INTRODUCTION

Hepatitis C virus (HCV) infection is a major concern for the public health worldwide in both developing and developed countries. Transmission of HCV infection is mainly by exposure to infected devices and tools despite rigid hygienic control, infected blood or blood products, hemodialysis, intravenous (IV) drug abuse, and organ transplantation. The estimation of national prevalence and ways of transmission of HCV should be completed in order to allow the national authorities to prioritize preventive measures and have the best and most appropriate use of available resources. Epidemiological surveys on the roles of potential risk factors, such as injections for medications, vaccinations, medical procedures, tattooing, and injections outside of medical settings, have shown a wide geographical variation with major implications for the populations and potential management, prevention, and control plans. Prospective investigations have revealed that about 80% of the acute hepatitis C cases progress to chronic infection; about 10%-20% of these cases will develop chronic liver disease complications, like liver cirrhosis, within two to three decades of onset, and about 1%-5% will end up with liver cancer.

As shown by Dialysis Outcomes and Practice Patterns Study (DOPPS) there has been wide variation in the prevalence of HCV among different dialysis units and countries with mean HCV prevalence of 13.5% and variation among countries is 2.6-22.9%. In India very wide range in the prevalence rate has been reported in dialysis population which is 4.3-45.2%11. In Punjab HCV prevalence is estimated to be 5%12.

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 occur. For detection of anti-HCV antibody there is current use of third generation ELISA which has shown greater sensitivity.
and specificity in patients undergoing Hemodialysis\(^8\). 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\(^24,15\).

A reactive or indeterminate/equivocal antibody test should be followed by HCV RNA testing to determine occult infections\(^16,17\). In India among anti-HCV negative patients with end-stage renal disease, occult HCV infection has been reported in 56.25% patients in those on hemodialysis\(^18\) and 42.2% in those who had received renal transplantation\(^19\). Very few studies have been done in this part of country. So this study has been undertaken with the following aim and objectives.

**Aims and Objectives**

To study prevalence of Hepatitis C Infection (HCV) by Real Time Polymerase Chain Reaction in Chronic Renal Disease patients undergoing hemodialysis in tertiary care hospital.

**MATERIALS AND METHODS**

The study was conducted in a tertiary care centre for a period of two years. The sample taken was plasma separated from blood. 5 ml blood was aseptically collected in vial by venipuncture in two vacutainer tubes containing dipotassium ethylene diamine tetra acetate (EDTA). The tube was centrifuged at 2500 rpm for 10 minutes and the supernatant plasma was stored at -20°C for further processing of HCV-RNA viral load.

240 patients were recruited for the study. PCR for quantitative detection of HCV-RNA was performed in all patients. HCV-RNA was quantified using standard RNA extraction and real time amplification kits using Taqman principle along with quantitation standards.

**Inclusion Criteria**

Patients of Chronic Renal Disease undergoing hemodialysis were recruited for study.

**Exclusion Criteria**

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

**Statistical Analysis**

Data was collected using a structured proforma. Data entry was done in Microsoft Excel spreadsheets. Data analysis was done using Statistical Package for Social Sciences (SPSS Ver. 18.0). Percentages were calculated for all categorical variables. P value was calculated for categorical outcomes. A p-value < 0.05 was considered as statistically significant.

**RESULTS**

A total of 240 Chronic Renal Disease patients (161 male and 79 female patients) who underwent haemodialysis were tested for HCV RNA out of which 128 (53.33%) were positive and 112 (46.67%) were negative.

**Quantitative distribution of HCV-RNA viral load**

The HCV-RNA quantification (viral load) was categorized in four groups in 128 total positive samples out of 240, very low positive (<1000 IU/ml), low positive (1000-50000 IU/ml), intermediate positive (50000-100000 IU/ml) and high positive (>100000 IU/ml). It is given in Fig.1.

![Fig 1 Quantitative distribution of HCV-RNA viral load (n=128)](image)

**Correlation between HCV-RNA positivity and duration of hemodialysis (n=240)**

Out of 128 HCV-RNA positive patients, it was observed that there were 7 (5.46%), 89 (69.53%) and 32 (25%) patients who were on hemodialysis for <2 yrs, 2-4 yrs and >4 yrs respectively. The maximum (56.33%) and minimum (20%) HCV-RNA positivity was in patients who were on hemodialysis for 2-4 years and <2 years respectively. There had been statistically significant correlation between HCV-RNA positivity and duration of hemodialysis (P value <.0001) (Table 1).

![Table 1 Correlation between HCV-RNA positivity and duration of hemodialysis (n=250) (Figures in parenthesis show percentages)](table)

**Correlation between HCV-RNA positivity and Blood Transfusion (n=250)**

Out of 240 patients there were 76 (31.67%) patients had history of blood transfusion. In these 76 blood transfused recipients 29 (38.16%) patients were HCV-RNA viral load positive. It was also observed that there were 99 (59.78%) patients positive for HCV-RNA viral load but do not have history of blood transfusion. There was statistical significant correlation observed between HCV-RNA positivity and blood transfusion (P value 0.006). (Table-2).
Table 2 Correlation between HCV-RNA positivity and Blood Transfusion (n=250) (Figures in parenthesis show percentages)

<table>
<thead>
<tr>
<th>History Of Blood</th>
<th>HCV-RNA</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>70 (40.22)</td>
<td>99 (59.78)</td>
<td>169 (100.00)</td>
</tr>
<tr>
<td>Yes</td>
<td>42 (55.26)</td>
<td>29 (44.74)</td>
<td>71 (100.00)</td>
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<td>Total</td>
<td>112 (46.67)</td>
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Distribution of HCV-RNA viral load and ALT

In our study the HCV–RNA quantification was evaluated in 240 patients. The biochemical parameter i.e. Alanine aminotransferase (ALT) was also recorded in all these 240 samples. Out of 240 positive HCV RNA patients total number of patients who showed increase in ALT (>40 U/L) were 62 (25.83%) and normal ALT (<40 U/L) in 178 (74.17%) patients. Out of 62 patients with increase in ALT levels (>40 U/L), positive HCV RNA viral load was observed in 58 patients (93.54%) while 65 (36.51%) patients with positive HCV-RNA viral load was seen in 178 patients with normal ALT levels (Fig. 2).

Table 2: Correlation between HCV-RNA positivity and Blood Transfusion (n=250) (Figures in parenthesis show percentages)

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Fig 2 Distribution of HCV-RNA viral load and ALT (n=240)

Fig. 2 depicts distribution of HCV-RNA viral load and ALT which shows significant statistical correlation (P value - 0.005). In a study done in United States the highest rate of infection was observed in the cohort age 45-49 years (7.1% among men and 2.3% among women). The NHANES also showed an anti-HCV prevalence of 0.9% in persons older than age 60 years, and a peak greater than 4.0% in age-specific prevalence, which shifted from persons between 30 and 39 years of age to persons between 40 and 49 years of age during the last decade.

The study showed a male predominance. The number of male patients was 161 (67.08%), whereas the females were 79 (32.92%). Male to female ratio was 2:1. It was also observed that the HCV positivity was higher in case of males (54.97%). In the Faridkot district of Punjab the prevalence of HCV was found to be more in males (67%) compared to females (33%). This may be due to the fact that in India, males seek health care services earlier than females.

In a study done in a tertiary care hospital in Ludhiana, Hepatitis C infection was found to be more prevalent in males as compared to females.

Total 240 patients were evaluated, in which 76 (31.67%) patients had history of blood transfusion which were again male predominant (53.16%). Among the age groups of <41 yrs, 41-60 yrs, >60 yrs the history of blood transfusion was seen in 62 (25.08%), 65 (59.78%) and 11 (68.75%) patients respectively. Maximum blood transfused recipients patients were of age group 41-60 yrs. Prior to 1992, blood transfusions carried a high risk of HCV infection, approximately 15-20% with each unit transfused. In 1988, 90% of cases of posttransfusion hepatitis were due to NANBH viruses which was later found out to be due to HCV. The screening of blood has reduced the rate of post transfusion hepatitis C by a factor of about 10,000; to a current rate of 1 per million transfusion.

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In our study out of 76 patients who were blood transfusion recipients there were 15 (19.74%) patients with positive anti-HCV antibody and 29 (38.16%) patients with positive HCV-RNA viral load.

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A total of 240 patients were evaluated for HCV-RNA viral load and they were categorized into three groups according to duration of hemodialysis: <2 yrs, 2-4 yrs and >4 yrs. Majority of patients belonged to patients on hemodialysis for 2-4 years were 163 (67.91%). The maximum (56.33%) and minimum (20%) HCV-RNA positivity was in patients who were on hemodialysis for 2-4 years and <2 years respectively (Table 1). The duration of hemodialysis in patients with or without blood transfusions is also an independent risk factor.
CONCLUSION

The increased sensitivity of the latest generation of HCV assays has dramatically reduced the risk of HCV transmission by blood components and reduced the time between acquisition of infection and detection of anti-HCV antibodies (the ‘serologic window’). In suspected acute HCV infection, a negative anti-HCV test does not exclude HCV infection. After an exposure to HCV, HCV RNA can be detected within short period, whereas antibodies to HCV are detectable in longer period. HCV-RNA is thus, probably the only choice of diagnosis to accurately determine the epidemiology and management of HCV infection.

Therefore in this scenario, detection of HCV-RNA in serum by polymerase chain reaction (PCR) assay should be considered as the gold standard for the diagnosis of HCV infection.

References


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