STUDYING THE PREVALENCE OF HEPATITIS B & HEPATITIS C VIRUS INFECTION ALONG WITH ITS ASSOCIATED RISK FACTORS IN HEMODIALYSIS PATIENTS

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Abstract
Background: Haemodialysis is a primary mode of therapy for patients with end stage renal diseases. Haemodialysis as it has an extracorporeal technique is associated with increased risk of parentally transmitted viruses including the Hepatitis B virus and Hepatitis C virus. Methodology- This study was undertaken in Department of Microbiology P.D.U Medical College, Rajkot from January 2017 to December 2017. The sample size taken was 100 patients. Patients fulfilling the inclusion criteria were enrolled for this study. Detection of HBsAg by RAPID test and detection of anti-HBC antibody by RAPID test was done. Results- In our study 41 patients were given blood transfusion and 59 patients were not given blood transfusion. All reported HBsAg or HCV positive cases were from haemodialysis patients who have received multiple blood transfusion. This suggests that blood transfusions are significant risk factors for HBV and HCV transmission. Conclusion- Blood transfusions are significant risk factors for HBV and HCV transmission.

INTRODUCTION
Haemodialysis is a primary mode of therapy for patients with end stage renal diseases. Haemodialysis as it has an extracorporeal technique is associated with increased risk of parentally transmitted viruses including the Hepatitis B virus and Hepatitis C virus. Hepatitis B virus is the most important causative agent of transfusion-associated hepatitis. Humans are the only reservoir of Hepatitis B virus (HBV). The virus particle (virion) is a small complex double-shelled structure having an external diameter of 42 nm with a nucleocapsid core and lipoprotein coat. This particle was first discovered by Dane and his colleagues in 1970 and thus is called Dane particle. It represents the complete HBV. The nucleocapsid core is 27 nm in diameter. It replicates in the nuclei of infected hepatocytes and possesses a distinct antigen called hepatitis core antigen (HBCAg). The virion core antigen contains DNA polymerase and double stranded DNA molecule. HCV is the most common cause of post-transfusion non-A Non-B hepatitis in the developed world. 50% of HCV infection is reported to progress to chronic infection of which 20% may progress to cirrhosis and hepatocellular carcinoma. HBV and HCV infections are the most common causes of liver disease in hemodialysis patients and pose problems in the management of the patients in the renal dialysis units. Chronic renal failure (CRF) patients do not clear these viral infections efficiently, and several outbreaks of hepatitis have occurred in these settings. The prevalence of HBV and HCV viruses is highly seen in HD population and it may be due to cross infection from other patients due to sharing of common equipments and requirements of multiple blood transfusions. Synergistic infection with HBV and/or HCV in patients with ESRD predisposed the patient for accelerated progression of the disease. The prevalence of HCV infection among patients on hemodialysis is high and varies between countries (2–60%) and between dialysis units within a single country. As these viral infections have common modes of transmission, the presence of co-infection in patients is not rare and is relatively high in transfusion recipients. The introduction of HBV vaccination, isolation of HBV-positive patients, use of dedicated dialysis machines, and...
regular surveillance for HBV infection dramatically reduced the spread of HBV in this setting. However, the relatively low acceptance of and response rates to the HBV vaccine among dialysis patients likely contributes to ongoing transmission, as does the need for vaccine boosts to maintain antibody to HBsAg (anti-HBs) at protective levels. Maintenance haemodialysis patients, hemophiliacs, and thalassemics form a major risk group for HBV and HCV infections owing to the frequent use of blood products and haemodialysis apart from the multiple invasive medical procedures to which these patients are exposed. With this background we carried out a study to find out the sero-prevalence of HBV and HCV in patients undergoing haemodialysis in a tertiary care centre in Rajkot.

MATERIALS AND METHODS

This prospective, single centre, observational & comparative type of study was undertaken in Department of Microbiology P.D.U Medical College, Rajkot from period January 2017 to December 2017. The sample size taken for this study was 100 patients. The dialysis unit of P.D.U Hospital, Rajkot has eight haemodialysis machines. Among these, one is dedicated for HBV and HBV/HCV co-infected patients and one machine is dedicated for HCV positive patients. Both the machines are placed away from the rest of the machines in an isolated room, so as to avoid cross contamination. The dialyzers of the patients are reused. Reprocessing of the dialyzers of the HBV / HCV positive patients are done in a separate room, away from the rest of the patients.

Patients with chronic renal disease who undergoing haemodialysis and completing one year of study period were included in this study.

Patients with acute renal disease who undergoing haemodialysis and those who were Hepatitis B and Hepatitis C seropositive were excluded from the study.

Blood samples received in microbiology laboratory from HD unit of P.D.U Hospital, Rajkot were included in study. Serum samples from these patients are routinely sent to microbiology laboratory at 1-2-month interval for HBsAg & HCV Ab testing. These samples were tested for presence of HBsAg & HCV Ab by Rapid Method. Any patient positive for HBsAg or anti HCV or to both were dialyzed on the dedicated machine. Statistical analysis will be performed using Microsoft Excel and other statistical analytic software.

A) Detection of HBsAg (By HEPA™ CARD)

**Procedure**

Bring the specimen and pouch containing the HEPA™ CARD to room temperature prior to testing. Remove one test card from the pouch and place it on a clean flat surface. With the help of the dropper provided, add one drop of serum/plasma into the sample well. Add 2-3 drops of assay buffer into the sample well of the test device. Let the reaction to proceed until the appearance of positive line and control line up-to 20 minutes. Read the results after 20 minutes. Strong positive reaction may be visible within 20 minutes. If questionable results are obtained and HBV infection is suspected, the test should be repeated on a fresh serum specimen after 4 weeks. As with all diagnostic tests, a definitive clinical diagnosis should be based on the result of a single test, but such result should be interpreted only after all clinical and laboratory findings have been evaluated.

**Results**

- **Negative**
  - If a distinct pink purple line is formed only at the control zone marked ‘C’ (control line), the test result is negative.
- **Positive**
  - If a distinct pink-purple line is formed at the test zone marked ‘T’ (test line) and the control zone marked ‘C’ (control line) the test result is positive, indicating that the sample contains Hepatitis B Antigen.
- **Invalid**
  - If a distinct pink-purple line is not formed at the control zone marked ‘C’, the test result is invalid.

B) Detection of Anti–HCV

**Immunochromatographic assay**

Bring the test specimen and test device to room temperature prior to testing. Remove one test card from the pouch and place it on a clean flat surface. Using the dropper provided add one drop of serum/plasma sample (approx. 30µl) then two drops of buffer (approx. 60µl) immediately into the sample well. Avoid overflowing. Read the results within 15 minutes. Strong positive reaction may be visible within 5 minutes. Do not read results after 20 minutes. If questionable results are obtained and HCV infection is suspected, the test should be repeated on a fresh serum specimen after 4 weeks.

Result interpretation is same as the results of test for detection of HBsAg (By HEPA™ CARD).

RESULTS

A total of 100 samples from Dialysis patients attending hemodialysis unit at P.D.U. Hospital, Rajkot were screened for Hepatitis B Virus infection, Hepatitis C Virus infection as under:

- Detection of HBsAg by RAPID test.
- Detection of anti-HBC antibody by RAPID test.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>HBsAg Negative</th>
<th>HBsAg Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>16-30</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>31-45</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>46-60</td>
<td>43</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 1: Prevalence of HBsAg among hemodialysis patient: Age wise.**

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Table 2: Prevalence of HBsAg among hemodialysis patient: Sex wise.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive (n=100)</th>
<th>Negative (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table 2: shows that there was 1 male seropositive for HBsAg.

Table 3: Prevalence of HCV among hemodialysis patient.

<table>
<thead>
<tr>
<th></th>
<th>Positive (n=100)</th>
<th>Negative (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4%</td>
</tr>
</tbody>
</table>

Table 3: Shows that there are 4 samples positive for HCV out of 100 samples, so overall prevalence for HCV is 4%.

Table 4: Prevalence of anti-HCV antibody among hemodialysis patient: Age wise.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>HCV Negative</th>
<th>HCV Positive</th>
<th>HCV Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>16-30</td>
<td>18</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>31-45</td>
<td>21</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>46-60</td>
<td>44</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>≥ 61</td>
<td>9</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>96</td>
<td>4</td>
<td>4%</td>
</tr>
</tbody>
</table>

Table 4: Shows that 2 HCV positive patients are present in the age group 16-30 years and 31-45 years.

Table 5: Prevalence of Hepatitis B and Hepatitis C virus co-infection among haemodialysis patients.

<table>
<thead>
<tr>
<th>HBsAg Positive</th>
<th>HCV Positive</th>
<th>Hepatitis B &amp; Hepatitis C virus co-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5: Shows that none of the sample shows HBsAg and HCV coinfection.

Table 6: Prevalence of HBsAg among hemodialysis patient: Blood transfusion wise.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>HBsAg Positive (n=100)</th>
<th>Percentage (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT</td>
<td>41</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Non-BT</td>
<td>59</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>1</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table 6: Shows that 1 HBsAg positive haemodialysis patient is found with history of blood transfusion.

Table 7: Incidence of HCV among hemodialysis patient according to Blood transfusion.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>HCV +</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT</td>
<td>41</td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td>Non-BT</td>
<td>59</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>4</td>
<td>4%</td>
</tr>
</tbody>
</table>

Table 7: Shows that 4 HCV positive haemodialysis patients were found with history of blood transfusion. The patients who tested positive for the HBsAg and HCV had multiple times blood transfusion.

DISCUSSION

Comparison of Prevalence of HBsAg among haemodialysis patient

In the Indian context, HBV prevalence in HD patients range from 3.4% to 45% which is higher in comparison to the prevalence of around 4.7% in the general population.\(^{[20,21]}\)

This study shows HBsAg prevalence rate of 1% among haemodialysis patients, which is lower than studies conducted by Fahri Yakaryilmaz\(^{[22]}\) (13.30%), Anwar K\(^{[23]}\) (10.63%), Karina Salvatierra\(^{[22]}\) (8.72%), A E 0 Otedo\(^{[25]}\) (8.00%), Dr. Ramin\(^{[25]}\) (7.02%). Some other studies have also shown higher prevalence rate in comparison to this study. Our study result is comparable to that of studies conducted by Rubina Malhotra\(^{[22]}\) (01.52), Kranthi Kosaraju\(^{[22]}\) (1.52%). Our study result is slightly higher than those of studies conducted by Abumwais JQ \(^{[29]}\) (0.0%).

Highest prevalence is among age group 46-60 years(1%).

A previous study from India has also reported a prevalence of 11. 5% in the HD population.\(^{[30]}\) In our study we reported a prevalence of HBV of 1% in the HD population.

Comparison of Prevalence of HBsAg among haemodialysis cases: sex wise

Study conducted by Dr. Ramin\(^{[25]}\) (5.94%), Kranthi Kosaraju\(^{[22]}\) (1.29%) and our study (1%) reported higher prevalence of HBsAg among male
haemodialysis patients. Shantanu Prakash[24] (1.61%) reported equal proportion of HBsAg among male and female haemodialysis cases. Karina Salvatierra[25] (5.23%), Filiz Kizilates[26] (1.33%) have reported higher prevalence of HBsAg among female haemodialysis cases. 

Comparison of Prevalence of Hepatitis C among haemodialysis patient

In our study out of 100 samples only 4 samples were positive for Anti-HCV antibodies with prevalence of 4% which is low compared to other studies conducted by Rubina Malhotra[27] (33.58%), Anwar K[28] (25.53%), Abumwais JQ[29] (24.68%), Fahri Yakaryilmaz[30] (20.21%). Some other studies have also shown higher prevalence rate in comparison to this study. Our study result is comparable to other studies conducted by GA Reddy[31] (5.97%), Filiz Kizilates[32] (5.49%), A E Otedo[33] (5.00%) . Our study result is higher than some other studies conducted by Kranthi Kosaraju[34] (1.11%), Md Jamil[35] (1.38%), Davood Yadegaryan[36] (3.06%).

Highest prevalence is among age group 16-30 & 31-45 years (2%).

In India the HCV prevalence rate in the general population is 1.85%[37] Literature from various Indian studies report a variability in HCV prevalence even in the HD population in the range 4.3% to 46% [36,37].

Comparison of Prevalence of HCV among haemodialysis cases: sex wise

Study conducted by Kranthi Kosaraju[38] (8.13%), Shantanu Prakash[39] (5.91%), Karina Salvatierra[40] (8.13%) reported higher prevalence of HCV among male haemodialysis patients. Abumwais JQ[41] (12.99%), Dr. Ramia[42] (3.24%) reported nearly equal prevalence of HCV among male haemodialysis cases. In our study we found that HCV infection was more common in females undergoing HD.

Table 8: Comparison of Prevalence of HBsAg among haemodialysis cases with Blood transfusion.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Study</th>
<th>HBsAg +</th>
<th>Total Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kranthi Kosaraju[34]</td>
<td>02(0.11%)</td>
<td>1710</td>
</tr>
<tr>
<td>2</td>
<td>Present Study</td>
<td>01(1.00%)</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Md Jamil[43]</td>
<td>11(2.17%)</td>
<td>507</td>
</tr>
<tr>
<td>4</td>
<td>Shantanu Prakash[44]</td>
<td>04(2.15%)</td>
<td>186</td>
</tr>
<tr>
<td>5</td>
<td>Karina Salvatierra[45]</td>
<td>07(4.07%)</td>
<td>172</td>
</tr>
<tr>
<td>6</td>
<td>Dr. Mohammed A[46]</td>
<td>05(5.00%)</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>Pradip Bhauunik[47]</td>
<td>03(0.82%)</td>
<td>165</td>
</tr>
<tr>
<td>8</td>
<td>A E O. Otedo[48]</td>
<td>08(0.80%)</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Filiz Kizilates[49]</td>
<td>18(1.33%)</td>
<td>1347</td>
</tr>
<tr>
<td>10</td>
<td>Dr. Ramin[50]</td>
<td>10(5.40%)</td>
<td>185</td>
</tr>
<tr>
<td>11</td>
<td>Cuong Minh Duong[51]</td>
<td>07(6.19%)</td>
<td>113</td>
</tr>
</tbody>
</table>

Table shows that most of the above studies (including our study) reported higher prevalence of HBsAg among blood transfused haemodialysis patients. In our study we found that HBV infection was more common in patients who had history of repeated blood transfusions. The HBV infection correlated with the duration of dialysis and blood transfusions and may, therefore, have been acquired through haemodialysis or blood transfusion. Although blood for transfusion is screened for HBV, but low level of HBsAg may not be detected by ELISA and blood transfusion still remains a major risk factor for transmission of HBV infection.

Comparison of Prevalence of HCV among haemodialysis cases with Blood transfusion

Study conducted by A E O. Otedo[42], Dr. Mohammed A[43], Shantanu Prakash[44], Dr. Ramin[45], Cuong Minh Duong[46] and our study reported higher prevalence of HCV among blood transfused haemodialysis cases. Study conducted by Kranthi Kosaraju[34], Md Jamil[43], Karina Salvatierra[45], Pradip Bhauunik[46] have reported higher prevalence in non-blood transfused haemodialysis cases.

Although blood for transfusion is screened for HCV infection, but low level of Anti HCV Antibody may not be detected by ELISA and Blood transfusion is remained as an important risk factor for HCV infection among haemodialysis patients.[42] This study shows HCV positive patients were more likely to receiving multiple blood transfusions. This suggests the number of blood transfusions are significant risk factors for HBV and HCV transmission, has been reported by others.[42]

Comparison of Prevalence of Hepatitis B and Hepatitis C virus co-infection among haemodialysis patients

Md Jamil[43] and Kranthi Kosaraju[34] have reported HBV and HCV co-infection of 0.2% and 0.1% respectively in their studies where as Fahri Yakaryilmaz[40] reported HBV- HCV rate of 3.7%. In our study none case of HCV HBV co-infection found. This may be due to difference in sample size and demographic area among the different studies.

Since both of these viruses share a common mode of transmission, we looked for the occurrence of coinfections among the cases studied. Dual infection with HBV and HCV, though rare, occurs more frequently in certain risk groups. The risk is greater among the CRF patients due to the frequent exposure to blood from transfusions and extracorporeal circulation during haemodialysis. In India dual infection of HBV and HCV has been reported to be in the range of 3%–3.7% of patients.
In our study 41 patients were given blood transfusion and 59 patients were not given blood transfusion. All reported HBsAg or HCV positive cases were from haemodialysis patients who have received multiple blood transfusion. This suggests that blood transfusions are significant risk factors for HBV and HCV transmission. Therefore, universal infection control precautions are very important for prevention of HBsAg and HCV transmission in haemodialysis unit. These may include isolation of HBsAg and HCV positive patients in a special unit or at least special section in the haemodialysis unit with a special dialysis staff; disinfecting all dialysis unit surfaces especially monitors hardly after each patient treatment; disinfecting hands and changing gloves and gowns between patient contacts; preparing medications outside the dialysis unit; using of items, medications, or instruments that are dedicated for use only for one patient. Dialysis staff must also review their practices and increase their vigilance in order to decrease HBsAg and HCV transmission.

ELISA test may not detect Low level of HBsAg and Anti HCV Ab in early stage of HBV/HCV infection in donor and in this case detection by PCR may be helpful in blood bank.

Our results are comparable with some of the studies conducted in India and abroad. Safe transfusion practices, enhanced vaccination coverage and proper disinfection procedures of HD equipments should be undertaken to reduce the burden of parentally transmitted hepatitis virus in the Haemodialysis patients.

We believe our data could help health professionals to deal better with haemodialysis patients. We also believe our data reinforces the need of prevention programs on HBV and HCV transmission, which also lead to reduction in prevalence of HBV and HCV.

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