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Original Article

Prevalence of Hepatitis B virus infection among the multi-transfused thalassemic and hemophilic patients receiving RCC & FFP

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ABSTRACT

Background: Patients with thalassemia and hemophilia require frequent blood transfusions, which increase their risk of hepatitis B virus (HBV) infection. As a major global health problem, HBV can lead to chronic liver disease and hepatocellular carcinoma, making multi-transfused patients particularly vulnerable. **Objective:** To assess the prevalence of Hepatitis B virus infection among the multi- transfused thalassemic and hemophilic patients receiving red cell concentrate (RCC) & fresh frozen plasma (FFP). **Methods & Materials:** A cross-sectional study was conducted in the Departments of Transfusion Medicine and Virology at BSMMU, Dhaka, over one year following IRB approval. Fifty multi-transfused patients (25 hemophilia and 25 thalassemia) receiving RCC or FFP were enrolled. Data were collected using a structured questionnaire after informed consent, and blood samples were tested for HBsAg and Anti-HBc (Total). Data were analyzed using SPSS version 22. **Results:** Among the participants, 22.0% were Anti-HBc (total) positive, while all were HBsAg negative. Anti-HBc positivity was significantly associated with a history of receiving blood transfusions from multiple centers other than BSMMU ($p<0.05$), whereas no significant association was found with hepatitis B immunization history ($p>0.05$). **Conclusion:** Anti-HBc positivity was found in 22.0% of multi-transfused thalassemic and hemophilic patients, and was significantly associated with a history of receiving blood transfusions from multiple centers other than BSMMU.

Keywords: *Thalassemia, Hemophilia, HBV, Anti-HBc, TTI*

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INTRODUCTION

Transfusion-transmitted infections (TTIs) remain a major public health problem worldwide, particularly among multi-transfused patients such as those with thalassemia and hemophilia [1,2]. Thalassemia is a hereditary blood disorder common in the "thalassemia belt," which includes Mediterranean countries, the Middle East, and South Asian nations such as Bangladesh, India, and Sri Lanka [3]. Patients with transfusion-dependent β-thalassemia major (BTM) and HbE-β-thalassemia (EBT) require regular blood transfusions and are therefore at high risk of transfusion-associated viral infections. Among TTIs, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common causes of chronic viral hepatitis and hepatocellular carcinoma in multi-transfused patients [4]. Although strict screening procedures have greatly reduced TTIs in developed countries [5], developing countries still face significant challenges in ensuring blood safety due to limited resources and inadequate screening systems [6]. Bangladesh lies within the global thalassemia belt, with approximately 9176 new cases each year [7]. These patients require frequent transfusions, often every 7–120 days, which increases the risk of acquiring TTIs when proper donor screening is not consistently implemented [8,9].

Regular transfusion therapy has improved survival and quality of life in hereditary hemolytic anemia, but it carries the risk of blood-borne viral infections, particularly hepatitis B [10]. HBV infection remains a major global health problem, affecting more than 2 billion people, with about 400 million chronic carriers [11]. Studies have reported high rates of HBsAg and anti-HBc positivity among multi-transfused children in countries such as Egypt [12].

Hemophilia patients are classified as having mild, moderate, or severe disease depending on clotting factor deficiency [13]. Management often involves replacement therapy using fresh frozen plasma (FFP), cryoprecipitate, or clotting factor concentrates. However, repeated transfusions significantly increase the risk of TTIs, including HBV, HCV, HIV, and syphilis [14,15]. In many developing countries, including China, high costs limit access to recombinant clotting factors, making transfusion of FFP or cryoprecipitate the primary treatment option [16]. As the risk of viral infection increases with repeated transfusions, this study aims to determine the prevalence of hepatitis B virus infection among multi-transfused thalassemic and hemophilic patients receiving RCC and FFP.

OBJECTIVES

The objective of this study was to assess the prevalence of Hepatitis B virus infection among multi- transfused thalassemic and hemophilic patients receiving Red Cell Concentrate (RCC) & Fresh Frozen Plasma (FFP).

METHODS & MATERIALS

This was a hospital-based cross-sectional study conducted in the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The study was carried out over a twelve-months period from March 2021 to August 2023.

Sample Selection

Inclusion criteria

- Patients with thalassemia who received transfusions by RCC >10 times.
- Patients with hemophilia received transfusion of FFP >10 times.
- Thalassemic and hemophilic patients of aged 5-40 years

Exclusion criteria

- Age group <5 years or >40 years.
- Thalassemic patients who received transfusions other than RCC.
- Hemophilic patients who received a transfusion other than FFP.
- Patients known to be hepatitis B virus positive before starting blood transfusion.
- Patients who did not give consent.

Data Collection Procedure

A total of 50 multi-transfused patients receiving RCC and FFP were included in this study according to the inclusion and exclusion criteria. After explaining the purpose of the study, written informed consent was obtained from each participant or their authorized guardian. During the one-year study period at the Department of Transfusion Medicine, BSMMU, Dhaka, 3 ml of venous blood was collected from each participant under aseptic conditions. The samples were sent to the Virology Department, aliquoted, and stored at −20°C until analysis. Serum samples were tested for hepatitis B surface antigen (HBsAg) and total anti-HBc using enzyme-linked immunosorbent assay (ELISA) with a fully automated analyzer (Abbott Laboratories, USA). Demographic and clinical data, including age, sex, and transfusion history, were recorded using a predesigned questionnaire and hospital records.

Ethical Considerations:

Ethical approval was obtained from the Ethical Review Committee of Bangabandhu Sheikh Mujib Medical University (BSMMU). Written informed consent was obtained from all participants or their legal guardians, and confidentiality of patient information was strictly maintained.

Statistical Analysis

Data were analyzed using SPSS version 22. Categorical variables were presented as frequency and percentage, and associations were assessed using the chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Table I shows the distribution of participants by demographic characteristics showed that out of the total 50, the majority were in the age groups of ≤10 years 19(38.0%) and 11-15 years 9(18.0%). The mean age of the participants was 16.64 years. Most of the participants were male 40(80.0%) and from rural areas 29(58.0%). In terms of economic condition, the poor class had the highest representation 38(76.0%).

Table I
Distribution of participants by demographic characteristics (*n*=50).

Characteristics		Frequency (N)	Percentage (%)
Age in year	10	19	38.0
	11-15	9	18.0
	16-20	8	16.0
	21-25	5	10.0
	26-30	3	6.0
	31-35	2	4.0
	>35	4	8.0
	Mean ± SD	16.64±9.8	
	Range (min-max)	(6-40)	
Gender	Male	40	80.0
	Female	10	20.0
Socio Economic Condition	Middle	12	24.0
	Poor	38	76.0
Residence	Urban	21	42.0
	Rural	29	58.0

Table II shows distribution of the study patients by hepatitis seropositivity. All Hemophilia patients (100.0%) and Thalassaemia patients (100.0%) tested negative for HBsAg Titre. Among Hemophilia patients, 6(24.0%) tested positive for Anti HBc (Total) Among Thalassaemia patients, 5 (20.0%) tested positive.

Table II
Distribution of the participant by hepatitis seropositivity (*n*=50).

Hepatitis seropositivity		Hemophilic patients (n=25)		Thalassaemia patients (n=25)	
		n	%	n	
HBsAg Titre	Positive	0	0.0	0	0.0
	Negative	25	100.0	25	100.0
Anti HBc Total	Positive	6	24.0	5	20.0
	Negative	19	76.0	20	80.0

Table III shows association between demographic characteristics with Anti HBc (total) category. The mean age was younger in positive group with compared to negative group but there was no statistically significant (p>0.05) difference in age between the positive and negative groups. Most of the participants were male but no statistically significant (p>0.05) difference in gender between the positive and negative groups. The differences of socio-economic condition and residence in the frequencies and percentages between the groups were not statistically significant (p>0.05).

Table III
Association between demographic characteristics with Anti HBc (total) category (*n*=50).

Characteristics		Anti HBc-Positive (n=11)		Anti HBc-Negative (n=39)		P-value
		n	%	n	%	
Age in year	10	3	27.3	16	41.0	[a]0.631[ns]
	11-15	3	27.3	6	15.4	
	16-20	2	18.2	6	15.4	
	21-25	3	27.3	2	5.1	
	26-30	0	0.0	3	7.7	
	31-35	0	0.0	2	5.1	
	>35	0	0.0	4	10.3	
	Mean ± SD	15.4±5.3		17.0±10.8		
	Range (min-max)	(9-22)		(6-40)		
Gender	Male	9	81.8	31	79.5	[b]0.618[ns]
	Female	2	18.2	8	20.5	
Socio Economic Condition	Middle	2	18.2	10	25.6	[b]0.472[ns]
	Poor	9	81.8	29	74.4	
Residence	Urban	3	27.3	18	46.2	[b]0.221[ns]
	Rural	8	72.7	21	53.8	

ns =not significant; [a]p value reached from Unpaired t-test; [b]p value reached from Fisher's exact test

Table IV presents the association between the type of hemophilia with Anti HBc (total) category. The associations between the type of hemophilia with Anti HBc (total) category were not statistically significant (p>0.05), indicating that there was no strong evidence of an association between the type of hemophilia with Anti HBc (total) category.

Table IV
Association between the type of hemophilia with Anti HBc (total) category (*n*=25).

Type of hemophilia	Anti HBc-Positive (n=6)		Anti HBc-Negative (n=19)		P-value
	n	%	n	%	
A	4	66.7	18	94.7	0.132 [ns]
B	2	33.3	1	5.3	

ns =not significant; p value reached from Fisher's exact test

Table V presents the association between the type of Thalassemia with Anti HBc (total) category. The associations between the type of Thalassemia with Anti HBc (total) category was not statistically significant (p>0.05), indicating that there was no strong evidence of an association between the type of Thalassemia with Anti HBc (total) category.

Table V
Association between the type of Thalassemia with Anti HBc (total) category (*n*=25).

Type of thalassemia	Anti HBc-Positive (n=5)		Anti HBc-Negative (n=20)		P-value
	n	%	n	%	
BTM	4	80.0	10	50.0	0.244[ns]
Eβ	1	20.0	10	50.0	

ns =not significant; p value reached from Fisher's exact test

Table VI shows that there was no statistically significant association between transfusion-related variables and Anti-HBc (Total) status. Although Anti-HBc–positive patients had a lower mean age at first transfusion and a higher mean number of transfused units compared to Anti-HBc–negative patients, these differences were not significant (p>0.05). The usual interval between transfusions was also not associated with Anti-HBc (Total) positivity.

Table VI
Association Between Transfusion-Related Variables and Anti-HBc (Total) Status (*n* = 50).

Variable		Anti HBc-Positive (n=11)		Anti HBc-Negative (n=39)		P-value
		N	%	n	%	
Age at first transfusion (years)		1.67 ± 1.80		2.82 ± 2.94		0.225[ns]
Total units transfused		27.36 ± 19.12		23.82 ± 18.77		0.584[ns]
Usual transfusion interval	0.5 month	2	18.2	5	12.8	0.965[ns]
	1 month	7	63.6	24	61.5	
	2 months	1	9.1	5	12.8	
	3 months	1	9.1	4	10.3	
	6 months	0	0.0	1	2.6	

ns =not significant; p value reached from Fisher's exact test

Table VII shows the association between H/O transfusions other than BSMMU (Bangabandhu Sheikh Mujib Medical University), with Anti HBc (total) category. There was a statistically significant (p<0.005) association between H/O blood transfusion other than BSMMU with Anti HBc (total) category.

Table VII
Association between H/O receiving transfusions from multiple centers with Anti HBc (total) category (*n*=50).

H/O receiving Blood Transfusion from multiple center	Anti HBc-Positive (n=11)		Anti HBc-Negative (n=39)		P-value
	n	%	n	%	
Yes	11	100.0	10	25.6	0.001[s]
No	0	0.0	29	74.4	

s = significant; p value reached from Fisher's exact test

Table VIII shows the association between history of hepatitis B immunization with Anti HBc (total) category. There was no statistically significant (p>0.05) association between history of hepatitis B immunization with Anti HBc (total) category.

Table VIII
Association between history of hepatitis B immunization with Anti HBc (total) category (*n*=50).

H/O hepatitis B Immunization	Anti HBc-Positive (n=11)		Anti HBc-Negative (n=39)		P-value
	n	%	n	%	
Yes (n=35)	6	17.1	29	82.9	0.205[ns]
No (n=15)	5	33.3	10	66.7	

ns =not significant; p value reached from Fisher's exact test

DISCUSSION

Despite the implementation of rigorous infection screening protocols at Bangabandhu Sheikh Mujib Medical University (BSMMU) before every blood transfusion, some multi-transfused patients were still found infected with transfusion-transmitted infections such as hepatitis B virus (HBV) and hepatitis C virus (HCV). This situation raises concerns regarding the limitations of conventional screening methods. One possible explanation is the presence of asymptomatic donors in the window period, during which hepatitis B surface antigen (HBsAg) cannot be detected by routine immunological tests. Another possibility is occult HBV infection, characterized by the absence of detectable HBsAg despite the presence of HBV DNA in the liver or serum [17]. In addition, rapid immunochromatographic (ICT) screening methods used in some settings may have limited sensitivity in detecting certain HBV serotypes, which could result in missed infections [6]. To minimize these risks, nucleic acid testing (NAT) has been recommended because it detects HBV DNA and significantly reduces the window period, thereby improving the safety of blood transfusion [18].

This cross-sectional study aimed to determine the prevalence of HBsAg and Anti-HBc antibodies among multi-transfused thalassemic patients receiving red cell concentrate (RCC) and hemophilic patients receiving fresh frozen plasma (FFP). A total of 50 multi-transfused patients (25 thalassemia and 25 hemophilia) attending the Department of Transfusion Medicine, BSMMU, Dhaka, between July 2022 and June 2023 were included. All patients had received more than ten transfusions and were aged between 5 and 40 years.

In this study, the overall prevalence of Anti-HBc positivity was 22.0% among the study participants. Similar studies have reported varying prevalence rates of HBV infection among multi-transfused patients. Bhuyan et al. (2021) reported a prevalence of 3.37% HBV infection among thalassemia patients in Bangladesh [19]. Tognon et al. (2020) screened 29,713 donors and found an overall HBV infection prevalence of 10.8% [20]. Mishra et al. (2020) reported 1.5% HBsAg positivity among multi-transfused β-thalassemia major patients [21], while Al-Moshary et al. (2019) detected HBV infection in 4.87% of β-thalassemia major patients [22]. In Bangladesh, Chakrabarty et al. (2014) reported 6.5% HBsAg positivity among multi-transfused thalassemia patients [23]. However, some studies reported no HBsAg positivity, which is consistent with the findings of the present study [24].

In the current study, all participants were negative for HBsAg, although a considerable proportion showed Anti-HBc positivity, suggesting previous exposure to HBV infection. The mean age of the participants was slightly lower in the Anti-HBc positive group compared with the negative group; however, the difference was not statistically significant (p>0.05). Tognon et al. (2020) also reported that HBV infection was associated with younger age in transfused patients [20]. Most participants in this study were male, which is expected because hemophilia is an X-linked genetic disorder affecting males.

Socioeconomic status and place of residence did not show any statistically significant association with Anti-HBc positivity in this study. However, previous research in Bangladesh has suggested that lower socioeconomic status and overcrowded living conditions may contribute to higher HBV prevalence [25,26].

No significant association was observed between Anti-HBc positivity and factors such as type of hemophilia, type of thalassemia, age at first transfusion, total units of transfusion, or interval between transfusions (p>0.05). Similar findings were reported by Bhuyan et al. (2021) [19]. However, receiving blood transfusions from multiple centers outside BSMMU showed a statistically significant association with Anti-HBc positivity (p<0.005). This finding indicates that transfusions from different sources may increase the risk of exposure to inadequately screened blood [20,27].

Vaccination history also appeared to influence HBV exposure. A higher proportion of Anti-HBc negative participants had received hepatitis B vaccination, which supports the protective effect of immunization. Previous studies have also demonstrated that vaccination significantly reduces the risk of HBV infection among transfusion-dependent patients [28,29].

Overall, the findings of this study emphasize the importance of strict donor screening, improved blood safety practices, HBV vaccination, and the implementation of NAT-based screening methods to minimize the risk of transfusion-transmitted infections among multi-transfused patients [30].

CONCLUSION

This hospital-based cross-sectional study demonstrates that, although no active Hepatitis B virus infection was detected among multi-transfused thalassemic and hemophilic patients receiving RCC and FFP (as evidenced by 100% HBsAg negativity), a considerable proportion (22.0%) showed Anti-HBc (total) positivity, indicating previous exposure to HBV. This finding highlights the presence of occult or past HBV infection despite routine donor screening practices.

Most demographic factors, disease type (thalassemia or hemophilia), transfusion-related variables (age at first transfusion, total units transfused, and transfusion interval), type of blood component, and hepatitis B vaccination history were not significantly associated with Anti-HBc positivity. However, a history of receiving blood transfusions from multiple centers other than BSMMU emerged as a significant risk factor, underscoring the variability in transfusion safety standards across different facilities.

These results emphasize that standard HBsAg-based screening alone may be insufficient to fully prevent transfusion-transmitted HBV infection in multi-transfused patients. Incorporation of Anti-HBc testing and nucleic acid testing (NAT) into routine donor screening, along with strengthening centralized transfusion services and ensuring complete hepatitis B vaccination coverage, is crucial to further reduce residual HBV transmission risk. Continuous surveillance and improved transfusion safety measures are essential to protect this vulnerable patient population.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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