Original Article

# FREQUENCY OF VARIOUS HEPATITIS C VIRUS (HCV) GENOTYPES IN FAISALABAD AND SURROUNDING DISTRICTS

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## ABSTRACT

**Objective:**

To observe the frequency of hepatitis C virus genotypes and to use this information as a guide for the selection of antiviral treatment.

**Study Design:**

Cross-sectional analysis of the baseline data of hepatitis patients enrolled for the cohort study

**Place and Duration of the Study:**

Umar Hospital and Madina Teaching Hospital, Faisalabad, Pakistan from August 2010 to December 2012

**Methodology:**

Anti HCV antibody positive patients of both sexes with well preserved hepatic function, treatment naive willing to be treated with antiviral treatment were screened over the study period. Patients having uncontrolled hypertension, diabetes mellitus, severe depression, decompensated liver disease were excluded from the study. All the baseline variables like age, sex, weight, height, body mass index (BMI), address and phone number were recorded. A written consent was also obtained.

Genotype testing was done by real time polymerase chain reaction (PCR) and quantitative measurement of the viral load was also done. In this assay, patients RNA along with standards and controls was run with master mix including specific primers. All the results were recorded on pre-printed performa.

**Results:**

A total of 91 patients were enrolled. Male to female ratio was 1.02(46/45) and mean age was 26.5±3.3 years. Fifty-nine percent of the study population belonged to peripheral suburbs and surrounding districts of Faisalabad while 41% belonged to district Faisalabad. Of the 91% patients 86.8% had genotype 3a while second largest type belonged to Untypable variety 7.7%.

**Conclusion:**

Like other areas of Pakistan, the most common genotype in Faisalabad and surrounding districts is type 3a followed by Untypable type

**Keywords:** Hepatitis C, Genotype

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## INTRODUCTION:

Hepatitis C Virus (HCV) is one of the leading causes of chronic viral infection worldwide. According to estimation by World Health Organization (WHO), approximately 3% of the world population has been infected with HCV, amounting to 120-180 million people globally **(1, 2)**. About 3 to 4 million people are infected each year and it is estimated that between 65%-80% of people newly infected with HCV will progress to chronic hepatitis which is a leading cause of end stage liver disease and Hepatocellular Carcinoma (HCC). Consequently, HCV infection contributes significantly to worldwide morbidity.**(2)**

Practitioners in Pakistan have been cognizant of the havoc of hepatitis C in the community. Terms like "Pakistan a cirrhotic State" have been used in literature **(3)** in the last decade, but it is only in the recent years that viral Hepatitis C has been acknowledged as a major public health problem in the country. National hepatitis prevalence survey in Pakistan calculates Hepatitis C prevalence in general population to be 4.9% albeit with wide variations amongst the provinces and cities. It is really a worrisome fact that this number is far more than that of patients with AIDS, tuberculosis and malaria combined and is estimated to triple in 15years time**(4)**. Examination of the Phylogenetic tree of the 5'end of NS4 has confirmed the existence of six genotypes of HCV, with a second level of branching pointing to the presence of at least 50 subtypes. **(5)**

The major genotypes have been designated the numbers 1 to 6, whereas the subtypes are denoted by letters **(6)**. Although the six genotypes can be found worldwide, the different genotypes have widely different geographic distribution; for example, type 1, 2, and 3 are most commonly found in Europe and USA, type 4 in Egypt, Saudi Arabia and Africa, and type 1 and 6 in the Far East **(7-9)**. Improved methods of genotype distribution over the last few years have enabled the importance of genotype in the progression of HCV-related disease and response to antiviral therapy to be studied. Present data strongly indicate that the HCV genotype is an important determinant of response to treatment, but the effect of genotype on the disease progression has been harder to clarify. As a result of the advances in methodology, and recent results of large clinical trials of combination chemotherapy, knowledge of HCV genotype is now central to the clinician in the management of the patients with chronic hepatitis C. **(10)**

Present study on genotype was conducted with an intention to see the frequency of various genotypes in Faisalabad and surrounding districts and it will be used as a guide for the selection of the type of treatment in these patients. This will be a new indication, perhaps not cited in the literature before.

| **Variables** | **Faisalabad Frequency (%) Mean (S.D)** | **peripheries Frequency (%) Mean(S.D)** | **F-test/Chi-squired test (P values)** |
|---|---|---|---|
| Gender Male/Female | 16(17.5) 21(23) | 30 (33) 24 (26.4) | 1.76 (0.19) |
| Age (Year) | 40.08 (11.2) | 37.17 (11.1) | 1.49 (0.23) |
| Weight (Kg) | 73.35 (11.1) | 72.52 (13.5) | 0.10 (0.76) |
| Height (M) | 5.45 (0.3) | 5.49 (0.3) | 0.51 (0.48) |
| BMI | 27.05 (3.6) | 26.46 (4.6) | 0.43 (0.52) |
| Genotype Frequency (%) | | | 1.29(0.86) |
| 2a | 0(0) | 1(1.8) | |
| 2b | 1(2.7) | 1(1.8) | |
| 3a | 33(89.2) | 46(85) | |
| 3b | 1(2.7) | 1(1.8) | |
| Untypable | 2(5.4) | 5(9.2) | |

## MATERIAL AND METHODS:

This paper is presented as a cross-sectional analysis of the base line data of hepatitis patients. The study was conducted at Umar Hospital civil lines and Madina Teaching Hospital, University Medical and Dental College, Faisalabad from August 2010 to Dec 2012. All the patients visiting these two places who had no contraindication to treatment to HCV treatment and were positive for HCV antibodies were screened for their genotype and viral loads. This test was only done on those patients who were willing to be treated later. Patients of both sexes with well preserved hepatic function and having no gross co morbidity were included in the study. Patients were explained about the nature, price and purpose of doing the test and a written informed consent was obtained. All variables like age, sex, height, weight, address and phone number and BMI were recorded.

## GENOTYPE TESTING:

Patient's blood was drawn at the laboratory by a phlebotomist and genotype was done at Bio Best lab canal road Lahore by a trained microbiologist. The test was done by real time polymerase chain reaction (PCR) which is based on kinetic measurement of PCR product during its exponential phase, which allows high rate viral detection providing highly sensitive, specific and reproducible quantification without contamination risk by simple automated (makino and cheunjmicrobial) methods. In this assay, patients RNA along with standards and controls was run with master mix including specific primers, Taqman probes and enzymes etc. In state of the art real time PCR system which by defining threshold cycles automatically calculates starting of samples. All the results were recorded on the pre-printed performas and results were recorded at lab as well as by the researcher during the study.

## RESULTS:

Ninety one patients were observed with male to female nearly equal ratio of 1.02(46/45). Mean age of the patients was 26.5±3.3 years; mean weight was 72.9±12.5kg and mean height was 1.6±0.08M. Mean BMI was 26.8±4.7 (Table 1, 2). Fifty-nine percent (54/91) of the people were from the peripheral suburbs and surrounding districts of Faisalabad while 41% (37/91) belonged to proper Faisalabad district (Table 3).

## AREA WISE DISTRIBUTION OF THE STUDY POPULATION:

The study population was divided into Faisalabad and surrounding districts, which included Nankana Sahib, Jhang, Chiniot, Shiekhupur, Hafizabad, Gujranwala, Sahiwal and OKara. Faisalabad district was divided into central Faisalabad and its major suburbs like Tandlianwala, Jaranwala, Chak jhumra, Samundri, Gojra and Dajkot. Sixty three subjects (69.2%) belonged to Faisalabad while 28 patients (30.73%) belonged to surrounding districts. After Faisalabad, the second largest number of patients were from district Nankana Sahib [n=15 (16.5%)].

## GENOTYPE DISTRIBUTION:

Of the 91 patients, 79 patients (86.8%) belonged to 3a variety while 7 patients (7.7%) were of Untypable variety. Two patients belonged to 3b and 2 (1.8%) to 2b type while 2a genotype was seen in only 1 (0.9%) patient. The results show that 3a is the predominant genotype (87%) in this area of Punjab followed by Untypable variety (Table 4, 5).

## DISCUSSION:

Results of the study reveal that predominant genotype in the district of Faisalabad and its surrounding areas are 3a followed by Untypable variety. This is quite in line with other published Pakistani studies which reveal that commonest genotype is type 3 followed by Untypable and type 1 (11-18). About 5% of the population had multiple serotypes. Positivity of multiple serotypes may be due to transient or occult super infection or ineffective immunity in patients with chronic HCV infection. The untypability of serotypes can be due to many reasons and lack of antibodies to NS4 proteins has been found in some cases (19,20). Khalid et al observed that genotype 3 is the most common type of HCV comprising of 61.5 % of the study population in Buner Swat [29].

Ansarie et al (12) has an interesting observation that 2.3% of the patients who have multiple serotypes also have a serotype 3 added up with other serotypes. Ahmed A et al studied further subtypes of genotypes 3 which showed that 49.5% cases had genotype 3a, 33.7% had genotype 3b, 8.7% had mixed, 4.5% had Ib and 3.6% were untypeble[31]. Abbas et al also observed that genotype 1 is very rare in our country[32].

Azhar et al. (27) reported from Bahawalpur district that Untypable serotype is more common in patients with cirrhosis.

Knowledge about prevalent HCV genotype in a particular geographic region has important implications, infecting genotype often determines the cause and severity of the disease process **(28,21)**. Recent studies account for the pivotal role of HCV genotype in determining the duration and efficacy of antiviral therapy **(22,23)**. HCV genotype determines the prognosis after orthotropic liver transplantation and the regional distribution of genotype influences the configuration of diagnostic assays and vaccine design. Additionally, since transmission of HCV infection is unknown in about 39% of the patients **(24)**, genotype distribution is a useful tool for tracing source of infection. HCV subtypes may have a bearing on disease progression, antiviral response and over all prognoses. But according to Akhund et al, there is no correlation of genotype with histological grading and staging of chronic hepatitis C[34].

For example, subtype 1b is associated with progression to chronicity **(25)** and subtype 3b is associated with poor response to widely used Alfa interferon.

Data about genotype distribution from various areas of Pakistan also reveal that commonest genotype is 3a followed by Untypable variety. Study by Azhar MA **(11)** from Bahawalpur revealed 70% type 3 and 16% as Untypable, Ansari N **(12)** from Karachi, type 3 as 77% and Untypable 17%, Khokhar N **(13)** from Islamabad had 64% type 3, Zuberi SJ **(14)** and Mumtaz K **(26)** from Karachi showed a prevalence rate of type as 79.5% and 87.8% respectively. Nasir J **(16)** from Rawalpindi had a frequency of 3a as 60%. Worldwide type 1 & 2 are commonly seen in North and South America as well as Europe, type 4 in Egypt and 5 & 6 genotype are prevalent in China and Japan etc.

Fariyar et al studied HCV genotypes in IRAN and concluded that 53.84% patients had genotype 1, 3.88 % had genotype 2, 23.08 % had genotype 3 and 13.4 % had genotype 4. Mixed infection with genotypes 1 and 3 was seen in 5.77 %[33]. A study conducted by Abro et al in United Arab Emirates revealed that Among the UAE nationals, 49.2% had genotype 1, 34.6% had 3, 14.5% had 4 and only 1.6% patients had genotype 2. High (68.4%) of genotype 1 was also observed in Irani nationals, while, 96.4% Egyptians had genotype 4 and 77.2% Pakistanis had genotype 3[30]. Ahmed et al observed that genotype 3 is the most prevalent in Pakistani population so combination antiviral therapy can be given without checking for genotype[31].

## CONCLUSION:

Since type 3a is the most prevalent genotype in Pakistan as depicted by various, published studies hence it is concluded that routine genotype testing shall not be done in all cases because of its prevalence which in 80% of the cases is going to be type 3a. It will save national funds and time and will be cost effective. This will also guide us towards a cheaper treatment with regular or standard interferon which shows a very good response as regards to Rapid Virological Response (RVR) and End of Treatment Response (ETR) etc, because type 3 is very responsive to standard interferon. Most importantly, this will be very cost effective for a poor country like Pakistan where disease is rampant and there are insufficient health care facilities sponsored by the state.

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