# Evaluation of the Antibody Response against Hepatitis C Virus Infection in Patients on Maintenance Hemodialysis (MHD): A Pilot Study

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## ABSTRACT

Majority of the patients with end stage renal disease (ESRD) are on maintenance hemodialysis (MHD) in Bangladesh. Dialysis patients are at high risk for contracting blood borne infection including hepatitis C virus (HCV) infection. The aim of this study was to evaluate the antibody response of hepatitis C virus infection in patients on MHD by detecting different viral markers in blood. A total of 88 patients with chronic kidney disease (CKD)were recruited from BIRDEM and BSMMU during the period from June 2006 to June 2007. Of them 63 patients on MHD and 25 predialysis patients were taken as cases and controls respectivly. Anti-HCV antibody were positive in 38% of dialysis patients but none of the controls were positive for Anti-HCV. When HCV positive MHD patients (38%) were compared to HCV negative MHD patients (62%), it showed that HCV positive patients had longer duration of dialysis ($24\pm25$ vs $9\pm6$ months, $p<0.001$), increased number blood transfusions ($29\pm34$ vs $10\pm9$ units, $p<0.004$) and elevated serum alanine aminotransferase level ($35\pm23$ vs $20\pm9$ U/L, $p=0.001$). Implementing comprehensive infection control program by routine screening of the CKD patients, safe blood transfusion program, reducing transfusion of blood by use of erythropoietin and proper disinfection and cleaning of hemodialysis units may reduce the infection by HCV Virus.

**Key words:** Hepatitis C virus, Antibody response, Hemodialysis

## Introduction

End-stage renal disease (ESRD) subjects on maintenance hemodialysis (MHD) are at high risk for hepatitis C virus infection[1]. The process of hemodialysis requires vascular access for prolonged period and many patients depend on multiple blood transfusions for correction of anemia[2].

The prevalence of hepatitis C virus (HCV) infection among ESRD patients on MHD is persistently higher than the general population[3]. Predisposing factors are immunocompromised patients on long-term hemodialysis[4], older age, black race, diabetes, concomitant hepatitis B virus (HBV) infection, prior renal transplant and alcohol or drug abuse, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), recurrent cellulitis or gangrene[5]. Risk factors associated with HCV infection among hemodialysis patients include history of blood transfusion, the volume of blood transfused and years on dialysis[6]. Previous blood transfusion, number of hemodialysis centers visited and years on dialysis are the major risk factor independently associated with higher rates of HCV infection[7]. HCV accounting for 20%-30% of acute hepatitis, 75%-85% chronic hepatitis[8]. Most hemodialysis patients with newly acquired HCV infection have elevated serum alanine aminotransferase (ALT) levels[2]. In chronic HCV infection, 60-70% patients showed persistence of fluctuating ALT elevation, indicating active liver disease[9].

Approximately, 170 million world populations have been infected with hepatitis C virus infection[10]. Seroprevalence of anti-HCV was found to be 4%-9.93% in India in two studies[11,12]. In Pakistan 5.31% were positive for anti-HCV[13]. One study showed that 24.8% intravenous drug users of Bangladesh were positive for HCV infection[14]. In Bangladesh, HCV seropositivity among professional blood donors was 1.2%[15]. Seropositivity was 6.8% in type 2 diabetic patients[16] .The prevalence of HCV infection was 7% in predialysis and 20.2% of hemodialysis patients in Turkey[17] .

The anti-HCV prevalence was 43%, 37.2%, 19%, and 16.4% in hemodialysis patients in China, Brazil, Japan and Hong Kong respectively[18,19] . In another study the prevalence of anti-HCV among hemodialysis patients in different countries were reported as in Netherlands 3.3%, Italy 29.0%, Tunisia 41.0%, Egypt 80.0%, Saudi Arabia 68%, Australia 5.9%, and Taiwan 60.0%[20]. In Pakistan and India the prevalence of anti-HCV was 23.7% and 9.93% in hemodialysis patients respectively[21,22]. In Bangladesh, 6% of all patients on MHD were serologically positive for hepatitis C virus infection shown in an earlier study[23] but a recent one showed that it has increased to 7.1%[24].

There are no study reports on immune status against Hepatitis C virus (HCV) infections among predialysis and dialysis patients in Bangladesh. Therefore, this study was undertaken to evaluate the antibody status of HCV in predialysis and dialysis patients followed up in two selected tertiary renal care centers.

## Materials and Methods

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

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 arteriovensous (AV) fistula considered as cases and 25 chronic renal failure patients attending Nephrology out-patient departments of BIRDEM and BSMMU and CRF (chronic renal failure) patients follow-up

project who were not on dialysis (predialysis) were considered as control group.

Five ml of 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 $-20^0$C and assayed within fifteen days of collection.

Serological markers for hepatitis C (Anti-HCV) was performed by commercial third generation enzyme-linked immunosorbent assay kit (Origin: Diasorin, Italy). Serum ALT was measured by kinetic method.

All the relevant data were compiled on a master table first and then analysed using software with statistical package for social science (SPSS) version 13. Percentages were calculated to find out proportion of the finding. Results were expressed as mean$\pm$SD or in percentage where suitable. Level of significance was expressed as p value and $p < 0.05$ was   considered significant.

## Results

Table I shows that different baseline parameters like age, duration of diabetes (DM Duration), duration of chronic kidney disease (CKD Duration) and related 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:** Baseline Parameters of Study Subjects

| Parameters | Cases (n=63) | Controls (n=25) | p Value |
|---|---|---|---|
| Age (yrs) | 54$\pm$11 | 57$\pm$10 | 0.28 |
| M/F | 36/27 | 15/10 | 0.80 |
| DM Duration(yrs) | 12$\pm$6 | 5$\pm$2 | 0.92 |
| CKD Duration(yrs) | 6$\pm$4 | 4$\pm$3 | 0.53 |
| S. Cr (mg %) | 9$\pm$2.5 | 4$\pm$2 | 0.001 |
| ALT (U/L) | 25$\pm$17 | 20$\pm$16 | 0.30 |

*M/F- Male /Female; DM- Diabetes Mellitus; CKD-Chronic Kidney Disease; S Cr-Serum Creatinine; ALT-Alanine amino transferase Data are expressed as mean (SD); p value reached from student's t test.*

**Table- II:** Distribution of patients by anti-HCV

| Anti - HCV | | Subject | | Total | |
|---|---|---|---|---|---|
| | | Cases | Controls | | p value |
| | | n=63 (%) | n=25 (%) | n=88 (%) | |
| Positive | | 24(38.1) | | 24 (27.3) | 0.001 |
| Negative | | 39 (61.9) | 25 (100) | 64 (72.7) | |

*Anti-HCV - Antibody to hepatitis C virus; p value reached from chi square test.*

**Table- III:** Distribution of MHD Patients by anti-HCV

| Parameter | Positive n=37 (59%) | Negative n=26 (41%) | p Value |
|---|---|---|---|
| DM Duration (yrs) | 14±6 | 13±8 | 0.65 |
| Dialysis Duration (m) | 11± 8 | 20±26 | 0.09 |
| BT (total units) | 11± 10 | 26±34 | 0.02* |
| BT (units/month) | 1.1± 1.1 | 1.6±1.3 | 0.05 |
| Dialyzer Reuse | 2±1 | 3±1 | 0.17 |
| Anti HCV "+ve" | 32% | 46% | 0.29 |

*DM- Diabetes Mellitus; BT-Blood Transfusion; ALT- Alanine amino transferase Data are expressed as mean (SD); p value reached from student's t test.*

**Table- IV:** Comparisons of dialysis patients according to the use of Erythropoietin

| | Positive n=24 (38%) | Negative n=39 (62%) | p Value |
|---|---|---|---|
| DM Duration (yrs) | 12±6 | 15±8 | 0.14 |
| Dialysis Duration (m) | 24± 25 | 9±6 | 0.001 |
| BT (total units) | 29± 34 | 10±9 | 0.003 |
| Dialyzer Reuse | 3±1 | 2±1 | 0.24 |
| ALT (U/L) | 35±23 | 20±9 | 0.001 |

*DM- Diabetes Mellitus; BT-Blood Transfusion; Anti-HCV - Antibody to hepatitis C virus*
*Data are expressed as mean (SD); P value reached from student's t test.*
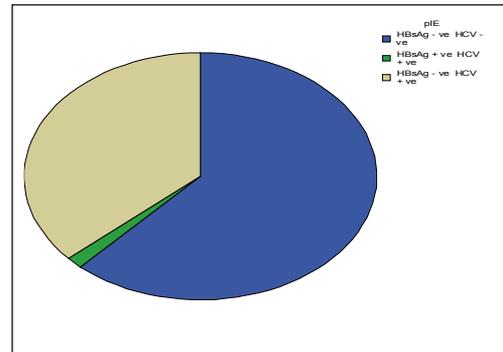


**Figure 1:** Pie diagram showing prevalence of Hepatitis B virus and Hepatitis C virus among dialysis (MHD) patients

Result from table II shows a statistically significant difference between cases and controls in terms of anti-HCV p<0.001 indicating that positive anti-HCV was high among the cases 38.1(%). However, no positive cases were found among the controls.

Table III indicateds that the positivity of anti-HCV increases with longer duration of maintenance haemodialysis and higher number of blood transfusions. Also the positive patients had a relatively higher serum level of alanine aminotransferase.

Table IV shows a statistically significant difference in respect of total units of blood transfusion recieved between two groups, indicating that erythropoietin users patients had less blood transfusion (11±10 units) needed than the negative ones (26±34 units, p=0.02). This table also shows that anti-HCV positivity between two groups was similar.

Figure 1 shows that 36.5% patients had only HCV infection, 1.6% patients had both HBV and HCV infection and 61.9% had none of the infection.

## Discussion

In the present study, anti-HCV positivity was observed to be significantly higher in the hemodialysis patient (38%) and no positive anti-HCV case was found in CRF patients without dialysis which is in agreement with the study of Lopez-Alcorocho et al[25]. Some authors reported lower anti-HCV positivity (4.4%) in CRF patients

on hemodialysis when compared with CRF patients without dialysis (17.1%)[26]. Another study observerd similar prevalence of anti-HCV (7%) among CRF patients on hemodialysis. A high positivity was also reported in Sudanese patients on hemodialysis (23.7%)[27]. These differences in observations among dialysis subjects could be related partly to screening protocols and the sensitivity of the tests employed. Higher prevalence of anti-HCV in MHD subjects may be due to the fact that dialysis units of the two institutions involved in this study have not been using separate dialysis machine for known anti-HCV positive cases. Again higher number of blood transfusion in MHD patients for anemia correction might be the risk factor for this high infection rate. Transfusion should be restricted to minimum by the administration of recombinant human erythropoietin in CKD patients to minimize viral infections. No anti-HCV positive patient was found in predialysis group may be for the reason that they had no history of blood transfusion. In our study, high anti-HCV positivity was also significantly associated with duration of maintenance hemodialysis (MHD). Previous studies have also indicated that the duration of dialysis treatment is clearly correlated with HCV positivity[27,7]. In the present study, the duration of dialysis in HCV positive patients was higher compared to HCV negative patients.

When biochemical markers associated with viral infections were investigated it was found that serum alanine aminotransferase (ALT) was relatively higher in anti-HCV positive patients. There are reports showing association between ALT and anti-HCV positivity[26]. It has also been shown that the greater the elevation in liver enzymes, higher is the probability of histological evidence of liver disease. Liver biopsy may confirm the presence of active liver disease in these patients.

Hepatitis C virus infection was high in dialysis patients. Implementing comprehensive infection control program by routine screening of the CKD patients, and proper disinfection and cleaning of hemodialysis units may reduce the infection Rate.

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