ORIGINAL ARTICLE

GENOTYPE VARIATION OF HEPATITIS C VIRUS IN DISTRICT BUNER SWAT

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Objective: To determine the frequency of various genotypes of Hepatitis C virus in District Buner, Swat. **Methods:** This Descriptive case series study was conducted at District Headquarter Hospital Daggar, and Bilal Medical Trust, Pir Baba, Swat, Pakistan from January 2007 to June 2008. A total of 400 patients, 154 Male and 246 Female aged 16–65 years (Mean age 31±13 years) without clinical and ultrasonographic evidence of cirrhosis, and with positive Hepatitis C Virus (HCV) antibodies by ELIZA, and HCV RNA detected by PCR were included. Hepatitis C virus genotypes were checked in serum by real time PCR (RT-PCR). **Results:** Genotype 3 was the most common type accounting for 61.5% of the study population. Amongst the subtypes, 182 (45.5%) had genotype 3a and 64 (16%) had genotype 3b. Thirty-five (8.7%) patients had genotype 1a, 12 (3%) had genotype 1b. Twenty-six (6.5%) patients had genotype 2a. Four (1%) patients had genotype 2b. Three (0.75%) patients had genotype 4a, and 1 (0.25%) had genotype 6a. Sixty-four (16%) patients had mixed infections. Forty (10%) patients had genotype 3a and 3b, 12 (3%) patients had genotype 1a, 1b, and 9 (2.25%) patients had genotype 2a and 1c, 2 (0.5%) had genotype1a, 3a and 1 patient (0.25%) had genotype1a, 3b. Nine (2.25%) patients had untypable genotype. **Conclusion:** Genotype 3 with its subtypes 3a and 3b is the commonest Hepatitis C virus present in District Buner, Swat. The others in order of decreasing frequency are genotype 1a, 2a and mixed types.

Keywords: Hepatitis C virus, HCV, Genotypes, District Buner

INTRODUCTION

Hepatitis C Virus (HCV) infection is one of the major blood-borne infections worldwide. Hepatitis C is comparable to a 'viral time bomb'. WHO estimates that about 200 million people (about 3% of the world population) are infected with Hepatitis C Virus (HCV) and 3 to 4 million persons are newly infected each year. At least 85% of infected persons become chronically infected and about 70% develop chronic hepatitis. Chronic Hepatitis C in return leads to liver cirrhosis and hepatocellular carcinoma (HCC).[1,2]

HCV is a spherical, enveloped, single-stranded RNA virus belonging to *Flaviviridae* family and genus *Hepacivirus*. HCV is highly heterogeneous and is classified into 11 different genotypes, of which 6 are major genotypes and are further classified into many subtypes based on the genomic sequence heterogeneity. Genotype of HCV is the intrinsic characteristic of the infecting virus strain and does not change over time.[3]

Two types of virological assays, including serological and molecular biology-based assays are in use for the diagnosis and management of HCV infection. Serological assays detect genotype-specific antibodies for the serological determination of HCV genotype (serotyping) while tests analysing the sequence of HCV genomes are called genotyping assays.[4] The latter are molecular biology based techniques and include PCR with genotype specific primers, restriction fragment length polymorphism assay and hybridization techniques.[5] The serotyping assay has low specificity and sensitivity, especially for serotype 3 which is predominant in Pakistan and lacks the capability of HCV subtyping, and can only be achieved by genotyping assays. In addition, serotyping assay cannot differentiate between current and resolved HCV infection.[6]

These genotypic diversities of HCV have distinct clinical consequences. The major determinant of outcome of therapy is HCV genotype. Different genotypes vary in their responsiveness to interferon/ ribavirin combination therapy. With the combination of peginterferon and ribavirin, patients with genotype 1 achieve sustained response rates of 40% to 45%, compared with rates approaching 80% with genotypes 2 or 3.

Importantly, patients with HCV genotype 1 achieve higher rates of response with 48 weeks than with 24 weeks of therapy, whereas patients with genotypes 2 and 3 are adequately treated with a 24-week course. Furthermore, patients with genotypes 2 and 3 require only 800 mg of ribavirin daily to achieve optimal response rates, whereas 1,000 to 1,200 mg daily is needed for patients with genotype 1.[7] Such heterogeneity hinders the development of vaccines, since vaccine antigens from multiple serotypes will probably be necessary for protection.[8]

Genotypes 1–3 have a worldwide distribution. Types 1a and 1b are the most common worldwide, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently detected than Type 1. Type 3 is endemic in South-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and Central Africa. Type 5 is almost exclusively found in South Africa, and genotype 6 is distributed in Asia.[9,10]

The objective of this study was to find out frequency of various genotypes of HCV in District Buner, Pakistan.

MATERIAL AND METHODS

This descriptive, case series study was conducted in District Headquarter Hospital Daggar, and Bilal Medical Trust, Pir Baba, District Buner, Pakistan, from January 2007 to June 2008. A total of 400 consecutive patients presenting to Outpatient Departments were included. Patients with age range 16–65 years, having positive anti-HCV by 3rd generation ELIZA and HCV RNA detected by PCR were included. This included both treatment naïve patients and non-responders or relapsers to previous specific antiviral therapy. Patients with clinical and/ or ultrasonographic evidence of cirrhosis were excluded from the study.

These patients had further test of their serum by Real-time PCR (RT-PCR) mostly in a single reference laboratory for determination of genotype of HCV. In this assay purified patient's RNA along with standard and control was run with master mix including specific primers, Taqman probes and enzymes in the RT-PCR detection system.

Data was entered into a pre-designed Performa. All data are presented as mean values or number of patients and $p \leq 0.05$ was considered as significant.

RESULTS

Women outnumbered men in our study by a significant proportion. Out of 400 patients there were 246 women and 154 men. The mean age of the study population was 31±13 years. Women were younger (Mean age 29 years) than men (mean age 34 years).

Genotype 3 was the most common accounting for 61.5% of the study population. Out of 400 patients, 182 (45.5%) had genotype 3a and 64 (16%) had Genotype 3b. Thirty-five (8.75%) patients had genotype 1a, and 12 (3%) had genotype 1b. Twenty-six (6.5%) patients had Genotype 2a, and 64 (16%) had mixed infections. Nine (2.25%) patients had untypable genotype (Table-1 and 2).

Table-1: Frequency of genotypes

Genotype	Subtype	n (%)
1	1a	35 (8.7)
	1b	12 (3)
	1c	0
2	2a	26 (6.5)
	2b	4 (1)
	2c	0
3	3a	182 (45.5)
	3b	64 (16)
	3c	0
4	4a	3 (0.75)
5	5a	0
6	6a	1 (0.25)
Mixed		64 (16)
Undetermined		9 (2.25)
	Total	**400 (100)**

Table-2: Mixed infection

Subtypes	n (%)
3a+3b	40 (10)
3a+1a	2 (0.5)
1a+1b	12 (3)
1a+3b	1 (0.25)
2a+1c	9 (2.25)
Total	64 (16)

Genotype 3 was more common in female as compared to male patients (55% *vs* 45%) and genotype 1 was slightly more common in male but the difference was not statistical significance.

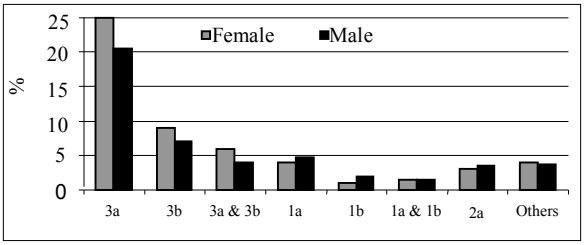

Figure-2: Gender distribution of different genotypes

DISCUSSION

Currently, many decisions for the treatment of Hepatitis C are based on genotype which, in addition to viral load, is the most important predictor of response to therapy.[11] It has become increasingly apparent that fixed treatment durations might not be appropriate for all patients. Also the side effects and high cost of antiviral therapy forced investigators to evaluate reduced duration of treatment. Based on the new evidence that fast viral clearance is highly predictive of SVR, a week 4 negative HCV RNA by a sensitive molecular assay was recently utilised as a criterion for shortening the duration of treatment to 12–16 weeks for genotypes 2 and 3, and 24 weeks for genotype 1 patients.[11–13] Shorter course of treatment up to 12 weeks in genotype 2 and 3 has also bee suggested to reduce the cost in resources constrained situation.[14]

In our study genotype 3a and 3b was the commonest subtype, followed by genotype 1a and 2a. Almost similar prevalence of different genotypes has been confirmed in studies from Khyber Pakhtunkhwa.[15,16] The most detailed study was

conducted by Idrees and Riazuddin[15] in 2008, who performed genotyping of 3,351 patients and reported genotype 3a in 48.23% and 3b in 19% from NWFP. Genotype 1a accounted for 6.56%, genotype 2a 7.56% and mixed infection 5.10% in their series. There is higher percentage of mixed infection in our study compared to their study accounting for some disparity in prevalence of different genotypes.

In 2004, a panel of 30 top gastroenterologists of our country met at a conference and reported that 75%–90% of HCV patients in Pakistan had genotype 3a.[17] This is higher figure for genotype 3a compared to our data. Studies from Bahawalpur region of Punjab province showed 71.4% and 69.6% prevalence of genotype 3.[18,19] Genotype 1 accounted for 14.7% of the total patients. Thirty-five patients (8.7%) had Genotype1a, 12 (3%) had Genotype 1b and 12 (3%) patients had Genotype 1a, 1b.[19]

Ahmed *et al*[20] in a study from Faisalabad, reported that 81% of patients had genotype 3 while only 9.5% had genotype 1. Genotype 3 was reported in 87% and genotype 1 in 1.07% in another study from Faisalabad region.[21] Hakim *et al*[22] reported from Karachi in 2008 that 51% of HCV patients had genotype 3a; 24% had 3a/3b co-infection and 16% had genotype 3b, while similar results were also reported from Balochistan by Afridi *et al*[23] who stated that 50% of HCV patients had genotype 3a followed by 3b and 1a. The results of latter two studies favour lower frequency of 3a as reported by us.

The frequency of genotype 1 is also higher in our study as compared to others from Pakistan. This may be due to difference in study population. Studies from India have reported higher frequency of genotype 1 (13.8% and 18.8%).[24,25] Internationally HCV genotype 1 is more prevalent especially in Northern Europe and America.[26,27]

Surprisingly our female patients were more as compared to their male counterparts. This is despite the conservative nature of our society. One explanation could be that males are more likely to be away from their home thereby utilising the facilities in big cities. Also female patients are more exposed to illicit injection for minor illnesses, mostly on their demand. HCV infection was also more commonly reported by Rauf *et al* in female population, i.e., 68% female vs. 32% male in IDPs from Swat.[16]

The study though representing a small area of Pakistan, has certain implications. The treatment schedule of chronic HCV infection varies with the genotype being treated. Accordingly determination of HCV genotype is considered important before initiation of therapy. However, as the large majority of our patients are infected with HCV genotype 3, testing for HCV genotypes is not done routinely prior to initiation of therapy. In patients who are non-responders to an initial course of interferon and ribavirin therapy, further workup including HCV genotype is initiated to determine the genotype and viral load.[28] However, this conventional approach needs to be revisited in view of studies including ours showing higher percentage of patient with genotype 1 in our patients and other studies recommending shorter duration of treatment.

CONCLUSION

HCV genotype 3a and 3b are the commonest types found in our population. However genotype 1a and 1b alone or in combination also accounts for a significant proportion of our patients. The latter group of patient requires a different approach as regard to their treatment. It is worthwhile doing the genotype in order to tailor specific treatment to individual patient.

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