Abstract

Aims & objectives: To determine the ratio of incidence and genotyping of HCV infection in various population of Mirpur, AJK. This was done by taking into account different modalities with reference to gender, age and socio-economic perspective. The screening was done by RDT and then it was confirmed by PCR method. The rtPCR was done at Genetic & Molecular Center Lahore. These tests were done at BSL 2 laboratory which was the criteria determined by risk assessment done by PI and the scientific officer of the facility. The main objective was to find out the exact incidence of HCV infection with reference to different typable and untypable strains prevalent in Mirpur, Azad Kashmir. In addition to make the laboratory professionals aware of different biosafety levels with respect to different risk groups of these organisms (according to Biorisk Management CWA15793-2008) handled and potentially present in the laboratory. The study will also establish the genotype most prevalent in these parts of AJK, and Punjab, Pakistan. In addition the introduction of Biorisk Management concurrently with this study will lead to professional development of laboratory technologists and technicians in terms of Biosafety and Biosecurity.

Place and duration of study: The sample collection was done at district and tehsil level, 100 patients were included in the study, which comprised of Barnala, Bhimber, Kotli, inclusive of Dina and Jhelum as patients were also reporting from these border line cities. This is a multicenter study October 2014 to September 2015; it was developed at Department of Pathology, MBBS Medical College, its affiliated DHQ Hospital Mirpur and Genetic & Molecular Center Lahore.

Keywords: rtPCR; HCV; Genotypes; Biorisk Management

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 disease in Pakistan [1]. The methods of detecting HCV are to screen the persons with identifiable risks. The primary source of HCV infection is infected blood or blood products. The injection drug users are at high risk of developing HCV infection, another sources of HCV transmission also include sexual contact with infected partner or multiple sexual partners or sex workers and in addition exposure to contaminated blood among laboratory and health care professionals [2]. It is not likely that HCV can spread from house hold activities except those that may have exposure to blood like sharing a razor or toothbrush. It is not spread by simple hugging and sharing of plates and cups [3]. Persons with hemophilia, should be tested for HCV infection, in other situations like acupuncture, body piercing and barbers also carry a substantial risk.

In clinical practice the initial test done is enzyme alt, HCV antibodies and if positive subsequent HCV RNA levels is known to be useful in providing and monitoring HCV management [4]. The HCV is a risk group 2/3 organism and the risk assessment methods applied with reference to this study determined it to be a BSL 2 level 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) and has been approved by FDA [5,6]. HCV genotyping does not predict the outcome of infection but it does provide information on outcome and duration of the treatment [7-10]. It can be performed by direct sequence analysis, by reverse hybridization to genotype specific oligonucleotides probes, and by restriction fragment length polymorphism RFLP [11,12]. In this study an attempt was made to characterize on molecular basis the genotype of HCV most prevalent in individuals who have been diagnosed positive for HCV antibodies and also HCV RNA by PCR.
Material and Methods

Local guidelines were developed in the light of WHO guidelines for Biosafety, hazard identification and risk assessment was performed using Biorisk Management guidelines CWA 16393-2012. The technicians were informed of 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 laboratory. It was made possible to do all the manipulations at BSL 2 simultaneously training the laboratory professionals in Biorisk management. The patients were reporting from these areas were categorized and analyzed for HCV infection on the basis of age, gender, signs and symptoms baseline ICT and LFT’s. Subsequently their PCR was done 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.

Results

It was found out that out of 100 cases with HCV infection the genotype ratio was as follows:

- 52 cases with 3a type
- 20 cases with 3b type
- 10 cases with 1a type
- 06 cases were untypable

It was also noted that the female population was more affected then the male population, 65% in comparison to 35% of male patients Figure 1. The categorization with reference to age revealed that younger group found to be more involved then the elderly group starting from 41-60 years. In addition the most frequent genotype that was determined was 3a followed by 3b Figure 1.

Discussion

The Hepatitis C Virus infection has been a foremost danger for the professionals who are exposed to many different invasive techniques while performing diagnosis and management of the patients [1]. The diagnostic criteria for this infection have been grouped in to two broad categories:

i. Serological means or assays that detect antibody to Hepatitis C virus
ii. Molecular assays and procedures that can detect quantify and also characterize HCV.

Serological 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) [13]. 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 [14,15]. Third-generation anti-HCV tests (EIA-3 and RIBA-3, respectively) contain antigens from the HCV core, nonstructural 3, nonstructural 4, and nonstructural 5 genes [11]. Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring the antiviral response to therapy. Optimal HCV PCR assays at present have a sensitivity of less than 100 copies of HCV RNA per milliliter of plasma or serum [15]. Two main technologies exist for assessing HCV RNA levels or viral load.

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 [16]. 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 [17].

i. Genomes within infected person.
ii. Genotype refers to genetic structure or makeup of living organisms.

The Hepatitis C virus 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 that 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 [18,19].

Worldwide, HCV genotype 1 is most common, accounting for 60 percent of cases. In the United States, 75 percent of all HCV infections are genotype 1; genotypes 2,3, and 4 are less common in the US, and other genotypes are rare. It is possible to infected with more than one HCV genotype; this is most likely among injection drug users, 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 our study it was revealed that the most frequent genotype that was determined was 3a followed by 3b which is prevalent in the sub continent. It was found out that Genotype 3a is very common among the participants of this project which was conducted in Mirpur, AJK and its adjacent zones. This determined the prevalence of any one strain in this community.

Initial findings reveals that persons infected with different genotypes respond differently to treatment [3]. HCV Keeping in view the recent infection rate with reference to HBV the HCV infections are more in Pakistan, it may be the case that there are no vaccine for it and simultaneously the efforts to curb this problem is limited. In addition the clinical laboratories are not following the Biosafety in their laboratories and may be that factor is also contributing that is Laboratory acquired infections for an increase rate of infection. As HCV is RG 2 level organism it’s handling at open bench for diagnosis can be contributing factor in the community of Pakistan. It requires involvement of public and as well as private sector to give awareness, education and also mange the drug menace which is still a major handicap in Pakistan.

In this study we have tried to focus on genotype and the gender and age group which are more affected keeping in view that this information may be of some relevance from those areas which are very remote and there is no information with regard to importance of data generation and epidemiological study which pin point the source infection. It was also important that 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.

**Conclusion**

It was concluded that:

i. There is high prevalence of 3a genotype in Mirpur and its adjacent cities.

ii. The high incidence in younger female age group belonging to reproductive age may be due to following:

iii. It also suggest that the there are not enough hygienic measures in maternity homes while the process of deliveries whether they are NVD, forceps or LSCS.

iv. 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 their knowledge.

v. In effective disposal of disposable syringes and reuse to other person after injection to one patient is also a contributing major factor.

vi. Drug addiction is also considered as major contributor.

vii. 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 at short.

viii. Integration of Biorisk Management with other ISO standards was also done.

**Outcomes**

i. The study was able to determine the prevalence rate on gender basis which was important on local basis and can be 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. This inclination may be the reason discussed above in the conclusion, it will be worthwhile to further confirm the cause and target the root cause more effectively in these remote areas of Pakistan.

ii. The genotype 3a was found to be more in the affected patients which certainly determined the management of these infections in a more targeted manner.

iii. In addition introduction of Biorisk Management was very encouraging and the professional showed keen interest in this scientific discipline and was eager to learn the key concepts of it.

**References**


