

Study on Efficacy of Hepatitis B Immunization in Vaccinated Beta-thalassemia Children in Tehran

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Abstract

Objective: In thalassemic children, HBV infection is common, thus immunization against HBV will reduce and prevent the rate of infection. The aim of this study was to evaluate the efficacy of HBV immunization and the prevalence of HBV infection in beta-thalassemic children in Tehran.

Methods: To assess the efficacy of immunization and determine the immune response of children with beta-thalassemia, sera of 99 children who had received three doses (10/20 µg) of recombinant HBV vaccine in months 0, 1, 6, were selected and tested for HBsAg, HBsAb and anti-HBc by ELISA method. Also, these sera were tested for HBV DNA using nested-PCR method.

Findings: In 99 beta-thalassemic children, 89 (89.9 %) were anti-HBs positive (responders) and 10 (10.1%) anti-HBs negative (non-responders). 3 (3.03%) were anti-HBc positive and 1(1.01%) was HBsAg positive. HBV DNA was not detected in any of them.

Conclusion: Our results have revealed that hepatitis B vaccine is highly immunogenic for thalassemic children and particularly well tolerated.

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Key Words: Beta-thalassemia; Vaccination; Immunization Hepatitis B; Iran

Introduction

Thalassemia is one of the most common genetic diseases in the world. It is a major health problem, brings much morbidity, early mortality and great deal of misery for a family both financially and emotionally. Patients with thalassemia major should receive blood transfusion regularly to maintain optimal

hemoglobin (Hb) level. They do not survive for more than 5 years without blood transfusion. Such transfusion will increase exposure to hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV)^[1,2]. Transmission of hepatitis B virus (HBV) infection through donated blood is more common than hepatitis C virus (HCV) infection (1:60000 vs. 1:103000)^[3].

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There are more than 2 billion people with hepatitis B infection worldwide with 350 million people as hepatitis B virus carriers.

At least 35% of Iranians have been exposed to HBV and 3% are chronic carriers. The prevalence of HBsAg in general population is 1.7% and among blood donors 0.5%^[4].

Beta-thalassemia is very common in Iran and some Mediterranean countries. There are at least 800 new cases yearly detected in Iran. Most of these patients need blood transfusion which increases the risk for hepatitis. The prevalence of HBsAg among beta thalassemic patients varies from zero to 1.5%^[5]. Hence, prevention in this high risk group is of great importance^[6].

On the other hand, thalassemic patients may have iron overloading due to chronic blood transfusion which could lead to impaired immune response toward vaccination^[7].

Therefore, determination of immune response in multi-transfused patients is very important.

Active immunization through the hepatitis B vaccination before exposure to virus is the most effective way to prevent infection. Based on the serum levels of anti-HBs, subjects are categorized as good responder (anti-HBs >100IU/ml), low responder (anti-HBs 10-100 IU/ml) and non-responder (anti-HBs <10IU/ml).

After vaccination, anti-body will appear in serum and remains for a long period of time. In 50% of cases, level of the anti-body is not detectable after vaccination and they need to be revaccinated^[8]. In a study from 217 thalassemic patients who were vaccinated against HBV, 18.4% were non-responders^[5].

The aim of this study was to evaluate the efficacy of HBV vaccine and the prevalence of HBV infection in children with thalassemia major disease in Tehran.

Subjects and Methods

Patients: In a cross-sectional study, sera of 99 beta-thalassemic children who had received three doses (10/20 µg) of recombinant HBV vaccine in months 0, 1 and 6 were selected. These children were studied at virology lab of Iranian Blood

Transfusion Organization (IBTO) during 2007-2008.

The study population included 55 males (55.1%) and 44 females (44.9%), aged 3 to 12 years (mean 7.2 ± SD 2.56). On the basis of their age, the patients were classified into 3 groups of 1-5, 6-10, 11-15 years old. About half (59.6%) of our study population was in the second group (6-10 year-olds). In the first group, there were 15 males (55.5%) and 12 females (44.4%), in the second group there were 31 males (52.5%) and 28 females (47.4%) and in the third group there were 8 males (66.6%) and 4 females (33.3%). Venous blood specimens were obtained from all the patients. Serums were separated and aliquots stored at -80°C until tested. This study was approved by the medical ethics committee of IBTO.

ELISA test: Serological markers of HBV infection such as HBsAg, and antibodies to hepatitis B core antigen (anti-HBc) and to HBsAg (anti-HBs) were tested using commercial enzyme linked immunosorbent assay (ELISA) kit (Enzygnost, Dade-Behring, Germany) according to the instructions of the manufacturer.

HBV DNA extraction: HBV DNA was extracted from all serum samples with the high pure viral nucleic acid kit (Roche, Germany) according to the instructions of the manufacturer. For suspension of HBV DNA, 30 µL elution buffer was used.

Nested-PCR: Nested-PCR was performed on all of thalassemics extracted DNA using amplification of a segment of the HBV surface antigen.

The S gene sequence was amplified by nested-PCR on the most conserved regions. In brief, 5µL of the resuspended DNA was added to an amplification mixture containing 2µL of 10x PCR buffer for each sample, 0.5µL dNTP, 0.2µL for each of outer primers, 0.3 µL (1.5U) of taq polymerase (Roche, USA) and 11.8 µL water (total volume of 20 µL).

The PCR profile was an initial 3 min denaturation at 94°C, followed by 35 cycles of amplification including denaturation for 45 s at 94°C, annealing for 60 s at 53°C, and extension for 90 s at 72°C. Strand synthesis was completed at 72°C for 6 min. A 1µL of the first round PCR products was then used for the second round

PCR under the same condition but with the inner primers and water was 15.8 μ l. PCR products were analyzed by horizontal gel electrophoresis on 2% agarose gel in TAE 1x buffer at 85 V/1h. A single 585 bp band was preserved in all cases after the second PCR^[9].

Statistical analysis: statistical analysis was done through χ^2 test using SPSS software ver. 11.5 with $P < 0.05$.

Findings

A total of 99 thalassemic children with mean age 7.2 ± 2.56 (range 3 to 12) years consisting of 55 males and 44 females were investigated in this study. Depending on patients' status, the average blood transfusions in these children was 3-4 times per year. 89 (89.9 %) children were anti-HBs positive (responders) and 10 (10.1%) anti-HBs negative (non-responders); 6 of them were in age group 1-5, and 4 in age group 6-10 year-olds.

1 (1.01%) was HBsAg positive and 3 (3.03%) were anti-HBc positive; all of them were in age group 6-10 year-olds. HBV DNA was not shown in any of cases and none of them was (HBV-DNA) PCR positive (Table 1).

On the basis of the immune status, the thalassemic patients were classified into 4 categories: 87 (87.87%) were immune to HBV via vaccination, 2 (2.02%) were immune to HBV via natural disease (past infection), 1 (1.01%) was carrier of HBV and 10 (10.1) were not immune to HBV (Table 2).

There was no meaningful association between sex and age with anti-HBs response, HBs Ag and anti-HBc in our study.

Discussion

Thalassemic patients are considered to be one of the high risk groups suffering from post-transfusion viral infection such as HBV.

Several studies with controversial results regarding immunity level and duration of acquired immunity from hepatitis B vaccination have been performed in different countries. 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^[10]. In Taiwan, 15 years after the vaccination of neonates, 75% were anti-HBs positive, but the level was not determined. The rate of seropositivity of anti-HBc was 2.9%^[11]. 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 a study in Iran, there was no difference between the immune response of the two sexes, but in others, the immune response in females was more than that in males. The reason could be related to differences in average weight of girls and boys.

In Kerman, Iran, from 215 children with major thalassemia, 34.8% were no responders and the remaining were either low or good responders^[12]. In a study in South Khorasan province, of 38 children with major beta-thalassemia, 16 patients (42.1%) were anti-HBs positive and 22 patients (57.9%) anti-HBs negative^[13].

In our study, 89.9% of these children were HBs anti-body positive (responders) and 10.1% negative (non-responders). Our results showed that HBV vaccine is immunogenic and safe in multitransfused thalassemic patients. This study is in close agreement with the study which has reported on 150 vaccinated children between 1-4.5 years of age. In the same group, decrease in

Table 1: Markers of HBV infection in thalassemic patients

	Positive Number (%)	Negative Number (%)	Total Number (%)
HBsAg	1 (1.01%)	98 (97.99%)	99 (100%)
Anti-HBc	3 (3.03%)	96 (95.97%)	99 (100%)
Anti-HBs	89 (89.9%)	10 (10.1%)	99 (100%)
(HBV-DNA) PCR	0	99 (100%)	99 (100%)

Table 2: Immune status of thalassemic patients with HBV infection

	HBsAg	HBsAb	HBcAb	HBV-DNA	Number (%)
Immune to HBV (vaccination)	-	+	-	-	87 (87.87)
Immune to HBV (natural disease)	-	+	+	-	2 (2.02)
Non immune to HBV	-	-	-	-	10(10.1)
Carrier of HBV	+	-	+	-	1 (1.01)

serum anti-HBs level with age was observed. When the anti-HBs titer falls to less than 10MIU/l HBV infection may occur but this is always subclinical and usually without detectable serum HBsAg. The suspected poor response to hepatitis B vaccine in these children, is due to their immunological abnormalities and frequent transfusion therapy (such as iron overloading)^[5]. Anti-HBc alone may be present in relatively high titers after the disappearance of anti-HBs and indicating recent infection. Anti-HBc is the only anti-body detected after self limited infections in 10% of patients, who never acquire detectable anti-HBs. In addition, anti-HBc is produced in person exposed to HBV and may be a good marker of past HBV infection^[14]. In one study in India, from 70 children with thalassemia, 75.7% had titers exceeding 10IU/l (responders) and 24.3% were non-responders. The seroprevalence of HBV marker (HBsAg and anti-HBc) was 5.7 % and 20%. HBV DNA was detected in 22 of 70 (31.4%) of these thalassemic children^[15].

In a study in South Khorasan province, none of these patients were positive for HBsAg and anti-HBc anti-body^[13]. In our study, 3.03% of our samples were anti-HBc positive and 1 (1.01%) was HBsAg positive (carrier of HBV) but none of them was HBV DNA positive. These results showed that in Iran the prevalence of HBV infection in beta thalassemic patients is lower than in general population^[4].

In our study in a region with intermediate HBV prevalence, we have shown frequency of anti-body response to HBV vaccine in a cohort of thalassemic children. 89.9% of these children were HBs anti-body positive and 10.1% negative. A recent study on 416 vaccinated thalassemic patients (mean age 25.6 yr) who

had low titer of HBsAb and received a booster dose showed that the numbers of positive vicinal patients have been increased from 46.9 % to 69.4%^[16]. Also, in close agreement with our study, this report revealed that response rate to vaccination was more than 95% after complete course (3 doses) in healthy individuals but failure to fulfill vaccination seems a problem in chronic transfused patients^[16]. Therefore, additional studies such as screening of thalassemic pediatric patients should be strictly followed to determine the efficacy of vaccination to protect these patients from HBV infection. To challenge with HBV, it is necessary to measure anti-HBs antibody level and according to their need, booster dose vaccination should be given especially in a high-risk group like thalassemic children.

In our study, there are a few limitations. The sample size was limited to patients who were referred to our lab and all sickle thalassemia and alpha-thalassemia patients were excluded from this study. There was little information about ferritin level and liver enzymes which indicated an iron load in thalassemic patients. In addition, many factors such as genetic features, HLA structure and T cell subgroups may influence response to the hepatitis B vaccination.

Conclusion

Our results have revealed that hepatitis B vaccine is highly immunogenic for children with thalassemia and particularly well tolerated. According to our findings most thalassemic

patients (87.87%) were immune to HBV (responders) and 10.1 % of them were not immune to HBV (non-responders). However, in non-immune to HBV (non-responder) revaccination is recommended.

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Conflict of Interest: None.

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