PREVALENCE OF HCV INFECTION IN PATIENTS ON HAEMODIALYSIS: SURVEY BY ANTIBODY AND CORE ANTIGEN DETECTION

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Abstract

Purpose: The present study was undertaken to determine the prevalence of HCV infection by antibody testing and HCV core antigen (HCVcAg) determination by ELISA in haemodialysis patients and to evaluate the HCV c Ag assay in the detection of HCV infected patients on haemodialysis. Materials and Methods: A total of 151 chronic renal failure patients on haemodialysis from May 2003 to October 2004 were studied. One hundred patients out of 151 were followed for 2-5 months. All the patients were tested for anti HCV and HCV core antigen once a month. Anti HCV ELISA positive specimens were confirmed by RIBA. Results: The overall prevalence of HCV infection was 13.23%. Antibody positivity was observed in 9.93% and HCVcAg alone was detected in 2.64%. One patient (0.66%) was initially positive for core antigen and later seroconverted. Conclusions: Screening for HCV antibodies alone does not exclude infection with HCV in patients on haemodialysis and HCVcAg may be a useful test for identifying HCV infected patients on haemodialysis in the early phase of infection before seroconversion.

Key words: Chronic renal failure, HCV c Ag, seroconversion

Chronic renal failure patients on haemodialysis (HD) are at high risk for blood borne infections because of prolonged vascular access and the potential for exposure to infected patients and contaminated equipment. Infection due to hepatitis viruses is one such infection that continues to be a major concern in dialysis setting. Development of diagnostic testing for hepatitis B confirmed a high incidence and prevalence for HBV infection both in patients and staff in HD units. Introduction of vaccination, isolation of hepatitis B virus (HBV) positive patients, dedicated dialysis machines and regular surveillance for HBV infection dramatically reduced the spread of HBV infection. Patients on haemodialysis have a high risk of acquiring HCV infection. Transfusion of unscreened blood, duration of dialysis and nosocomial transmission within haemodialysis unit are implicated as the main transmission routes of HCV infection in haemodialysis patients. HCV infection in patients on HD has been associated with greater morbidity and mortality.

The prevalence of antibodies to HCV (anti HCV) in HD patients ranges world wide from 1% in UK to 62% in Portugal and is highly variable between different countries in the same locality. There is also great variability in HCV testing practices in dialysis centre. Data on prevalence of HCV among Indian haemodialysis patients are scanty. The prevalence of anti HCV in patients on haemodialysis from India is reported to be in the range of 3-45%.

Virological diagnosis and monitoring of HCV infection are based on two categories of laboratory tests, namely serologic assays detecting specific antibody to HCV (indirect test) and assay that can detect, quantify or characterise the component of HCV viral particles (direct tests), such as HCV RNA. Antibody detection tests are considered important tools to assess the magnitude of HCV infection in patients on HD. The window phase in HD patients can be longer as these patients are immunocompromised and the anti HCV ELISA test alone may fail to detect the infected patients in the acute phase of the disease.

HCV RNA detection is widely accepted as a gold standard test in the diagnosis of HCV infection in HD patients. However there have been concerns that wide spread use of PCR may be limited by availability, the need for meticulous specimen handling and concerns regarding reproducibility. Many of these reservations have been addressed by introduction of automated PCR assays shown to be accurate and suitable for the diagnosis and monitoring of viral load in patients infected with HCV.

The latest break through in diagnosing early HCV infection is by detecting the HCV core antigen (HCVcAg) that is present during the early stage of infection when anti HCV antibodies have not been produced. A highly sensitive enzyme immunoassay for HCVcAg has been developed by Ortho Clinical Diagnostics. There is a positive correlation between the concentration of core antigen and HCV RNA in anti HCV negative specimens. This study was undertaken to find out the prevalence of HCV infection and utility of the HCVcAg assay in the early diagnosis of HCV infection in patients on HD.
Materials and Methods

Study design and patients

The study was performed in the department of microbiology in collaboration with department of nephrology of the Nizam's Institute of Medical Sciences (NIMS). The study protocol was approved by the ethics committee of NIMS. A total of 151 chronic renal failure patients on haemodialysis from May 2003 to October 2004 were studied. Eleven out of 151 patients were anti HCV positive before enrolling them in to the study and were excluded.

Patients follow up program

Of the remaining 140 / 151 patients, 40 were on HD for a short period i.e., less than one month and could not be continued in the study. The actual study comprised of the remaining 100 patients who were followed for two to five months.

Haemodialysis unit

The haemodialysis unit has one routine haemodialysis area and two isolated areas, one for HBsAg positive patients and other for anti HCV positive patients. The routine haemodialysis area has six haemodialysis machines, HBsAg positive area has one machine and anti HCV positive area has one machine. All the patients underwent serological testing for HBsAg, anti HCV and HIV before initiating the dialysis. Patients who were negative for HBsAg and anti HCV before initiating dialysis were dialyzed in routine haemodialysis area. Patients who were positive for anti HCV and / HBsAg before initiating the dialysis were dialyzed on dedicated machines in the respective isolated areas. Any patient who seroconverted to HCV or HBV during haemodialysis treatment was shifted to the respective isolated area.

All haemodialysis machines were chemically disinfected between each dialysis session. Dialyzer membranes and tubings were reused. Reuse areas and areas for storage of dialyzers are separate for HBsAg and anti HCV positive patients and for patients who are negative for both. In these three different areas (routine haemodialysis area, HBsAg positive area, anti HCV positive area) dedicated nursing staff were posted to look after each patient.

Specimen collection

Blood samples were drawn from the patients before the start of the first haemodialysis and every month there after. Serum was separated within two hours after blood sampling. All the serum samples were divided in to 0.5 mL aliquots and stored at -20°C.

Serology

All the 100 patients were tested once monthly for anti HCV, HCV core antigen with Ortho HCV 3.0 ELISA test system and Ortho antibody to HCV core antigen ELISA test system (Ortho clinical diagnostics) respectively. Anti HCV ELISA positive specimens were confirmed with supplemental anti HCV serological tests by using CHIRON RIBA HCV 3.0 SIA. All core antigen ELISA positive specimens were retested in duplicate and the specimen was considered repeatedly reactive for HCV core antigen if either or both duplicate determination(s) was (were) reactive.

Results

The status of HCV positive patients among the 100 study patients is shown in table 1. The initial specimens of (i.e., before initiating haemodialysis) three of the 100 patients were positive for anti HCV antibodies and they were not followed up further.

Four other patients were positive for HCVcAg. Two out of four patients were positive for HCVcAg in the initial specimen that was collected before initiating the haemodialysis. They were continued to be followed up for seroconversion. Both these patients were negative for anti HCV in the first month and second month follow up specimens. However, these patients were lost to further follow up beyond two months. In the remaining two patients, one patient was positive for HCVcAg in the first month follow up specimen and the other patient in the fourth month follow up specimen. The next follow up specimens of these two patients were negative for anti HCV. There was only one patient out of the 100 patients that had both demonstrable HCVcAg and anti HCV. The initial specimen of this patient was positive for HCVcAg and the first month follow up specimen was positive for anti HCV.

Out of 151 patients, 15 patients were anti HCV positive (9.93%). All anti HCV positive specimens were positive in supplemental anti HCV tests (RIBA). In the RIBA most of the anti HCV positive patients showed antibody response to NS3 antigen band compared to core antigen band. The overall prevalence of hepatitis C (HCV antibody positivity and / or HCVcAg Positivity) was 13.23%. The prevalence data are given in table 2.

Discussion

It is well known that haemodialysis patients are at high risk for development of hepatitis C infection. However, the data on the prevalence of anti HCV among Indian haemodialysis patients is scanty. Salunkhe et al in 1992 reported 45%, Chadher et al in 1993 reported 12.1%, Sumathi et al in 1993 reported 37.5%, Agarwal et al in 1999 reported 42%, and Jaiswal et al in a study from 1992-2000 reported prevalence of 30%. The prevalence of HCV infection among the haemodialysis patients at our institute is 13.23%.

The performance parameters of the testing method used have a direct impact on the detection of hepatitis C and thus
can lead to differences in the prevalence data. In the early 1990s, the first generation HCV antibody testing kits were introduced using NS4 antigen repertoire as the solid phase. These tests were further improvised with the addition of NS3 and the core regions of the viral genome. This second generation ELISA assay had a higher sensitivity and specificity over the earlier one. At present, the third generation ELISA assays use highly purified antigens with the addition of NS5 region of HCV genome and have the highest sensitivity and specificity. The antigens are either a purely recombinant (Ortho), or purely synthetic peptides (UBI), or a mixture of recombinant and synthetic peptides (General Biologicals). The sensitivity and specificity of these 3rd generation kits depend on the type of antigen used.

With the advent of the molecular techniques, the circulating virus (viraemia) can now be detected by HCV RNA measurement. This testing is used for early detection (before seroconversion) and also is essential for confirmation of active HCV infection and monitoring of antiviral therapy. Several authors reported high prevalence of HCV infection among haemodialysis patients by using anti HCV and HCV RNA detection. The antigens are either a purely recombinant (Ortho), or purely synthetic peptides (UBI), or a mixture of recombinant and synthetic peptides (General Biologicals). The sensitivity and specificity of these 3rd generation kits depend on the type of antigen used.

More recent studies have shown that the HCV core antigen is detectable throughout viraemic preseroconversion period. Studies in HCV seroconversion series have shown that the average time to detection of core antigen is 1-2 days after the appearance of RNA. Peterson et al reported that HCV core antigen appears substantially earlier than the HCV antibody during early phase of HCV infection and simultaneously with HCV RNA in most seroconversion series. It became undetectable in a large proportion of seroconversion specimens after the development of anti HCV. In our study we detected four HCV infected patients with core antigen. Core antigen was negative in the 15 anti HCV positive specimens. HCVcAg in patients on haemodialysis, such responses have to be confirmed using RT-PCR for RNA. However, one can probably say that the expression of antigen and antibody in immunocompromised patients is very inconsistent, irregular and may fluctuate.

Table 1: Anti HCV and Core Ag results of HCV infected patients

<table>
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<tr>
<th>Patients</th>
<th>Serostatus</th>
<th>Initial</th>
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Table 2: Prevalence of Hepatitis C virus (HCV) antibodies and HCVcAg in 151 haemodialysis patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number (%)</th>
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<tbody>
<tr>
<td>HCV antibody positive</td>
<td>15 (9.93)</td>
</tr>
<tr>
<td>HCVcAg positive</td>
<td>4 (2.64)</td>
</tr>
<tr>
<td>HCV antibody and HCVcAg positive</td>
<td>1 (0.66)</td>
</tr>
<tr>
<td>HCV antibody and / or HCVcAg positive</td>
<td>20 (13.23)</td>
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LFU: lost to follow up.
patients (patient 4 in table 1), became anti HCV positive in the fourth month follow up specimen. The initial specimen, first, second and third month follow up specimens were negative for core antigen. We are in agreement with Courouse et al that in all preseroconversion phase specimens we can not detect core antigen. The antibody positivity in the absence of the antigen is probably because the antigen kit may not be sensitive enough to detect low and falling levels of antigen that may be present before the seroconversion. In this patient we started collecting the samples only three months before seroconversion and hence the antigen status is not known in this patient before three months. Hence we do not know the exact stage of infection in this patient.

Devesa et al observed restricted antibody reactivity to Hepatitis C Virus synthetic antigens in patients on haemodialysis. In the RIBA strip NS3 and NS5 antigen bands are recombinant proteins and the core and NS4 bands are of synthetic antigens. This could be one of the reasons for greater antibody response towards NS3 antigen among our haemodialysis patients.

In conclusion, the prevalence of HCV infection in our haemodialysis patients with anti HCV detection alone was 9.93% but when we used HCVcAg along with anti HCV ELISA for diagnosis of HCV infection the prevalence was 13.23%. Screening for HCV antibodies alone does not exclude infection with HCV in patients on haemodialysis and HCVcAg may be useful and an early test for identifying antibody negative HCV infected patients on haemodialysis.

References


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**ANNOUNCEMENT**

**SIXTH NATIONAL CONFERENCE
SOCIETY FOR INDIAN HUMAN AND ANIMAL MYCOLOGY (SIHAM)
17TH - 22ND JANUARY 2006
HYDERABAD**

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- **19th January 2006**: CME on “Anti-Fungal Chemotherapy: From the Laboratory to the Bed side”
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  - Meet the Expert sessions: Topics to be decided.
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