

Prevalence of Hepatitis C genotypes in District Swabi, Khyber Pakhtunkhwa

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Abstract: The present study was conducted to determine the prevalence and genotype of Hepatitis C in District Swabi. Blood samples were collected from 100 seropositive patients from various hospitals of District Swabi, out of which, 93 samples were found positive by qualitative analysis through PCR. Positive samples were genotyped using Nested PCR. It was observed from the analysis of positive samples that 1a, 1b, 2a, 3a, 3b along with mixed genotypes are prevalent in Swabi. The most prevalent genotype was 3a with the rate of 73.13% followed by 3b with 11.82% rate and 1b to be the least common genotype at rate of 1.10%. From the present study it was concluded that HCV genotypes 1a, 1b, 3a and 3b are distributed in various parts of Swabi and genotype 3a is the most frequent genotype.

Keywords: Hepatitis C, Genotypes, PCR.

INTRODUCTION

Hepatitis C is infectious disease of liver caused by HCV (Chen *et al.*, 2006), which is the 2nd most common cause of viral hepatitis. As per 2004 report of WHO 785,000 dies all over the world annually due to Hepatitis C (Idrees and Riazuddin, 2008) and 3.3% of world population i.e. 200 million people are HCV infected (Simmonds, 1999).

Hepatitis C virus is spherical shape having enveloped, single stranded RNA and belongs to genus Hepacivirus and Flaviviridae family. It has 11 different genotypes owing to its highly heterogeneous nature. Amongst 11 genotypes 6 are major genotypes (Simmonds, 1999).

HCV infection can be diagnosed by serological assays and molecular biology -based techniques. Serotyping is performed to detect genotype specific antibodies which are less specific and sensitive (Pawlotsky, 1999).

Genotyping which is molecular biology based technique is used to examine the sequence of HCV genome and performed through PCR, RFLP and Hybridization techniques (Naz *et al.*, 2007)

According to different reports approximately 10million people are infected with Hepatitis C in Pakistan which is an alarming situation (Raja *et al.*, 2008) and it may be correlated with the lack of health education and less awareness about safe surgical and dental procedures (Qureshi *et al.*, 2009).

In Pakistan the Prevalence of HCV reported in different

regions is highly capricious and it also goes the same for different groups of the same locality (Muzaffar *et al.*, 2008). As per different studies prevalence of HCV was 5.31% in Islamabad (Masood *et al.*, 2007), in different areas of Punjab it was reported to be 0.4-31.9% (Umar and Bilal, 2012 and Qureshi *et al.*, 2010), 4-6% was noticed in Sindh province (Kazmi *et al.*, 1997), while in Khyber Pakhtunkhwa 1.1-9% was witnessed (Khan *et al.*, 2004), in Quetta 1.5% of prevalence was reported (Bosan *et al.*, 2010) and in Gilgit Baltistan province it was 25.7% (Tariq *et al.*, 1999).

MATERIALS AND METHODS

Study population

This study was conducted from 2012 till 2014. Blood samples were collected from 100 HCV Positive Patients from different private clinics and D.H.Q. Hospital, Swabi and were tested in Biotech Molecular Diagnostic Laboratory Rawalpindi.

All the methods and procedures adopted in the completion of present study were approved by the Ethical Committee of Abasyn University, Peshawar, KPK, Pakistan.

Sample collection

A 5ml blood sample was taken from each patient and was allowed to clot, followed by centrifugation at 5000rpm for 5 minutes for serum isolation.

RNA extraction

HCV RNA was extracted from the samples by using Qiagen kit according to manufacturer instruction and extracted RNA was stored at -20°C.

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All the samples were tested for qualitative analysis of HCV using PCR. A 10ul of Extracted RNA was used to for the synthesis of Complimentary DNA. The 5'NCR of viral RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (M-MLV RTase, Fermentas) in a total reaction volume of 20 µl in ABI Veriti 96 well thermocycler for 42°C for 55minutes followed by 70°C for 10 minutes.

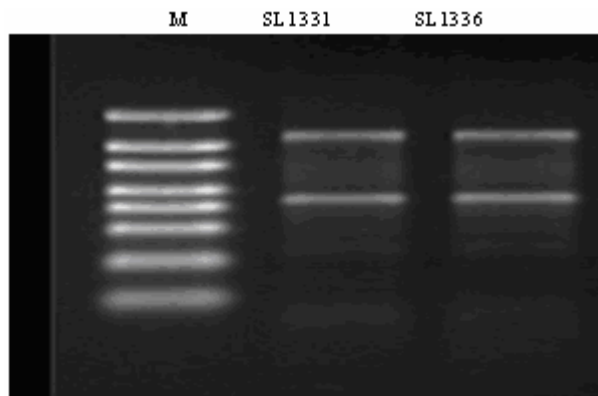


Fig. 1: Gel Electrophoreses of Extracted RNA
L-1 L2 L3L4L5 L6 L7

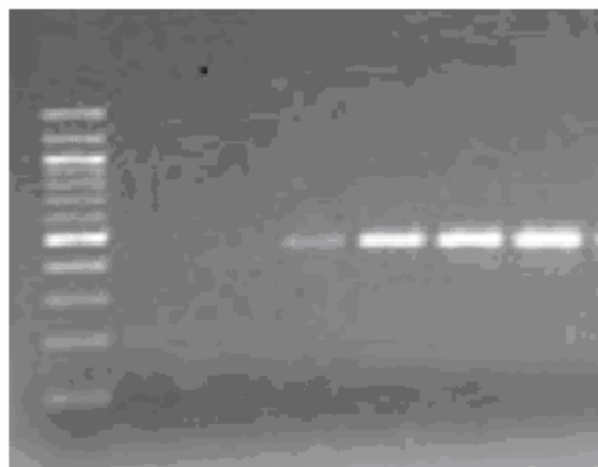


Fig. 2: PCR product of HCV Qualitative runs on Agarose Gel

Table 1: Prevalence of HCV in male and female in district swabi

Patients	HCV Positive	HCV Negative	Total
Male	48	4	52
Female	45	3	48
Total	93	7	100

Qualitative analysis of HCV was carried out by using amplified cDNA, First round of PCR was performed using sense and antisense primers Second round PCR (nested PCR) was performed by using the first round product with inner sense and antisense primer using Taq

polymerase (Fermentas) for 35cycles. The amplified DNA fragments were electrophoresed on 1.5% agarose gel and were visualized under UV light of Wealtec gel doc system for specific bands of HCV. Out of 100 patients, 93 proved positive. Seven samples were HCV negative.

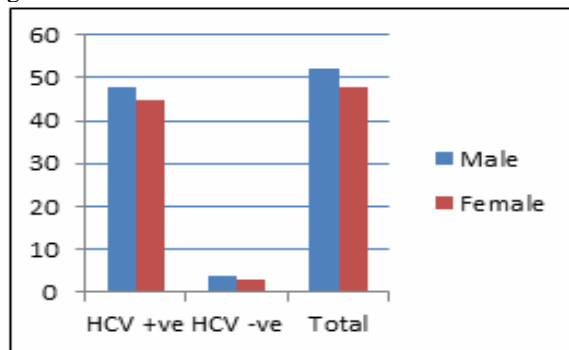


Fig. 3: Gender wise HCV Prevalence

Frequency of different genotypes were calculated by analyzing genotypic data 3a genotypes was found to be most common accounting for 73.11% of studied population and 1b was least frequent genotype found 1.10% of the population under study.

Table 2: Frequency Distribution of HCV Genotypes amongst the Positive Sample

Genotypes	Total	Frequency
1 a	8	8.60
1 b	1	1.10
2 a	2	2.15
3 a	68	73.11
3 b	11	11.82
Mixed	3	3.22
Total	93	100

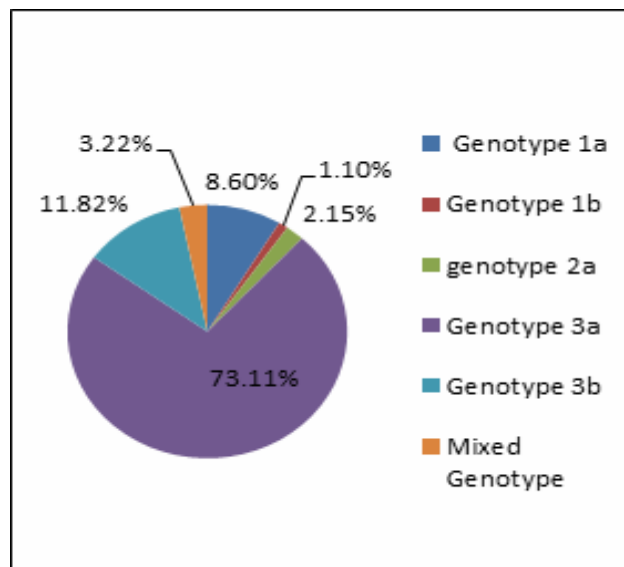


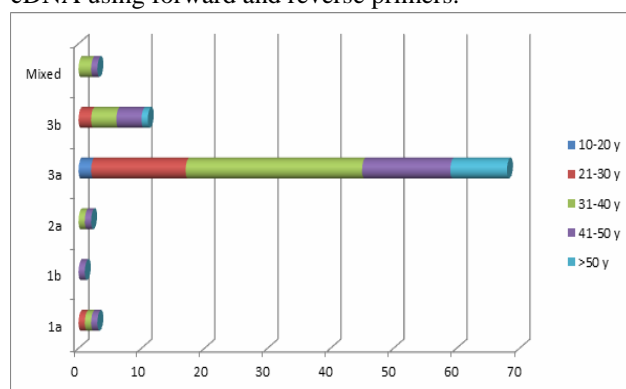
Fig. 4: Distribution of genotypes (n=93)

Table 3: HCV genotypes distribution in different age group

Genotype	Age Groups(years)					Total
	10-20	21-30	31-40	41-50	51 >	
1a	-	1	5	1	1	8
1b	-	-	-	1	-	1
2a	-	-	1	1	-	2
3a	2	15	28	14	9	68
3b	-	2	4	4	1	11
Mixed	-	-	2	1	-	3
Total	2	18	40	22	11	93
%age	2.15	19.3	43.02	23.65	11.83	100

Amongst different age groups, 31-40 years age group was found to be more effected with different HCV and 3a was found more prevalent while age group below 20 years was less effected having only 3a genotype

Positive samples were processed for HCV genotyping by the method described by Ohno *et al.*, 1997, with some necessary modifications. Genotyping of Positive samples was carried out by transcribing 10 μ L of viral RNA to cDNA through M-MLV RT at 42 $^{\circ}$ C for 60minutes. First round PCR was performed with 4 μ L of the synthesized cDNA using forward and reverse primers.

**Fig. 5:** Age wise distribution of different genotypes

The product of first round PCR was amplified in the second round as Mix1 and Mix 2. Mix 1 and Mix 2 contained primers for genotype 2a, 2b, 3b, 1b and genotype 1a, 3a, 4, 5a and 6a, respectively. The second round PCR products were electrophoresed on 1.5% agarose gel with 100bp DNA marker and analyzed under UV light. Genotype confirmation was based on specific PCR bands.

RESULTS

Out of 100 samples 93 samples were found positive on PCR. Out of total 48males 45females were positive.

DISCUSSION

Hepatitis C virus infection is of great concern worldwide and the prevalence in developing countries including

Pakistan is increasing day by day. A previous study conducted by Umar and Bilal, 2012 reported that in Pakistan about 10million people are infected with HCV. Genetically the virus is heterogeneous in nature which leads its genome to produce different genotypes. According to Pawlotsky, 1999 different genotypes of HCV may have different clinical manifestation in the infected individuals in terms of severity. A multivariable logistic regression analysis performed by Rubbia *et al.* 2004 in reported chronic HCV patients that chronic hepatitis C progression is affected by Steatosis.

In the present study out of 100 HCV positive samples, 93 cases were HCV positive on qualitative PCR based analysis. Out of total PCR positive samples, 48.7% were genotype 3a, 20.2% were 3b, 10.1% were 1a, 6.7% were 2a, 5.0% were 1b, and 0.8% was genotype 4. Furthermore, 5.9% had co-infection and 2.5% samples showed no result.

Among the positive sample collected from District Swabi genotype 3 is the most prevalent one. The same pattern of distribution was also reported from other studies conducted in Bangladesh, Northern and Southern India and Nepal. A study conducted by Afridi *et al.*, 2009 documented that genotype 3 has more than 45% prevalence while in the present study it is 48.7%. The existence of some untypable samples shows that the present genotyping methods used previously by Idrees *et al.*, 2008 fails and it is dire need of the day that some other techniques like the cloning and sequencing should be used to identify these novel genotypes. These methods will also be helpful for diagnostic purpose. It is concluded that 1a, 1b, 3a and 3b genotypes of HCV are prevalent in different areas of Swabi with genotype 3a the most common genotype circulating in Swabi. Furthermore ignorance and lack of awareness regarding proper diagnosis, good prognosis and treatment of HCV are very poor in the area.

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