

Immune Status Against Hepatitis B Infection in Children with Thalassemia Major in Pakistan: A Single-Centre Study

Misbah Fakh, Faisal Basheer*, Qamar uz Zaman Khan, Fasih Hashmi**, Hafsa Asad

Pak Emirates Military Hospital/National University of Medical Sciences (NUMS), Rawalpindi Pakistan, *Combined Military Hospital Bahawalpur/National University of Medical Sciences (NUMS) Pakistan, **Allama Iqbal Open University, Islamabad Pakistan

ABSTRACT

Objective: To assess the immunogenicity of the Hepatitis B Vaccine in children with thalassemia.

Study Design: Prospective longitudinal study.

Place and Duration of Study: Pak Emirates Military Hospital, Rawalpindi Pakistan from Aug 2018 to Jan 2019.

Methodology: After ethical approval and informed consent, 150 diagnosed patients with thalassemia were selected from the Thalassemia centre at Pak Emirates Military Hospital Rawalpindi. The patients' transfusion, vaccination history and clinical data were obtained. The patient's sera were tested for Anti-Hepatitis B surface antibodies (anti-HBs) by ELISA. The patients who showed seroprotection (anti-HBs titer >10 IU/L) were taken as a case group.

Results: Out of 150 patients, only 22% (33 patients) showed seroprotection (anti-HBs titer >10 IU/L). Children younger than four years had significantly positive anti-HBs (p -value=0.067) at a 5% significance level. Time since the last vaccination was also a significant factor, with the 3.76 ± 3.072 years range exhibiting better protection ($p=0.002$).

Conclusion: Protective Anti-HBs titer was reduced after the age of 4 years in our patients, so we recommend screening patients with thalassemia after four years to assess the need for a booster dose.

Keywords: Beta-thalassemia, Hepatitis B, Immune status.

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INTRODUCTION

Thalassemia is the most prevalent disorder worldwide, affecting a single gene. The prevalence is documented worldwide, up to 3% in the general population and 7% of pregnant ladies.¹ Approximately 1.5% of the population is estimated to be carriers of β -thalassemia, with 50–60,000 new patients with thalassemia being born each year.² In Pakistan, thalassemia's carrier rate is about 5–8% which differs province-wise.³ Thalassemia is transfusion-dependent anaemia. The quality of life of thalassaemic patients remains very poor in developing countries like Pakistan with multiple contributing factors, including the financial burden of regular transfusion, poor compliance with chelation and its side effects, society bias, and emotional and psychological impacts of the disease,⁴ Transfusion acquired infections such as hepatitis B, hepatitis C, HIV, CMV have been a major problem, particularly in multi transfused thalassaemia patients due to lack of awareness and poor donor screening practices (sub-optimal or deficient quality control, reliance on rapid testing in some settings, etc.).⁵ The prevalence of

Hepatitis-B in the general population in Pakistan is estimated to be 2.76%,^{6,7} beta thalassemia patients being the high-risk group due to chronic transfusion therapy. The expanded immunization program (EPI) in Pakistan recognized the lifelong burden of the Hepatitis-B virus and added Hepatitis-B immunization to the EPI schedule in 2009. This study aimed to determine the immunogenicity of the Hepatitis-B vaccine in children with thalassemia, the most prevalent transfusion-dependent anaemia in our country, on chronic transfusion therapy belonging to various age groups.

METHODOLOGY

The prospective longitudinal study was conducted at Pak Emirates Military Hospital Rawalpindi from August 2018 to January 2019. Ethical approval was sought (Letter No. A/28/PEMH/19/EC/05). Informed consent was taken from all the patients.

Inclusion Criteria: Beta thalassemia patients less than 19 years old, on regular transfusion therapy, vaccinated as per EPI after 2009, were included in the study through consecutive sampling.

Exclusion Criteria: Patients who received a booster dose of vaccine, intermedia patients of beta thalassemia, already treated for Hepatitis-B infection and with comorbid conditions such as immunodeficiency were excluded in the study.

Correspondence: Dr Misbah Fakh, House No. 554, Lane No. 6, Street No. 18, Chaklala Scheme III, Rawalpindi, Pakistan
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For sample size calculation (WHO sample size calculator), we use one sample single proportion test based on the proportion of exposed/cases in the population. The prevalence of exposed cases were 22%,⁸ keeping an absolute precision of 7%, the required sample size was 135. One hundred and fifty beta thalassemia patients were included in the study.

The patients were registered at the Thalassemia Centre in Pak Emirates Military Hospital (PEMH), Rawalpindi Pakistan. All the patients' history was documented, especially the age at diagnosis and start of regular transfusion therapy, the interval between the transfusions, surgical procedure done for spleen removal and comprehensive physical examination. The laboratory tests on a 5ml blood sample included a complete blood picture, liver enzymes, bilirubin and ferritin levels.

The tests were performed with fully informed consent. Anti-HBs titers were also performed. Enzyme-linked immune absorbent assay (ELISA) kits for anti-Hepatitis-B surface antibody (anti-HBsAb) in human serum or plasma samples (G.B. Lippincott Company, USA) were used for quantitative estimation of anti-hepatitis-B surface antibody (anti-HBs Ab) titer. The results were interpreted as per manufacturer instructions. Anti-hepatitis-B surface antibody (anti-HBsAb) titer >10IU/L was considered positive, and <10 IU/L was considered negative.

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Frequency and percentages were used to describe the qualitative variables of the study, whereas mean and standard deviation were used for quantitative variables. We used the chi-square or likelihood ratio test to compare the proportion of hepatomegaly, jaundice, splenomegaly and splenectomy. In contrast, independent sample t-test was used to compare the average age, ferritin level, number of transfusions and years since the last vaccination between the two Groups. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

We selected 150 diagnosed beta-thalassemia major children aged less than 19 years. Out of 150, eighty-four (56.0%) were males, and 66(44.0%) were females. The mean age of 150 children was 7.14±4.11 years. Thirtythree patients (22.0%) had positive anti-HBs, while the remaining 117(78%) were negative (Table-I). Out of 150, one hundred and nine patients (72.7%) had hepatomegaly, 73(48.7%) were affected by jaundice,

84(56.0%) had splenomegaly, and 25 patients (16.7%) had splenectomy done (Table-II).

Table-I: Descriptive Statistics (n=150)

Variables	Categories	Frequency (%)
Age Groups	Toddlers (1-3) years	31 (20.7)
	Pre-School (4-6) years	47 (31.3)
	School age (6-11) years	46 (30.7)
	Adolescent (12-19) years	26 (17.3)
Gender	Female	66 (44.0)
	Male	84 (56.0)
Anti-HBs	Positive	33 (22.0)
	Negative	117 (78.0)

Table-II: Clinical Signs and Symptoms or Acute Infection (n=150)

Variables	Categories	Anti-HBs (+ve) (n=33) n(%)	Anti-HBs (-ve) (n=117) n(%)	<i>p</i> -value
Hepatomegaly	Yes	16(14.7)	93(85.3)	<0.01
	No	17(41.5)	24(58.5)	
Jaundice	Yes	12(16.4)	61(83.6)	0.109
	No	21(27.3)	56(72.7)	
Splenomegaly	Yes	10(11.9)	74(88.1)	0.001
	No	23(34.8)	43(65.2)	
Splenectomy	Yes	7(28.0)	18(72.0)	0.428
	No	26(20.8)	99(79.2)	

The mean ferritin level was 2901.89±1492.92 ng/mL, the average number of transfusions was 84.94±50.74 and the average time since the last vaccination was 5.19±3.07 years recorded in our data.

To test the difference in ferritin level, average number of transfusion and average number of years since last vaccination for positive and negative anti-HBs titers, we used an independent sample t-test. The results showed that the years passed since the previous vaccination significantly affected the anti-HBs level, and the immunity against Hepatitis-B decreased with time. In addition, the high frequency of HBV markers proportional to the number of transfusions indicates a greater chance of acquiring the infection through blood products that miss detection during routine screening (Table-III).

Table-III: Comparison of Age, Time since Vaccination, No. of Transfusion, and Ferritin Level to Anti-HBs Result (n=150)

Variables	Anti-HBs (+ve) (n=33)	Anti-HBs (-ve) (n=117)	<i>p</i> -value
Age (years)	5.98±4.83	7.47±3.84	0.067*
Ferritin level (ng/mL)	2530.18±1623.38	3006.74±1444.13	0.106
Number of Transfusion (per year)	71.57±58.56	88.71±47.91	0.087**
Number of years since last Vaccination	3.76±3.072	5.59±2.96	0.002*

DISCUSSION

Thalassemia major patients depend on regular blood transfusions to sustain life and expensive chelation therapy and other medical management. As a result, thalassaemia is a major healthcare challenge, places great psychological and financial stress on the affected families, and is a huge burden on the national healthcare delivery system.⁷ Although the mean survival has increased recently, quality of life remains a big question mark.⁸ Frequent blood transfusions have their hazards. Blood products are screened for a variety of blood-borne diseases, but even then, the risk of parenteral infection is very high. These patients subsequently suffer from various conditions, including chronic hepatitis and chronic liver disease due to hepatitis B and C virus transmission.

They pose a significant burden on the already under-resourced health system of developing countries like Pakistan.⁹ The Hepatitis B vaccine protects against hepatitis B infection and subsequent hepatocellular carcinoma, which are both the causes of chronic liver disease. Currently, the therapeutic options for treating chronic HBV infection are difficult to implement and not fully effective, leaving room for further improvements. Vaccination is an easy and cost-effective measure to prevent disease and conditions. Additionally, vaccination eliminates the incidences of persistent HBV infection and chronic liver disease and diminishes the pool of chronic carriers, thus limiting transmission of infection to susceptible contacts.¹⁰ Chronic hepatitis secondary to hepatitis B virus is a major cause of chronic liver disease and hepatocellular carcinoma. The infection can be perinatally acquired or during the lifetime due to multiple risk factors, transfusion being an important one.¹¹ The Hepatitis B vaccine protects against hepatitis B infection and subsequent hepatocellular carcinoma, both the causes of chronic liver disease, especially in children who acquire the infection during infancy and early childhood.¹² Introducing the Hepatitis vaccine in the EPI program has effectively cut down the disease burden in disease-prevalent regions like Pakistan. If done according to the guidelines, the vaccination induces an immune response in >90% of the population. As part of the expanded immunization program (EPI), it is administered at 6, 10 and 14 weeks. These three doses produce a protective response described as anti-hepatitis B surface antibody (anti HBsAb) titers >10 IU/L in greater than 90% of children and adults. However, after ten years, the immune response loses its efficacy,

especially in high-risk children and adults, and some studies suggested a booster dose.¹³ One of the studies also found that 50% of patients undergoing repeated or frequent transfusions show undetectable levels of anti-hepatitis B surface antibody (anti-HBsAb) titers, necessitating the need for booster and, in some cases, revaccination.¹⁴ Booster doses ensure a sustained immune response. Without booster doses, the immune response decay rate is 10% per year.¹³

Normal immune response in good responders (anti-HBs > 100IU) declines from 75-28% one month after the vaccination to one year after. However, the protective titers (anti HBs >10 IU) are found in 93.7% of the population one month after the vaccination. One year after the vaccination, protective antibody titers were found in 82% of the patients. This decline creates a marked difference in protective titers between children <3 years of age and children >3 years of age in the general population.¹³

Compared to the general population, the results in the high-risk group show marked variability in the immune response. Among these high-risk groups, chronic transfusion therapy and hemodialysis patients are of special interest. Studies show that patients undergoing chronic transfusions are better protected than hemodialysis patients (80% vs 38%). One of these studies also documented the effect of a booster dose of hepatitis vaccine on immunity status and found that a booster dose increased the seroprotection rate from 46.9% to 69.4%.^{14,15}

Our study showed that only 22% of the vaccinated thalassaemic patients undergoing chronic transfusion therapy had adequate protective anti-hepatitis B surface antibody (anti-HBsAb) titers. These results are comparable to other studies, especially one from Egypt that showed that 26% of thalassemia patients had protective anti-hepatitis B surface antibody (anti-HBsAb) titers but is in contrast to the Iranian study that documented only 34% of the children as non-responder meaning anti-HBsAb level <10.¹⁶ In addition, in another study from Egypt, inadequate response to the vaccine was also reported compared to the age and sex-matched healthy controls (36% vs 90%) in children suffering from diseases requiring repeated blood transfusions for causes other than thalassaemias such as sickle cell anaemia and leukemia.¹⁵

In our study, we found out that children less than four years of age had a high percentage of anti-hepatitis B surface antibody (anti-HBsAb) titers compared to children older than four years of age. These

results are like the previous studies that showed a high percentage of immune-protected beta thalassemia children (42.1%) under three years of age. However, a blunted immune response was observed in children aged 3-6 years (13%). This blunting of immune response was also observed in the general population of Chinese children, with 75% within two years of vaccination vs 48.2% seven years after vaccination. Nevertheless, the decline in immune response was greater for thalassemia patients than for the general population.¹⁶ Our results are comparable to other studies that conclude that children undergoing chronic transfusion therapy gradually lose protective antibody titers and become vulnerable to developing hepatitis B infection. Although the timeline of decline in immunity is variable, these studies document that a significant drop occurs after the age of three years.^{15,16}

Although 78% of our patients lacked the protective antibody response, none of them was found to be hepatitis B surface antigen (HBsAg) positive. These results are comparable to other studies that report the very low prevalence of chronic hepatitis infection in thalassemia children.¹⁷

In good centres in Pakistan, proper screening procedures for blood product transfusion are in place, but unfortunately, the number of such centres in Pakistan is less. Therefore, most patients suffering from thalassemia depend on blood banks in the public sector, are working in utter resource constraints and cannot provide adequate screening for blood products. In other developing countries, due to the improvement of screening and safety procedures in blood product transfusion, the rate of hepatitis B surface antigen (HBsAg) positivity has declined from 16.5 to 2% over 20 years thalassemia patients. This decline can be attributed to the introduction of immunization against hepatitis B as well as improved facilities in blood banks.¹⁸

LIMITATIONS OF STUDY

The limitation of our study was the lack of records of the initial response to the vaccination. So exact cause of this decline in immune response at around three years of age cannot be ascertained. These low tiers can be due to a decline in immune response or poor response to the initial vaccine series.

RECOMMENDATIONS

We recommend screening all thalassemia patients undergoing chronic transfusion therapy at three years of age for protective anti-hepatitis B surface antibody titers (anti-HBs Ab), considering a drop of protective

antibody titers at this age, and recommend a booster vaccine in children if the titers are found to be in non-protective range. We also recommend documentation of anti-HBs titers in infants newly diagnosed as thalassemia major to document the immune response and take necessary precautions for the non-responders.

CONCLUSION

Due to chronic transfusion therapy, Thalassemia children were at increased risk of developing hepatitis B infection by transfusion being a significant risk factor and blunted immune response to hepatitis B vaccine as compared to the general population. Our study found that the number of years has passed since the last vaccination, and the frequency of patients with seroprotective levels of anti-HBsAg antibody decreases. This study also finds out that despite the low levels of seroprotective antibodies in the majority of patients greater than four years of age, the prevalence of hepatitis B is very low. Although better screening of blood products can play a crucial role in limiting the spread of hepatitis B infection, in developing countries like Pakistan, these facilities are not available widely.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

MF: Conception, study design, drafting the manuscript, approval of the final version to be published.

FB: Study design, drafting the manuscript, critical review, approval of the final version to be published.

QZK: Critical review, approval of the final version to be published.

FH: Data acquisition, data analysis, interpretation of data, approval of the final version to be published.

HA: Drafting the manuscript, interpretation of data, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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