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NON RESPONDERS TO HEPATITIS B VACCINE AMONG CHRONIC KIDNEY DISEASE PATIENTS ON HAEMODIALYSIS IN A TERTIARY CARE HOSPITAL.

Immunology

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ABSTRACT

Introduction: Hepatitis B Viral infection is a serious threat to dialysis patients. The prevalence of HBV is more among hemodialyzed (HD) patients than the general population and these patients have a tendency to become chronic carriers of HBV due to defective immune system.

Aim and Objectives: The aim of this study was to identify the non responders to the Hepatitis B vaccine in chronic renal failure patients on hemodialysis.

Materials and Methods: Blood samples were collected from seventy seven patients on hemodialysis and they were screened for HBsAg and Anti-HBs titres.

Results: Of the total 77 haemodialysis patients 18 (23.37%) were females and 59 (76.63%) were males. Of the 77 patients 40 were non responders (Anti HBs Ag titre <10 IU/ml), 16 were poor responders (Titre between 10 – 100 IU/ml) and 21 were good responders (Titres >100IU/ml).

Conclusion: As the nonresponders among the dialysis patients is high and the antibody titre falls with time in these patients, regular monitoring of Anti-HBs is necessary and booster doses are to be administered whenever necessary.

KEYWORDS

Blood borne virus, vaccine non responders, antibody titres

INTRODUCTION:

Infection with hepatitis B Virus (HBV) is a major health threat worldwide. Hepatitis B Virus is an enveloped 42 nm diameter partially double stranded DNA virus belonging to the family Hepadnaviridae. Humans are the only natural hosts. The mode of transmission in adults is by the parenteral, sexual and transplacental routes. The chance of chronicity of Hepatitis B after acquiring depends on when the infection is acquired (which has got to do with the Immunocompetence of the subject). When infection is acquired early in life (less immunocompetent), they likely develop into chronic hepatitis while when infection is acquired in adults (immunocompetent), most of them clear the virus spontaneously and only < 5% develop chronic hepatitis[1,2]. Hepatitis B is a hundred times more transmissible than human immunodeficiency virus (HIV). The virus is abundant in body fluids and can survive up to a week at room temperature on fomites, the surfaces of utensils, medical supplies, and other objects and most infected patients remain asymptomatic [3].The major envelope protein of HBV is the hepatitis B surface antigen (HBsAg). HBsAg was the first identified blood marker for active HBV infection and was originally called 'Australia antigen'[4,5]. HBsAg had become the main determinant used for HBV vaccines. The vaccine is made from the yeast *Saccharomyces cervisiae* and is composed of physio chemically purified non-glycosylated molecule of HBsAg which is adherent to aluminium hydroxide and preserved with thiamersol[6]. In the general population, seroprotection is defined by an Anti HBs antibody titre>10 mIU/ml.

The prevalence of HBV is more among hemodialysed (HD) patients compared to the general population and these patients have a tendency to become chronic carriers of HBV due to a defective immune system. Once infected, 50 to 60% of HD patients become chronic carriers of HBV and also increase the risk of transmission of HBV to other hemodialysis patients, medical personnel and family members[6]. Hepatitis B vaccination showed strong seroprotection against infection for at least 15 years in all age groups of general population, although antibody levels were decreased among chronic kidney disease patients in a shorter duration.[7] The waning of protective anti-HBs antibodies was detected in 26% of HD patients lost during 6 – 36 months of observation.[8] It is largely the result of a general immune-compromised state that is characteristic of advanced renal failure. The factors leading to defective immunity are diabetes mellitus, azotaemia, age, sex, malnutrition, erythropoietin deficiency, ineffective dialysis, failure to complete the full course of HBV vaccine. Additional risk factors include infection with HCV or HIV due to transfusion of blood or blood products[6]. The number of patients on haemodialysis is growing rapidly worldwide. Patients on chronic haemodialysis are considered high risk because many diagnostic and therapeutic procedures carried out on these individuals increases the probability of HBV infection. Thus, the primary means of protection for individuals on dialysis is a targeted vaccination strategy against HBV. Therefore HBV immunization is highly recommended for patients suffering from chronic kidney disease (CKD), whether dialysis dependent or not. Improvements in quality of medical care and infection control measures followed in haemodialysis units also contribute to protection against infection. Patients on dialysis have lower antibody titres[4]when compared to normal individuals. HBV vaccination stimulates specific antibody production by the B cell activation, which is mediated by CD8+ cytotoxic T cells and CD4+ helper T cells. There are several ways where uraemia causes diminished immune response. Patients on dialysis have lymphocytopenia. CD4+ lymphocyte count is important to provide antibody production subsequent to vaccination.[9] Sengar et al showed that an immune response to HBsAg may be negatively associated with HLA8.[10] The objective of this study was to identify the non responders to Hepatitis B vaccination among the hemodialysis patients.

MATERIALS AND METHODS:

This cross sectional study was conducted on seventy seven patients on long term haemodialysis for at least 1 year at Chettinad Hospital and Research Institute, a teaching hospital at Kelambakkam in Kanchipuram district of Tamilnadu in India. A total of 59 male and 18 female patients with chronic kidney disease due to various etiological factors were included in the study and their blood samples were collected after obtaining informed consent. Haemodialysis was being performed in these patients two to three times each week, 4 to 4.5 hours per session, using single use dialyzers. The inclusion criteria were patients with renal failure requiring haemodialysis who had received full course of Hepatitis B vaccine. Their vaccination status was checked from their previous immunisation records. All hemodialysis patients who did not have any evidence of protective HBsAb titre of more than or equal to 10 mIU/ml had been vaccinated with Engerix B 40 µg intramuscularly (IM) into the deltoid. The schedule was 0, 1, 2 and 6 months. Blood samples were collected and processed in the

clinical Microbiology laboratory of Chettinad Hospital for detection of Hepatitis B surface antigen (HBsAg) and antibody to hepatitis B surface antigen (Anti-HBs), in all these hemodialysis patients. Detection of HBsAg was performed by the Enzyme linked immunosorbent assay(ELISA) method using Erba Lisa SEN HBsAg kit [11]and Anti-HBs titres were determined by the ELISA method using HBsAb kit [12] (DiaPro Diagnostic Bioprobessrl, Italy).

HBsAg ELISA

Qualitative determination of Hepatitis B Surface antigen in patient serum by Enzyme Linked Immunosorbent Assay (ELISA)method was performed using the kit Erba Lisa SEN HBsAgas per the manufacturer's protocol. In brief, all reagents and test specimens were brought to room temperature before starting the procedure. 100µl of sample diluent was added to the blank, and 25µl of sample diluent to rest of the wells. 75µl of control and test specimens were pipetted into the respective wells. 50µl of conjugate was added to each well following which the plate was covered and incubated at 37°Cfor 60 minutes. Wash procedure was carried out using the ELISA washer. 50µl of color reagent was added to all wells and incubated for 15minutes in the dark at room temperature. The optical density was read at 450nm within 15 minutes of adding stop solution. The cut off value was calculated by using the kit formula and absorbance of all the wells higher than the cut off value were considered as reactive.

Anti HBs ELISA:

Quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in the patient serum was done by Enzyme immune assay – Diapro Diagnostics, according to the manufacturers' protocol. Micro plates coated with highly purified HBsAg specifically captures anti HBsAg antibodies to the solid phase in the first incubation with sample. After washing, captured antibodies were detected by HBsAg labelled with peroxidase (HRP) that specifically binds the second available binding site of these antibodies. The enzymes specifically binds to the wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and this was detected by an ELISA reader. Samples with a concentration lower than 10 WHO mIU/ml were considered negative for anti HBsAg antibody. Samples with a concentration higher than10 WHO mIU/ml were considered positive for anti HBsAg antibody.

Chi square test was used for statistical analysis.

RESULTS:

77 patients undergoing haemodialysis were included in this study, of which 18 (23.37%) were females and 59 (76.63%) were males (Figure 1).The mean age of the patients was 53±18 years. The duration of haemodialysis was 4-5 years. The distribution of responders and non responders is given in Figure 2. The primary cause of chronic renal failure was also established in these dialysis patients: diabetic nephropathy in 39 patients (51%), hypertensive nephropathy in 20(26%), chronic glomerulonephritis in 14 (18%) and glomerulopathy of unknown origin in 4 (3%) (Table1). There was no significant difference in the Anti HBs antibody levels between male and female, diabetic, hypertensive and other subjects (p= N.S) which is shown in Table 2. 37 (48%) patients had antibody titre of ≥ 10 IU/ml and the remaining 40(52%) patients had titres ≤ 10IU/ml (Figure.2). Out of 37 (48%) responders 10 were females and 27 were male (Table 2). Among the 37 responders, 22 (59%) were good responders with HBsAb level of ≥ 100 IU/ml and 15 (40%) were poor responders with the HBsAb level ≥10 IU/ml but less than 100 IU/ml (Figure 3). The non responders had titre values of 0 IU/ml in 34 out of 40 (85%) and less than 10 IU/ml in 6 out of 40 (15%) patients.2 of the non responders were HBsAg positive.

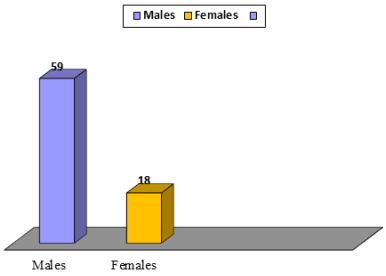

Figure 1: Gender wise distribution of the haemodialysis patients

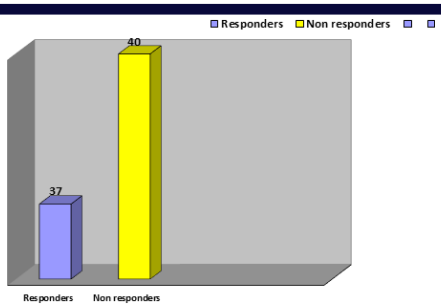

Figure 2: Distribution of the Responders and Non responders

Table 1: Distribution of patients according to causes of renal failure

Cause of Renal Failure	Total	Non responders <10 IU/ml (n=40)	Responders	
			Poor responders (n=15)	Good responders (n=22)
Diabetic nephropathy	39	19	8	12
Hypertensive nephropathy	24	8	10	6
Chronic glomerulonephritis	11	6	3	2
Others	3	3	0	0

Table 2: Age wise distribution of the responders and the non responders to Hepatitis B vaccination

Variables		Non responders <10 IU/ml (n=40) 52%		Responders			
				Poor responders (n=15)		**Good responders (n=22)**	
		Male	Female	Male	Female	Male	Female
Age (yrs)	>80	0		0		1	
	61-80	5		3		4	
	41-60	19		8		3	
	21-40	7		2		6	
	<20	1		0		0	
Gender M/F		32	8	13	2	14	8

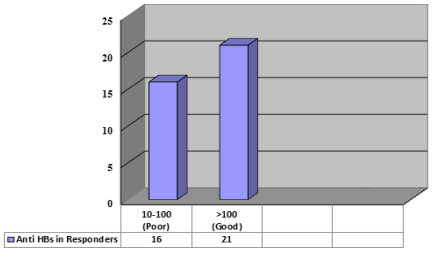

Figure 3: Anti HBs Titres in Responders (n=37)

DISCUSSION:

This study identifies the percentage of haemodialysis patients protected against Hepatitis B virus. Hepatitis B vaccination schedule, commonly used in the general population was three doses of 20 µg recombinant vaccine doses at zero, one and six months. But the haemodialysis patients have to be vaccinated against HBV using recombinant HBV vaccines given at zero, one, two and six months in the dose of 40 µg intramuscularly. Some studies correlate the vaccine response with the gender and found females respond well to vaccination while the males are less prone for seroconversion.[13] However, in our current study and also in the study by Crosnier et al[14],the gender difference among the dialysis patients was not statistically significant . In our study 37 (48%) haemodialysis patients had seroconversion after vaccination out of which 27% (10) were females and 73% (27) were males. However another study showed a seroprotection rate of 67% out of 118 being high responders.[13] Also, Stevens et al [15] found a much higher seroconversion rate totalling 89% (76% male and 100% female dialysis patients) with the same vaccine, dose, and schedule of vaccination which shows a higher rate than our study. Most of the chronic renal failure patients who were admitted for haemodialysis had a history of Diabetes mellitus, hypertension and chronic glomerulonephritis.

Persons who developed an anti-HBs titer below 10 IU/L are referred to as non responders. An Anti-HBs titer of ≥ 10 IU/L is considered as positive seroconversion. Persons who develop an Anti-HBs titer between 10 and 100 IU/L are referred to as responders. In some countries Anti-HBs titer ≥ 100 IU/L is considered as a seroprotective titre.[16] Other studies like Salwa Ibrahim et al[13], Costa et al[16] observed that persons with titre>100 IU/L were protected for many years and they did not require booster doses whereas, titre of individuals with 10-100 IU/L were closely monitored and might require booster doses to prevent from future infection. Patients with non protective titers were asked to repeat the vaccination schedule.

CONCLUSION:

Since there is a high percentage of non responders to HBV vaccination among the dialysis patients and because antibody titre falls with time in patients on prolonged dialysis, regular monitoring of Anti-HBs has to be performed. The only protective measure against Hepatitis B virus for these people is vaccination. Therefore, further booster doses are to be administered to those patients whose Anti-HBs titres have fallen below10 mIU/ml. Attempts can be made to increase the immune response among the non responding dialysis patients to HBV vaccination by administering adjuvants like interferon, erythropoietin, immune stimulants etc along with the vaccine.[17]

Recommendations for further study

Analysis at genetic level can also be done to identify the existence of a genetic basis for the poor immune response in some chronic kidney disease patients. Other Hepatitis B viral markers like HBcAg, HBeAg and others can also be assessed and correlated.

REFERENCES:

1. Poland GA, Jacobson RM. Prevention of hepatitis B with the hepatitis B vaccine. New England Journal of Medicine. 2004 Dec 30;351(27):2832-8.
2. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clinical Infectious Diseases. 1995 Apr 1;20(4):992-1000.
3. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiologic reviews. 2006 Jun 5;28(1):112-25.
4. Nahar K, Jahan M, Nessa A, Tabassum S. Antibody responses after hepatitis B vaccination among maintenance haemodialysis patients. Bangladesh Medical Research Council Bulletin. 2011; 37(3):88-91.
5. Unger JK, Peters H. Hepatitis B in chronic kidney disease: Moving toward effective prevention. Kidney international. 2008 Apr 1; 73(7):799-801.
6. Gasim GI, Abdelhaleem Bella IA. Immune response to hepatitis B vaccine among patients on hemodialysis. World journal of hepatology. 2015 Feb 27;7(2):270.
7. Grzegorzewska AE. Hepatitis B vaccination in chronic kidney disease: review of evidence in non-dialyzed patients. Hepatitis monthly. 2012 Nov;12(11).
8. Tsouchnikas I, Dounousi E, Xanthopoulou K, Papakonstantinou S, Thomoglou V, Tsakiris D. Loss of hepatitis B immunity in hemodialysis patients acquired either naturally or after vaccination. Clinical nephrology. 2007 Oct 1;68(4):228-34.
9. Vaccine recommendations for patients of chronic kidney disease. Indian J Nephrol. 2005; 15(Suppl 1):S72–4.
10. Sengar DP, McLeish WA, Sutherland M, Couture RA, Rashid A. Hepatitis B antigen (HBAg) infection in a hemodialysis unit. I. HL-A8 and immune response to HBAg. Canadian Medical Association Journal. 1975 Apr 19; 112(8):968.
11. Package Insert HBs Ag ELISA kit- Erba Lisa SEN HBsAg kit
12. Package insert- Anti HBs Ab ELISA kit -DiaPro Diagnostic Bioprobessrl, Italy
13. Ibrahim S, el-Din S, Bazzal I. Antibody level after hepatitis-B vaccination in hemodialysis patients: impact of dialysis adequacy, chronic inflammation, local endemicity and nutritional status. Journal of the National Medical Association. 2006 Dec;98(12):1953.
14. Crosnier H, Jungers P, Courouce Am, Laplanche A, Benhamou E, Degos F, Lacour B, Prunet P, Cerisier Y, Guesry P: Randomised Placebo-Controlled Trial Of Hepatitis B Surface Antigen Vaccine In French Haemodialysis Units: II. Haemodialysis Patients. Lancet 1:797—800, 1981
15. Stevens CE, Szmuness W, Goodman AL, Weseley SA, Fotino M: Hepatitis B vaccine: Immune responses in haemodialysis patients. Lancet 2:1211—1213, 1980 11.
16. Costa NC, Canhestro MR, Soares CM, Rodrigues JS. Monitoring of post-vaccination anti-HBs titles vaccine in children and adolescents in the pre-dialysis of chronic kidney disease. JornalBrasileiro de Nefrologia. 2017(AHEAD):0-.
17. R. K. Gupta and G. R. Siber, "Adjuvants for human vaccines. Current status, problems and future prospects," Vaccine, vol. 13, no. 14, pp. 1263–1276, 1995