PREVALENCE AND DETECTION OF GENOTYPE OF HEPATITIS C VIRUS IN CHRONIC RENAL DISEASE PATIENTS UNDERGOING HAEMODIALYSIS IN TERTIARY CARE HOSPITAL IN PUNJAB

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ABSTRACT

BACKGROUND
Hepatitis C virus (HCV) infection is the commonest blood borne infection among haemodialysis patients. Despite reduction of Hepatitis C prevalence after recognition of the virus and testing of blood products, haemodialysis patients still comprise a high risk group. Hepatitis C virus is detected on the basis of serology, liver function profile and molecular methods. Genotyping is an important tool in epidemiological study, pathogenesis and reaction to antiviral therapy.

MATERIALS AND METHODS
The haemodialysed patients with appropriate symptoms were tested for Hepatitis C Virus (HCV) infection, between July 1, 2015 and February 18, 2016. Blood was aseptically collected and processed for detection of Anti-HCV antibody by ELISA in Department of Microbiology, CMC and H, Ludhiana. Quantitative detection of HCV-RNA was done by Real Time Polymerase Chain Reaction (RT-PCR) and if this was positive genotyping was done.

RESULTS
A total of 52 Chronic Renal Disease patients (39 male and 13 female patients) who underwent haemodialysis were tested for HCV RNA, out of which 10 (19.23%) came as positive. In 3 patients Genotyping has result of 2 patients with genotype 1 (66.67%) and 1 patient with genotype 4 (33.33%). Patients with Chronic Renal Disease with HCV infection had received more number of dialysis sessions (biweekly) as compared to those without HCV infection. Currently, Hepatitis C virus is most frequently found in patients with Chronic Renal Disease patients undergoing haemodialysis. The diagnosis of HCV is confirmed by detection of HCV-RNA by Polymerase Chain Reaction.

CONCLUSION
In the present study, it was seen that duration and frequency of dialysis is significantly longer among HCV positive patients as compared to HCV negative patients. Attention should be given to strict adherence to infection control measures in dialysis setting.

KEYWORDS
Haemodialysis Patients, HCV, Genotype, PCR, ELISA.


BACKGROUND
Hepatitis C infection is now acknowledged as an issue of major public health importance for most countries in the world.[1] Hepatitis C virus is a blood-borne pathogen that appears to be endemic in most parts of the world. There are however substantial geographic and temporal variations in the incidence and prevalence of HCV infection, largely due to differences in regional risk factors for the transmission of HCV. The World Health Organisation (WHO) estimates that the global prevalence of HCV infection averages 3% or around 170 million infected persons worldwide. However, population-based surveys are not available for most parts of the world and prevalence estimates are based on testing of selected populations such as blood donors. Prevalence of confirmed EIA positivity in blood donors ranges from less than 0.1% in Northern Europe to 0.1 - 0.5% in Western Europe, North America, parts of Central and South America, Australia and a few regions of Africa.

Intermediate rates (1 - 5%) have been reported from Brazil, Eastern Europe, the Mediterranean area, the Indian subcontinent and parts of Africa and Asia.[2]

Hepatitis C Virus (HCV) infection remains frequent in patient receiving long-term dialysis, both in developed and less developed countries. The natural history of HCV infection in dialysis patients remains incompletely understood; controversy continues even in patients with intact kidney function. Determining the natural history of HCV remains difficult for several reasons: the disease has a very long duration, it is mostly asymptomatic and determining its onset may be difficult. Additional factors can modify the course including coinfection with HBV, HIV and alcohol use. Because treatment is widely used, future natural history studies of chronic HCV may not be possible as easily documented onset
of infection, that is post-transfusion HCV no longer occurs.[3,4] Serologic testing has clearly demonstrated that HCV infection is highly prevalent among ESRD patients and is a serious cause of increased morbidity and mortality in this group. Failures of HCV screening, excessive exposure to blood and blood products, nosocomial transmission of HCV in HD units and long dialysis duration are the main determinants of increased risk of HCV infection in the HD patient group.[3] The worldwide prevalence of HCV infection among HD patients varies widely with estimates ranging from 5% to approximately 60% depending on geographic location.[6-9]

The standard clinical and serological techniques are not very sensitive and specific in monitoring the diagnosis and rate of progression of chronic hepatitis. Early stages of the infection are missed, because the antibodies develop only after six weeks of infection and the tests for anti-HCV antibody may be negative in the initial period before the seroconversion occurs.[10] For detection of anti-HCV antibody, there is current use of third generation ELISA which has shown greater sensitivity and specificity in patients undergoing haemodialysis.[11] Detection of HCV RNA by reverse transcriptase PCR has been used as the ‘gold standard’ to identify current HCV infection.[12] Third-generation anti-HCV ELISA is the screening test for the diagnosis of HCV infection. It has shown better performance than the previous two generations of anti-HCV tests with a mean window period of 70 days. The confirmation of HCV is by the detection of HCV RNA in serum by Polymerase Chain Reaction (PCR) assay, which appears earlier than the anti-HCV antibodies by several weeks or months (becomes detectable 1 - 3 weeks after exposure).[13-14]

The HCV genotype analysis has not only effect on disease presentation but is also valuable for antiviral therapy, counselling and the proper management.[15] Very few studies have been done in Northern part of country. So this study has been undertaken with the following aims and objectives.

**Aims and Objectives**

1. To study prevalence of Hepatitis C Virus (HCV) infection by ELISA, Real Time Polymerase Chain Reaction in Chronic Renal Disease patients undergoing haemodialysis in tertiary care hospital.
2. To study prevalence of genotype of Hepatitis C Virus (HCV) in these positive patients.

**MATERIALS AND METHODS**

All patients after ethical clearance were briefed about the study and willing consent was signed. A total of 52 Chronic Renal Disease patients (39 male and 13 female patients) who underwent haemodialysis were tested for Hepatitis C Virus (HCV) infection, between July 1, 2015 and February 18, 2016. The studied population ranged in age from 30 years to 75 years (Average 67.5 years). The samples taken will be blood serum (for ELISA) or plasma (for PCR). The minimum volume of sample was 5 mL for ELISA and PCR. From 5 mL of blood 2 - 3 mL of plasma or serum were used for the test. Blood was aseptically collected in sterile vacutainers and processed for detection of Anti-HCV antibody by ELISA. If the test is positive for ELISA (seropositive), it will be further tested for HCV-RNA viral load by real time PCR. Detection of Anti-HCV antibodies was done by ELISA by kit from the J. Mitra and Co. Pvt. Ltd. (Sensitivity: 100%, Specificity: 99.73%). The third generation HCV Microlisa, a qualitative enzyme-linked immunosorbent assay and was used for the detection of antibodies against HCV (anti-HCVs) in serum or plasma.

Quantitative detection of HCV-RNA was done by Real Time Polymerase Chain Reaction (RT-PCR) and genotyping done in positive cases.

HCV-RNA was quantified using standard RNA extraction and real time amplification kits using Taqman principle along with quantitation standards.

**Inclusion Criteria**

Adult Patients of Chronic Renal Disease undergoing haemodialysis were recruited for study.

**Exclusion Criteria**

All other confirmed patients of hepatitis including alcohol and drug-induced hepatitis (anti-tubercular drugs, halothane) were excluded.

**RESULTS**

A total of 52 Chronic Renal Disease patients (39 male and 13 female patients with average age of 65.5 years) who underwent haemodialysis were tested, out of which 3 patients (5.76%) came positive for anti-HCV antibody and 10 (19.23%) came positive for HCV RNA; 25 patients (48.079%) were anti-HCV antibody positive, but negative for HCV RNA; 10 patients (23.25%) were anti-HCV negative, but positive for HCV RNA. HCV Genotyping was done in 3 patients among which 2 patients were positive for genotype 1 (66.67%) and 1 patient for genotype 4 (33.33%). Patients with Chronic Renal Disease with HCV Infection had received more number of dialysis sessions (biweekly) as compared to those without HCV infection. In this observational study, we studied the prevalence of kidney disease patients having hepatitis C infection, who were admitted to our hospital for haemodialysis [Table 1] [Figure 1].

<table>
<thead>
<tr>
<th>HCV Test</th>
<th>Number (Sample Size 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV Positive</td>
<td>3 (5.76%)</td>
</tr>
<tr>
<td>HCV RNA Positive</td>
<td>10 (19.23%)</td>
</tr>
<tr>
<td>HCV RNA Negative</td>
<td>42 (80.76%)</td>
</tr>
<tr>
<td>Anti-HCV Positive</td>
<td>HCV RNA Positive 3 (10.0%)</td>
</tr>
<tr>
<td>Anti-HCV Negative</td>
<td>HCV RNA Positive 7 (13.46%)</td>
</tr>
</tbody>
</table>

**Table 1. Distribution of HCV in Haemodialysis Patients in Tertiary Care Hospital in Punjab**

![Figure 1. Distribution of HCV in Haemodialysis Patients in Tertiary Care Hospital in Punjab (n = 52)](image-url)
DISCUSSION

Haemodialysis patients are at high risk for the development of hepatitis C infection. There is inadequate data about the prevalence of HCV infection in haemodialysis patients. In a study done in Andhra Pradesh, the prevalence of HCV infection among haemodialysis patients is 23.5% with viraemia of 18.01%.[16] A study in Mexico shows HCV viraemia in 5% patients with anti-HCV prevalence of 6.7%.[17] In a study in Brazil, the prevalence of anti-HCV was found to be 39%.[18] In the current study, the HCV viraemia was found to be 19.23% with anti-HCV prevalence of 5.76% among haemodialysis patients. Also according to the current study, the length of time spent on dialysis has contributed to the prevalence of HCV infection.

The tests for anti-HCV antibody may be negative in the initial period before the seroconversion occurs; therefore, there are negative results in serology in spite of HCV viraemia in haemodialysis patients.[19] In our study, 13.46% patients were anti-HCV negative despite the presence of HCV viraemia.

The tests may be with positive serology and negative viraemia, which occurs both in immunosuppressed and immunocompetent state. This occurs as a result of three factors – non-establishment of carrier state (commonly seen in renal transplant patients), whereas other two factors like low level of undetectable viraemia and intermittent viraemia are seen in dialysis settings.[19–20] In current study, HCV viraemia was not detectable in 25 patients with positive serology. Several physiopathogenetic mechanisms have been proposed to explain the intermittent HCV viraemia like heparin interference with the PCR assay used for the detection of HCV RNA,[21] mechanical extraction of viral particles adhering to dialyser membrane and induction of interferon production, hepatocyte growth factor or other cytokines with antiviral properties by the haemodialysis procedure.[22]

Genotype 1 is most prevalent followed by genotype 3 worldwide (30.01% of total) and its prevalence is highest in Southern Asia. In patients of United States, the prevalent major HCV genotypes are genotypes 1a and 1b.[23] In our study, HCV genotype 1 was found to be most predominant (66.67%) followed by genotype 4 (33.33%).

The level of kidney function in the CKD population plays a crucial role on the pharmacokinetics of antiviral drugs targeted at HCV. Kidney filtration and catabolism have a significant contribution to the clearance of IFN and ribavirin; thus, there is the need to make appropriate dosing adjustment and caution.[24]

Currently, Hepatitis C virus is most frequently found in patients with Chronic Renal Disease patients undergoing haemodialysis. Third-generation anti-HCV ELISA is the screening test for the diagnosis of HCV infection. It has shown better performance than the previous two generations of anti-HCV tests with a mean window period of 70 days.[25]

The duration of therapy depends on the genotype of the Hepatitis C virus. Combined therapy with both pegylated interferon and ribavarin will have to be given for 24 weeks in genotype 3 patients and 48 weeks genotype 1 patients. The sustained response is less in case of genotype 1 as compared to genotype 3. Systemic screening of ALT and anti-HCV in haemodialysis patients is strongly recommended (Monthly for ALT and 6 monthly for anti-HCV). The diagnosis of HCV is confirmed by detection of HCV-RNA by Polymerase Chain Reaction.

CONCLUSION

In the present study, it was seen that duration and frequency of dialysis is significantly longer among HCV positive patients as compared to HCV negative patients. Attention should be given to strict adherence to infection control measures in dialysis setting. All dialysis unit should apply universal precautions and use dedicated dialysis equipment for anti-HCV positive patients. It is important for the designers of dialysis units to create an environment that makes infection-control procedures easy to implement. Adequate hand-washing facilities must be provided and the machines and shared space should make it easy for staff to visualise individual treatment stations.

REFERENCES