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Prevalence of Hepatitis C Genotypes in Bhimber (AJK)

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KEY WORDS:

- Hepatitis C
- Genotypes
- Prevalence
- Bhimber
- AJK (Azad Jammu and Kashmir)
- HCV
- Viral Hepatitis
- Infection Rates

ABSTRACT

Background: The HCV genotyping provides valuable epidemiological therapeutic information. It is rightly rectifying the importance of genotype proficiency before clinical therapy. In the connection this particular study will be valuable for the health care providers and clinicians in designing the therapeutic strategies to cope this manic disease. The aim of the study is to investigate the prevalence of genotype present in District Bhimber.

Materials and Methods: For this purpose, 100 HCV antibody positive samples were collected and analyzed using Real Time PCR.

Results: In our findings the prevalence of HCV in patients having age group greater than 40 was high. Most prevalent genotype is 3a 96(64%) followed by 1b 15(10%) and 3b 12(8%). 3a is found to be the prevalent genotype of the young patients and 1b among old patients. We have not found any correlation between viral load and gender. Intermediate viremia is seen in 3a genotype.

Conclusion: This research underscores the importance of HCV genotyping for tailored therapy. It reveals a high prevalence of genotype 3a among younger patients in District Bhimber, with genotype 1b more common in older individuals. No gender-viral load correlation was found, but intermediate viremia occurred in genotype 3a cases. It is also concluded that more work is needed in order to determine the genetic diversity and characterization of genotype among gender.

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Introduction

Hepatitis C virus belongs to family flavivaridae and genus hepacivirus causing blood born disease hepatitis C. Hepatitis C infection is a public health disease worldwide affecting 170 to 200 million people each year[2]. According to world health organization Pakistan is ranked second in the countries with high rates of chronic infections. Unsafe injections are the common cause of HCV transmission in these countries. According to WHO, Pakistan is ranked among the top syringe consuming countries. Prevalence of HCV infection in Pakistan is significantly higher when compared with neighboring countries[3,4,5]

Major risk factors for HCV transmission are blood, blood products and body fluids. Genome of hepatitis C virus is approximately 9.3Kb. It is positive single stranded RNA virus. Choo et al in 1991 discovered the genome of the virus encoded by polyprotein, which after lysis by proteolytic enzymes produces structural and non-structural proteins. Non-structural proteins lie towards N terminal region of the genome and mostly involved in RNA replication[6,7]. HCV has high mutation rate because RNA polymerase does not have proof reading ability. There are approximately 6 genotypes and almost several subtypes[8]. More prevalent genotype among Pakistani population is 3a followed by 3b and 1a. There is a strong association between genotype 3 and hepatocellular carcinoma[9]. Genotype 3 involves in HCV induced steatosis. It is difficult to diagnose an acute hepatitis during initial stage. Clinical signs and symptoms within six months from the onset of disease considered as acute hepatitis. HCV RNA can be detectable within 1-2 weeks and rises speedily for the period of first few weeks of the onset of infection[10].There

is less chances of liver failure during acute phase. 30-20% individuals may have chances to clear the infection and 70-80% may enter into chronic infection.

Frequency of HCV genotypes in Pakistan

Pakistan has 3a genotype followed by 3b and 1a. Percentage of chronically infected individuals in Pakistan is 30% more than other countries[11]. Genotype 3a shows strong relationship with chronic infection by suppressing the apoptotic effect. Programmed cell death is a natural phenomenon responsible for the elimination of viral infections. Anti-apoptotic effect was seen by core region and NS5A but till date it is not clear which protein has anti apoptotic affect[12].

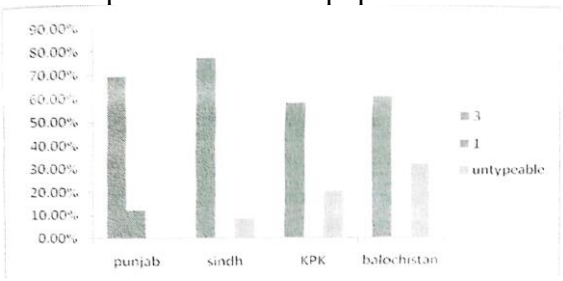

Figure 1: Distribution of HCV genotypes in 4 provinces of Pakistan

Materials and Methods

100 nHCV-antibody positive samples were collected from Atique diagnostic laboratory Bhimber, Azad Kashmir for detecting the prevalence of HCV. Antibody tests were performed by immune chromatographic technique. Then qualitative test was done by PCR. Out of these patients, 75 were HCV-RNA positive and 25 were HCV-RNA negative.55 were females and 20 were males out of 75 HCV-RNA samples. For genotyping analysis, HCV-RNA was extracted by using Amplisen HCV genotype-FRT PCR kit. For RNA extraction, 450 ul of lysis solution and 10ul of IC were added in the reaction tube of 1.5 ml. Plasma sample of 100ul was added in these tubes and vortex them thoroughly.

Negative control of about 100ul was added in a tube labeled as negative control and vortex it. After that sorbent of 25ul was added per each tube and vortex them thoroughly. Those vortex tubes were incubated at room temperature for 10 minutes. The supernatant was discarded after centrifugation and added 500ul of washing solution 1 in each tube. Then vortex

and centrifuged each tube at 10,000 g for one minute. Each tube was washed with washing solution 3 and 4 by vortex and centrifuged at the same rate as discussed above. In the last step, RNA was dissolved in a RNA buffer. Viral load was determined by the RT-PCR through Scacae Biotechnology. It was a one step process in which DNA synthesis and amplification take place in the same reaction mixture. Complementary DNA was synthesized according to protocol of the kit (Amplisen HCV genotype-FRT PCR). This kit is used for the qualitative detection of HCV genotypes by means of real time hybridization-fluorescence detection. For this, we used hot start method which reduces the chances of non-specific amplification.

Genotyping by RT-PCR using Amplisen kit

According to the protocol of the kit 25ul of the solution was prepared for genotyping. For that purpose three reaction mixtures were prepared by adding 65ul of RT-PCR-mix-2-FEP/FRT and 6ul of polymerase (TaqF) per each tube with PCR-mix-1-FRT HCV for genotypes 1b/3, PCR-mix-1-FRT HCV for genotypes 1a/2, PCR-mix-1-FRT HCV for genotype 4/IC. After vortex 12.5ul of the prepared mixture was added in PCR tubes. And transfer in it 12.5ul of the mixture having cDNA for making 25ul of the solution.

It was a twostep process. In a first step, cDNA was synthesized and in then genotyping was done in the second step. During PCR, denaturation was done at 95

°C for 15 mints for one cycle. Then amplification was done at 60 C° for 40 second. Two types of dyes were used here FAM and JOE. Peaks for FAM were green in color and for JOE yellow in color. Peaks for genotype 1b were given by FAM in a tube having reaction mixture for 1b and 2.

Results

200 CV-antibody positive patients were selected from Atique lab Bhimber Azad Kashmir. Positivity test was done by immune chromatographic technique. Out of these 200 positive patients 150 were HCV-RNA positive and 50 were HCV-RNA negative. 111 were females and 39 were males out of 150 HCV-RNA samples. Out of the 150 HCV-RNA positive patients 27 were untype able. Remaining 123 typeable patients,96(64%) was with genotype 3a, 15(10%) and 12(8%) with genotype 1b and 3b respectively. Genotype 3a was Predominant in both males and females, but after that 3b was found to be more common in males in comparison to females whom have shown 1b to be more prevalent. Two age groups i.e. less than forty and greater than forty were also related to genotypes and results showed that younger male patients were infected by genotype 3a while 1b was prevalent in older female patients. Viral load was categorized as low (60,0000), intermediate (60, 0000-80, 00000) and high (80,00000) (table.2). A patient with genotype 3a had intermediate vermeia rather than other genotypes. No significant association was seen between gender and viral load. Viral load was present in the same ratio among males and females.

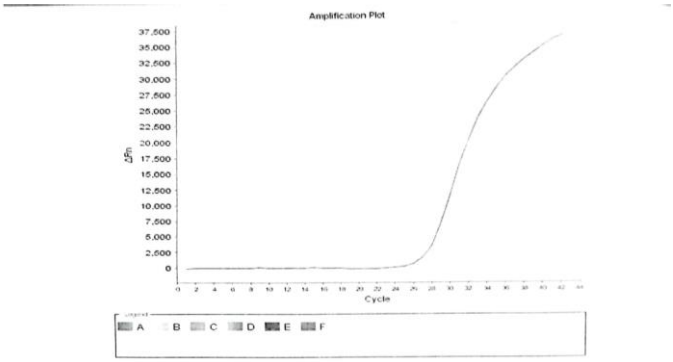

Figure 3: Peak with FAM dye shows the presence of genotype 1b in reaction mixture having PCR-mix-1-FRTHCV genotypes 1b/3.

Subtype	Male			Female		
	<40	>40	Total	<40	>40	Total
1b	Nil	Nil	Nil	0	15	15
3a	16	2	18	40	38	78
3b	5	7	12	Nil	Nil	Nil
untypeable	5	4	9	10	8	18
Total	26	13	39	50	61	111

Table 1: HCV viral load categories and its distribution in genotypes

Gender		Viral load		
		Low	Moderate	High
Males	Total	10 (25.64%)	21 (53.84%)	8 (20.51%)
	? 40	7	15	4
	> 40	3	6	4
Females	Total	41 (36.93%)	46 (41.44%)	24 (21.62%)
	? 40	16	26	08
	> 40	25	20	16

Table 2: Viral load

Discussion

In this particular study we have not observed equal distribution of the genotypes among gender[11]. There is a significant difference between gender and different genotypes. 1b was the prevalent genotype of the females and 3b among the males, however genotype 3a was present in same ratio among females and males. Contradict to the previous study, we depict a

strong association among genotype and gender which is only supported by the study conducted in Libya where prevalent genotype among the females were 1b while 4a among the males[12]. Results show the high prevalence of genotype 3a among the young patients. While its ratio declines as the age group increases. Our observation about the prevalence of 1b in female is that they are potentially more exposure of blood transfusion rather than males during C-section of their delivery. 3a shows intermediate viremia as compared to other genotypes. So, patients infected with this type of genotype give better response against therapy and require short term duration of treatment. No significant distribution was seen between viral load and gender Viral load was high among the older patients. Results of our study on the basis of viral load match with the resul of Ali et al. According to our study prevalent genotype in district District Bhimber AJK is 3a followed by 1b and 3b like all other regions of the Pakistan.

Conclusion

In conclusion, this research underscores the significance of HCV genotyping in informing therapeutic approaches. By highlighting the prevalence of genotypes in District Bhimber, it offers crucial insights for healthcare providers. Our findings reveal a high prevalence of HCV among individuals over 40, with genotype 3a being the most common, particularly among younger patients. Additionally, genotype 1b is prevalent among older patients. Despite no correlation between viral load and gender, intermediate viremia is noted in genotype 3a cases. This study emphasizes the necessity of genotype proficiency prior to clinical therapy, guiding clinicians in designing effective therapeutic strategies against this challenging disease

Declaration Of Interest

The authors declare no conflict of interest.

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Author's Contribution

1. U.R.: Created concept and design of the research, prepared initial draft, collected data, interpreted the results and generated discussion and conclusion.
2. S.K., N.R. A.M., N.Z., A.A: Data collection.
3. K.S., S.M., J.I.: Proof reading.