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TRANSFUSION TRANSMITTED VIRAL HEPATITIS B AND HEPATITIS C INFECTION STATUS AND ASSOCIATED RISK FACTORS IN MULTI TRANSFUSED CHILDREN WITH BETA THALASSEMIA

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Abstract

Introduction: Hemoglobinopathies, including β-thalassemia, are frequent worldwide, particularly in South Gujarat, India. Regular blood transfusions, which are required to manage thalassemia, increase the risk of transfusion-transmitted infections (TTIs), specifically Hepatitis B (HBV) and Hepatitis C (HCV). This study will look at the frequency and stages of HBV and HCV infections in multi-transfused thalassemia children in South Gujarat.

Aim: To determine the proportion of transfusion-related HBV and HCV infections in multi-transfused thalassemia children, to evaluate the stages of Hepatitis B and Hepatitis C infection and to evaluate risk factors associated with transfusion transmitted HBV and HCV.

Materials and Methods: An observational, cross-sectional study was carried out at a tertiary care facility in South Gujarat from July 2019 to October 2020. Sixty-six pediatric patients aged one to fifteen years with thalassemia who had received at least two blood transfusions or more who were either admitted to pediatric wards for routine blood transfusions or attended the outpatient department for regular care in the previous year were included. Serological assays were used to assess Hepatitis B and C infections, and positive cases were confirmed using HbeAg, Anti-HBc antibody and HCV RNA PCR. Data were examined for correlations between infection rates and a variety of parameters.

Results: Of the 66 patients, 1.5% tested positive for HBV, while 9.1% tested positive for HCV. HBV positive patient did not have active viral replication on further testing. All HCV positive cases had undetectable virus levels. Higher transfusion frequency was strongly linked with higher Hepatitis B($p=0.017$) and Hepatitis C prevalence ($p=0.0001$). Elevated blood ALT and ferritin levels were seen in HCV-positive patients, indicating that they are potential indicators for Hepatitis C infection.

Conclusion: The study found a low HBV prevalence but a significant HCV prevalence among multi-transfused thalassemia children. Frequent blood transfusions are a significant risk factor for Hepatitis B and Hepatitis C. Newer techniques for early detection of Hepatitis B and Hepatitis C virus are needed to prevent TTI's. It is recommended to improve blood screening techniques and check serum ALT and ferritin levels on a regular basis. Larger, multi-centre investigations are needed to validate these findings and evaluate transfusion safety practices.

Keywords: Hepatitis B and C, Beta Thalassemia, Blood Transfusion, TTI

Introduction

Hemoglobinopathies, particularly β-thalassemia, represent some of the most common monogenic diseases globally, affecting about 7% of the world's population. India has a significant burden of these disorders, with β-thalassemia being the most prevalent single-gene disorder in the country.[1,2] Gujarat, especially the South Gujarat region, shows a higher prevalence of hemoglobinopathies compared to other Indian states, with the β-thalassemia trait affecting 1.95% of the population.[2,3] For children with transfusion-dependent thalassemia, regular blood transfusions are essential for survival; however, this life-saving treatment carries inherent risks, particularly the risk of Transfusion-Transmitted Infections (TTIs). [3,4]TTIs, including Hepatitis B, Hepatitis C, HIV, Syphilis, CMV, and Malaria, are significant threats to multi-transfused children.

Of these, Hepatitis B and C are particularly concerning due to their potential to cause chronic or fatal liver diseases. The incidence of TTIs is closely linked to the prevalence of these infections within the blood donor community. India, which houses one-fifth of the global population, contributes significantly to the worldwide burden of Hepatitis B, with an estimated 40 million carriers, representing 10-15% of the global HBV carrier population.[5] The challenge is further intensified by the presence of low viremia and mutant strains of Hepatitis B in the Indian population, which are not easily detectable using routine ELISA tests, making post-transfusion hepatitis a significant concern.

Moreover, the burden of Hepatitis C remains high, particularly among high-risk groups and those receiving frequent blood transfusions. The proportion of anti-HCV positivity among multi-transfused patients in India is notably high, reaching up to 24.06%.[6] Given these significant health risks, this study is designed to address the need for evaluating the effectiveness of current blood donor screening protocols in multi-transfused thalassemia patients. Specifically, this hospital-based study aims to determine the occurrence and stages of transfusion-transmitted Hepatitis B and C infections among multi-transfused thalassemia children in South Gujarat. The specific objectives of this study were: to determine the proportion of transfusion-related Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections in multi-transfused thalassemia children and to evaluate the stages of Hepatitis B and Hepatitis C infection (acute, chronic, carrier) in the infected children.

Materials and Methodology

This observational, cross-sectional study was conducted in a tertiary care hospital in South Gujarat from July 2019 to October 2020. The study focused on pediatric patients aged 1 to 15 years diagnosed with thalassemia major, thalassemia intermedia, or sickle-beta thalassemia. These patients were either admitted to pediatric wards for routine blood transfusions or attended the outpatient department for regular care.

A total of 66 patients were included in the study, selected using a convenient sampling technique. The study targeted pediatric patients requiring routine blood transfusions, all of whom had received two or more transfusions in the past year. Inclusion criteria consisted of patients aged 1 to 15 years with a diagnosis of beta thalassemia major, beta thalassemia intermedia, or sickle-beta thalassemia, and a history of two or more blood transfusions within the last year. Patients unwilling to participate and those without complete medical records were excluded from the study.

Ethical approval was obtained from the institutional Human Research Ethics Committee. Informed consent was obtained from the parents or guardians of the participants, and verbal assent was sought from the children. Strict confidentiality was maintained, with access to patient data restricted to the investigator and study guide. Data analysis was performed anonymously to protect patient identities.

The study included the recording of detailed medical histories, focusing on Hepatitis B vaccination status, the number of transfusions per year, and the type of healthcare facility where transfusions were received. Clinical examinations were conducted to assess associated morbidities and to evaluate drug history for chelating agents and their compliance. Blood samples were collected under aseptic conditions for laboratory analysis. Hepatitis B testing included tests for HBsAg, HBeAg and Anti-

HBc antibodies, while Hepatitis C screening involved Anti-HCV antibody testing, with positive results confirmed by HCV RNA PCR. Data entry was performed using MS Excel and analyzed with SPSS version 20.0. The study calculated the proportion of each viral infection and assessed associations with potential risk factors. Results were communicated to the patients' families and treating units to ensure appropriate follow-up and management.

Results

An observational, cross-sectional study was conducted to estimate the proportion of transfusion-transmitted viral Hepatitis B and Hepatitis C infections in multi-transfused children with thalassemia at a tertiary care center in South Gujarat during 2019-2020. During data collection period, a total of 80 patients received care at this institute. Of these, 14 did not meet the inclusion criteria, so 66 patients were ultimately included in the study.

In current study, more than two-thirds of the patients 69.7% were boys (n=46), while 30.3% patients were girls (n=20) and boy: girl ratio of 2.3: 1. Mean age of the patients was 9 ± 3.5 years (range: 2 to 15 years). Around one-thirds patients 33.3% were 6 - 9 years old, among boys (n=46), majority of the patients (32.6%) were 10 – 12 years old, followed by 6 - 9 years old (28.3%). However, among girls (n=20), majority of the patients (45%) were 6 - 9 years old, followed by 10 – 12 years old (25%), <6 years old (20%) and 13 - 15 years old (10%). In current study, most of the patients (57.6%) belonged to Hindu religion, while (42.4%) patients belonged to Muslim religion. In current study, more than three-fifths of the parents of patients (60.6%) had consanguineous marriage while remaining (39.4%) had non-consanguineous marriage. Some of the patients (39.4%) had hepatosplenomegaly in per abdominal examination, while some (36.4%) had only splenomegaly and remaining (24.2%) had no abnormality. [Table 1]

Table 1: Demographics and Clinical Characteristics of thalassemia patients (n=66)

Characteristics	Boys [n=46 (%)]	Girls [n=20 (%)]	Total [n=66 (%)]
Age Distribution (in years)			
< 6 years	4 (66.7)	2 (33.3)	6 (9.1)
6 – 9 years	13 (59)	9 (41)	22 (33.3)
10 – 12 years	15 (68)	7 (32)	22 (33.3)
13 – 15 years	14 (87.5)	2 (12.5)	16 (24.2)
Mean Age + SD	9.2 + 3.72	7.95 + 3.81	8.93 + 3.63
Religion			
Hindu	25 (65.8)	13 (34.2)	38 (57.6)
Muslim	21 (75)	7 (25)	28 (42.4)
Consanguinity			
Consanguineous	29 (72.5)	11 (27.5)	40 (60.6)
Non-Consanguineous	17 (65.4)	9 (34.6)	26 (39.4)
Hepatosplenomegaly			
Hepatosplenomegaly	19 (73)	7 (27)	26 (39.4)
Splenomegaly	18 (75)	6 (25)	24 (36.4)
No Abnormality	9 (56.3)	7 (43.7)	16 (24.2)

Table 2: Distribution of Patients by Place of Blood Transfusion, ABTR, Medication History and number of blood transfusions per year (n=66)

Place of Blood Transfusion	
Variables	n (%)
Government Hospital	31 (47)
Non-Government	22 (33.7)
Any Blood Bank	13 (19.7)
Annual Blood Transfusion Rate [ABTR (ml/kg/yr)]	

<100	1 (1.5)
101-150	17 (25.8)
151-200	31 (47)
201-250	15 (22.7)
>250	2 (3)
Chelating agent and medication history	
T. Deferasirox	60 (90.9)
Tablet Deferasirox plus Injectable Deferoxamine	01 (1.5)
T. Hydroxyurea	1 (1.5)
None	4 (6.1)
Number of blood transfusions per year	
<10	3(4.5)
11-15	53(80.4)
16-20	7(10.6)
>20	3(4.5)

Table 3: Distribution based on relationship between age of patients and number of blood transfusions per year

Age group	<15 times blood transfusion/year	>15 times blood transfusion/year	Chi square	P value
0-5	11(16.6)	1(1.5)	11.47	0.003
6-10	15(22.8)	12(18.2)		
11-15	9(13.6)	18(27.2)		

Most of the patients were receiving blood transfusions from government hospitals (47%), some patients (33.3%) took blood transfusions from Non-Government (Private sectors/ NGOs /Public private partnership blood banks) while others (19.7%) patients took blood transfusion from any of the blood banks. More than four-fifths of the patients (80.4%) required 11 – 15 times blood transfusion per year, while (10.6%) required 16 – 20 times blood transfusion per year, 4.5% require >20 times blood transfusion per year and 4.5% required ≤ 10 times blood transfusion per year.

Number of blood transfusion received by patients more than 15 times increases in 11-15 years age group where as in 6-10 years it is higher in less than 15-time BT per year. The chi square value of above table is 11.47 and p value is 0.003. There is statistical significance between the age of the patient and number of times of blood transfusion. As the age of the patient increases, blood transfusion requirement also increases. Majority of the patients have ABTR of 150 – 200 ml/kg/year (47%), followed by (25.8%) who require 100 – 150 ml/kg/year, (22.7%) patients were require 200 – 250 ml/kg/year, 3% patients were require > 250 ml/kg/year and 1.5% patients required <100 ml/kg/year. Most of the patients (90.9%) were taking tablet Deferasirox as Iron chelating drug, while 1.5% were using Tablet Deferasirox + Injection Deferoxamine as Iron chelating agent, 1.5% patients were using Tablet Hydroxyurea for associated sickle cell trait and 6.1% patients were not taking any medication for Iron chelation. [Table2]

Table 4: Association of Hepatitis B and C with Demographics and Transfusion Frequency

Variables	**Hepatitis B positive n (%)**	**Hepatitis B negative n (%)**	**Total n (%)**	**Hepatitis C positive n (%)**	**Hepatitis C negative n (%)**
Gender					
Male	01 (2.17)	45 (98.3)	**46 (69.7)**	2 (4.3)	44 (95.7)
Female	00 (0)	20 (100)	**20 (30.3)**	04 (20)	16 (80)
Total	**01 (1.5)**	**65 (98.5)**	**66 (100)**	**06 (9.1)**	**60 (90.9)**

Chi-square	0.44			**4.13**	
p-value	0.5			**0.04**	
Religion					
Hindu	1 (2.7)	37 (97.3)	**38 (57.6)**	03 (7.9)	35 (92.1)
Muslim	0 (0)	28 (100)	**28 (42.4)**	03 (10.7)	25 (89.3)
Total	**01 (1.5)**	**65 (98.5)**	**66 (100)**	**06 (9.1)**	**60 (90.9)**
Chi-square	**0.75**			**0.15**	
p-value	**0.38**			**0.69**	
Consanguineous Marriage					
Yes	01 (2.5)	39 (97.5)	**40 (60.6)**	05 (12.5)	35 (87.5)
No	0 (0)	26 (100)	**26 (39.4)**	01 (3.8)	25 (96.2)
Total	**01 (1.5)**	**65 (98.5)**	**66 (100)**	**06 (9.1)**	**60 (90.9)**
Chi-square	0.66			1.428	
p-value	0.41			0.232	
Frequency of blood transfusion					
< 15 times	00	52	52	01	51
> 15 times	01	13	14	05	9
Total	**01 (1.5)**	**65 (98.5)**	**66 (100)**	**06 (9.1)**	**60 (90.9)**
Chi-square	5.7			**15.0**	
p-value	0.017			**0.0001**	

Out of the total 66 patients, only 1 patient (1.5%) was positive for Hepatitis B surface antigen and rest (98.5) were negative for Hepatitis B surface antigen. Hepatitis B surface antigen positive patient was further tested for HBeAg and Anti-HBc IgM antibody, which were both non-reactive.

Out of total 66 patients, 6 patients (9.1%) tested positive for Anti-HCV antibody and 60 patients (90.9%) tested negative for Anti-HCV antibody. All the patients who tested positive for Anti-HCV antibody (n=06) were confirmed by HCV RNA PCR. All the patients had undetectable viral levels. Mean age of patient having Hepatitis C was 9.83 + 2.14 years. In current study, total 1.5% males were affected with Hepatitis B and none of the females were affected with Hepatitis B. Findings related to Hepatitis B infection, determinant positive for Hepatitis B was found to be low and was not statistically related with possible demographic characteristics such as gender, religion and relation with p value 0.5, 0.38 and 0.41, respectively.

Findings related to Hepatitis C infections, determinant positive for Hepatitis C was found to be low and was not statistically related with possible demographic characteristics such as age, religion and marriage within certain kin groups. Moreover, females are more likely than males to be positive for Hepatitis C.

Additional relationships were found between the frequency of blood transfusions and the rate and seropositivity of Hepatitis B and Hepatitis C infection, p=0.017 and p = 0.0001 respectively. It was more likely that those who had more than fifteen blood transfusions per year would turn out to be Hepatitis B and Hepatitis C positive. [Table 4].

Table 5: Liver function test among beta thalassemia patients

	n	**S. ferritin**	**ALT**
		Mean + SD	**Mean + SD**
Hepatitis C positive	06	3456.67 + 2345.54	31.67 + 8.45
Hepatitis C negative	60	2726.1 + 1892.14	28.67 + 7.98
Hepatitis B negative	65	2817.02 + 1932.76	29.17 + 7.84

The liver function test findings among beta thalassemia patients showed that Hepatitis C positive patients had higher mean serum ferritin levels (3456.67 ± 2345.54) than those who are Hepatitis C

negative (2726.1 ± 1892.14) and Hepatitis B negative (2817.02 ± 1932.76). ALT levels are slightly higher in Hepatitis C positive patients (31.67 ± 8.45) than in Hepatitis C negative (28.67 ± 7.98) and Hepatitis B negative patients (29.17 ± 7.84). [Table 5].

Discussion

The study's participants had a mean age of 9 ± 3.5 years, which is consistent with previous findings by ***B. Hossain et al.*** (9.4 + 3.20 years) and ***H. Bhavsar et al.*** (6.84 + 3.78 years).[7,8] The similar mean age supports consistency in patient characteristics across research by matching typical demographics seen in pediatric studies. Haemoglobinopathies like thalassemia major are diagnosed at an early age and require regular blood transfusions since childhood. The findings support the typical observation that thalassemia patients are diagnosed early in life, necessitating ongoing monitoring and treatment throughout their youth.

The gender distribution in the present study, with 69.7% males is consistent with findings from other studies, indicating a predominance of males in thalassemia cases. ***Patel NA et al.*** reported 62.14% males,[9]***K. Mishra et al.*** found 67.8% males,[10] and ***Manisha et al.*** observed 63.6% males.[5]This male preponderance across studies suggests a consistent trend where thalassemia is more prevalent in males, likely due to genetic and demographic factors that affect the distribution of hemoglobinopathies.

In the present study, 57.6% of participants were Hindu and 42.4% were Muslim. This differs from the findings of ***Manisha et al.***, where 81.8% of participants were Hindu, with a smaller proportion of 16.7% Muslim participants.[5] The lower percentage of Hindu participants in the current study compared to ***Manisha et al.*** indicates potential regional or demographic differences in the prevalence of thalassemia.[5] These variations could be attributed to different socio-cultural or genetic factors influencing the distribution of hemoglobinopathies in different populations.

The proportion of consanguineous marriages among the parents of participants in the present study was 60.6%. This is higher compared to ***Patel NA et al.***, who reported 42.37%,[9] but lower than ***R. Kiani et al.***, who found an 81.81% prevalence of consanguineous marriages.[11] These variations suggest regional or cultural differences in the prevalence of consanguinity, which may influence the occurrence of hemoglobinopathies such as thalassemia.

In the current study, over half of the patients (47%) received blood transfusions from government sources, whereas one-third used non-government sources such as the private sector, non-governmental organizations (NGOs), or public–private partnerships. The remaining 19.7% of patients chose transfusions from different blood banks due to their convenience. This proportion is similar to that reported by ***Manisha et al.***, who found that 44% of patients received transfusions from government sources, while the remaining 56% used a combination of blood banks.[5]

In the present study, the proportion of Hepatitis B infection was 1.5%, with a single male patient affected and no female patients, which is comparable to the 0.79% found in ***P. Gugnani et al.***, where only one male patient was affected.[12] Significantly higher proportion of seropositivity for Hepatitis B is found in some studies like ***Muhhamad et al.***[13]***, R. Mahmoud et al.***[14] and ***H Bhavsar et*** **al.** [8] 7.87%, 4.11% and 6% respectively. Whereas some study ***M Sindhu et al***. showed lower proportion of seropositivity for Hepatitis B as 0.72%.[15] The differences in the sero-proportion of Hepatitis B across world and among various studies in India may be due to variation in geographical distribution of viral infections, differences in the proportion of viral load and stages among blood donors, different sensitivity and specificity of blood tests used for screening of donated blood, implementation of more efficient and superior NAT testing of donated blood in some centers only.

For Hepatitis C, the present study reported a 9.09% prevalence, with 3.03% in males and 6.06% in females, whereas ***P. Gugnani et al.*** reported a higher overall prevalence of 13.4%, with 6.3% in males and 7.1% in females.[12] The differences in Hepatitis B and C infection rates between two studies could be due to multiple factors like variations in sample size, demographic characteristics, geographic location, blood transfusion practices, and study methodologies.

The number of blood transfusions received by the HCV-positive group in the present study was higher than that of the HCV-negative patients. This finding is supported by the study conducted by ***Bhavsar et al.***, which also suggested that an increased number of transfusions raises the risk of Hepatitis C.[8]

Conclusion

This hospital-based study clearly shows the incidence and progression of Hepatitis B and C infections among multi-transfused thalassemia children in South Gujarat. It discovers a low frequency of Hepatitis B (1.5%) and a significant prevalence of Hepatitis C (9.09%), with a higher rate of infection among individuals who have more frequent blood transfusions. While Hepatitis B infections were rare, the study emphasizes the significance of regular blood screening and surveillance for transfusion-transmitted diseases. The study indicates that high blood ALT and ferritin levels are strong indicators of Hepatitis C infection, implying that these parameters can be used as surrogate markers. This emphasizes the importance of enhanced blood donor screening processes and more diligent surveillance for Hepatitis B and C among multi-transfused thalassemia patients.

Recommendations

To lower the risk of transfusion-transmitted illnesses, it is critical to improve blood screening procedures by combining more sensitive Hepatitis B and C testing over and above the routine testing strategies. Additionally, frequently monitoring serum ALT and ferritin levels in multi-transfused thalassemia patients can aid in the early detection of Hepatitis C and improved management of iron overload. Larger, multi-center studies could help to validate these findings and analyze the efficacy of various transfusion safety methods in varied circumstances.

Limitations

The study's small sample size may limit the generalizability of the findings to a larger population. Furthermore, because this was a single-center study, the findings may not be representative of practices or infection rates in other hospital settings. The cross-sectional strategy, which collects data at a single point in time, further restricts the capacity to track changes or development in Hepatitis infections over time.

Conflict of Interest:
None

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