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Serum Anti-Hbs-Ag in Stable Hemodialysis Patients and its Relationship with Various Demographic and Biochemical Data

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Abstract

Introduction: To evaluate the relationship between various biochemical, nutritional and demographic factors with immune response to hepatitis B vaccine in maintenance hemodialysis (HD) patients. *Material and Methods:* A retro-prospective study was carried out on 68 patients undergoing maintenance hemodialysis .Patients were vaccinated against hepatitis B virus with an intramuscular hepatitis B vaccination schedule, 40 micrograms at 0, 1, and 6 months. We also selected 32 age matched normal healthy persons who had vaccinated against hepatitis B previously to compare the antibody production with HD patients. *Results:* The value of serum antibody level against hepatitis B surface antigen (HBs) in hemodialisis patients and healthy persons were 35±55(median=5.5) and 135±71 (median=175) mIU/ml respectively. There was a significant deference between mean serum antibody level against HBs antigen of hemodialysis patients and normal subjects ($p<0.001$), there were not any significant differences of antibody production against HBs antigen between males and females or diabetic and non diabetics. There were no correlation between serum antibody level against HBs-Ag and serum albumin and also with body mass index. There were not significant correlation between anti-HBs antibody level and age, amounts of hemodialysis, duration of dialysis, dialysis adequacy, serum ferritin level and serum lipids. There were not also significant correlation between anti-HBs antibody level and serum parathormone, calcium, phosphorus, serum hemoglobin and hematocrit level. *Conclusion:* In this study, there was not significant correlation between serum antibody level against hepatitis B surface antigen and various nutritional and demographic factors of patients under regular hemodialysis.

Keywords: Hepatitis B; Hepatitis B vaccine; Hemodialysis; Malnutrition, Anti-HBs antibody.

Introduction

High susceptibility to various infections like hepatitis B virus (HBV) causes increased morbidity or mortality in patients with end stage kidney disease. HBV vaccination is recommended for all patients undergoing hemodialysis; however, antibody response is much lower than in healthy individuals [1]. In a study of 15 hemodialysis wards, it was found a serum hepatitis B surface antigen (HBs- Ag) prevalence of 16.8 percent in those patients [2]. It has been found that 30-40% of hemodialysis patients fail to produce antibodies against HBs-Ag antigen after vaccination towards hepatitis B virus [3]. Indeed hepatitis B vaccine response rates of 34-88% have been reported among hemodialysis patients [4], hemodialysis patients also have an inability to maintain adequate antibody titers during time [5]. The

results of various studies showed that maintenance hemodialysis (HD) patients are at risk of malnutrition [6-8] which is associated with a reduced life expectancy mainly because of cardiovascular and infectious complications [6,10]. Protein-energy intake is often due to inappropriate dietary restrictions and anorexia which promot malnutrition in most patients under hemdialysis [8-13]. Because of the importance of accurately identifying hemodialysis patients capable of transmitting hepatitis B, we aimed to consider various biochemical, nutritional and demographic factors which could affect response to HBV vaccination in maintenance hemodialysis patients.

Material and Method

Patients

This cross-sectional study was carried out on patients with end-stage renal failure (ESRD) undergoing maintenance HD with acetate basis dialysate and polysulfone membranes. The study was conducted in hemodialysis center of Hajar Medical, Educational and Therapeutic Center of Shahrekord in Iran in 2006 to 2007.

According to the severity of the secondary hyperparathyroidism, each patient was under treatment for secondary hyperparathyroidism with oral active vitamin D3 (Rocaltrol), calcium carbonate, and Rena-Jel(sevelamer) at various doses. According to the severity of anemia, patients were given intravenous iron sucrose (venofer) at varying doses after each dialysis session. All patients were also given folic acid 3 mg daily, L-carnitine, 750 mg daily, B-complex tablets, and 2000 units of intravenous Eprex [recombinant human erythropoietin (rHuEPO)] after each dialysis session routinely. Exclusion criteria were the presence of any active or chronic infection or using any antibiotics or NSAIDs and infection of hepatitis C virus.

In this study we also selected 32 ages matched normal healthy persons who had vaccinated against hepatitis B previously to compare the antibody production with HD patients.

Hepatitis B Vaccination

Patients' vaccination was started before the study through a schedule of 0, 1, and 6 months. They received 2 ml (40μg) of Euvax Hbs-Ag (hepatitis B recombinant vaccine which contains a preparation of the surface antigen of the hepatitis B virus) by intramuscular deltoid injections. Normal age matched subjects were received from the same vaccine on a schedule of 0, 1, and 6 months and 4-5 years had past from their last vaccinations. The local ethics committee approved the study and informed consent was obtained from all patients.

Laboratory Methods

Blood samples were collected after overnight fasting for complete blood count (CBC), levels of serum calcium (Ca), phosphorus (P), Alkaline phosphatase (ALP) iron, albumin (Alb), and ferritin [radioimmunoassay (RIA)] and also lipid profile were measured using standard methods. Intact serum parathormone (iPTH) was measured by the RIA method using DSL-8000 kits from the USA (normal range of values is 10-65 pg/ml).Antibodies to hepatitis B surface antigen (anti-HBs) were determined by the ELISA technique with Dialab kits (manufactured in Austria) at least 6 months after completion of initial vaccination series to assess response to vaccination. A subject had responded to the vaccine if the anti-HBS level was >10 mIU/ml. Those with levels 10–100 mIU/ml were termed 'poor responders', whereas those with levels >100 mIU/ml were termed 'good responders' [1].For measurement of adequacy of HD, urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data (14). .Duration and frequency of HD treatment were calculated from the patients' records. Body mass index (BMI) calculated using thestandard formula (post-dialysis weight in kilograms/ square height in meters, kg/m. The duration of each HD session was 4 hours.

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD), median and range values. Comparison between the groups was performed using Student's t test. Statistical correlations were assessed using partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at p value < 0.05.

Results

Of 68 patients (female=19, male=49), 18 were diabetics.

The mean age of the patients was 53 ± 18 years.

The mean duration of patients on HD was 27 ± 29 months (Median: 22.8 months).

Table 1, shows the parameters of the study patients.

The mean value of antibody titer in hemodialisis patients and healthy persons were 35±55 (median=5.5) and 135±71 (median=175) mIU/mL respectively (Table 1).

Table 1. Data of the patients

N=68	Range	Median	Mean±SD
Age (year)	66	52	53±18
DH (month)*	179	22.8	27±29
Amounts of HD (numbers of dialysis)	4762	166.5	282±582
BMI	25.3	22.4	22.7±4.4
URR (%)	35	56	55.3±6.8
Ca (mg/dl)	5	8.5	8.5±1.1
P (mg/dl)	7.7	6.6	6.6±1.9
IntactPTH (Pg/ml)	1457	204	322±311
Alb (g/dl)	1.6	3.8	3.7±0.4
Hgb (g/dl)	5.8	18	10±2
Hct %	27	32	33±5
ALP(IU/l)	853	106	166±162
Ferritin ng/dl	2145	699	688 ±499
Iron micg/dl	1308	95	305±352
Cholesterol (mg/dl)	54	150	150±54
Tg (mg/dl)	454	104	136±83
Anti-HBS	184	5.5	35±55

*DH = duration of hemodialysis

We did not find any significant differences of antibody production against hepatitis B surface antigen between males and females or diabetic and non diabetics (p = N.S).

Figure 1, shows that there was a significant deference between mean serum antibody level against hepatitis B surface antigen of hemodialysis patients and normal subjects (r < 0.01).

There was not significant correlation between serum albumin and serum antibody against hepatitis B surface antigen, also there were not any significant correlation between anti-HBs antibody level with age, amounts of hemodialysis, duration of dialysis, BMI, URR, serum ferritin level and serum lipids (p = N.S.). In this study also there were not any significant correlation between HBs antibody level and serum iPTH, Ca, P, ALP, serum hemoglobin and hematocrit level too(p = N.S.).

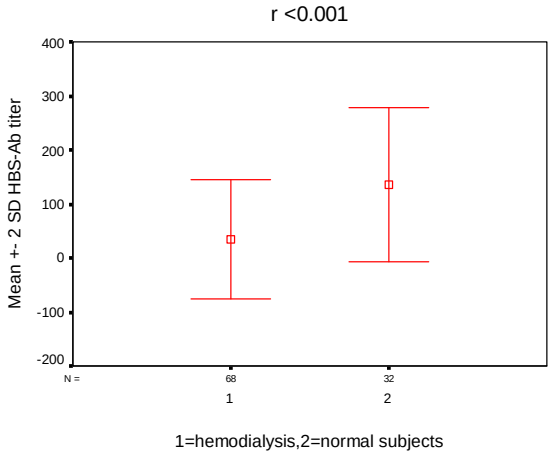

Figure 1. Significant difference between mean serum antibody level against hepatitis B surface antigen and normal subjects (r<0.01)

Discussion

In this study we found that there was a significant deference between mean serum antibody level against hepatitis B surface antigen of hemodialysis patients and normal subjects, there were not any significant differences of antibody production against hepatitis B surface antigen between males and females or diabetic and non diabetics. there was a significant deference between mean serum antibody level against HBs antigen of hemodialysis patients and normal subjects($p<0.001$), there were not any significant differences of antibody production against HBs antigen between ales and females or diabetic and non diabetics. There were not correlation between serum antibody level against HBs-Ag and serum albumin and also with BMI. There were not significant correlation between anti-HBs antibody level and age, amounts of hemodialysis, duration of dialysis, dialysis adequacy, erum ferritin level and serum lipids. There were not also significant correlation between anti-HBs antibody level and serum parathormone, calcium, phosphorus, serum hemoglobin and hematocrit level. Patients with chronic kidney disease suffer from defective host defenses, which are directly the result of the renal impairment, in addition to those dependent on the primary illness leading to the renal failure [15]. Although vaccination can provide significant protection against hepatitis B virus infection, vaccine response rates are low and unpredictable among hemodialysis patients [5]. In adults with normal immune status, vaccine response rates are as high as 95%, and antibody to hepatitis B surface antigen (anti-HBs) remains above protective levels in 40-87% of persons for as long as 15 years. Only 34-88% of vaccinated hemodialysis patients develop protective levels of anti-HBs, and limited data are available that assess the persistence of this response [5]. Due to the impaired immune response, hemodialysis patients are given larger doses of the vaccine and frequently require revaccination or booster doses to produce and maintain adequate anti-HB titers [16-19]. Given the limited efficacy of hepatitis B vaccine in hemodialysis patients, it would be useful to identify factors that may influence vaccine response in these patients. Several studies have attempted to identify such factors. Previuos studies have shown that unresponsiveness to HBV vaccine is multifactorial, and related to the presence of several interacting factors.Studies showed that response rates were greatest among hemodialysis patients younger than 40 years [5] and sex may also influence vaccine response [20], however, other studies as well our results have failed to show that sex or hemodialysis dose influence response to hepatitis B vaccine [5]. Ibrahim et al. [21] studied the evolution of HBsAg antibody (HBs-Ab) after primary vaccination in 29 HD patients and showed no significant correlation with age, duration of HD therapy, serum albumin. They found responders to primary vaccination had significantly higher levels of urea reduction ratio. They also showed the response to hepatitis-B vaccination among hemodialysis patients neither correlated with age, systemic inflammation nor nutritional status but efficient hemodialysis was associated with good response to hepatitis-B vaccine. Kara et al. in a retrospective study on 34 HD found, the immune response of the HBV vaccine was low in the HD patients and it was affected by several factors such as nutritional status [22]. Lombardi et al. [23] considered 35 HD patients who underwent anti-HBV vaccination and classified them to responders or non-responders. They evaluated the relationship of anthropometric and impedenziometric parameters with antibody production and found that these parameters were higher in responders than in non responders, but the difference did not reach statistical significance [23]. Dacko et al. [24] to find the factors contributing to the reduced antibody response to hepatitis B vaccination in peritoneal dialysis patients and the influence of nutritional status [as assessed by serum albumin] and dialysis adequacy on the development of hepatitis B antibodies examined 32 continuous ambulatory peritoneal dialysis/continuous cycling peritoneal dialysis patients. They found that patient age, sex, months on peritoneal dialysis, and race were not different among converters and nonconverters. Median serum albumin and final weekly Kt/V were not different among converters and nonconverters. They concluded that nutritional status, residual renal function, and weekly Kt/V in nutritionally replete and well-dialyzed peritoneal dialysis patients do not predict response to hepatitis B vaccine [24]. Fabrizi et al. [25] on 118 chronic HD patients who were received recombinant hepatitis B vaccine found a statistically significant difference between responder and non-responder patients with regard to nutritional parameters such as serum total proteins and mean levels of transferrinemia. They also found that the number of diabetic patients was significantly increased in the nonresponder group and also patients with persistent antibodies were younger and had a shorter duration of HD treatment compared to those responders who rapidly lost anti-HBs [25] . To identify factors implicated in the vaccine response, Peces et al. [26] conducted a study on vaccinated 80 seronegative patients on HD . The result of this study showed that there were no differences between responders and nonresponders concerning sex, time on

HD, HD dose, nutritional status, hemoglobin level, HD membrane, iPTH level and calcitriol treatment (26). Kovacic et al. [27] conducted a study to see how dialysis efficacy affects response to hepatitis B virus (HBV) vaccination. Study subjects consisted of 30 patients on chronic haemodialysis. Subjects were divided into groups according to the level of HBsAb: non-responders and good responders. The group of responders had a significantly more efficient dialysis (Kt/V) than the group of non-responders. The group of good responders had a significantly better Kt/V than the group of non-responders. Kt/V values showed a significantly positive correlation with the HBs-Ab level. They concluded that HBV vaccination reaction was weaker in patients on chronic haemodialysis with inefficient dialysis. Efficient haemodialysis significantly improves the response to vaccination with recombinant HBs-Ag [27]. To assess if malnutrition influences the response to the hepatitis B virus vaccine Fernández et al. [1] considered sixty-four HD patients and found increase in age negatively influences the formation of antibodies, serum albumin and predialysis blood urea concentration are positively correlated with the formation of antibodies and responders had significantly higher levels of serum albumin and prealbumin and predialysis blood urea than non-responders. Patients with serum albumin levels between 3 and 3.5 g/dl were non-responders in a higher percentage (87.5%) than those with serum albumin levels between 4.5 and 5 g/dl (18.8%). Authors concluded that malnutrition negatively influences the response to the hepatitis B virus vaccine in haemodialysis patients [1]. Very recently Ocak et al. [28] to investigate whether haemodialysis patients who suffering from diabetes mellitus could be considered at risk for the development of the protective antibodies to hepatitis B vaccination conducted a study on forty-nine HD patients which were divided into two groups: group A (19 diabetic patients) and group B (30 non-diabetic patients), after vaccination group A found to have a lower protective antibody rates than the patients in group B .They showed that diabetic patients on HD may carry a greater risk of not seroconverting than non-diabetic ones for antibody response to HB vaccination [28]. Sorkhi et al. in a study on 62 HD patients showed duration of haemodialysis had no effect on response to vaccination [29]. In an another study conducted by Ramezani et al. [30] on 54 HD patients could not found any correlation between anti-HBs titers and serum hemoglobin, serum albumin, triglycerides, cholesterol, fasting blood sugar, PTH, efficiency of dialysis, duration of dialysis treatment, sex, smoking, percentage of subjects with DM. They concluded that unresponsiveness to HBV vaccine is multifactorial, and related to the presence of several modulators [30].

Conclusions

This study was designed to examine the relationship between various biochemical, nutritional and demographic factors with immune response to hepatitis B vaccine in maintenance hemodialysis (HD) patients. We could not show any significant correlation between serum antibody level against hepatitis B surface antigen and various nutritional and demographic factors of patients under regular hemodialysis. However, previous authors showed different results, some of them were in accordance with our study. We concluded that factors related to serum antibody level against hepatitis B surface antigen were different in this group of patients and different factors might be responsible for antibody production and more multicentre studies need to clearly define these related factors.

Ethical Issues

This study was approved by the ethical committee of Shahrekord University of Medical Sciences.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Hamid NASRI carried defined the aim of research and the design of experiment and carried out the experiments. Mohammad-Reza ARDALAN participated in the design of the study and performed the

statistical analysis. Azar BARADARAN coordinate and heped to draft the manuscript. All authors read and approved the final manuscript.

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