and subtypes.

Genotypes	Subtypes	Males	Females	Total
1	1a	286 (10.24%)	169 (7.73%)	455 (9.13%)
	1b	187 (6.69%)	88 (4.02%)	275 (5.52%)
2	2a	92 (3.29%)	138 (6.31%)	230 (4.62%)
	2b	34 (1.22%)	23 (1.05%)	57 (1.14%)

3	3a	1396 (49.96%)	985 (45.04)	2381 (47.80%)
	3b	556 (19.90%)	455 (20.80%)	1011 (20.30%)
4	4	82 (2.93%)	33 (1.51%)	115 (2.31%)
5	5a	2 (0.07%)	1 (0.05%)	3 (0.06%)
6	6a	1 (0.04%)	0 (0.00%)	1 (0.02%)
Untypable	Untypable	91 (3.26%)	161 (7.36%)	252 (5.06%)
Mixed	Mixed	67 (2.40%)	134 (6.13%)	201 (4.04%)
Total		2794 (56.09%)	2187 (43.91%)	4981 (100%)

Table-2: Demonstration of mixed HCV subtypes.

Mixed Subtypes	Males	Females	Total
3a & 3b	17 (25.37%)	49 (36.57%)	66 (32.84%)
3a & 2a	10 (14.93%)	31 (23.13%)	41 (20.40%)
3a & 2b	7 (10.45%)	13 (9.70%)	20 (9.95%)
3a & 1a	16 (23.88%)	19 (14.18%)	35 (17.41%)
3a & 1b	9 (13.43%)	10 (7.46%)	19 (9.45%)
3a & 4	3 (4.78%)	1 (0.75%)	4 (1.99%)
2a & 1a	3 (4.78%)	3 (2.24%)	6 (2.99%)
2a & 2b	2 (2.99%)	8 (5.97%)	10 (4.98%)
Total	67 (33.33%)	134 (66.67%)	201 (100%)

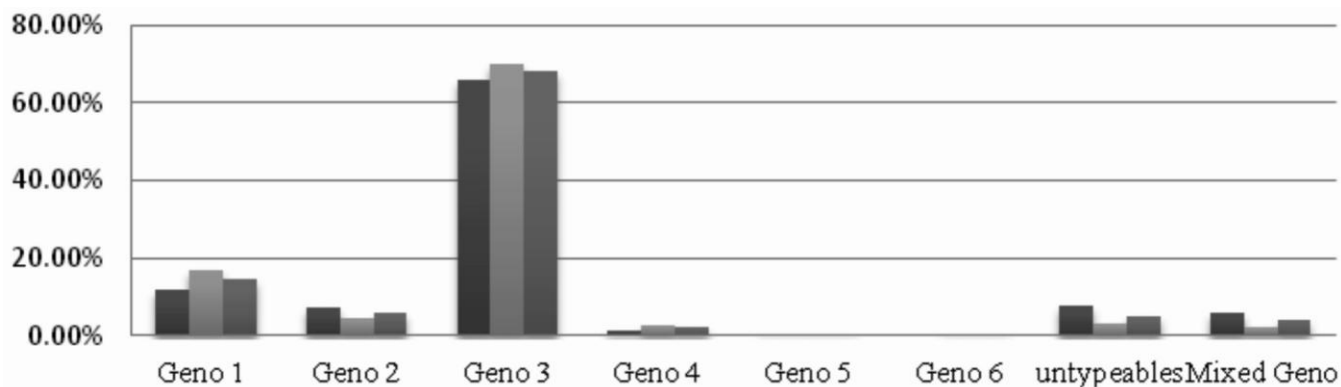

Fig. 3: Presentation of HCV Genotypes in Male and Female Patients.

Table 3: Presentation of different HCV genotypes reported from different areas of Pakistan.

Reference	Year	Province	Population Groups	HCV genotype (%)					
				Geno-1	Geno-2	Geno-3	Geno-4	Geno-5	Geno-6
Tong <i>et al</i> [25]	1996	Punjab	Patients with CLD, HCC	6.66	-	80	-	-	-

Shah <i>et al</i> [22]	1997	Sindh/Karachi	Chronic Hepatitis	6.66	2.23	87	2.22	-	-
Moatter <i>et al</i> [21]	2002	Different areas of Pakistan	Patients with HCV	14	-	68	-	-	-
Akhtar and Moatter[26]	2004	Sindh/Karachi	Households of Thalassemic Children	-	-	100	-	-	-
Iqbal <i>et al</i> [23]	2007	Punjab(Lahore)	Chronic Hepatitis	4.87	9.32	73.85	2.48	-	-
Idrees and Riazuddin[20]	2008	Different areas of Pakistan	Patients with HCV	11.51	8.41	67.46	1.49	0.18	0.12
Afridi <i>et al</i> [27]	2008	Baluchistan	Patients with HCV	7.14	-	60.72	-	-	-
Akhund <i>et al</i> [28]	2008	Sindh	Patients with HCV	3.06	-	75.87	-	0.87	-
Husain <i>et al</i> [29]	2009	Punjab (Faisalabad)	Patients with HCV	1.09	-	87.10	-	-	-
Ahmad <i>et al</i> [30]	2010	Punjab(Lahore)	Patients with HCV	23.61	-	59.09	13.70	-	-
Baig <i>et al</i> (44)	2014	Jamshoro/Hyderabad (Sindh)	Patients with HCV	7.50	18.70	72.90	0.9	-	-

Table 4: Changing epidemiology of different HCV genotypes in last five years (Nov 2007 to Nov 2015).

Genotypes	Subtype	Nov 2007- Nov 2009 (%)	Nov 2009- Nov 2011 (%)	Nov 2011- Nov 2013 (%)	Nov 2013- Nov 2015 (%)	Total (%)
1	1a	25 (4.47)	59 (5.31)	150 (10.12)	221 (12.08)	455 (9.13)
	1b	21 (3.76)	45 (4.05)	88 (5.94)	121 (6.62)	275 (5.52)
2	2a	31 (5.55)	52 (4.68)	64 (4.32)	83 (4.54)	230 (4.61)
	2b	7 (1.25)	13 (1.17)	17 (1.15)	20 (1.09)	57 (1.14)
3	3a	295 (52.80)	575 (51.8)	685 (46.2)2	826 (45.16)	2381 (47.80)
	3b	112 (20)	258 (23.2)	303 (20.45)	338 (18.48)	1011 (20.30)
4	4	10 (1.79)	22 (1.98)	37 (2.5)	46 (2.52)	115 (2.31)
5	5a	0	1 (0.09)	2 (0.14)	0	3 (0.06)
6	6a	0	1 (0.09)	0	0	1 (0.02)
Untypable	Untypable	35 (6.26)	41 (3.69)	71 (4.79)	105 (5.74)	252 (5.06)
Mixed	Mixed	23 (4.11)	44 (3.96)	65 (4.38)	69 (3.77)	201 (4.04)
Total		559 (100)	1111 (100)	1482 (100)	1829 (100)	4981

Table5: Possible source of infection of different HCV genotypes (n=4981).

Major Genotypes	Subtypes	Blood/ Blood Product Transfusion (%)	General/ Gynecological Surgery (%)	Dental Surgery (%)	Barbers (%)	Piercing/ Tattooing (%)	Infected Family Members (%)	Unknown Reasons (%)	Total (%)
1	1a	28 (13.15)	54 (5.38)	59 (5.320)	32 (5.76)	19 (20.21)	132 (23.12)	131 (9.13)	455 (9.13)
	1b	31 (14.55)	45 (4.49)	41 (3.69)	20 (3.60)	26 (27.66)	24 (4.203)	88 (6.14)	275 (5.520)
2	2a	10 (4.70)	75 (7.48)	58 (5.23)	10 (1.80)	7 (7.45)	5 (0.876)	65 (4.53)	230 (4.62)
	2b	2 (0.94)	13 (1.30)	11 (0.99)	5 (0.90)	2 (2.13)	3 (0.525)	21 (1.46)	57 (1.14)
3	3a	61 (28.64)	465 (46.40)	543 (48.90)	317 (57.02)	14 (14.89)	321 (56.22)	660 (46.03)	2381 (47.80)
	3b	22 (10.33)	208 (20.70)	287 (25.90)	129 (23.20)	7 (7.45)	66 (11.56)	292 (20.36)	1011 (20.30)
4	4	16 (7.51)	19 (1.89)	22 (1.98)	8 (1.44)	4 (4.26)	3 (0.525)	43 (3.00)	115 (2.31)
5	5a	0	1 (0.10)	2 (0.18)	0	0	0	0	3 (0.06)
6	6a	0	0	0	1 (0.18)	0	0	0	1 (0.02)
Untypable	Untypable	11 (5.16)	46 (4.59)	38 (3.42)	23 (4.14)	9 (9.57)	8 (1.401)	117 (8.16)	252 (5.06)
Mixed	Mixed	32 (15.02)	77 (7.68)	49 (4.41)	11 (1.98)	6 (6.38)	9 (1.576)	17 (1.19)	201 (4.04)
Total		213 (4.28)	1003 (20.14)	1110 (22.28)	556 (11.16)	94 (1.89)	571 (11.46)	1434 (28.78)	4981 (100)

DISCUSSION

Although in this study the most frequent HCV genotype in Pakistani population was found genotype 3 (68.10%) particularly its subtype 3a (47.80%) that was also in accordance with previous studies.^{20,24} However in last eight years (2007 to 2015) the prevalence of this type was observed on decreasing side (72.80% to 63.64%). On the other hand, the gradual increase was observed in HCV genotype 1 (1a+1b) i.e. 8.23% to 18.70% (Table 4). The increase in type 1 was examined more than hundred percent which was highly significant (p = 0.001). The same situation was observed in type 4 which increased from 1.79% to 2.52% as demonstrated in table 4, which is in agreement with the previous reports.^{20,24} HCV genotype 3 is the most responding type to Interferon therapy²⁵⁻²⁷ and most of the population of this region is infected with this genotype. Proper management of HCV patients infected with this genotype can be helpful to wipe out the HCV infection of the population

of this area as compared to those areas where type 1 and 4 are prominent and are not friendly to Interferon therapy.²⁵⁻²⁶

In 201 (4.04%) HCV infected patient’s samples, more than one genotype was found. These mixed genotypes showed the exposure of the HCV infected per-sons to more than one sources of HCV infection which indicates unawareness of disease in these patients. Untypable cases may be due to the low viral load or new HCV genotype which may not be classified by this method. Untypable HCV cases were also indicated in an earlier study done in Pakistan.²⁰

In the present study significant difference (p = 0.005) in males and females was observed regarding the distribution of different HCV genotypes, which was contrasted to the previous study done in this area in which no significant difference was observed.²⁰ It was interesting to see that in males the ratio of genotype 1 and 4 was higher as compared to females, while the situation was contrasted in genotype 2, where the ratio

Table 6: Foreign tour history of Hapatitis C patients infected with different HCV genotypes.

Genotype	Subtype	Tour to Middle East		Tour to Europe/USA		No Foreign tour (%)	Total
		Male (%)	Female (%)	Male (%)	Female (%)		
1	1a	63 (13.85)	43 (9.45)	89 (19.56)	47 (10.33)	213 (46.81)	455
	1b	51 (18.54)	27 (9.82)	62 (22.55)	37 (13.45)	98 (35.64)	275
2	2a	11 (4.78)	2 (0.87)	12 (5.22)	3 (1.30)	202 (87.83)	230
	2b	3 (5.26)	2 (3.51)	4 (7.02)	2 (3.51)	46 (80.70)	57
3	3a	58 (2.44)	55 (2.31)	132 (5.54)	14 (0.59)	2122 (89.12)	2381
	3b	22 (2.18)	12 (1.19)	16 (1.58)	9 (0.89)	952 (94.16)	1011
4	4	32 (27.83)	9 (7.83)	19 (16.52)	4 (3.48)	51 (44.34)	115
5	5a	1 (33.33)	0	1 (33.33)	0	1 (33.33)	3
6	6a	0	0	1 (100)	0	0	1
Untypable	Untypable	33 (13.09)	11 (4.37)	19 (7.54)	13 (5.16)	176 (69.84)	252
Mixed	Mixed	1 (1.99)	15 (7.46)	9 (4.48)	11 (5.47)	165 (82.09)	201
Total		275 (5.52)	176 (3.53)	364 (7.31)	140 (2.81)	4026 (80.83)	4981

of this genotype was higher in females. As mentioned before in this part of the study, genotype 1 and 4 are not common genotypes of this region as compared to genotype 2 and 3, which indicates that the source of infection in females may be local while in males foreign source may be possible, because most of the male population of this area visits to foreign countries for business, job and religious purposes as compared to females.

Most of our population visits Saudi Arabia and Iran for religious purposes in which male and female ratio may be same, but a large number, mostly the males visits or stay there especially in Saudi Arabia and UAE for earning purposes, where HCV genotype 1 and 4 is the most common type in the HCV infected population.²⁸⁻³⁰ So these countries may be the main source of transmission for genotype 1 and 4. The source of genotype 1 may also be Europe and USA, where this type is most common.³¹⁻³⁴ So, future planning to block these probable sources of transmission is the need of the hour. For that, proper screening of those people who return after a long stay in above mentioned countries should be mandatory.

The probable transmission source of HCV infection in this study revealed that most of the people infected with genotype 1 and 4 had a foreign tour history as compared to those people infected with genotype 2 and 3 which provides strength to the above mentioned possibility. It was also important to see that mixed genotypes were more common in females especially in those mostly exposed for surgery purposes. Most of those females had history of more

than one gynecological surgeries, which indicates that surgery may be the major source of infection. In single genotype infected population the most common source was suspected barbers and dentists, which was also indicated in previous studies.³⁵ It shows lack of awareness in our population about HCV transmission sources. It is also important to mention here that only the male population visited the barbers for hair cutting and shaving purpose. So, the data was collected only from the male population not from the females regarding the possible source of HCV infection in this category. Blood trans-fusion and needle source was less common in this study that was contrasted to the previous study.³⁵ It may be because of proper screening and new needle use awareness, which was a good sign to overcome this infection.

Out of 4981 HCV infected individuals 11.46% had a history of HCV infected family members (Table 5). In this region the joint family system and cousin marriage are very common. Because Hepatitis C disease remained in silent form and infected persons looks healthy which may transfer their infection silently to other family member living with.³⁶ This can potentially hap-pen through the sharing of razors, toothbrushes, or any sharp instruments that carry HCV infected blood.³⁷ Therefore, it is important to keep each person's personal use items such as toothbrush and razor in separate areas of the bathroom and each item should be clearly labeled. In this way, the accidental use of the potentially HCV infected household item may be prevented. The proper screening of joint family members should be done to elucidate the infected member. In

28.78% cases no possible source of HCV transmission was found. In those cases no family member was infected and never visited the barbers for a shave and had no blood transfusion or needle contact history but were infected with HCV. It clearly indicates that there are others, as yet unidentified modes of transmission.

In conclusion a decrease in genotypes 2 and 3 especially subtype 3a and gradual increase in genotypes 1 and 4 in this population was examined. This situation is alarming because genotype 1 and 4 are not so friendly to Interferon therapy as genotype 2 and 3. Therefore, future treatment strategy should be directed towards type 1 and type 4. The probable source of transmission was observed in those cases that had a history of repeated general or dental surgery and visited to barbers. So a proper strategy on the government level to monitor the standard sterilization of surgical instruments in hospitals and awareness campaign for barbers and family members is the need of the hour, which can be helpful in reducing the chances of trans-mission.

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