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Frequency of Hepatitis B, C, and Human Immunodeficiency Virus (HIV) among Children with Transfusion-Dependent Thalassemia at the National Institute of Child Health, Karachi, Pakistan

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ABSTRACT

OBJECTIVE: To evaluate the current screening and transfusion practices for blood-borne infections and the frequency of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) among children with transfusion-dependent thalassemia (TDT).

METHODOLOGY: This cross-sectional study was conducted at the Pediatric Outpatient Department of the National Institute of Child Health, Karachi, Pakistan, from February to July 2025. A total of 230 children aged ≥1 year with TDT major were analyzed. Demographics, disease duration, transfusion history, therapy type, HBV vaccination status, and presenting complaints were recorded. Data were entered and analyzed using IBM SPSS Statistics version 26.0.

RESULTS: Of a total of 230 patients, 127 (55.2%) were male, and the mean age was 8.6±4.1 years. HCV infection was detected in 29 (12.6%), and HBV in 6 (2.6%) children, while no HIV case was identified. HCV-positive children were relatively older (10.9±3.8 vs. 8.3±3.0 years, p<0.001), had longer disease duration (8.2±3.3 vs. 6.3±3.6 years, p=0.008), higher monthly transfusions (2.9±0.7 vs. 2.6±0.6, p=0.015), less HBV vaccination (58.6% vs. 77.1%, p=0.032), had fatigue (72.4% vs. 50.7%, p=0.029), lower hemoglobin (7.2±0.9 vs. 7.7±1.0 g/dl, p=0.012), and higher ferritin (3204.5±910.4 vs. 2584.1±762.8 ng/ml, p=0.001). HBV-infected children were less likely to be vaccinated (16.7% vs. 76.3%, p=0.001).

CONCLUSION: This study highlighted a significant burden of HCV and, to a lesser extent, HBV infection among transfusion-dependent beta-thalassemia children, with strong associations to older age, longer disease duration, higher transfusion frequency, and incomplete vaccination.

KEYWORDS: Children, hepatitis B, hepatitis C, human immunodeficiency virus, thalassemia.

INTRODUCTION

Beta-thalassemia major is one of the most severe inherited hemoglobinopathy, caused by mutations in the β-globin gene on chromosome 11 [1]. Although the disorder is most prevalent in populations of the Mediterranean basin, it is also frequently encountered in Southeast Asia and Africa[2]. Globally, an estimated 270 million individuals are carriers of thalassemia, of whom approximately 80–90 million (1.5% of the world population) specifically carry β-thalassemia mutations. Each year, about 300,000–400,000 infants are born with serious hemoglobin disorders, including nearly 23,000 with β-thalassemia major. Notably, up to 90% of these births occur in low- and middle-income countries[3,4].

In Pakistan, β-thalassemia major is one of the most common genetic disorders. The estimated carrier frequency is 5–7%, corresponding to a carrier pool of nearly 9.8 million individuals[5]. According to the Thalassemia Federation of Pakistan, the prevalence of β-thalassemia trait is approximately 6%, with more than 50,000 registered patients across treatment centers nationwide[6]. Management of this condition largely depends on lifelong, regular blood transfusions, resulting in millions of blood units being collected worldwide annually[7,8]. However, repeated transfusions place patients at considerable risk of transfusion-transmitted infections (TTIs) [9,10]. While the implementation of stringent donor blood screening protocols has nearly eliminated TTIs in developed nations, developing countries such as Pakistan still face challenges due to resource constraints and inconsistent adherence to recommended safety standards.

Different studies from Pakistan worked on hepatitis B or C virus or HIV among beta-thalassemia major children and reported different prevalence. A recent Pakistani study by Ahmed et al from Baluchistan worked on multiple hepatitis B and C viruses among beta-thalassemia major children and reported that 2.8% of children had hepatitis B virus (HBV) and 18.3% children had hepatitis C virus (HCV), two of whom were infected with both HBV and HCV[11]. Another Pakistani study by Khalil S 2016[12] from Rawalpindi examined the prevalence of HBV and HCV in children with beta-thalassemia major and reported 5.0% of children with HBV and 38.7% of children with HCV. Beta-thalassemia major patients are dependent on blood transfusions and are at risk of developing TTIs. This can limit their life expectancy. Therefore, blood donors should always be properly screened. The current study was planned to determine the frequency of HBV, HCV, and HIV among children with beta-thalassemia major undergoing regular blood transfusions.

METHODOLOGY

This cross-sectional study was conducted at the outpatient department of the Pediatric Department of the National Institute of Child Health, Karachi, Pakistan, from February to July 2025, after obtaining approval from the ethical review board of the institution (letter number: IERB-54/2024). A sample size of 230 was calculated using the OpenEpi sample size calculator, assuming an anticipated HCV prevalence of 18.3% in children with beta-thalassemia major,11 with a 95% confidence interval and a 5% margin of error. A non-probability consecutive sampling technique was adopted for sample selection. The inclusion criteria were children of any gender aged ≥ 1 year diagnosed with beta-thalassemia major. Exclusion criteria included children with any comorbid conditions unrelated to β-thalassemia or its complications. Beta-thalassemia major was defined as the presence of two abnormal beta-globin genes, diagnosed through molecular genetic testing and/or hemoglobin electrophoresis. Informed written consent was obtained from the parents/caregivers of the children.

Demographic details of each eligible child, including gender and age, were noted. Each child was asked about beta-thalassemia, including the duration of disease, the total number of blood transfusions, the frequency of transfusions per 4 weeks, and the type of therapy. Vaccination status regarding HBV was documented. Any presenting complaints were also noted. Approximately 3-5 mL of venous blood from each child was collected in an aseptic environment and sent to the institutional laboratory for evaluation of haemoglobin, HBsAg, anti-HCV antibodies, and HIV-1/2 antibodies by ELISA. The polymerase chain reaction test was performed on all those children in whom HBV, HCV or HIV were positive by ELISA.

The statistical analysis was performed using "IBM-SPSS Statistics" version 26.0. The qualitative variables were presented as frequencies and percentages. A Shapiro-Wilk test was used to assess the normality of the quantitative data. For the representation of quantitative variables, means and standard deviations (SD) were calculated. Effect modifiers such as gender, age groups, duration of beta-thalassemia disease, type of therapy, and HBV vaccination status were controlled for by stratification to assess their effect on outcome using the chi-square test, with p-values < 0.05 considered significant.

RESULTS

In a total of 230 transfusion-dependent beta-thalassemia children, the mean age was 8.6±4.1 years. There were 127 (55.2%) male children and 103 (44.8%) female children. The prevalence of HCV infection was detected in 29 (12.6%) children, while HBV infection was detected in 6 (2.6%) children. No case of HIV infection was detected among the study participants. Children who were HCV-positive were significantly older (10.9±3.8 years vs 8.3±3.0 years, $p<0.001$). The duration of disease was significantly longer among HCV-positive children (8.2±3.3 vs 6.3±3.6 years, $p=0.008$). The mean number of transfusions per month was significantly higher in HCV-positive children (2.9±0.7 vs 2.6±0.6, $p=0.015$). A significantly lower proportion of HCV-positive children had received the HBV vaccine compared to HCV-negative children (58.6% vs. 77.1%, $p=0.032$). Fatigue was significantly more common among HCV-positive children (72.4% vs. 50.7%, $p=0.029$). HCV-positive children had significantly lower mean hemoglobin (7.2±0.9 g/dl vs 7.7±1.0 g/dl, $p=0.012$), and higher mean serum ferritin levels (3204.5±910.4 ng/ml vs 2584.1±762.8 ng/ml, $p=0.001$) than HCV-negative children (**Table I**).

Table I: Comparison of demographic, clinical and laboratory characteristics between transfusion-dependent thalassemia children with and without hepatitis C virus infection (N=230)

Characteristics		HCV infection (n=29)	No HCV infection (n=201)	P-value
Gender	Male	17 (58.6%)	110 (54.7%)	0.693
	Female	12 (41.4%)	91 (45.3%)	
Age (years)		10.9±3.8	8.3±3.0	<0.001
Duration of disease (years)		8.2±3.3	6.3±3.6	0.008
Monthly transfusions		2.9±0.7	2.6±0.6	0.015
Chelation therapy		27 (93.1%)	194 (96.5%)	0.375
HBV vaccinated		17 (58.6%)	155 (77.1%)	0.032
Presenting complaints	Fatigue	21 (72.4%)	102 (50.7%)	0.029
	Pallor	19 (65.5%)	97 (48.3%)	0.082
Hemoglobin (g/dl)		7.2±0.9	7.7±1.0	0.012
Serum ferritin (ng/ml)		3204.5±910.4	2584.1±762.8	0.001

Children with HBV infection were significantly less likely to have received the HBV vaccine (16.7% vs. 76.3%, p=0.001). There were no statistically significant differences in mean hemoglobin (7.3±1.0 vs 7.6±1.2 g/dl, p=0.545) or mean serum ferritin levels (3061.0±729.7 vs 2641.8±794.3 ng/ml, p=0.202) between children with and without HBV infection. **Table II** presents the comparison of characteristics of thalassemia children with and without HBV infection.

Table II: Comparison of demographic, clinical and laboratory characteristics between transfusion-dependent thalassemia children with and without hepatitis B virus infection (N=230)

Characteristics		HBV infection (n=6)	No HBV infection (n=224)	P-value
Gender	Male	3 (50.0%)	124 (55.4%)	0.795
	Female	3 (50.0%)	100 (45.6%)	
Age (years)		9.6±4.2	8.6±4.1	0.556
Duration of disease (years)		7.8±2.6	6.4±4.0	0.395
Monthly transfusions		2.8±0.8	2.7±0.6	0.690
Chelation therapy		6 (100%)	215 (96.0%)	0.116
HBV vaccinated		1 (16.7%)	171 (76.3%)	0.001
Presenting complaints	Fatigue	5 (83.3%)	118 (52.7%)	0.137
	Pallor	5 (83.3%)	111 (49.6%)	0.102
Hemoglobin (g/dl)		7.3±1.0	7.6±1.2	0.545
Serum ferritin (ng/ml)		3061±729.7	2641.8±794.3	0.202

DISCUSSION

This study documented the frequency of HCV and HBV among transfusion-dependent children with beta-thalassemia major at 12.6% and 2.6%, respectively. No case of HIV infection was identified. The observed HCV prevalence of 12.6% aligns with the report of Bhuyan GS et al.[9], who identified HCV in 13.5% of transfusion-dependent beta-thalassemia patients using ELISA screening, and closely parallels the frequency reported by Wohab MA 2025[13], who found HCV positivity in 8.5% of their cohort from Bangladesh. The frequency in this study remains substantially lower than figures reported by Kousar T 2021[14], who detected HCV in 33.3% of multi-transfused children at a tertiary care hospital, and by Khalil S 2016[12], who reported that 38.7% of thalassemia patients were HCV-positive. Studies from other regions in Asia, such as Agarwal S 2017[15] and Mukherjee K 2017[16] documented HCV prevalence rates as high as 24%. The disparity in HCV prevalence across centres can be attributed to several factors, including differences in blood donor selection, transfusion safety protocols, the sensitivity of the screening assays used, and the duration of exposure to transfusions. In settings where nucleic acid amplification testing (NAT) and sensitive serological methods are implemented, new HCV infections are reduced. A study from Turkey demonstrated that the introduction of more sensitive second-generation assays led to a substantial decline in new HCV infections, reflecting the impact of improved blood safety practices[17].

This study found that HCV-positive children were older (10.9±3.8 years vs 8.3±3.0 years), had longer disease duration (8.2±3.3 years vs 6.3±3.6 years), and received more frequent monthly transfusions (2.9±0.7 vs 2.6±0.6). Agarwal S 2017[15] reported higher HCV prevalence among older children and those with increased transfusion numbers, with significant associations between advancing age, cumulative transfusion burden, and HCV infection. Mahmoud RA 2016[18] also documented a strong correlation between HCV seropositivity, increasing patient age, disease duration, and serum ferritin levels, findings consistent with the current results. Such associations are biologically plausible, given the cumulative risk of exposure with each transfusion and the potential for imperfect screening, especially before the widespread implementation of highly sensitive diagnostic assays. These findings underscore the need for periodic re-evaluation of blood screening policies and reinforce the clinical imperative to use of nucleic acid-based testing to minimize residual risk.

The present study found that HCV-positive children had higher mean serum ferritin levels (3204.5±910.4 ng/ml) compared to those without HCV (2584.1±762.8 ng/ml). This observation is consistent with data from Agarwal S 2017[15] who documented rising serum ferritin levels among HCV-positive thalassemia children, likely reflecting greater transfusion exposure and potentially increased hepatic iron loading in the context of chronic HCV. This highlights the dual challenge of iron overload and viral hepatitis in transfusion-dependent populations, both of which contribute to increased morbidity, risk of hepatic fibrosis, and overall poorer quality of life[19,20].

This study found a strong association between incomplete hepatitis B vaccination and the presence of both HCV and HBV infection. Naz R et al.[21] found that 73.9% of affected thalassemia patients were not immunized against HBV, and this subgroup bore the highest burden of infection, especially those undergoing more than 25 annual transfusions. Prasad et al.,[22] also described an association between incomplete immunization and increased risk of both HBV and HIV.

The prevalence of HBV infection in this study (2.6%) was lower than that reported in some regional studies, such as Bhuyan GS et al. [9] (3.4%) and Kousar et al.[14] (5.9%) and considerably lower than the figure documented by Altaf W 2025[23], where 9.9% of children tested positive for HBV. Studies from Turkey (0.75%)[17] and Iran (0%)[24] where rigorous

blood donor selection and sensitive screening tests are routine, have shown significantly lower prevalence of HBV infection among thalassemia children.

No cases of HIV were detected in the present study. This finding mirrors the results of Bhuyan GS et al. [9], Wohab MA 2025[13] and Mukherjee K 2017[16], who also reported a 0% HIV prevalence among their thalassemia patients. Prasad GJ 2015[22] reported HIV prevalence of 3.4% among 237 children in India. The non-existence of HIV burden may be attributed to stricter HIV screening, mandatory testing of donor blood, and the overall lower population prevalence in the pediatric age group in South Asia[25,26]. These results support the effectiveness of current HIV blood safety protocols in Pakistan and neighboring countries.

Several important clinical and public health implications arise from these findings. The persistence of TTIs in transfusion-dependent children, despite ongoing advances in donor screening, highlights the need for robust and integrated hemovigilance systems. Routine post-transfusion surveillance for HBV and HCV should be standard, with a strong emphasis on catch-up and booster HBV immunization for unvaccinated children. Education of healthcare workers and families about the importance of vaccination and regular monitoring is essential for risk reduction.

The cross-sectional design precludes the establishment of a temporal relationship between transfusion events and infection acquisition. Prospective cohort studies with regular longitudinal sampling would allow for the capture of incident TTIs and more robust risk assessment. The use of non-probability consecutive sampling may introduce selection bias, although it reflects real-world clinical practice in high-volume transfusion centers. The reliance on parental recall for some vaccination data and presenting complaints may introduce recall bias. The study also did not assess other TTIs such as cytomegalovirus or parvovirus B19, nor did it evaluate the impact of TTI positivity on liver function or long-term morbidity.

CONCLUSION

This study highlighted a significant burden of HCV and, to a lesser extent, HBV infection among transfusion-dependent beta-thalassemia children, with strong associations to older age, longer disease duration, higher transfusion frequency, and incomplete vaccination. These findings support strengthening local blood safety by implementing nucleic acid testing alongside serological screening to reduce window-period transmission, and by ensuring universal, documented HBV immunization.

Ethical Permission: Department of Pediatric Medicine, National Institute of Child Health, Karachi, Pakistan, ERC approval letter No. IERB-54/2024.
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AUTHOR CONTRIBUTION

Shaikh AQ: Data collection and synthesis, responsible for data's integrity, proofreading, and approval for final publication.
Naeem B: Conception and design, drafting, critical revisions, approved for publication.
Lashari S: Conception and design, drafting, critical revisions, approved for publication.
Hussain W: Drafting, proofreading, critical revisions, approved for final publication.
Fatima H: Literature review, proofreading, critical revisions, approved for final publication.
Aman L: Data analysis, proofreading, critical revisions, approved for final publication**.**

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