Original Article

Evaluation of the antibody response against Hepatitis B Virus infection in patients on maintenance hemodialysis: A Pilot Study
S Shahin1, Khoybar A2, A Farhana3, K Matira4

Abstract
Objective: This study was undertaken to evaluate the antibody response of hepatitis B virus infection in patients on maintenance hemodialysis (MHD) by detecting different viral markers. Method: Study subjects comprised a total of 88 chronic kidney disease (CKD) patients from Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and Bangabandhu Sheikh Mujib Medical University (BSMMU). Of them 63 patients on MHD and 25 predialysis patients served as cases and controls respectively. Clinical history was taken and serological markers for HBV (HBsAg, Anti-HBs, and Anti-HBc) were determined by using ELISA.

Results: Hepatitis B virus was positive in 1.6% of maintenance hemodialysis (MHD) patients and in 16% of controls (p<0.02). Anti-HBc antibody was positive in 62% of dialysis patients and 72% of controls (p=NS) and the positivity was significantly associated in dialysis subjects with longer duration of dialysis (18 ± 22 vs. 10 ± 7, months, p<0.04), multiple units of blood transfusions (22 ± 29 vs. 10 ± 12, units, p<0.04) and more reuse of dialyzer (3 ± 1 vs. 2 ± 1, times, p<0.03) than the negative ones. Among MHD patients 84% were vaccinated against HBV with a schedule of 3 (79%) and 4 (21%) doses and protective antibody titer (>10 IU/L) was found in 57%. None of the controls were vaccinated but 66% had protective titer indicating post exposure natural immunity. Conclusions: Hepatitis B virus positivity was significantly higher among the predialysis subjects compared to dialysis group.

Key words: Hepatitis B virus, Antibody response, Hemodialysis

Introduction
End-stage renal disease (ESRD) subjects on maintenance hemodialysis are at high risk for hepatitis B virus infection1. Parenteral route is the major route for HBV transmission2. The process of hemodialysis requires vascular access for prolonged period3. Furthermore, hemodialysis patients are immunosuppressed4 that increases their susceptibility to infection requiring frequent hospitalization and surgery, which again increases their risk for exposure to nosocomial infections3. Although vaccination is routinely recommended in ESRD patients, antibody response to vaccination is suppressed and its level rapidly declines among patients on chronic dialysis due to the decreased immunological responses5. The prevalence of chronic hepatitis B virus (HBV) infection is high (>8%) in sub-Saharan Africa, most of Asia and the Pacific Islands, intermediate prevalence (2 to 7%) regions include the

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Amazon, southern parts of Eastern and central Europe, the Middle East and the Indian sub-continent, low prevalence (<2%) regions include most of Western Europe and North America. In India, HBV prevalence was 8.8% and 14.2% in predialysis and hemodialysis group respectively. In Turkey, prevalence of HBV was 10.5% and 13.3% in predialysis and hemodialysis patient’s respectively. In Bangladesh, around 12% of all patients on MHD were serologically positive for hepatitis B virus infection, has been shown in a recent study. So far no study has been conducted to see the seroprevalence of HBV in CKD (predialysis) patients in Bangladesh.

Therefore, this study was undertaken to evaluate the antibody status of HBV in predialysis and dialysis patients followed-up in two selected tertiary renal care center.

**Subjects and methods**

**Study design**

This cross sectional study was carried out in the Department of Immunology, BIRDEM, Dhaka and Nephrology Department of BSMMU during the period of June 2006 to June 2007.

**Study subjects**

Eighty-eight patients were finally included in this study. Of them 63 end stage renal disease (ESRD) patients who were on maintenance hemodialysis for at least 3 months and getting dialysis through arteriovenous (AV) fistula considered as cases and 25 chronic renal failure (CRF) patients attending Nephrology Out-patients departments of BIRDEM and BSMMU and ‘CRF patients follow-up project’ who were not on dialysis (predialysis) considered as control group.

**Sample collection and preservation**

Five milliliter blood was taken from the arterial channel immediately after pricking the fistula during dialysis session in MHD patients and labeled with a known serial number for each patient. In controls fasting samples were taken. Serum sample were preserved at -20°C and assayed within fifteen days of collection.

**Laboratory analysis**

Serological markers for hepatitis B (HBsAg, Anti-HBc, Anti-HBs) were assessed using commercial third generation enzyme-linked immunosorbent assay kit (Diasorin, Italy). Serum creatinine and alanine aminotransferase were assessed by standard laboratory method (Kinetic method).

**Statistical analysis of data**

All the relevant data were entered and then analyzed using the statistical package for social science (SPSS) version 13. Results were expressed as mean ± SD or percentage as appropriate. Level of significance was expressed as ‘P’ value and p<0.05 was considered as significant.
Results

Table I- Baseline Parameters of Study Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=63)</th>
<th>Controls (n=25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>54±11</td>
<td>57±10</td>
<td>0.28</td>
</tr>
<tr>
<td>M/F</td>
<td>36/27</td>
<td>15/10</td>
<td>0.80</td>
</tr>
<tr>
<td>DM Duration (yrs)</td>
<td>12±6</td>
<td>5±2</td>
<td>0.92</td>
</tr>
<tr>
<td>CKD Duration (yrs)</td>
<td>6±4</td>
<td>4±3</td>
<td>0.53</td>
</tr>
<tr>
<td>S Cr (mg %)</td>
<td>9±2.5</td>
<td>4±2</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>25±17</td>
<td>20±16</td>
<td>0.30</td>
</tr>
</tbody>
</table>

DM = Diabetes mellitus, results are expressed in mean ± SD on percentage where suitable, M/F = Male/Female, CKD = Chronic Kidney Disease, S Cr = Serum Creatinine, ALT = Alanine Aminotransferase

Table II - Distribution of patients by hepatitis B virus infection

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Cases</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1 (1.6%)</td>
<td>4 (16%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Negative</td>
<td>62 (98.4%)</td>
<td>21 (84%)</td>
<td></td>
</tr>
</tbody>
</table>

HBsAg – Hepatitis B surface antigen
P value reached from chi square test; p<0.05 considered significant

Figure 1- Distribution of patients by anti-HBc total

Note: Figure 2 above indicated that among cases and controls, anti-HBc positive was 61.9% (n=39) vs. 72% (n=18) (P=NS) and this was not significantly different between the two groups
Table III- Distribution of Cases (MHD patients) by anti-HBc total

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Positive (n=39)</th>
<th>Negative (n=24)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>52±10</td>
<td>58±11</td>
<td>0.03</td>
</tr>
<tr>
<td>CKD Duration (yrs)</td>
<td>6±4</td>
<td>5±2</td>
<td>0.41</td>
</tr>
<tr>
<td>DM Duration (yrs)</td>
<td>14±7</td>
<td>13±7</td>
<td>0.28</td>
</tr>
<tr>
<td>Dialysis Duration (m)</td>
<td>18± 22</td>
<td>10±7</td>
<td>0.03</td>
</tr>
<tr>
<td>BT (total units)</td>
<td>22± 29</td>
<td>10±12</td>
<td>0.03</td>
</tr>
<tr>
<td>BT (units/month)</td>
<td>1.4± 1.2</td>
<td>1.1±1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Dialyzer Reuse</td>
<td>3±1</td>
<td>2±1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CKD- Chronic Kidney Disease, DM – Diabetes mellitus, BT- Blood transfusion, m - months
Results are shown in mean ± SD

Table IV - Level of immunity against hepatitis B virus in cases (MHD)

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated (n=53, 84%)</th>
<th>Non-vaccinated (n=10, 16%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective (&gt;100IU/L)</td>
<td>11 (19%)</td>
<td>4 (57%)</td>
<td></td>
</tr>
<tr>
<td>Low protective (10-100IU/L)</td>
<td>20 (39%)</td>
<td>-</td>
<td>0.74</td>
</tr>
<tr>
<td>Non protective (&lt;10IU/L)</td>
<td>22 (42%)</td>
<td>3 (43%)</td>
<td></td>
</tr>
</tbody>
</table>

P value reached from chi square test; p<0.05 considered significant

Different laboratory parameters were similar between cases (MHD patients) and controls (predialysis patients). The only difference was in serum creatinine (S. Cr) level and this was higher in MHD patients (p<0.001) (Table I).

The proportion of positive hepatitis B virus infection was found to be higher among the control (16%) compared to case (1.6%) and the difference was statistically significant (p<0.02) (Table II).

Results showed no significant association between positivity of anti-HBc with duration of chronic renal failure and duration of diabetes mellitus (Table III). However, data shows higher preponderance of positive anti-HBc among the patients with prolonged duration of maintenance haemodialysis (p<0.03) and number of total units of blood transfusion (p<0.03). A statistically significant association was also found between anti-HBc status and number of reuse of dialyser (p<0.02) indicating the positivity of anti-HBc was high among the patients with more reuse of dialyser. Data analysis also indicated that the mean age of the positive anti-HBc total was found to be low (52.21±10.2 years) compared to negative anti-HBc total (57.96±10.8 years) and the difference was significant (p<0.05). Result showed that 84% of the cases (dialysis patients) were vaccinated and 16% of them non-vaccinated (P<0.001). No significant difference was seen in proportion
of the patients with protective and non-protective titers among the vaccinated and non-vaccinated subjects. Vaccination schedule was 3 doses in 79% and 4 doses in 21% of cases (Table IV).

**Discussion**

Bangladesh has an intermediate prevalence of hepatitis B virus infection with a 4% HBsAg positive population. In a previous study, 3.5% prevalence rate of hepatitis B virus infection in pregnant women of Bangladesh was seen. It was observed in the present study that HBsAg was positive in hemodialysis patients in lower frequency (1.6%). Lower prevalence of HBsAg in MHD patients probably due to routine screening for HBsAg before selection of patients for MHD. Furthermore, we selected patients from two hemodialysis units where HBsAg positive patients were not accepted for hemodialysis to minimize the risk of spreading of HBV infection. This notion was similar to. Moreover, 84% of our dialysis patients were vaccinated which might also contribute to low number of HBsAg positive cases in MHD group. On the other hand, HBsAg was found in higher frequencies in our predialysis diabetic CKD patients (16%). In diabetic patients HBV infection has been shown about 14% in a study and in predialysis patients it ranged from 8-10% in studies from India and Turkey. Higher prevalence of HbsAg in our predialysis patients may be attributed to the fact that these patients had not been undergone routine screening for HBsAg and vaccination. Although, other factors like, frequent hospitalization, history of injections (including insulin), poor nutritional status (metabolic derangement) leading to suppressed immune response etc. remain common for both groups. Therefore, HBV vaccination alone seems to make the difference of HBsAg status observed in both groups.

We found 62% of the dialysis patients and 72% controls are anti-HBc positive, which is higher than some other reports showing around 40% but similar to one showing 76%. Positive anti-HBc total always indicate a remote HBV infection and is the most valuable single serologic marker in diagnosis of HBV infection even when HBsAg remains negative. We found positivity of anti-HBc total higher among the patients with more reuse of dialyzer, Qadi et al. reported reuse of dialyzer is one of the risk factors for viral transmissions. Similarly increased duration of dialysis and higher number of blood transfusion has been shown to be associated with increased frequency of HCV infection, which was seen in our patients too. It is possible that our patients became more anti-HBc positive due to higher blood transfusion and longer duration of dialysis.

Majority of our MHD patients had vaccination (84%) but analysis found that only 19% have protective immunity, 39% low protective immunity and 42% have non protective immunity indicating that approximately half of the dialysis patients had no protection despite vaccination which is probably due to immunosuppression. In present study in all immunity groups, majority of the patients were more than 50 years ages. It has been suggested that advanced age reduces the response against HBV vaccine in hemodialysis patients. In our study response against hepatitis B vaccine to attain a protective titer was in 58% subjects. This relatively low response may be due to higher age, presence of diabetes and lower doses of vaccination schedule as majority (79%) took three-dose regimen. Some studies showed that vaccine response is 64% with 3 doses whereas 86% with 4 doses.
We can conclude that Hepatitis B virus positivity was significantly higher among the predialysis subjects compared to dialysis group. Anti-HBc antibody was positive in two-third of the study subjects. With a three-dose vaccine schedule only half of the dialysis patients could attain protective antibody titer. According to standard statistical formula, a large sample size should have been taken to reflect the picture of whole population. However, as this is a Pilot study sample size was confined at 88 subjects.

Acknowledgement
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References


infection in Turkish hemodialysis patients. Ren Fail 2006; 28: 729-35.


“Allah has not sent down a disease except that He also sent down its cure: whoever knows it (the cure), knows it, and whoever is unaware of it (the cure), he is unaware of it.” (the medicine) while those who are ignorant of it are unaware of it.” [An-Nasai’, Ibn Mājah, Al-Hakim and Ibn Hibban]