RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS AND INTRODUCTION OF BIORISK MANAGEMENT (CWA 15793-2008) AT MIRPUR DISTRICT, AZAD JAMMU AND KASHMIR

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ABSTRACT

The object of the study was to determine the ratio of incidence and genotyping of hepatitis C virus (HCV) infection with reference to different typable and untypable strains in various populations of Mirpur, Azad Jammu and Kashmir (AJK). This was done by taking into account different modalities with reference to gender, age and socio-economic perspective. A total of 88 patients were included in the study and the screening was initially carried out by rapid diagnostic tests (RDT) with further confirmation by polymerase chain reaction (PCR) method. The real time PCR (qPCR) was performed at Genetic and Molecular Center, Lahore. These tests were done at Biosafety Level 2 (BSL 2) laboratory (according to Biorisk Management CWA 15793-2008) which was the criteria determined by risk assessment performed by principal investigator and the scientific officer of the facility. The genotype 3a was found to be the most common type in affected patients which certainly determined the management of these infections in a more targeted manner. The study was able to determine the prevalence rate on gender basis which was important on local basis and can be the basis to initiate the study for finding out the reasons for the same. The age group most affected was found to be in the younger group. In addition, introduction of Biorisk Management was very encouraging and the professionals showed keen interest in this scientific discipline and were eager to learn the key concepts of it.

Keywords: Hepatitis C virus, qPCR, genotypes, biorisk management.

1. INTRODUCTION

The hepatitis C virus (HCV) is a major public health problem and a leading cause of chronic liver disease. HCV is also the leading cause of death form liver diseases in Pakistan1. The primary source of HCV infection is infected blood or blood products. The drug addicts are at higher risk of developing HCV infection. Other sources of HCV transmission may include sexual contact with infected or multiple sexual partners or sex workers, and exposure to contaminated blood among laboratory and healthcare professionals2. It is not likely that HCV can spread from household activities except those that may come in contact with blood, e.g. sharing a razor or toothbrush. Hepatitis C is not spread by simple hugging and sharing of plates and cups3. Persons with hemophilia should be tested for HCV infection. Acupuncturing, body piercing and barbers also carry a substantial risk of transmitting HCV infection through their utensils.

The methods of detecting HCV screen the persons with identifiable risks. In clinical practice the initial test done for the detection of HCV infection includes enzyme alanine aminotransferase (ALT) and HCV antibodies. If the initial tests are found positive, detection of subsequent HCV RNA levels are useful in providing and monitoring HCV management4. The HCV is a risk group 2 organism and the risk assessment methods applied with reference to this study determined it to be a Biosafety Level 2 (BSL 2) as the study was focused on genotyping and other serological methods. HCV RNA can be detected in the blood using amplification procedures such as
polymerase chain reaction (PCR)⁵ that has been approved by FDA⁶. HCV genotyping does not predict the outcome of infection but it does provide information on the outcome and duration of the treatment⁷⁻¹¹. It can be performed by direct sequence analysis, by reverse hybridization to genotype specific oligonucleotides probes, and by restriction fragment length polymorphism (RFLP)¹².

In this study an attempt has been made to characterize on the molecular basis the genotype of HCV most prevalent in individuals who have been diagnosed positive for HCV antibodies and also HCV RNA by PCR.

2. MATERIAL AND METHODS
Local strategy was developed in the light of WHO guidelines for biosafety, hazard identification. Risk assessment was performed using Biorisk Management Guidelines CWA 16393-2012, which is continuation of CWA 15793. The technicians were informed about the potential biohazards related to this project. HCV is a risk group 2 organism and the aims and objectives of this study relate it to BSL 2. It was made possible to do all the manipulations at BSL 2 by simultaneously training the laboratory professionals in biorisk management. The patients reporting from different areas were categorized and analyzed for HCV infection on the basis of age, gender, signs and symptoms, immunoassay chromatography test (ICT) and liver function test (LFT). Subsequently, their PCR was carried out to verify the infection and genotyping was performed to differentiate between various strains for the purpose of management modalities. In this study main focus was age, gender and genotype of patients who was determined positive by PCR.

2.1. Place and Duration of Study
The sample collection was done at district and tehsil level. A total of 88 patients were included in the study which were from Barnala, Bhimber, Kotli, inclusive of Dina and Jhelum as patients were also reporting from these border line cities. This was a multicenter study conducted from October 2014 to September 2015 at the Department of Pathology, Mohtarma

Benazir Bhutto Shaheed (MBBS) Medical College, its affiliated Divisional Headquarters (DHQ) Hospital, Mirpur and Genetic and Molecular Center, Lahore.

3. RESULTS
Out of 88 cases with HCV infection, the genotype ratio was found as follows: 52 cases with 3a type, 20 cases with 3b type, 10 cases with 1a type, 06 cases were untypable. The most frequent genotype was found to be 3a followed by 3b (Fig. 1). It was also noted that the female population (65%) was more affected then the male population (35%). The categorization with reference to age revealed that younger group (20-40 years) is more affected to HCV infection then the elderly group (41-60 years) in both males and females (Fig. 2).

Fig. 1. Common genotypes of HCV in Mirpur, AJK.

4. DISCUSSION
The HCV infection has been a foremost danger for the professionals who are exposed to many different invasive techniques while performing diagnosis and management of the patients¹. The diagnostic criterion for this infection has been grouped into two broad categories:
i. Serological assays that detect antibody to HCV.
ii. Molecular assays and procedures that can detect, quantify and characterize HCV.

Seralogical assays have been subdivided into screening tests for anti-HCV such as the enzyme immunoassay (EIA) and supplemental tests such as the recombinant immunoblot assay (RIBA). Three generations of anti-HCV tests have been developed and each generation has resulted in an improvement in the sensitivity of detecting anti-HCV. Supplemental anti-HCV tests are designed to resolve false-positive testing by EIA and are appropriately used in low-prevalence settings in which false-positive anti-HCV tests remain a problem. Third-generation anti-HCV tests (EIA-3 and RIBA-3, respectively) contain antigens from the HCV core, nonstructural 3, 4, and 5 genes. Detection of HCV RNA in patient specimens by PCR provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring the antiviral response to the therapy. Optimal HCV PCR assays at present have a sensitivity of less than 100 copies of HCV RNA per ml of plasma or serum. Two main technologies exist for assessing HCV RNA levels or viral load i.e. quantitative PCR and branched-chain DNA test. Quantitative PCR is the most sensitive test for determining hepatitis C viral load whereas the branched-chain DNA test appears to be the most precise method. Major limitations of the current tests are inadequate dynamic range and high variability of PCR-based assays, and poor sensitivity of the branched-chain DNA test. Molecular tests have also been developed to classify HCV into distinct genotypes. The clinical importance of HCV genotype determination remains a subject for future investigation.

The HCV has more than six different genotypes, which are numbered in the order of their discovery. Each of these genotypes has many subtypes, which were lettered in the order they were discovered. It is important to find out which hepatitis C genotype you have, because it determines both the type of treatment and the length of treatment. HCV genotype also helps to predict the likelihood of curing. Across the globe HCV genotype 1 is the most common accounting for 60% of cases. In the United States, 75% of all HCV infections are genotype 1. On the contrary, genotypes 2, 3 and 4 are less common in the US and other genotypes are rare. It is possible to get infected with more than one HCV genotype; this is most likely among drug addicts and people who received contaminated blood products (before 1987 when viral inactivation started), or a blood transfusion (before 1993 when effective screening procedures were instituted).

In this study, it has been observed that the most frequent genotype was 3a followed by 3b (Fig. 1) which is prevalent in the subcontinent. It was found that genotype 3a is very common among the participants of this project which was conducted in Mirpur, AJK and its adjacent zones. This indicates the major prevalence of 3a strain in this community. Initial findings reveal that persons infected with different genotypes respond differently to treatment. Keeping in view the recent infection rate with reference to hepatitis B virus, the HCV infections are more in Pakistan. It may be due to the fact that there is no vaccine for it and simultaneously the efforts to curb this problem are limited. In addition, the clinical laboratories are not following the Biosafety protocols. This may be the factor that is also contributing towards an increase in the rate of infection.

As HCV is risk group level 2 organism, its handling on open benches for diagnosis could be the contributing factor towards its spread in the community of Pakistan. In order to control its spread, involvement from both public and private sectors is required to give awareness, education and also manage the drug menace which is still a major handicap in Pakistan. In this study we have tried to focus on the genotype and also on the gender and age group as this information may be of some relevance from those areas which are very remote. There is no information available with regard to importance of data generation and epidemiological study which pinpoint the source of infection. Through this study, we were able to transmit knowledge in
three different institutes with respect to biorisk management (CWA 15793-2008) and its integration with other related ISO standards.

5. CONCLUSION
It is concluded that there is high prevalence of 3a genotype in Mirpur and its adjacent cities. The high incidence in younger female age group, belonging to reproductive age, might be due to the fact that there are not enough hygienic measures in maternity homes during the delivery process whether they are normal vaginal deliveries, forceps or lower section caesarian section. In addition, proper education with regard to safe sex is not available which makes them vulnerable to their partners who may be suffering from HCV infection without knowing it. Drug addiction and ineffective discarding of disposable syringes or their reuse is also a major contributing factor. It is emphasized that more information with reference to above points may be provided to the people of these remote areas which is composed mostly of hilly terrain and where basic health amenities are insufficient.

REFERENCES


