Original Article

Frequency of Hepatitis B Infection in Vaccinated Children with β-Thalassemia Major Receiving Multiple Transfusions

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Abstract

Objective: To determine the frequency of hepatitis B infection in vaccinated children with beta thalassemia major receiving multiple blood transfusions.

Methods: This was a cross-sectional study with a non-probability consecutive sampling technique conducted at the Department of Pediatrics, Cantonment General Hospital & Holy Family Hospital, Rawalpindi, from January 26, 2022, to July 26, 2022. A total of 245 children diagnosed with beta thalassemia major and receiving regular transfusion therapy were enrolled. Venous blood samples were collected and tested for hepatitis B surface antigen (HBsAg) using an enzyme-linked immunosorbent assay (ELISA). Demographic and clinical data were recorded, including age, gender, and number of transfusions. Quantitative variables, such as age and transfusion frequency, were expressed as mean ± standard deviation (SD), while qualitative variables, including gender and hepatitis B positivity, were expressed as frequencies and percentages.

Results: The mean age of patients was 9.56±4.3 years. Of the total, 170 (69.4%) were male and 75 (30.6%) were female. Children receiving fewer than eight transfusions annually were 136 (55.6%), while those receiving eight or more were 109 (44.4%). Hepatitis B infection was detected in 20 children (8.2%).

Conclusion: Hepatitis B infection is still prevalent in vaccinated children with transfusion-dependent beta thalassemia major and is more frequent in those receiving a higher number of blood transfusions. Strengthening blood donor screening programs and considering booster vaccination strategies may help reduce this burden.

Keywords: Beta thalassemia major, Blood transfusion, Child, Hepatitis B, Vaccination

Contributions:
SS, STM, - Conception, Design
STM, - Acquisition, Analysis, Interpretation
STM, JAK, RA - Drafting
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Introduction

Hepatitis B virus (HBV) infection remains one of the most important public health problems worldwide, despite the development of a safe and effective vaccine more than four decades ago. Globally, nearly 296 million people are living with chronic HBV infection, and the disease continues to contribute significantly to liver-related morbidity and mortality, particularly cirrhosis and hepatocellular carcinoma.[1,2] The burden is especially high in low-and middle-income countries, including Pakistan, where HBV is endemic.

Children with transfusion-dependent beta thalassemia major are particularly vulnerable to HBV infection because of their lifelong dependence on regular blood transfusions.[3] In Pakistan, the HBV vaccine was incorporated into the Expanded Program on Immunization (EPI) in 2009, and donor screening has become routine; however, sporadic cases of HBV infection are still being reported in this group.[4,5]

It is well established that HBV vaccination provides protective antibody responses in over 90% of healthy children, and strict transfusion safety protocols have significantly reduced the risk of infection in many developed countries.[6,7] Yet, the persistence of HBV infection among vaccinated children with thalassemia raises critical questions. It remains unclear whether these cases are due to primary vaccine failure, waning immunity over time, the emergence of HBV escape mutants, or lapses in blood transfusion safety practices.[8,9]

Available literature from Pakistan has reported variable prevalence rates of HBV infection in thalassemia patients. However, most of these studies were conducted before the nationwide implementation of EPI or were limited to single centers with small sample sizes.[10,11] Thus, contemporary multicenter data reflecting the current situation in vaccinated thalassemia patients are lacking.

This knowledge gap has important implications. Without robust and updated evidence, it is difficult to determine whether existing preventive measures are sufficient or if additional strategies such as booster vaccination, periodic serological monitoring, or further strengthening of donor screening are needed in high-risk populations.

The rationale of the present study was to address this deficiency in knowledge by estimating the frequency of HBV infection in vaccinated children with beta thalassemia major receiving regular blood transfusions. By filling this gap, the study aimed to provide evidence that could guide improvements in vaccination policies and transfusion safety practices in Pakistan. Therefore, the objective of this study was to determine the frequency of HBV infection in vaccinated children with beta thalassemia major who were receiving multiple blood transfusions.

Materials And Methods

This cross-sectional study using a non-probability consecutive sampling technique was conducted in the Department of Pediatrics, Cantonment General Hospital, and Holy Family Hospital, Rawalpindi, over a period of six months from 26th January 2022 to 26th July 2022. The sample size was calculated using the OpenEpi

online calculator with an anticipated prevalence of 4.12%, a confidence level of 95%, and a precision of 2.5%, yielding a required sample size of 245 children. Ethical approval for the study was obtained from the Institutional Ethical Review Board via letter No. 02, dated 20/01/22. Written informed consent was obtained from the parents or guardians of all participating children, and confidentiality was maintained by coding all study data without identifiers. Children of either gender aged 1 to 15 years with a diagnosis of beta thalassemia major, confirmed by haemoglobin electrophoresis showing HbF >90% before transfusion, and who had received complete HBV vaccination under the Expanded Program on Immunization (EPI) schedule were included. Children with thalassemia minor, those with a prior diagnosis of hepatitis B or hepatitis C, a history of surgery, or chronic liver disease defined as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels >50 IU/L were excluded from the study. Data were collected by trained pediatric residents under the supervision of consultant pediatricians. After obtaining consent, a venous blood sample of 3 mL was drawn aseptically and labeled with patient identifiers. Samples were transported immediately to the hospital laboratory, kept at room temperature, centrifuged, and tested within two hours using an enzyme-linked immunosorbent assay (ELISA) for hepatitis B surface antigen (HBsAg). Laboratory results were recorded as either positive or negative. Demographic data, including age, gender, and transfusion history, were documented in a structured data collection form. Children receiving more than eight transfusions per year were classified as receiving multiple transfusions. Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 24. Quantitative variables such as age and annual number of transfusions were expressed as mean ± standard deviation(SD), while qualitative variables such as gender and hepatitis B status were presented as absolute numbers with corresponding percentages. The chi-square test was applied to compare categorical variables. Statistical significance was set at a two-sided p-value of <0.05. Exact p-values and 95% confidence intervals (CI) were reported where appropriate.

Results

A total of 245 patients were enrolled in the study. The mean age of patients was 9.56±4.3 years. Of the 245 patients, 170 (69.4%) were males and 75 (30.6%) were females. With respect to transfusion history, 136 patients (55.6%) had received fewer than 8 transfusions, whereas 109 patients (44.4%) had received more than 8 transfusions (Table 1).

Table 1: Demographic Characteristics of Study Population (N = 245)

		Frequency	Percent
Age			
	1-10 years	128	52.2%
	11-15 years	117	47.8%
Gender			
	Male	170	69.4%
	Female	75	30.6%
Number of Transfusions			
	<8	136	55.55%
	≥8	109	44.44%

Hepatitis B surface antigen (HBsAg) was detected in 20 patients (8.2%) (Figure 1). All positive cases were observed in children with more than 8 transfusions (20 of 109; 18.3%), while no positive cases were identified among those with fewer than 8 transfusions (0 of 136; 0%). This association was statistically significant (p = 0.00) (Table 2).

On age-based analysis, 8 of 128 children (6.3%) aged 1–10 years and 12 of 117 children (10.3%) aged 11–15 years tested positive for hepatitis B, though the difference was not statistically significant (p = 0.257). According to gender, 15 of 170 males (8.8%) and 5 of 75 females (6.7%) were positive, with no significant difference between groups (p = 0.570) (Table 3).

Table 2: Comparison of Hepatitis B Status by Number of Transfusions: N=245

Hepatitis B	No. of Transfusions	No. of Transfusions	Total	P Value
	<8	**>8**		
Positive	0	20	20	
	0%	18.34%	8.2%	**<0.001**
Negative	136	89	225	
	100%	81.65%	91.8%	

Table 3: Age and Gender Stratification of Hepatitis B Cases: N=245

		Hepatitis B Positive	Hepatitis B Negative	P Value
Age				
	1-10 years (N128)	8(6.3%)	120(93.7%)	**0.257**
	11-15 years (N117)	12(10.3%)	105(89.7%)	
Gender				
	Male (N170)	15(8.8%)	155(91.2%)	**0.570**
	Female(N75)	5(6.7%)	70(93.3%)	

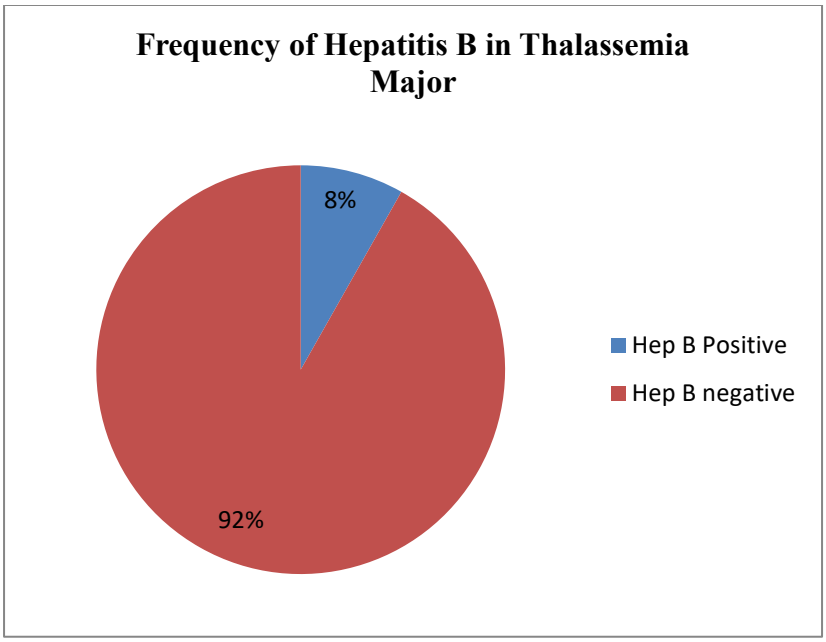

Figure 1: Frequency of Hepatitis B cases in thalassemia patients (N=245)

Discussion

In the present study, hepatitis B surface antigen was detected in 8.2% of cases, with a clear association between infection and the number of transfusions received. Infection was observed exclusively in those with more than eight transfusions, while none of the patients with fewer than eight transfusions tested positive. When the effect of age was considered, hepatitis B infection was found in 6.3% of children aged 1–10 years and 10.3% in those aged 11–15 years, though this difference did not reach statistical significance. These findings indicate a possible cumulative risk with advancing age, consistent with longer transfusion exposure, though the relatively small subgroup sizes may have limited statistical power. Thus, transfusion frequency emerges as the only variable with a strong and statistically significant effect in this cohort.

Our results align with previously published data. Ehsan et al. reported a prevalence of hepatitis B ranging between 0.66% and 7.4% in a pooled analysis of 14 studies, with risk rising in older children and those with higher transfusion requirements.[10] The slightly higher prevalence in our study (8.2%) compared to the pooled estimates may reflect limitations in local donor screening. Similarly, Naz et al. demonstrated an incidence of 11.3% among children receiving more than 25 transfusions annually,[11] which is close to the 18.3% positivity rate observed in our group with more than eight transfusions, both highlighting the cumulative risk. Nadir et al. also observed that 17.8% of children receiving eight or more transfusions were hepatitis B Positive,[12] almost identical to our results, further supporting the strength of this association.

Mukhtar et al. documented an overall prevalence of 8.4% in thalassemic children,[13] nearly the same as our observed rate, reinforcing that the burden in Pakistan remains substantial. Cacopardo et al. from Italy reported an 8% prevalence among transfusion-dependent thalassemics,[14] which mirrors our findings. However, studies from Palestine and India have reported lower prevalence rates of 0.7% and 1.5% respectively.[15,16] The discrepancy likely reflects stricter donor screening, nucleic acid testing, and robust transfusion safety measures in those countries, whereas lapses in donor selection and limited use of molecular screening persist in our region.

Data from Iran further illustrate the variability in prevalence. Allamehzadeh et al. found hepatitis B positivity in 8.4% of thalassemic patients,[17] which corresponds with our data, while Farshadpour et al. reported 3.17% seropositivity and 1.59% occult infection,[18] and Fatemeh et al. observed 1.16% occult infection.[19] The lower prevalence in these Iranian studies compared to our results may be attributed to effective vaccination coverage, improved blood safety, and detection of occult infections through advanced methods. These comparisons highlight that while the prevalence of hepatitis B among thalassemia patients is declining in developed and some middle-income countries, it remains relatively high in Pakistan due to systemic weaknesses in transfusion services.

The consistent finding across studies is that the risk of hepatitis B infection correlates strongly with the number of transfusions. The lack of positive cases among patients with fewer than eight transfusions in our study further confirms this dose–response relationship. Although age and gender did not significantly influence infection in our cohort, other studies have reported higher rates in older age groups, [11,12,] which is biologically plausible due to cumulative transfusion exposure.

This study is limited by its single-center design, relatively small sample size in subgroup analyses, and the absence of molecular testing, such as PCR, which could identify occult infections. Multicenter studies with larger samples and molecular diagnostic tools are required to determine the true burden of both overt and occult hepatitis B infection in thalassemia patients.

In conclusion, hepatitis B remains a significant transfusion-related infection among vaccinated thalassemia children in Pakistan, with prevalence strongly associated with the number of transfusions. The results are comparable to regional studies but higher than those reported from countries with stringent transfusion safety measures. These findings highlight the urgent need for stricter donor screening, widespread use of nucleic acid testing, reinforcement of vaccination with booster doses, and patient education to prevent transmission in this vulnerable group.

Conclusions

Hepatitis B infection is still prevalent in vaccinated children with transfusion-dependent beta thalassemia major and is more frequent in those receiving a higher number of blood transfusions. Strengthening blood donor screening programs and considering booster vaccination strategies may help reduce this burden.

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