

HEPATITIS C VIRUS GENOTYPE DISTRIBUTION AMONGST HCV POSITIVE PATIENTS PRESENTING AT A PRIVATE TERTIARY CARE HOSPITAL OF PESHAWAR, KHYBER PAKHTUNKHWA

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ABSTRACT

Introduction: Hepatitis C, a liver disease caused by the Hepatitis C virus (HCV), often results in chronic hepatitis, cirrhosis and hepatocellular carcinoma. HCV has different genotypic geographic distribution as well as genetic diversity and heterogeneity. The present study was conducted to find out the frequencies of different HCV Genotypes in patients referred to a tertiary care hospital of Peshawar, Khyber Pakhtunkhwa, Pakistan, and to determine correlations, if any, between Genotype, age, viral load, gender and nationality.

Materials & Methods: The present descriptive study was conducted in the Pathology laboratory of Rehman Medical Institute (RMI), Peshawar over a period of 8 months (August 2015 to March 2016). Blood samples of 36 HCV positive patients (by ELISA) were included for study. RNA samples of HCV positive patients were subjected to genotyping using Single Linear Array HCV Genotyping strip (Roche Diagnostic) by hybridization method. The Linear Array HCV Genotyping Strip results were read visually by comparing the pattern of blue bands to a reference table of Genotype patterns. HCV genotyping data were analyzed by SPSS version 16.0. Viral load association with HCV Genotypes, age, gender and nationality were assessed by Chi-square test with $p < 0.05$ considered significant.

Results: Of 36 patients, 24 (70.6%) were from Pakistan and 10 (29.4%) from Afghanistan. The ages of these subjects ranged from 20-60 years with male to female ratio of 1:1. Amongst them, HCV Genotype was positive in 34 (94.4%) cases. The most frequent Genotype found was Genotype 3. Genotype 3 alone was found in 24 (70.6%) patients and Genotype 1 alone in 01 (02.9%) patient. The combination of Genotype 3 and 4 was found in 06 (17.6%) patients, while the combination of 1 and 3 was found in 03 (08.8%) patients. No association was found between Genotype and viral load, age, gender and nationality.

Conclusion: HCV Genotype 3 was the most frequent genotype, similar to other studies carried out in different regions of Pakistan.

Key Words: Polymerase Chain Reaction; Genotype; Hepatitis C Virus; Hepatitis, Chronic; Hepatic cirrhosis; Carcinoma, Hepatocellular.

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INTRODUCTION

Hepatitis C Virus (HCV) mainly affects hepatocytes, resulting in chronic hepatitis, cirrhosis and hepatocellular carcinoma.¹ About 170-200 million people around the world are thought to be affected by HCV infection. HCV has different Genotype distribution and has genetic diversity and heterogeneity.² Approximately 17 million people in Pakistan are estimated to be infected with HCV.³ Approximately 350,000 people die of diseases caused by HCV worldwide.⁴ Now it is considered that distinct Genotypes of HCV predict sustained viral response and they are epidemiological markers indicating the source of infection in given population.^{5,6} With help of phylogenetic analysis various Genotypes and subtypes have emerged, with 30% nucleotide differences in their sequences,⁷ and about 20-25% of nucleotide variation present in the subtypes of HCV in the nonstructural coding region.^{8,9} In 2005, six Genotypes and 100 subtypes emerged with help of phylogenetic analysis.¹⁰ In 2013, whole HCV genome was sequenced and by 2014, a 7th Genotype was discovered.¹¹ Genotype 2 and 3 have better therapeutic response as compared to Genotype

1,4,5,6,7. This variation is popular and constant over geographical distribution. So finding the Genotypes of HCV is important in predicting the duration of the treatment as well as prognosis.¹²⁻¹⁵

Determination of the subtypes of HCV Genotype is important even in the treatment of emergent resistant cases.¹⁶ Poor therapeutic response with Genotype 1 is tackled with introduction of oral antiviral drugs that improves the sustained viral response. It is being said that the treatment of HCV infection is entering into a newer era with less side-effects and interferon-free treatment.¹⁶ Currently, the treatment for Genotype 1 seems to be better than Genotype 3.^{17,18}

Knowing the importance of HCV Genotype and availability of rare data on HCV Genotype in Peshawar, Khyber Pakhtunkhwa led to the present study in order to analyze the limited data available in this context. The study was conducted to find out different Genotypes in HCV positive patients and find out any relation with other factors like viral load, age, sex and ethnic group.

MATERIALS & METHODS

The present descriptive study, based on retrospective data collection, was carried out at Rehman Medical Institute (RMI) Laboratory from August 2015 to March 2016. A total of 36 patients suffering from HCV infection underwent HCV genotyping; records of their genotyping were accessed for data collection keeping their identities confidential.

Patients who were additionally suffering from other blood transmissible diseases such as Hepatitis B and Human Immunodeficiency Virus (HIV) were excluded from the study. These patients came for primary consultation or they were referred from other community doctors

for genotyping after testing positive by anti-HCV antibody tests. RNA samples were extracted from the patients' serum. By using reverse transcription, extracted RNA samples were converted into cDNA and amplified through PCR using the Cobas Amplicor Analyzer (Roche Diagnostic). Genotyping was done by using single Linear Array HCV Genotyping Strip (Roche Diagnostic) Hybridization method in which denaturation solution was added to the amplification tubes to chemically denature the HCV amplicon to form single-stranded DNA.

The denatured amplicon was then subjected to Linear Array detection within two hours. Aliquots of denatured amplicon were then transferred to the appropriate well of the typing tray that contained hybridization buffer and a single Linear Array HCV Genotyping Strip, which was coated with a series of oligonucleotide probes specific for various HCV Genotypes. The biotin-labeled HCV amplicon hybridized to the Genotype-specific oligonucleotide probes only if the amplicon contained the matching sequence of the Genotype-specific probe.

After the hybridization reaction was completed, the Linear Array HCV Genotyping Strip was washed several times and detected by using Horse Radish Peroxidase Conjugate.

The Linear Array HCV Genotyping Strip was then read visually by comparing the pattern of blue bands to a reference table of Genotype patterns.

Genotype data were analyzed for descriptive statistics by SPSS 16.0. Associations of viral load with different HCV Genotypes and with age, gender, and nationality were assessed, using the Chi-square test; $p < 0.05$ was considered significant.

RESULTS

Of 36 patients previously tested positive for HCV by ELISA, 34 (94.4%) tested positive for HCV by genotyping; hence the results are shown for these 34 patients. Table 1 gives the demographic data of these 34 patients. Most

patients (25, 73.5%) were above age 40, with 09 (26.5%) patients below age 40; there were 17 males and 17 females (50% each); nationality was from Pakistan in 24 (70.6%) patients and from Afghanistan in 10(29.4%) patients.

Table 1: Demographic data of patients (n=34).

Demographic Variables	Frequency	Percentage
Age Groups (years)		
20—30	04	11.8
31—40	05	14.7
41—50	18	52.9
51—60	07	20.6
Gender		
Males	17	50.0
Females	17	50.0
Nationality		
Pakistan	24	70.6
Afghanistan	10	29.4

Table 2 gives the data for genotyping results. The most frequent genotype was 3 (24, 70.6%), followed by combination of genotypes 3 & 4 (06, 17.6%), combination of genotypes 1 & 3 (03, 08.8%) and Genotype 1 alone (01, 2.9%).

Regarding viral load, data are available for 26 patients; the high and medium groups had 09 (34.6%) each, while the low group had 08 (30.8%) cases.

Table 2: Genotyping data of patients (n=34).

Genotyping Data	Frequency	Percentage
Genotypes		
1	01	2.9
3	24	70.6
1 and 3	03	08.8
3 and 4	06	17.6
PCR (Viral Load) (n=26)		
Low (1,000,000)	08	30.8
Medium (>1,000,000 5,000,000)	09	34.6
High (> 5,000,000 25,000,000)	09	34.6
Very High (> 25,000,000)	-	-

Table 3 gives data for the viral loads found in different genotypes for 26 patients. Combined Genotypes of 3 & 4 (n=04) had the highest

mean viral load, followed by Genotype 3 (n=20); combined Genotypes 1 & 3 (n=01) had the lowest viral load.

Table 3: Distribution of mean viral loads by Genotypes (n=26).

Genotypes	Mean Viral Loads	S.D.
Genotype 1 (n=1)	313680.00	-
Genotype 3 (n=20)	4734219.80	4900128.804
Genotypes 1 and 3 (n=1)	37647.00	-
Genotypes 3 and 4 (n=04)	7668188.00	11323101.146
Total (n=26)	4834941.35	6055145.655

Table 4 provides data for the mean viral loads by gender, age groups and nationality. Though males had higher mean viral loads, the difference was not significant ($p=0.639$); similarly the age group of more than 40 years

had higher, but non-significant ($p=0.790$) mean viral loads. Patients from Afghanistan had higher mean viral loads, but the difference was not significant ($p=0.696$).

Table 4: Mean viral loads by gender, age groups, and nationality (n=26).

Groups	Viral Load (Mean \pm SD)	p value
Gender		
Male (n=14)	5365686.43 \pm 7277245.455	0.639
Female (n=12)	4215738.75 \pm 4468243.473	
Age Groups (years)		
40 (n=06)	5430939.50 \pm 4094303.890	0.790
> 40 (n=20)	4656141.90 \pm 6609532.323	
Nationality		
Pakistan (n=16)	4457865.44 \pm 6972045.583	0.696
Afghanistan (n=10)	5438262.80 \pm 4491516.457	

DISCUSSION

The pattern of distribution of HCV Genotypes varies throughout the world. Genotypes 1, 2 and 3 are widely spread all over the world.²⁰

Approximately 70% of chronic HCV infections in the USA²¹ are caused by genotype 1, 15-20% by genotype 2, 10-12% by genotype 3, 1% by genotype 4, and less than 1% by genotype 5 or 6. Of the genotype 1 infections, approximately 55% are genotype 1a and 35% 1b.

Similarly in Australia Genotype analysis was carried out,²² which demonstrated that HCV Genotype 1 was predominant in 55% of HCV positive patients followed by Genotype 3 in 38% of HCV positive patients; Genotype 2 was 7%. In a study from 2006, Ramia S, et al²³ mention that the most prevalent Genotypes in Europe are 1 and 2 followed by Genotypes 3. Two

patterns of HCV Genotype distributions are found in the Middle East.²³ Among Arabs (Egypt, Gaza, Jordan, Kuwait, Lebanon, Saudi Arabia and Syria), Genotype 4 is predominant, while among non-Arabs (Iran, Israel and Turkey), Genotype 1 is seen frequently.^{25,26}

The predominant Genotype reported in South Africa is Genotype 5 (54%) followed by Genotype 4 (19%) Genotype 1 (19%), and Genotype 3 (2%) in HCV positive patients.²³

Most regions of China show a high prevalence of HCV Genotype 1 followed by type 2 while in southern regions HCV Genotype 6 is common and in Southwest China Genotype 3 and 6 are prevalent.^{27,28}

Commonest Genotype throughout India is found to be Genotype 3, while in south India Genotype 1 is predominant.²⁹⁻³¹

A systemic review done in Pakistan³² on pattern of HCV Genotype geographic distribution showed that predominant Genotype was 3. The geographic distribution was such that, in Punjab it was 68.94%, in Sindh 76.88%, in KP 58% and 60.71% in Baluchistan. Also there was high prevalence of Genotype 1 (12.14%) in Punjab. HCV Genotype 3 has a good serological viral response to treatment and control and has short treatment duration as compared to Genotype 1 which has poor serological response to treatment and duration of treatment is prolonged.^{33,34} A study done in Lahore included 489 HCV positive patients,

among these cases the most common HCV Genotype was found to be Genotype 3a (80.77%), followed by Genotype 4 (4.9%) and Genotype 1 (9.6%).³⁵

A recent study (2014)³⁶ conducted in district Bannu of Khyber Pakhtunkhwa Province also confirmed high prevalence of Genotype 3a (38%) followed by Genotype 3b (21%).

CONCLUSION

HCV Genotype 3 was the most frequent genotype found in the present study. This finding agrees with other studies carried out in different regions of Pakistan. No correlation was found between Genotype and viral load, age, gender and nationality.

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