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Prevalence of Hepatitis C infection in young adults of district Bannu

Sajid Raza[1], Farhana Maqbool[1*], Zulfiqar Ahmed Bhatti[2], Malik Mujaddad- Ur-Rehman[1], Shahid Nadeem[3], Asif Raza[4], Nazia Murtaza[1]

[1]*Department of Microbiology, Hazara University Mansehra, 21300 KPK, Pakistan*
[2]*Department of Environmental Sciences, COMSATS, Abbottabad 22010, Pakistan*
[3]*Department of Genetics, Hazara University Mansehra, KPK, Pakistan*
[4]*Department of Pharmaceutics, Gomal University D I Khan, KPK, Pakistan,*

Abstract : *It is estimated that the people affected by Hepatitis C worldwide is around 250 million and chronic carrier of hepatitis B are around 400 million. In present study a total of 450 young adults apparently healthy from Bannu district were screened for anti-HCV antibodies by using Immunochromatographic kits. Among them the male young adults were 280 and female young adults were 170 of the age 15 to 26 years of age. The positive samples were further proceeds for ELISA. The ELIZA positive samples were further conformed for RNA (HCV) in the blood by real time polymerase chain reaction (RT-PCR). The PCR positive samples were further genotyped. The results indicated that 4.44% of young adults of district Bannu were actively infected with HCV. The prevalence of active infection was slightly more in male young adults (4.4%) as compared female young adults (3.5%). The prevalence was also higher in the age group 23 to 26 years which was 5.55% and lowers in the age group 15 to 17 years which was 2.70%. The most frequent circulating genotype in the study population was 3a followed by an unknown genotype>3b>2a> 1b.*

Keywords - : *ELISA, Genotype, HCV RNA, Immunochromatographic kit, RT-PCR*

I. INTRODUCTION

It is estimated that the people affected by Hepatitis C worldwide is around 250 million and chronic carrier of Hepatitis B are around 400 million [1, 2]. Hepatitis can also cause by other viruses which includes cytomegalovirus, yellow fever, Epstein-Barr virus etc, in addition to the hepatitis viruses. Hepatitis C virus causes an infectious disease [3].The symptoms of HCV infection is asymptomatic, chronic infection occur when infection established than leads to the scarring of the liver fibrosis and cirrhosis, generally apparent after many years and cirrhosis can developed liver failure. Other complication including liver cancer [4] .In 1989 hepatitis C virus was identified as the leading pathogen for non A, non B viral Hepatitis [5].The family flaviviridae was the family of hepatitis C virus, based on its similarity with pest viruses and Flaviviruses, but ranked as the first member of new genus called Hepacivirus [6] .The RNA virus is single stranded with 96kb length, enveloped, consisting of structural (core, E1, E2 and possibly P7) and non structural (NS2, NS3, NS4, A, N54 B, NS5A, and NS5B) protein [7, 8]. According to the centers for disease control HCV has six major genotypes and more than 50 subtypes of HCV. On the variations in the non structural viral genome in NS-5 this classification is based. The variation among the genotype of virus is 30-50%, while the variance 1-5 % in the nucleotide sequence from a single HCV infected patients [9]. F1 and F2 are first viral proteins that come in contact with the cells and are responsible HCV attachment with the cells [10]. Transmission can occur through sharing of needles by contact HCV infected blood. Blood transfusion sexual contact and during transplantation of organ but very often this could not occur. From HCV infected mother Hepatitis C may pass to her baby [11].In 20% to 56% of patient with chronic hepatitis interferon IFN- alone or in combination with ribavarin is the effective treatment for hepatitis C and sustained virological response occur in 20%-56% of chronic hepatitis patient [12,13,14]. The objective of the present was to screen the adults of Bannu district for hepatitis C viral infection using ICT Kits, ELISA, viral RNA by polymerase chain reaction (PCR) and genotype present in PCR positive samples. The significance study was to find out the prevalence of HCV in adults, active infection and genotype circulating in young adults which will help to determine the treatment strategy.

II. MATERIALS AND METHODS

2.1 Sampling

A questionnaire was used to collect the data that contain all the information including age, sex, history and residence of the person. All these young adults belonged to the various areas of the district Bannu (Table 1).

After initial information sterile syringe was used for the collection of the blood from the patient. 5ml of blood was taken from the patient and centrifuged for 5 minute at 11000 rpm and serum was separated and stored at -20°C for analysis.

2.2 Screening Test

The screening was done with the help immune chromatographic kit (InTec Product, Xieman) according to manufacturer instructions. The method for ELISA test for HCV was adopted from the protocol in the EIA (Enzyme immune assay) kit, anti HCV manual. RNA extraction and real time PCR method was adopted from the protocol in the HCV Real –TM Qual handbook. The PCR positive samples were further examined for the genotypes of HCV and the procedure was adopted from Ohno et al., [15]. For genotypes identification, multiplex-PCR reactions were used, which consist of two sets of primers each processed corresponding in the nested PCR. The primers details and PCR amplified fragments size associated with them are given in the figure 1.

2.3 Serum isolation

Serum samples used for genotyping, obtained from actively infected adults with hepatitis C virus. All of them detected by RT-PCR were sero positive for anti-HCV.

Figure 1 shows the positions of nucleotide and sequences and the primers used. As we are trying to detect the nine different subtypes, on the basis of differences in the sizes of the different bands the recognition primers were divided into two different types, so in the same gel no genotype-specific bands were analogous molecular size. HCV type 2a-specific sense primer was also added. It was noted that by deducing from the sequences obtainable, two bands have HCV type 2a isolates, one of 190bp and other 139 bp, whereas type 4 isolates may infrequently give rise to 190-bp band. Following primers were used for genotype analysis.

Forward Primer 5'- GGGAGGTCTCGTAGACCGTGCACCATG- 3'

Reverse Primer 3'- GAG (AC) GG (GT) AT (AG) TACCCCATGAG (AG) TCGGC-5'

Primers for genotype in mix 1 are 2a, 1b, 2b and 3b

Primers for genotype in mix 2 are 1a, 3a, 5a and 6a.

Mix 1

S7 5'- AGACCGTGCACCATGAGCAC-3'

S2a 5'- AACACTAACCGTCGCCCACAA-3'

G1b 5'-CCTGCCCTCGGGTTGGCTA (AG) -3'

G2a 5'-CACGTGGCTGGGATCGCTCC-3'

G2b 5'-GGCCCCAATTAGGACGAGAC-3'

G 3b 5'- CGCTCGGAAGTCTTACGTAC-3'

Mix 2

S7 5'- AGACCGTGCACCATGAGCAC-3'

G1a 5'- GGATAGGCTGACGTCTACCT-3'

G 3a 5'- GCCCAGGACCGGCCTTCGCT-3'

G4 5'- CCCGGGAACTTAACGTCCAT-3'

G5a 5'- GAACCTCGGGGGGAGAGCAA-3'

G6a 5'- GGTCATTGGGGCCCCAATGT-3'

Figure 1: Primers used in this research

2.4 Expected band size of the genotype specific bands amplified by PCR

The band sizes of genotype 1a, 1b, 2a, were 208 bp, 234 bp, 139 bp. The 190 bp amplicon also detected in HCV type 4 isolates. Band sizes of genotype 2b, 3a, 3b, 4, 5a, 6a were 337 bp, 232 bp, 176, 99 bp, 320 bp, 336 bp respectively.

The HCV isolated from 20 samples were genotyped. In this HCV genotyping system by using real time-PCR, from serum RNA was extracted with spin column kit (Sacace Biotechnologies, Italy) and with Moloney murine leukemia virus reverse transcriptase with random hexamer (GIBCO BRL, Gaithersburg, Md.). Reverse transcribed into cDNA, with the following parameters 2µL of this cDNA was amplified for 40 cycles: (denaturing) amplification at 94° C for 1 min followed by 20 cycles, (annealing) 45° C for 1 min and (extension) 72° C for 1 min followed by 20 extra cycles of 94° C for 1 min, 60° C for 1 min, and 72° C for 1 min. For the

second-round PCR, first-round PCR product of 0.5 ml was amplified for 30 cycles; each cycle consisted of 94° C for 1 min, 62° C for 45 s, and 72° C for 1 min.

III. RESULTS

450 healthy individuals of age range 15-26 years, males and females were selected. Among the total samples tested for the prevalence of HCV, there were 280 (62%) males, and 170 (38%) females from different areas of district Bannu (Table 2). The individuals were categorized into 3 age groups. These result showed that about 20 (4.44%) out of 450 individuals were found positive for anti HCV antibodies in their blood (Table 2).

The prevalence rate of anti HCV antibodies was higher among the age groups ranges 24-26, 21-23 which were 5.55%, 5.45% respectively and comparatively lower in 18-20, 15-17 age group which have 2.32% and 2.70% respectively (Table 2).

3.1 Quantitative analysis of HCV

HCV RNA was extracted from patient serum and performed by Real-time PCR within a single tube. Smart cycler carried out amplification and detection simultaneously using Taq man probes and detected through fluorescent reporter dye specific for PCR. The RNA extracted from the blood samples were subjected to real time PCR for the quantitative analysis with a positive control. The result of a selected positive sample with control is shown in the Fig 2a, 2b and 2c.

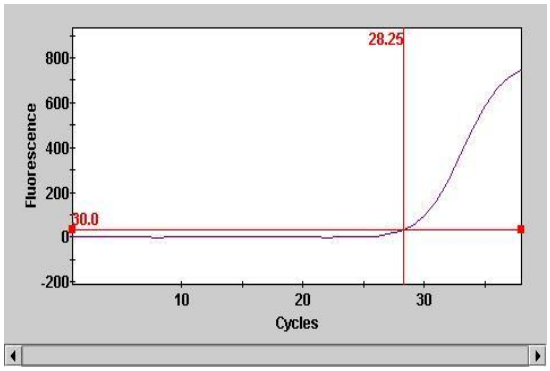

Figure 2a: Positive Control Graph

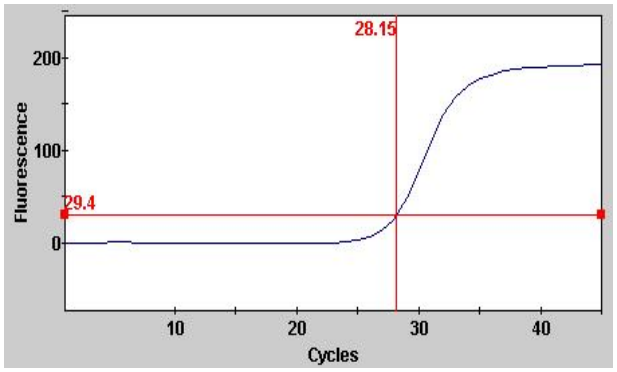

Figure 2b: Sample Graph

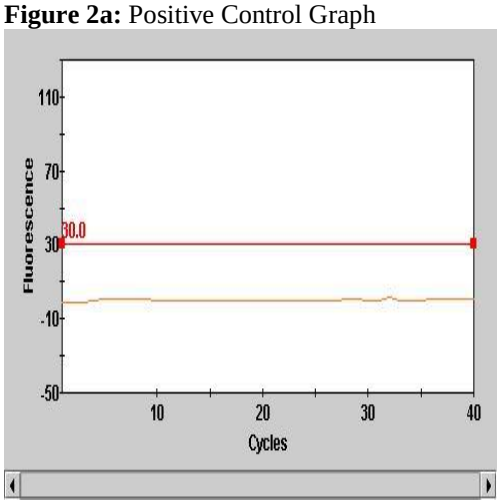

Figure 2c: Negative sample graph

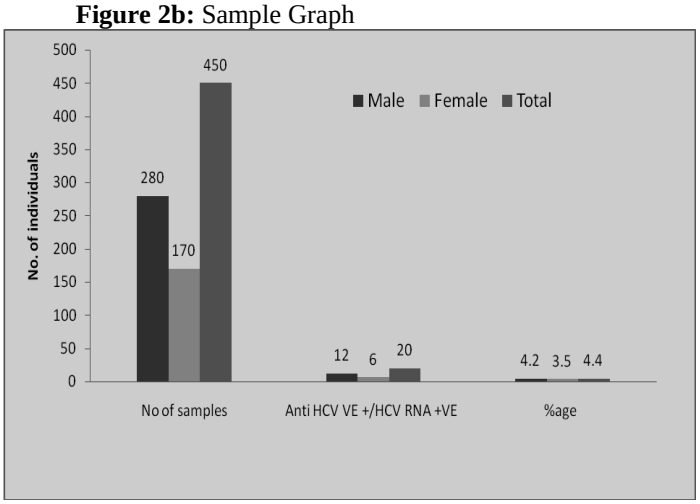

Figure 3: Distribution of HCV among female and male young adults of district Bannu

All the ELISA positive samples were analyzed by RT-PCR for HCV RNA. By using Real-time PCR, we develop and evaluated a rapid, sensitive, specific and reproducible for the detection of Hepatitis C Virus RNA in serum. The prevalence rates of anti HCV antibodies and anti HCV RNA in male young adults have 4.2% and have 3.5% among female adults of District Bannu (Table 3, Fig. 3). Table 4 showed HCV genotype frequency distribution among HCV positive cases. Of the total 20 HCV positive cases, the 3a genotype constituted 45% of the infection (Table 4). The most frequently circulating genotype of HCV in district Bannu is 3a and least frequently circulating genotype of HCV is 1b.

Table 1: Basic information about collected blood samples from District Bannu.

t	Areas	Samples	Males	Females
1	Bannu	280	170	120
2	Kaki	100	78	22
3	Domain	70	42	28
	Total	450	280	170

Table 2: Age-wise distribution of the anti- HCV antibodies and HCV

S.NO	Age Group(years)	Samples	ANTI-HCV +ve	HCV RNA +ve
1	15-17	74	2 (2.70%)	2 (2.70%)
2	18-20	86	2 (2.32%)	2 (2.32%)
3	21-23	110	6 (5.45%)	6 (5.45%)
4	24-26	180	10 (5.55%)	10 (5.55%)
	Total	450	20 (4.44%)	20 (4.44%)

Table 3: Sex- wise distribution of the anti- HCV antibodies and HCV RNA

S.No	Sex	Samples	ANTI-HCV +ve	HCV RNA +ve
1	Male	280	12 (4.2%)	12 (4.2%)
2	Female	170	6 (3.5%)	6 (3.5%)
	Total	450	20 (4.44%)	20 (4.4%)

Table 4: Different HCV genotype frequency distribution among the samples studied

S.No.	Genotypes	Total cases	Percentage
01	1a	-	-
02	1b	01	5%
03	2a	02	10%
04	2b	-	-
05	3a	09	45%
06	3b	04	20%
07	4	-	-
08	5	-	-
09	6	-	-
10	Unknown	04	20%
11	Total	20	100

IV. **DISCUSSION**

It is estimated that one fourth of a million deaths per year take place as a result of HCV associated with chronic liver disease [16]. A recent community based study in San Juan and between central and South America in 2001-2002 the approximate value of HCV was 6.3% [17]. The common prevalence of HCV was about 1% in Europe, but different amongst different countries [18]. In developed countries the approximate prevalence of HCV infection in dialysis patient was about 8% to 20% [19, 20] and much more in those countries which are not highly developed [21]. In 2001 in Saudi Arabia the anti HCV prevalence in dialysis patient was 43.9 % [22]. There are 2-5 million HCV-positive persons were estimated in Europe, the prevalence rate was up to 20% in the south of the country. In developing countries which also include Pakistan Hepatitis C viral infection is a big health problem and rapidly emerging infection disease [25, 26, 27]. In different regions and several groups of the same community the HCV prevalence may be different [28].The study which was carried out in the hospital the HCV prevalence rate was as 5.31% in Islamabad [29].

Standard protocol was required to study of the prevalence of active HCV and molecular screening of HCV in district Bannu. Highly sensitive method of detection was used in the present study. The study showed that the young adults of the district Bannu of the age (15-17) have the active infection approximately 2.70% (Table 2).The least absence of HCV in this age was due to a little exposure to transmission factor such as exposure to barber, blood transfusion and body piercing etc.5.55% was recorded in the age group 23-26 which is the highest among all other age group. Among street children homosexual behavior were excessive who are victimized sexually, in such behavior later on in order to raise their income they adopt commercial sex. Amongst the male homosexual population condom usage was very little and the prevalence rate of 17.24% ± 7.98 % was observed [30, 31].The histories of these young adults showed different route of transmission of HCV. The possible rout of transmission among these adults were dental surgery, blood transfusion, homosexual and heterosexual in both male and female, also prostitution in female were the highest risk factor among the adults of these age.

In 2009, Ali *et al.,* [32] studied the HCV prevalence in various age groups in which prevalence rate was more in males as compared to females in accordance with our study which was 12% in males young adults and 6% in females (Table3). The young female adults are little affected as compared to male young adults. The prevalence rate was 6.66% in young adults and in old population this rate was 8.92%. The female young adults were less infected with HCV was due to least exposure to risk factor responsible for HCV such as barber, also the estrogen hormones play an important role in clearance of HCV infection in female[33]. Studies have shown that immune system of the body play an important role in clearance of virus. From the body's-cells initial response when strong to HCV infection remove the virus or destroy the virus and weak initial response showed that with the passage of time HCV infection become strengthen and leads to the chronic infection [34]. Cytotoxic T-lymphocytes (CTLs or CD8) cells have evoke a potential factor in the development of chronic infection of HCV [35]. Immunologic disorder plays an important role in chronic hepatitis C which is approximately 38% [36]. Due to this reason our study showed that adults of age below 20 years were less infected due to strong immunity as compared to the age above 20 years. Over all study indicated that 2% young adults of the District Bannu have anti bodies against HCV in their blood and also actively infected with HCV detected by real time PCR. The most frequent genotype of HCV circulating was 3a 45%, followed by unknown genotype 20%; HCV genotype with 3b 20%, with 2a 10% and 1a 5%.The HCV genotype 1a, 4a, 5a, 6a and 2b were not observed among the 20 studied cases. In the present study the HCV genotype observed in some respect varies from the previous study, but common finding was that the most common genotype (45%) was 3a, while previously Hakim *et al*., 2008, Idrees and Riazuddin (2008*),* and Ahmad *et al*., 2010, have reported it as 51%, 49.05% and 55.09% respectively[36, 37, 38, 39]..

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