ORIGINAL ARTICLE

HCV Prevalence and its Predominant Genotypes in Sargodha Region of Pakistan

HAROON RIAZ, MUHAMMAD ZAHID LATIF, MUHAMMAD ATIF QURESHI, *ABDUL ROUF, RAHILA NIZAMI*[*]

ABSTRACT

Background: Hepatitis C (HCV) is known as one of the most deadly viral infections caused by a virus that belongs to the family Flaviviridae. It usually remains asymptomatic but in chronic cases may leads to liver cirrhosis.

Aim: To find out the prevalence of HCV in Sargodha region of Pakistan and to evaluate the frequency distribution of various HCV genotypes among those with HCV infection.

Method: Individuals, who visited/admitted at our affiliated medical facilities or our collection centers in Sargodha region of Pakistan, were selected for this study. All individuals had given their proper consents for this study and also filled the form related about their medical/clinical history. Serum samples that were found positive for anti-HCV on ICT screening kits (ACON Laboratories Inc., San Diego, USA) were sent to HealthCare Diagnostics and Research Centre, Lahore for molecular detection of HCV and its genotyping.

HCV RNA was extracted and reverse transcribed to synthesis cDNA that was further subjected to nested PCR for detection of HCV viral RNA. The multiplex PCR genotyping for HCV was done only for the samples with detected HCV-RNA. Results were analyzed accordingly.

Results: After initial screening for Anti-HCV antibodies, a total of 635 blood serum samples were proceeded for HCV detection through a qualitative nested PCR test. 206(32.44%) samples were found positive for HCV RNA. The prevalence was found higher among females (59.22%) than the males (40.73%).After multiplex PCR for HCV genotyping, it was found that 3a (41%) is the most prevalent HCV genotype following 2a genotype (19%). While the distribution of all other genotypes among those with HCV-RNA was in the following order; 1b (7%) > 1a (4%) > 2b (2%). No significant difference was seen in HCV prevalence regarding any gender or a particular age group.

Conclusions: The current study concludes that the HCV prevalence in Sargodha region was recorded higher than the officially stated statistics of Pakistan Government. It is proposed that governmental and non-governmental organization should initiate a mass scale awareness campaign and treatment measures in this region to eradicate this disease so that the misery of people could be cut down.

Keywords: Prevalence, Hepatitis C, HCV, HCV Genotypes, Sargodha.

INTRODUCTION

Hepatitis C (HCV) is known as one of the most deadly viral infection caused by a virus that belongs to the family Flaviviridae. It usually remains asymptomatic but in chronic cases may leads to liver cirrhosis.According to an estimation by the World Health Organization, Hepatitis C (HCV) is infecting approximately 3% of the world population. Worldwide HCV infected population is estimated about 170 million individuals while each year 3 to 4 million persons are diagnosed with HCV infection[1]. In Pakistan, hepatitis B surface antigen (HBsAg) andanti-hepatitis C virus (HCV) are positively diagnosed respectively in 2.5% and 4.8%of the population, reflecting an overall 7.6% infection rate in the general population. The prevalent high burden of chronic liver disease (CLD) is evident of the higher hepatitis infection rate[2]. According to WHO, the prevalence of HCV in Pakistan varies widely, ranging between 3–13%[1].Hepatitis C virus (HCV) is one of the most important infection in humans with notable clinical complications all over the world and after Hepatitis B virus, HCV is responsible for the second most cases of deadly of viral hepatitis[3]. Currently due to HCV heterogeneity, there are eleven identified genotypes. Out of these eleven, at least six major genotypes of HCV are frequently tested in different diagnostic centers around the globe. All of these genotypes are known to have several distinct subtypes[4]. These different genotypes and their subtypes are not known to differ greatly in their virulence or pathogenicity. But this diversity among a single virushave great medical and clinical

AzraNaheed Medical College, Lahore & Health Care Diagnostics and Research Centre, Lahore

**University of Management & Technology*

Correspondence to Dr Muhammad ZahidLatif, Director, Department of Medical Education & Assistant Professor Community Medicine AzraNaheed Medical College, Superior University Raiwind Road, Lahore Email: mzahidlatif@yahoo.com Cell: 0333-4428870

consequences.Heterogeneity and highly mutable nature of HCV is also responsible to hinder the development ofits vaccines. Moreover, these different genotypes are related to many aspects of HCV infections that includesthe management of clinical aspect of chronic HCV disease and its epidemiology[5]. Before starting any antiviral drug therapy, it is recommended to determine theHCV genotype as this genotype determination could predict the length of the treatment[6]. The role of viral genotype in the pathogenesis of liver disease is still not well established. Environmental, genetic, and immunological factors may contribute to a variable disease progression. It is reported that an individual infectedwith an HCV genotype infection has shown different response to interferon/ribavirin antiviral course than the individual infected with some other HCV genotype.This clinical significance of its genotyping has established the factthat it could play an important role to determine the clinical course of disease and its responsiveness to the interferon/ribavirin comboantiviraltherapy[7]. Many previous studies have reported that a patient with genotype 1 is less responsive to an interferon/ribavirinantiviral treatment than the patients infected with genotype 2 or 3 of HCV[8]. Therefore, it is important to consider the patient genotype before advising him an interferon standard therapy.

HCV genotypes 1, 2, and 3 areknown to be distributed world widely but their frequency of prevalence is greatly varied in various geographical regions. 1a and 1b genotypes of HCV are the most prevalent onesin Europe[9,10,11], in Japan[12] and in the United States[6] and these two genotypes also accounts for 60% of global HCV infection. HCV genotypes 2a and 2b are predominantly found in Japan, Europe and North America while genotype 2c is found exclusively in northern Italy. HCV genotype 4 is more frequently distributedin the Mid-East and Northern region of Africa[13,14], while genotypes 5 is usually not found outside of South Africa [15] while genotypes 6-11 are endemically found in different region of Asia but not as frequent as the other genotypes are found.

There are few studies available from Pakistan that suggest 3a as the predominant HCV genotype in various regions of the country[13,17,18]. There is no reliable study available that could evaluate the prevalence of various HCV genotypes in the region of Sargodha. Therefore, this study was designed to assist in evaluation and determination of the frequency of prevalence for various HCV genotypes that are found endemic in Sargodha region of Pakistan. This study will also help to assess the routes of its transmission.

METHOD

Sampling and ICT Blood Screening: After critically evaluating all of the ethical and research related issues, the ethics committee of the research center approved this study. The serum samples were collected from all the patients,with a suspected liver disease,admitted/visited our health facilities or collection centers in Sargodha region, Pakistan. At the time of blood sample collection, a specifically designed form was filled to get the patient's consent and the required medical/clinical information.

At first, all the serum samples were screened for HCV antibodies using Immuno-chromatographic test kit (ICT: ACON®, ACON Laboratories Inc., San Diego, CA, USA).Only the Anti-HCV positive serum samples were received at HealthCare Diagnostics & Research Centre, Lahore along with theform containing clinical/medical history and consent of the patients.

HCV RNAExtraction and Detection PCR: Nested reverse transcription (RT) PCR was done for the qualitative detection of HCV RNA using primers that correspond to the relatively conservative 5'UTRnon-coding region of the highly mutable HCV as described previously [19]. In short, HCV viral RNA was extracted from 200μL of serum sample by using viral RNA extraction kit (Gene JET Viral DNA/RNA Purification Kit, Thermo Fisher Scientific Inc. USA) as described by the manufacturer protocol. Verso 1-Step RT-PCR Kit (Thermo Fisher Scientific Inc. USA) was used to synthesized cDNA from the specified 5'UTR region of extracted viral RNA, using antisense primer. This kit is specifically designed to produce the dsDNA once reverse transcription is completed. So the primers for the first round were also added in the reaction mixture. Nested PCRs were executed with Taq polymerase (Thermo Fisher Scientific Inc. USA) and another set of primer. The amplified product for each samples were visualized on 2% agarose gel dyed with ethidium bromide over a UV transilluminator to identify the specific HCV PCR bands. On detection of HCV presence, the respective PCR positive samples were further proceeded for HCV genotyping.

HCV Genotyping: HCV genotyping was done by using a specific HCV genotyping protocol that is already described with details in one of our research group's previous study[19]. The short outline of this HCV genotyping method is that about 100 ng of HCV RNA was used to synthesize cDNA by reverse transcription using Verso 1-Step RT-PCR Kit (Thermo Fisher Scientific Inc. USA) and in the same PCR reaction the 470-bp fragment is also amplified from the non-coding region of HCV 5'UTR and itscore region. For nested PCR, two second-round multiplex

PCRs were performed using the first round PCR amplified product as a template, one with primer mixture-1 and the other with mixture-2. Mixture-1 had primers for genotypes 1a, 1b, 1c, 3a, 3c and 4 while Mixture-2had primers for genotypes 2a, 2c, 3b, 5a, and 6a primers. The PCR-amplified product was run on a 2% agarose gel to segregate the genotype-specific fragment along with a 50-bp DNA ladder (Thermo Fisher Scientific Inc. USA) and evaluated on a UV transilluminator. The identification of HCV genotype-specific PCR band was done to determine the respective HCV genotype for each of the sample.

RESULTS

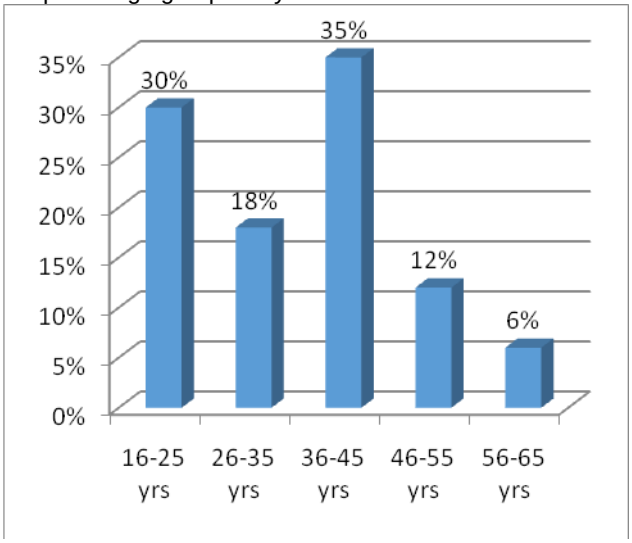
During the study period, a total of 635 anti-HCV positive blood sera samples were received at HealthCare Diagnostics and Research Centre, Lahore. Out of these samples, 206 samples were confirmed positive for HCV RNA by qualitative PCR. So the prevalence for HCV for these suspected individuals (symptomatic or asymptomatic) was found 32.44%. Out of these 206 patients with detected HCV-RNA, 122(59.22%) were females and 84 (40.73%) were males. The age-group of 36-45 years bear the largest number of HCV patients (37.08%) and smallest number of patients was in the 56-65 years age-group. A total of 87.55% patients belong were below 45 years of age (Graph1).

This study shows that except the age-group of 16-25, where male patients were in balance with the female patients as both have 30 patients in that group, in all other age-groups female patients have a significantly higher number of HCV patients(Graph 2).

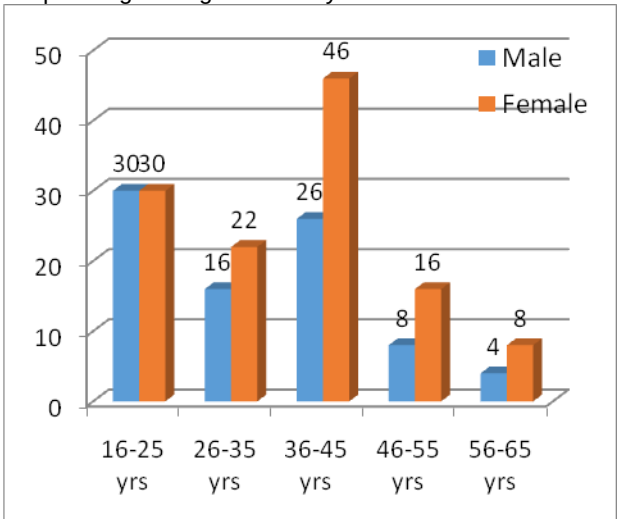
Genotypic PCR analysis of hepatitis C virus of the all the 206-HCV positive patients was carried out. The results shows that out of 206 patients 56 (27.71%) were un-typable for any genotype. This could be either due to low viral load of HCV or due to some genotypes that are still to be known. Among the rest samples, the genotype 3a has been found to be the most predominant strain in the population; 22% males and 20% of females have this genotype. The other strains detected were 2a of which 11% were males and 9% were female, 1a (both males and females were of 2%), 1b (2% were males and 5% werefemale), 2b (2% were females and none was a males), and none of the patient was detected with 3b, 4a, 5a and 6a genotype of HCV(Graph 3,4).

This study shows that there is no specific relationship of age-groups or genders in case of prevalence of different HCV Genotypes but female patients were found to have higher frequency of HCV infection.

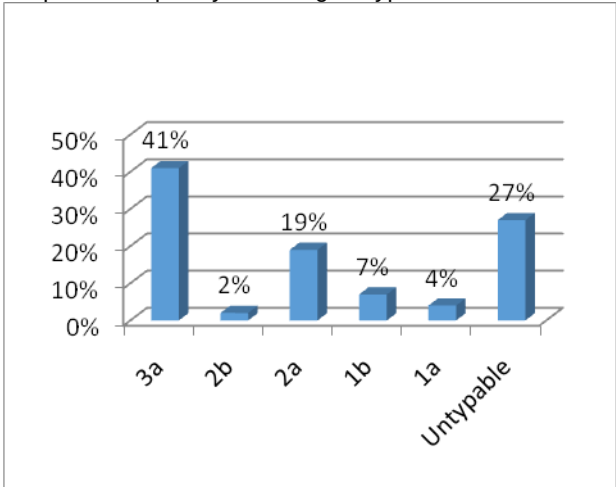
Graph -1: Age group analysis.



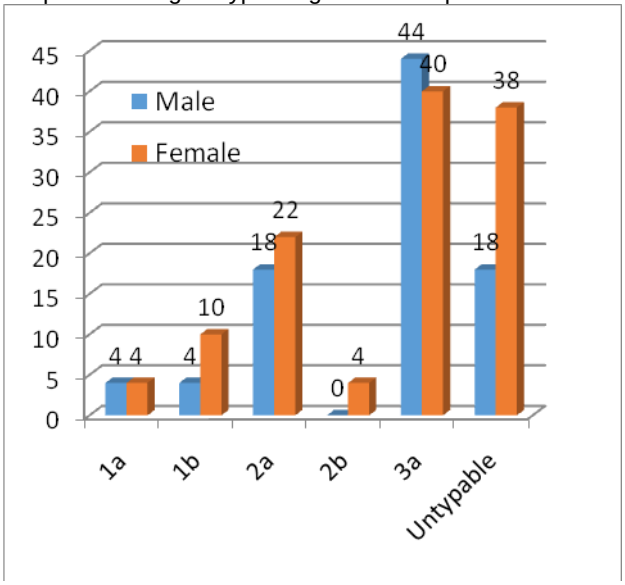
Graph 2: Age and gender analysis



Graph -3: Frequency of HCV genotypes.



Graph -4: HCV genotypes & gender wise prevalence.



DISCUSSION

HCV is an important cause of CLD (chronic liver disease) and hepatic cirrhosis in Pakistan and one of the major cause of early death. A recent study suggested that about 6% of the Pakistani population is infected with HCV[20].

In this study, we have tested 635 samples that were all positive for HCV antibodies and out of them only 206 were confirmed as infected by HCV by qualitative PCR. This shows that screening for anti-HCV with immuno-chromatographic test kit or by an anti-HCV ELISA for HCV infection are faultier and lack the required sensitivity[21]. Therefore, the detection of HCV by a PCR based methods is regarded the best possible option due to higher levels of specificity and sensitivity. HCV infection,like any other pathogen derived infection, is a classic example of thosediseases in which direct detection of the pathogenic viral agent is required for anerror-free diagnosis. We have analyzed the relationship between prevalence of HCV genotypes in different age groups and gender. Our results showed that HCV frequency is non-significant among the different age groups but we found it significantly higher in females as compare to males. These results are in consistence with the results of previously done research[19].

A detail analysis of our results suggests that the maximum patients fall in the age group of ≤ 50 and > 16 years of age in Sargodha region. These results are similar to a previous work which demonstrate that HCV prevalence was found highest in the age group of 16-50 years[22,23] but also contradict to a study that shows higher HCV distribution among older age group[24].We suggest two reason for these results, either the higher prevalence of HCV in younger age is due to their increased exposure to the risk factor or this could also be interpreted that due to an increasing awareness and early diagnosisof HCV resulted higher reported cases specially the urban areasof Pakistan like Sargodha.

In this recent study, we have also done HCV Genotyping for all those 206 samples that were found positive by qualitative PCR. We have found that genotype 3a is the most frequent among the HCV carrier individuals while 2a is second most prevalent genotypes among the studied individuals. These results are in agreement to previously done many studies on Pakistani population[19,25,26,27,28]. Determination of the HCV genotypes is of essential for the study of many aspects of HCV infection including pathogenesis, epidemiology and its responsiveness to the interferon/ribavirin antiviral therapy[29,30].

CONCLUSION

In this study, we have concluded that 3a is the most endemic HCV genotype in Sargodha region that is in consistence with the already established facts but the prevalence of HCV infection is found higher than the national average of HCV infection rate (7.6%) in anti-HCV positive patients.We also recommend that a patient with HCV infection should always have to be tested for HCV genotyping before the start of antiviral drug therapy from a reliable diagnostic center and ICT blood screening or ELISA for HCV antibodies is not at all a reliable method for HCV detection. We also proposed that governmental and non-governmental organization should initiate a mass scale awareness campaign and treatment measures in this area.

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