



Original Research Article

Evaluation of the Anti-Hepatitis B Immunity Status Post Vaccination in Patients on Renal Replacement Therapy

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ABSTRACT

Renal replacement therapy is a major risk factor for Hepatitis B virus infection. This study was conducted to determine the prevalence of hepatitis B virus infection in patients of chronic renal failure, especially in those on Renal Replacement Therapy and to evaluate the sero-conversion rate in patients of chronic renal failure on Renal Replacement Therapy (RRT) and those on Conservative Therapy, after immunization with 3rd and 4th doses of recombinant hepatitis B vaccine. The study was conducted in Department of Microbiology, JNMC, AMU, Aligarh on 110 patients of chronic renal failure with end stage renal disease on Renal Replacement Therapy, 31 patients of chronic renal failure on conservative therapy and 15 healthy controls. Blood samples were collected and HBsAg, IgM AntiHBc and antiHBs antibody were estimated by ELISA. Out of total 141 patients 9 (6.4%) were positive for hepatitis B virus infection. All the patients positive for hepatitis B virus infection were on Renal Replacement Therapy. 70.9% on RRT and all those on conservative therapy showed seroconversion after 3 doses of vaccine while 68.8% on RRT and all those on conservative therapy showed seroconversion after 4 doses of vaccine. Thus besides RRT, there are various risk factors for hepatitis B virus infection. Seroconversion to hepatitis B vaccine was lower in RRT patients compared to those on conservative treatment.

Keywords

Maternal mortality,
Perinatal mortality,
Cerebral palsy,
Asphyxia

Introduction

Hepatitis B infection is a major public health problem worldwide due to its long term sequelae which include chronic hepatitis, cirrhosis and hepatocellular carcinoma. India comes under intermediate zone of HBV prevalence with a carrier rate of 4.7% (John and Abraham, 2001) and has an estimated 42 million carriers, next only to china.

Hepatitis B virus infection has been a major complication in chronic renal failure patients and poses problems in management of such patients in renal dialysis units (Saha and Agarwal, 2001). Chronic renal failure patients do not clear the hepatitis B virus effectively (Reddy *et al.*, 2005). The prevalence of HBV in India is reported to range between 3.4–4.3% which is several

folds higher than the carrier rate in general population.

Chronic hemodialysis patients are at higher risk for infection because the process of hemodialysis requires vascular access for prolonged periods. In an environment where multiple patients receive dialysis concurrently, repeated opportunities exist for person-to-person transmission of infectious agents, directly or indirectly via contaminated devices, equipment and supplies, environment surfaces or hands of personnel. Furthermore, hemodialysis patients are immunosuppressed which increases their susceptibility to infection.

The most important modality for prevention of hepatitis B virus infection is induction of immunity by hepatitis B vaccine (Saha and Agarwal, 2001). Introduction of hepatitis B vaccination, isolation of hepatitis B positive patients, use of modern dialysis machines together with regular surveillance for HBV infection, have dramatically reduced the spread of HBV infection in dialysis units (Fabrizi *et al.*, 2002). When applied together with universal precautions, these measures have resulted in up to 10-fold drop in the number of new hepatitis B cases in hemodialysis patients in Western Europe and in the United States. In India the prevalence of HBV infection in hemodialysis units has decreased from 32% to 4.7%. It has been reported that vaccinated hemodialysis patients who maintained protective levels of anti HBs antibody had no HBV infection. The proportion of hemodialysis patients who develop a protective antibody response after vaccination was lower than in adults with normal immune status.

Thus, vaccination regime currently recommended for dialysis population is the accelerated vaccination regime. It involves

the intramuscular administration of three to four doses of vaccine over a period of 4 months. It is an easy and cost effective method to increase the response rate of hepatitis B vaccine in chronic renal failure patients in developing countries.

There are very few studies published about the seroprevalence of HBV infection among chronic renal failure patients on renal replacement therapy, especially from northern India. Keeping the low socioeconomic profile of majority of CRF patients from this part of the country, it is also important to know the efficacy and cost effectiveness of the higher potency and 4-dose schedule for chronic renal failure patients as compared to the lower potency and 3-dose vaccination schedules. The incidence and magnitude of infection with the increase in morbidity and mortality in chronic renal failure patients had made us to undertake this study.

Material and Methods

This study was conducted in Department of Microbiology to find out the prevalence of hepatitis B virus infection and post vaccination seroconversion in patients admitted in the Nephrology Unit of Jawaharlal Nehru Medical College Hospital for a period of 20 months. 110 patients of chronic renal failure with end stage renal disease (ESRD), 31 patients of chronic renal failure on conservative therapy, and 15 healthy controls for the immunization programme were included in the study. Patients with known cases of viral hepatitis and other morbid conditions were excluded from the study.

Patients were administered 20 µg and 40 µg of Gene Vac B, recombinant DNA hepatitis B vaccine (Serum Institute of India, Pune). All patients were given 4 doses of hepatitis

B vaccine intramuscularly at 0, 1, 2 and 6 months (as per CDC recommendations, 2006) after taking informed consent. The controls received 3 doses of 20 µg hepatitis vaccine at 0, 1 and 6 months.

Blood samples were collected for qualitative estimation of HBsAg and IgM AntiHBc. For quantitative estimation of AntiHBs antibody by ELISA test using HEPALISA kit (J. Mitra & Co Pvt. Ltd, New Delhi), blood samples were collected one month after 3rd dose and one month after 4th dose of hepatitis B vaccine.

Result and Discussion

Out of 141 patients of chronic renal failure, 92(65.4%) were males and 49(34.7%) females. In patients on renal replacement therapy (RRT) majority of patients 26(23.6%) were in the 31–40 years age group while the minimum number of patients 4(4.5%) were from 10–20 year age group. In patients treated conservatively majority 8(25.8%), patients belonged to 21–30 and 41–50 years age group.

Risk factors found in chronic renal failure patients for Hepatitis B virus infection are shown in Figure 1. In Renal Replacement therapy patients other than hemodialysis, majority 80(72.7%) of patients gave history of previous hospitalization. Similarly in those on conservative treatment majority 17(54.8%) were hospitalized previously. History of blood transfusion was present in 40(36.4%) of RRT patients and 10(32.2%) of those on conservative treatment.

Out of total 141 patients, 9 (6.4%) were positive for hepatitis B virus infection. All the patients positive for hepatitis B virus infection were on RRT. Majority of them were males from 21–30 years of age. None of the patient treated conservatively tested

positive for hepatitis B virus infection as shown in Table 1.

After excluding the 9 patients tested positive for hepatitis B virus infection, all the 132 patients were given 1st dose of vaccine. All of them turned up one month after the first dose and were given the 2nd dose. However, only 68.2% and 31.1% turned up for the 3rd and 4th dose respectively. Only 18.2% of the patients could be studied 1 month after receiving the 4th dose of vaccine for seroconversion.

In patients on RRT, out 31 of the patients who received full course of vaccine, 22(70.9%) of the patients showed seroconversion while 18(58.1%) had seroprotective antibody titres. All those on conservative treatment attained seroconversion after 3 doses of 20 µg vaccine while only 66.6% among them had seroprotection (Table 2). When 40 µg of vaccine was given all the patients on RRT and conservative therapy had seroconversion while 6(60%) and 2(66.6%) respectively showed seroprotection. In RRT group 35.5% of the patients administered 20 µg vaccine had antibody concentrations in the range of 10-100 mIU/ml and 22.6% had antibody concentrations of >100 mIU/ml. In conservatively treated 50.0% of the patients had antibody concentrations in the range of 10-100 mIU/ml and 16.6% attained higher antibody titres. When 40 µg of vaccine was given 20% of the patients on RRT had antibody titres between 10-100 mIU/ml and 40% had antibody titers of >100 mIU/ml. 66.6% of the patients among those treated conservatively had antibodies in the range of 10-100 while none of them could attain higher antibody titers.

After 4 doses of vaccine, 68.8% of the patients on RRT showed seroconversion and also had seroprotective antibody titres.

50.0% on conservative treatment attained seroconversion after 4 doses of 20 µg vaccine and 50.0% among them had seroprotection (Table 3). When 40 µg of vaccine was given all the patients on RRT and conservative therapy showed seroconversion. All the conservatively treated patients attained seroprotection while 87.5% of the RRT group was able to do so. When 4 doses of 20 µg of vaccine were given 18.8 % of the patients among the RRT group had antibody concentrations between 10-100mIU/ml while 50% of them attained higher antibody titers. In the conservatively had antibody titers of > 100 mIU/ml. 62.5% of the patients on RRT in comparison to 100 % of the patients treated conservatively had antibody titers more than 100 mIU/ml after 4 doses of 40 µg of vaccine.

Thus seroprotection rates attained were compared in patients on RRT, on Conservative treatment and in healthy controls. After 3 doses of 20 µg vaccine 58.1 % of the patients on RRT attained seroprotective levels of antibody titres while after 4 doses 60% were able to do so as compared to those treated conservatively where 66.6% of the patients were positive after 3rd and 4th doses. On increasing the amount of vaccine 60% and 87.5% response was seen after 3rd and 4th dose respectively. Whereas in those treated conservatively 50% and 100% of the patients attained seroprotective levels of antibody after 3rd and 4th dose of dose of 40 µg vaccine respectively. 92.3% of the healthy controls responded to 3 doses of 20 µg vaccine.

Hemodialysis is an important modality of therapy for patients of end stage renal failure; both as a lifelong maintenance hemodialysis as well as preparatory hemodialysis for renal transplantation. Being peculiar mode of therapy, the patients treated with hemodialysis are at a higher risk

of blood borne viral and other infections; both among themselves as well as between the hemodialysis staff and the patients (Saha and Agarwal, 2001). Moreover, dialysis patients have a tendency to become chronic carriers of HBsAg because of the defective immune responses associated with uremia (Huang, 2002).

Hepatitis B vaccination is recommended for all susceptible chronic hemodialysis patients. In fact, vaccination is recommended for pre-end stage renal disease patients before they become dialysis dependent and for peritoneal patients because they might require in centre hemodialysis (CDC, 2006).

In the present study, age of the chronic renal failure patients ranged from 10-70 years and the mean age was 42.18(±12.45). Among the patients on RRT 65.4% were males and 34.5% were females. Among the conservatively treated patients 64.5% were males and 35.5% were females.

In the present series of chronic renal failure patients on RRT, hemodialysis itself was the major risk factor for HBV infection. Other important risk factors found were history of previous hospitalization (72.7%), followed by blood transfusion (36.4%). Out of the total 141 patients of chronic renal failure, 9 patients were found to be positive for hepatitis B virus infection giving a prevalence of 6.2%. All these 9 were on RRT and none of the 31 chronic renal failure patients on conservative therapy showed evidence of HBV infection. Therefore, the prevalence of HBV infection in chronic renal failure patients on RRT was 8.2%(9 out of 110). Our results were similar to the findings of other workers. Prevalence of 7% was reported by Chandra *et al.* (2004), and 8% by Otedo *et al.* (2003).

Though, the frequency of HBs antigen carriers in patients on hemodialysis is low in developed countries, the prevalence and incidence rates of HBV infection among dialysis patients in less developed countries remain high. Patients on hemodialysis with chronic HBsAg carriage are typically anicteric, and rarely develop symptoms of hepatitis. Moreover the aminotransferase activity in dialysis population is usually depressed which hampers the recognition of HBV related liver disease among dialysis patients by biochemical tests (Fabrizi *et al.*, 2002).

There was a male preponderance for HBV infection in our study as out of 9 patients who tested positive for hepatitis B virus, 8(88.8%) were males and only 1(11.1%) was a female. This may be due to higher transmission rate of hepatitis B among young males (Singh *et al.*, 2003).

HBV infections have several modes of transmission of which transfusion of infected blood and blood products and perinatal transmission are most important. Other important modes include sexual transmission, tattooing and needle stick exposure (Sebastion *et al.*, 1990). Blood transfusion has been pointed out as an important risk factor for viral hepatitis within hemodialysis patients (Chanpong *et al.*, 2002). In the present study, 77.7% HBV positive patients have had blood transfusion in the past. Murthey *et al.* (2003) also reported that 77.9% of their HBV positive patients had a history of past blood transfusion.

Multiple blood transfusions and hemodialysis for a prolonged period of time are known to act as risk factors for acquiring hepatitis B virus infection, especially in centers with poor screening of blood for the virus prior to blood transfusion (Kapoor and Saxena, 2000).

The second part of the study comprised of post vaccination seroconversion among chronic renal failure patients. Seroconversion with hepatitis B vaccination was attained when antiHBs antibody concentration was at least 1mIU/ml, while seroprotection with hepatitis B vaccination was considered to be achieved when concentration of antiHBs antibody was ≥ 10 mIU/ml (Vijaykumar *et al.*, 2004).

The seroprotection rates attained by chronic renal failure patients on RRT was evaluated and compared with those on conservative treatment. Normal persons included in the vaccination programme served as healthy controls.

Forty one (31.1%) patients of chronic renal failure on RRT could be followed after three doses, out of them 31 patients were given 20 μ g vaccine and 10 patients were given 40 μ g vaccine. As seen in our study, 70.9% of the RRT patients who were administered 20 μ g vaccine attained seroconversion after 3 doses while 58.1%, achieved seroprotection with the antibody concentrations above 10 mIU/ml. All the patients on renal replacement therapy patients showed seroconversion after 3 doses of 40 μ g vaccine while 60% of the patients in this group had seroprotection. Therefore, in the present study on chronic renal failure patients, the seroprotection attained after 3 doses of 40 μ g vaccine was not significantly higher than after 3 doses of 20 μ g vaccine.

When 3 doses of 20 μ g vaccine were given to the patients treated conservatively all of them showed seroconversion while 66.6% had seroprotective levels of antibody. Therefore, in the present study, the seroprotection rate in the conservatively treated chronic renal failure patients was slightly better as compared to those on RRT.

Table.1 Hepatitis B virus infection among chronic renal failure patients (n=141)

CASES POSITIVE FOR HBV INFECTION					
ON RENAL REPLACEMENT THERAPY (n=110)			ON CONSERVATIVE THERAPY (n=31)		
HBsAg only	IgMAntiHBsAg only	Both HBsAg and IgMAntiHBsAg positive	HBsAg only	IgMAntiHBsAg only	Both HBsAg and IgMAntiHBsAg positive
6 (66.6%)	1 (11.1%)	2 (22.2%)	-	-	-

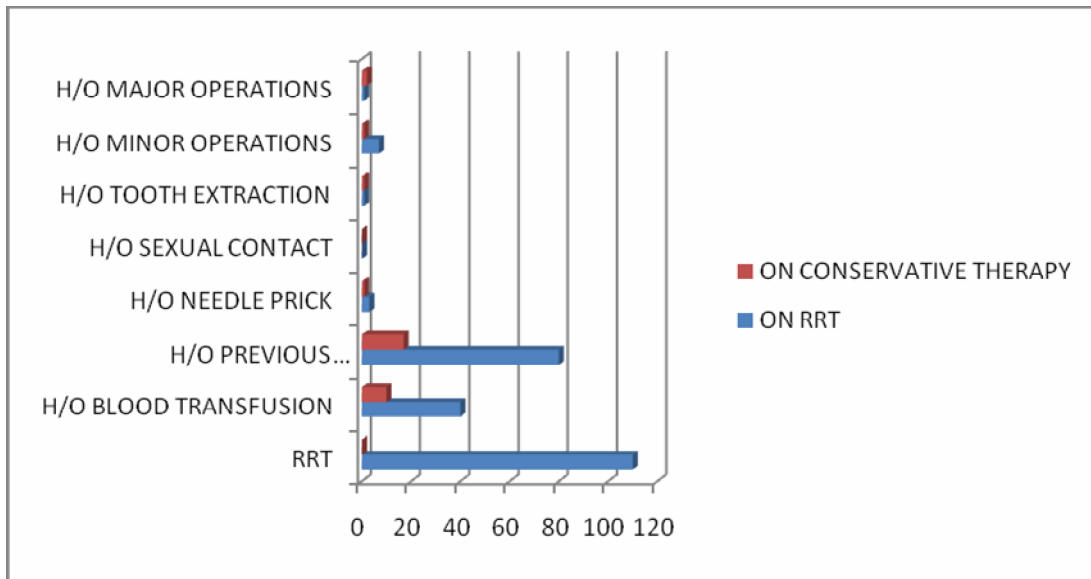
Table.2 Relationship between amount of vaccine and immune response after 3 doses of vaccine among chronic renal failure patients

Amount of vaccine	On RRT			On Conservative Therapy		
	No of cases	Seroconversion	Seroprotection	No of cases	Seroconversion	Seroprotection
Single (20µg)	31	22 (70.9%)	18 (58.1%)	6	6 (100%)	4 (66.6%)
Double (40µg)	10	10 (100.0%)	6 (60.0%)	3	3 (100.0%)	2 (66.6%)
Total	41	32 (78.1%)	24 (58.5%)	9	9 (100.0)	6 (66.6%)

Table.3 Relationship between amount of vaccine and immune response after 4 doses of vaccine among chronic renal failure patients

Amount of vaccine	On RRT			On Conservative Therapy		
	No of cases	Seroconversion	Seroprotection	No of cases	Seroconversion	Seroprotection
Single (20µg)	16	11 (68.8%)	11 (68.8%)	2	1 (50.0%)	1 (50.0%)
Double (40µg)	8	8 (100.0%)	7 (87.5%)	2	2 (100.0%)	2 (100.0%)
Total	24	19 (79.2%)	18 (75.0%)	4	3 (75.0%)	6 (75.0%)

Figure.1 Risk Factors found in chronic renal failure patients for Hepatitis B virus infection



Singh *et al.* (2003) also reported rates that seroprotection rates in those treated conservatively are better (81.2%) than those on dialysis (63.3%).

After 3 doses of 40 µg vaccine, 66.6% of the conservatively treated patients showed

protection while after 4 doses all of those treated conservatively were seroprotected.

24 (18.2%) patients of chronic renal failure on RRT turned up after 4 doses of hepatitis B vaccine. Out of these 24, 16 patients were given 20 µg vaccine and 8 patients had been administered 40 µg vaccine.

After 4 doses of 20 µg vaccine, 68.7% of the patients attained seroconversion and also had seroprotective antibody titres. After 4 doses of 40 µg vaccine all the patients showed seroconversion while 87.5% of the patients were seroprotected. After 4 doses of 20 µg vaccine only 50% of the conservatively treated patients showed seroprotection. The seroprotection rates were lower than the RRT group of patients. This may be due to small sample size in this group. All the conservatively treated patients given 4 dose of 40 µg hepatitis B vaccine were seroprotected.

However, antiHBs concentration in dialysis patients who received 4 doses of 40 µg were significantly higher than in those who received only 3 doses of 20 µg vaccine (0.05), which indicated a better protective immunity in favour of 40 µg vaccine. The dialysis patients are not able to respond to hepatitis B vaccination due to their depressed immunity and when they do not respond they develop low antibody titres and do not maintain adequate antibody levels over time, compared to healthy population (Jungers *et al.*, 1998). Despite the evidence for decreased efficacy the current recommendations are to vaccinate patients with end stage renal disease (Kruger *et al.*, 1999).

On the basis of the study, it was concluded that, Renal Replacement Therapy (RRT) itself is one of the major risk factor for the transmission of Hepatitis B virus infection. Seroconversion to hepatitis B vaccine is lower in RRT patients as compared to those on conservative treatment and healthy adults. Immune response to 4 doses of 40 µg in chronic renal failure patients was better in comparison to standard 3 doses of 20 µg vaccine and it decreases with age. Therefore, it is recommended that chronic renal failure patients should be vaccinated as

early as possible and definitely before putting them on any kind of RRT. It is further suggested that a double dose and an additional dose of hepatitis B vaccine should be administered in this group of patients.

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