RESEARCH PAPER

Requirement of a Booster Dose of Hepatitis B Vaccine in Children With Thalassemia After 5 Years of Primary Vaccination: A Prospective Study

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Objective: To determine anti-HBs antibody levels in multi-transfused children with beta-thalassemia major who had received primary hepatitis B vaccination ≥5 years ago, and to document their antibody response to a booster dose of hepatitis B vaccine. **Methods**: We included 85 children each of beta-thalassemia major and age-matched healthy controls, who had completed primary hepatitis B vaccination ≥5 years ago. Participants were assessed for anti-HBs titres, and those with beta-thalassemia major who were seronegative (titres<10 mIU/mL) were administered a single booster dose of hepatitis B vaccine. CD4 counts, serum levels of IL-2 and IFN-γ, and anti-HBs titres were evaluated at baseline and following booster dose of vaccine. **Results**: Seroprotection rates for hepatitis B after an average (SD) duration of 10.8 (3.8) years of completion of primary immunization were significantly higher among children with beta thalassemia major compared to healthy controls (72.9% *vs*. 52.9%, *P*=0.007). All the 23 seronegative children with beta-thalassemia major achieved seroprotection after a single booster dose of hepatitis B vaccine. **Conclusion**: A single booster dose of hepatitis B vaccine after 5 years of primary immunization is adequate to provide seroprotection to multi-transfused children with beta-thalassemia major.

Keywords: *Seroprotection, Infection, Immunity*.

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Children with thalassemia are particularly vulnerable to hepatitis B infection not only on account for multiple transfusions but also due to immunological derangements. The global prevalence rates for hepatitis B surface antigen (HBs Ag) positivity have been reported as 0.3% to 5.7% amongst children with thalassemia [1], with the rates being higher in Asians and South East Asians [2]. A few studies have shown these children to have immune dysfunction, especially cell mediated immunity, possibly due to iron overload [3-6]. Contrarily, a few studies have also shown that this group has a particularly active humoral immune response due to repeated antigenic stimulation [5-7]. The long term seroprotection following hepatitis B vaccination has been reported as to vary from 13-80% [8-11] in different studies and the need for booster doses remains controversial.

We conducted this study in Indian children with beta-thalassemia major who had completed primary hepatitis B vaccination five years or more back. The primary objective of study was comparing their seroprotection rates with those in age-matched healthy controls following primary hepatitis B immunisation. We also measured the responses to a single booster dose of hepatitis B vaccine, and their immune functions.

METHODS

This study was conducted in the thalassemia day care at a tertiary care hospital over a period of one year 17 months (December, 2013 to April, 2015). Eighty-five children with beta-thalassemia major and 85 age-matched healthy controls who had documentary evidence of completion of primary hepatitis B vaccination schedule with three doses given more than five years ago, and without subsequent booster were recruited. Children having evidence of hepatitis B infection or having known immunodeficiency like HIV infection, were excluded from the study. The control group included either the healthy siblings of the children with beta-thalassemia major or the healthy participants visiting the out-patient department for minor illnesses. A written informed consent was taken from the parents/guardians of all participants prior to recruitment in our study. An informed written assent was also obtained for all participants aged more than 12 years.

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Ethical clearance was obtained from the institutional ethics committee.

Participants were divided into three groups on the basis of the time since completion of primary vaccination: Group 1: 60-120 months, Group 2: 121-180 months, and Group 3: 181-240 months. A 2 mL venous blood sample was drawn for baseline investigations (anti-HBc IgG, HBs Ag, anti-HBs, anti-HCV) in all participants. HBs Ag, anti-HCV, and anti-HBc were assessed using ELISA based kits (Biored, France). Anti-HBs titres were measured using ELISA based kits (Diapro). Seroprotection was defined as anti-HBs ≥10 mIU/mL.

All seronegative children with beta-thalassemia major were tested for immunological functions before being administered a single booster dose of recombinant hepatitis B vaccine intramuscularly in a dose of 10 µg to those younger than 20 years, or 20 µg for subjects aged 20 years or older. Immunological functions as ascertained by absolute CD4 count, cytokine assay (IL-2, IFN-γ), and Mantoux test (intradermal test following 5TU). IL-2, IFN-γ and anti-HBs titres were measured in children with beta-thalassemia major before and 4-6 weeks after administration of the booster dose of hepatitis B vaccine.

A 2 mL fresh whole blood was drawn in heparinized tubes for assay of CD4+ cells by flow cytometry (BD FACS Count). Cytokines (IL 2, IFN-γ) were analyzed in sera using ELISA based kits (Diaclone).

The sample size was calculated based on study by Vahidi, et al. [10] and Yazdanpanah, et al. [12]. Seroprotective rate after more than five years of primary vaccination in all patients with beta-thalassemia major was 48.7% [10] and in children without thalassemia was 84% [12]. Considering 20% difference in seroprotection rates between children with beta-thalassemia major and controls as clinically relevant, a sample size of 85 in each group was needed with 80% power and 5% alpha error.

Statistical analysis: Chi-square test was used for comparing the proportion of seroprotection between children with beta thalassemia major and those without thalassemia. Unpaired student *t* test was used for comparing the anti-HBs titres between groups and also the serum ferritin levels between the seroprotected and seronegative children with beta-thalassemia major. SPSS software version 20 was used for analysis.

RESULTS

A total of 88 children with beta-thalassemia major aged 5-20 years were assessed for eligibility in the study; of which 85 (57 males) were included in the study; three subjects were excluded as two were HBsAg positive and one was anti-HBc positive. Eighty five age matched controls (56 males) were also recruited.

Participants in both groups were comparable with respect to mean (SD) age [11.4 (4.1) vs 11.2 (4.3) years; *P*=0.79] and gender [67% vs 66% males; *P*=0.87] in thalassemia group and the control group, respectively. The mean (SD) time lag between last dose of hepatitis B vaccine and inclusion in the study was 10.7 (3.7) years in the thalassemia group and 10.9 (3.9) years in the control group (*P*=0.34). The median (IQR) anti-HBs titres in the thalassemia group were higher than in control group [35.90 (9.3, 262.0) vs 14.40 (1.0, 55.8) mIU/mL; *P*=0.001]. Seroprotection rates were significantly higher in the thalassemia group (72.9% vs 27.1%; *P*=0.007).

A total of 23 children with beta-thalassemia major were found to lack seroprotective titers following primary hepatitis B vaccination. Their mean (SD) age was 11.9 (4.6) years with a mean (SD) time lag between completion of primary hepatitis B vaccination and estimation of anti-HBs titers as 11 (3.7) years. All of them were administered a booster dose of hepatitis B vaccine. Anti-HBs titers estimated after 4.7 (0.75) weeks were >10 mIU/mL for all children. The median (IQR) of anti-HBs titers before and after booster dose of hepatitis B vaccine was 5.1 (1.3-8.2) mIU/mL and 278.1 (170.1-353.0) mIU/mL, respectively; 22 children had anti-HBs titers > 100 mIU/mL.

The proportion of seroprotected children and time since primary vaccination did not show a statistically significant relationship (*P*=0.25). The proportion of seroprotected children in the beta-thalassemia major group after 60-120 months, 121-180 months and 181-240 months of primary vaccination were 77.1%, 74.3% and 60%, respectively.

Among the 23 seronegative children with beta-thalassemia major, CD4 counts were normal in all except

Table I Response to a Single Booster Dose of Recombinant Hepatitis B Vaccine in Children With Thalassemia Having Anti-HBs Titre <10 IU/mL After Primary Hepatitis B Vaccination (*N*=23)

Parameter	*Before booster dose*	*After booster dose*
Anti-HBs titers (mIU/mL)	5.1 (1.3-8.2)	278.1 (170.1-353.0)
Interleukin 2 levels (pg/mL)	0	0 (0-1422)
CD4 count (cells/mm^3)	869 (682-1050)	-

Values in median (IQR).

WHAT THIS STUDY ADDS?

- Majority of multi-transfused children with β–thalassemia major have seroprotective titers even after five years of primary vaccination.

two children, IL-2 was detectable in only two children and IFN-γ was undetectable in all children. Even following antigenic stimulus (HBV booster), only ten children had detectable IL-2 and five had detectable IFN-γ levels. There was no significant correlation of HCV infection (*P*=0.43), body mass index (*P*=0.06), serum ferritin level (*P*=0.77) and chelating agents [deferiprone (*P*=0.413), deferasirox (*P*=0.18), desferrioxamine (*P*=0.55)] with immune response to primary hepatitis B vaccination.

DISCUSSION

In this cross-sectional study, we found that nearly three-fourths of children with beta-thalassemia major had seroprotective titres even after five years of completing the primary hepatitis B vaccination. Further, the seronegative children with β-thalassemia major mounted an anamnestic response to a booster dose of hepatitis B vaccine.

Limitations of the present study include the lack of serial annual titres of anti-HBs in the participants to find out the exact time of fall to seronegative levels. The strengths of our study include its robust sample size. We also evaluated markers of cell mediated immunity in our thalassemic cohort. However, a comparison of CD4 counts and cytokine levels in seronegative children with beta-thalassemia major and control group was not possible due to financial constraints. This information would have offered more insights into this study.

Reported seroprotection in 72.9% children with beta-thalassemia major from other studies have ranged from 13% to 80% following 3 to 6 years of primary Hepatitis B vaccination [8-11]. The waning immunity following primary hepatitis B vaccination after every passing year has been reported even in countries with high prevalence of hepatitis B infection [13]. In a follow up study from Taiwan [13], it was shown that universal hepatitis B vaccination in infancy led to adequate protection up to 14 years of age and in the absence of a booster the hepatitis B surface antibody (anti-HBs) decayed at an annual rate of 10.2%, although the new infection rates did not differ in children who received and those who did not receive booster hepatitis B vaccine.

The higher number of children with thalassemia presenting with protective titres than healthy controls could be due to higher incidence of antigenic stimuli to which children with thalassemia are exposed following repeated blood transfusions. A few studies have suggested that a possible reason for having higher protection rate is due to passive transport of anti-HBs antibodies through the donor blood [14,15]. We feel that a need for a booster dose in such individuals needs to be determined on a case-to-case basis depending upon the risk and vulnerability to hepatitis B.

Following, a single booster dose, all the previously seronegative children in this study developed protective titres showing an intact humoral response, while the cell mediated response appeared blunted as demonstrated by low rates of detection of cytokines. Previously, few studies have demonstrated dysfunctional cell mediated immunity in these children [3-6].

Based on our findings and considering the increased risk of hepatitis B in children with beta-thalassemia major, we suggest regular assessment of anti-HBs titres following primary hepatitis B vaccination and recommend administration of a booster dose whenever indicated at the earliest.

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