Determination of Hepatitis B Surface Antibody Titer in Vaccinated Children with Major Thalassemia in Kerman-Iran

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ABSTRACT

Background: Thalassemia patients are more susceptible to hepatitis than the normal population due to the frequent blood transfusions. **Objective:** To determine the immune response of children with major ß-thalassemia, by measuring anti-hepatitis B surface antibody (anti-HBs Ab) following the last HBV vaccine injection. **Methods:** This study was carried out on 215 thalassemic children who received three standard intramuscular recombinant HBV vaccines. Children age ranged between 1-4.5 with a mean age of 3.37 years. Based on the time lapsed since last vaccine injection, the subjects were divided into three groups; 0-15 months, 15-30 months and 30-45 months, respectively. Based on the serum levels of anti-HBs antibody, subjects were categorized as: good responders (anti-HBs >100 IU/Lit), low responders (anti-HBs 10-100 IU/Lit) and non-responders (anti-HBs <10 IU/Lit). **Results:** The mean range of anti-HBs level in the above mentioned groups were 205.34, 128.8 and 54.25 IU/lit, respectively ($P<0.0001$). In girls, the mean antibody level was 104.2 and in boys it was 95.8 IU/Lit ($P>0.05$). Out of 215 selected individuals 75 (35%) were good responders, 65(30%) low responders and 75 (35%) non-responders. **Conclusion:** Standard HBV vaccination in thalassemic children results in an immune response in more than 65% of the subjects. Therefore, assessment of anti-HBs antibody level, 45 months after the last vaccination, is recommended.

Keywords: Hepatitis B Antibody, Thalassemia, Iran

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INTRODUCTION

There are more than 2 billion people with Hepatitis B infection worldwide, with 350 million people as hepatitis B virus carriers. Overall, 1.4%-6.5% of the total population living in different parts of the world and 2-4 million living in Iran are carriers (1,2). There are also about 20000 cases of thalassemia major in Iran. Kerman is one of the top 10 provinces of Iran with a high prevalence of thalassemia (3-7). Most of these patients need blood transfusions which increase the risk for hepatitis (5,8,9). Hence, prevention in this high risk group is of great importance. No comprehensive study has been done on the post-vaccination immunization status of thalassemia patients to determine the necessity of booster dose based on the serum antibody levels months to years after vaccination and, since the carriers of HBV are symptomless but can transfer the disease (10,11), the importance of this study becomes more obvious.

SUBJECTS AND METHODS

This is a cross-sectional study. A simple randomized sampling of all thalassemic patients aging from 1-4.5 years, who were under the care of thalassemia foundations in Kerman, was included in our study. According to the sample size in other studies and by assuming a 10% decrease in patients' antibody titer compared to normal and by using [n=z*z*p(1-p)/d*d] formula in which p=10%, a=0.05%(confidence level 95%) and d=4% (expected accuracy), 215 thalassemic patients were chosen. In this study children with Hepatitis B, or any other blood disease and those with impaired immune system were omitted. The required information were taken out of the patients' files with the variants of: sex, age at the time of splenectomy, use of Desferal, parents relationship, blood group, and the time elapsed from the last hepatitis vaccination. The exact dates of their vaccination were recorded and those with unclear dates of vaccination were omitted from the study. Five ml venous blood was obtained from each child with consent of their parents. After serum separation, anti HBs level was measured with Dynex, Behring ELISA processor instrument. Upon the measurement of anti-HBs, differentiation of patients with positive anti HBs antibody due to viral contact and those due to vaccination was possible. Measurment of anti-HCV was also performed on the blood samples. Whenever anti-HCV was positive, the case was replaced by another suitable sample. Out of the first 215 samples, 4 cases (1.85%) were omitted due to their anti-HCV positivity. The patients were then divided according to the time interval from the last vaccination dose into the following 3 groups:

Group 1: 0-15 months from the last vaccination
Group 2: 16-30 months from the last vaccination
Group 3: 31-45 months from the last vaccination

Anti-HBS was measured in IU/L and the results were classified into 3 groups as follows:

Group 1: cases with no immunity i.e serum antibody < 10 U/L
Group 2: cases with relative immunity i.e serum antibody >= 10-100 IU/L
Group 3: cases with complete immunity i.e serum antibody > 100 IU/L

Statistical analysis including Chi-Square, one sample t-test, Pearson independent sample t-test and correlations were performed by SPSS software.

RESULTS

The age of the patients ranged from 1-4.5 years, with a mean age of 3.37 years. Out of the total 215 cases, 104 were males (48.37%) and 111 were females (51.63%). In the first group, there were 16 males (44.4%) and 20 females (55.6%). The mean age in this group was 1.8 years. In the second group, there were 30 males (50%) and 30 females (50%) and their mean age was 2.9 years. In the third group there were 58 males (48.74%) and 61 females (51.26%). The mean serum anti-HBs level in 3 different groups was equal to 100.14 IU/L with the minimum of 9 and the maximum of 1964 IU/L (only one case) (Table 1-3). Antibody levels in the first, second and the third groups were 205.343, 128.8 and 54.252 IU/L, respectively. The level of serum anti-HBs in the 3 groups were significantly different using ANOVA and Bartlett tests ($P<0.0001$). The mean serum anti-HBs level of boys was 95.8 IU/L and that of girls was 104.2IU/L, with no statistically significant difference ($P>0.05$) (Table 1-3). In the first group, the mean serum anti-HBs level in boys was 210.06 and in the girls was 201.37 IU/L which had no significant difference ($P>0.05$). In the second group, the mean serum anti-HBs level in the boys was 146.93 and in the girls was 201.37 IU/L which was not significantly different ($P>0.05$). In the third group, the mean serum anti-HBs level in boys was 37.87 and in girls was 69.84 IU/L with no meaningful difference ($P>0.005$). The quantity distribution in all 3 groups was: in the total of 215 cases, 75 (34.88%) were no responders (anti-HBs<10), 65 cases (30.234%) were low responders (anti-HBs=10-100), and 75 cases (34.88%) were good responders (anti-HBs>100). In the first group only one case (2.8%) was no responder, but in the second and third groups there were 13 cases (21.6%) and 61 cases (51.3%) of no responders, respectively. Besides, in the first group, 6 cases (16.7%) were low responders, while in the second and the third group, 16 cases (26.7%) and 43 cases (37.4%) were low responders, respectively. On the other hand, in the first group, 29 cases (80.5%) were good responders, while in the second and third groups 31 cases (51.7%) and 15 cases (12.6%) were good responders, respectively. Chi-square test of our cluster sampling cases showed significant differences among the 3 groups ($P<0.005$) (Table3).

Table 1. Distribution of hepatitis B antibody titer versus duration of time after vaccination

Antibody / Time after vaccination (months)	<10 IU/L	10-100 IU/L	>100 IU/L	Total Cases
0-15	1	6	29	36
15-30	13	16	31	60
30-45	61	43	15	119
Total	75	65	75	215

X2= 68/5 P= 0.00000

Table 2. Distribution of hepatitis B antibody titer in female groups versus duration of time after vaccination

Antibody / Time after vaccination (months)	<10 IU/L	10-100 IU/L	>100 IU/L	Total
0-15	0	3	17	20
15-30	9	5	16	30
30-45	31	22	8	61
Total	40	30	41	111

X2= 38 P= 0.000000

Table 3. Distribution of hepatitis B antibody titer in male groups versus duration of time after vaccination

Antibody / Cases after vaccination	<10 IU/L	10-100 IU/L	>100 IU/L	total
0-15 months	1	3	12	16
15-30 months	4	11	15	30
30-45 months	30	21	7	58
Total	35	35	34	104

X2= 33/8 P= 0.000000

The results showed that the immunity of the patients decreased as the time interval after immunization increased. The difference between the data obtained from no responders, low responders, and good responders in the 3 groups were studied with Mantel Haenszel Test and was found to be statistically significant between the first and also between the second groups.($P<0.05$), between the first and the third groups and the second and the third groups ($P<0.005$). No significant difference was found regarding the antibody titers of the patients and their parents using Chi-square test ($p=0.64$). There was a significant difference between responder and non-responder cases with their age. As the age of the children goes up, the response rate decreases ($P<0.005$) (Table1).

DISCUSSION

Several studies with controversial results regarding the immunity level and the duration of acquired immunity from Hepatitis-B vaccination have been performed in different countries. A similar study has been performed on infants in our country, but there is no study on thalaessemic patients. In this study, the immunity level after Hepatitis-B vaccination and the long-term effects of the vaccination were studied. Out of 215 children with major thalessemia, 75 cases were no responders, and the remaining were either low or good responders. In cases with 0-15 month interval after their vaccination, 97%, were low or good responders. In the group with>30 months interval after vaccination, 49% were low or good responders. A study in Italy, on 56 thalassemic children with 3-dose Hepatitis-B immunization showed that anti HBs antibody level was <10 IU/L after 3 years, however, a booster dose raised it to >1000 IU/L (12). In another study on 114 patients with thalessemia major, 60 HBV negative cases were injected with Hepatitis-B vaccine with intervals of 0, 1 and 6 months. After 12 and 72 months, 93% and 80% of cases were low or good responders, respectively (13). While in present study, only 15 months after vaccination 58.6% of the cases were low or good responders. In a study on children in China, serum anti-HBs was 75% within 2 years of vaccination and decreased to 48.2%, 7 years post-vaccination (14). In Spain, serum anti-HBs level was evaluated 6.5 years after vaccination. In this study, 85% of the immunized patients had complete immunity after 6.5 years; therefore administration of booster dose was not advised (15). While in our study, only 35% were good responders 45 months after their vaccination. In Taiwan, 15 years after the vaccination of the neonates, 75% were anti-HBs positive, but the level was not determined (11). In another study in Spain on a pre-pubertal group, 50% of those vaccinated had serum protective anti-HBs level after 7.5-10 years. In their study, it was suggested to have a booster dose 10 years after the primary vaccination to acquire complete immunity (16). In a study on 150 vaccinated children between 1-4.5 years of age in our country, 136 children were anti-HBs positive and 14 cases were anti-HBs negative. In the same group, decrease in serum anti-HBs levels with age was ob-

served (19-20). In some studies, there was no difference between the immune response of the two sexes, but in others, the immune response in females was more than males (22). The reason could be related to differences in average weight of girls and boys. Different results obtained in our study could be explained on the selected target group that is β-thalassemia children. In conclusion, data of this study emphasize that children with major thalassemia are a high-risk group and it is advisable to measure serum anti-HBs level 45 months after the last vaccination and if necessary, give them a booster dose at that time.

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