



Original Research Article

Hidden burden: Hepatitis C virus infection among multi-transfused thalassemic children in Gujarat

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Abstract

Background: Thalassemia is a genetic hemoglobinopathy leading to chronic anemia, frequently requiring regular blood transfusions. Recurrent transfusions elevate the risk of transfusion-transmitted infections (TTIs), including hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, and syphilis. Iron overload also remains a serious complication. This study aimed to assess the prevalence of HCV infection in β -thalassemia major children receiving multiple transfusions.

Materials and Methods: A cross-sectional study was conducted at the Department of Microbiology, tertiary care centre, Vadodara, from February to July 2024. A total of 119 thalassemic children with regular transfusion history were included. Serum samples were first screened using the Qualisa third-generation ELISA for anti-HCV antibodies. All ELISA-reactive samples were further confirmed using qualitative real-time PCR to detect HCV RNA, ensuring diagnostic precision. Demographic and transfusion data were collected through caregiver interviews.

Results: Out of 119 participants, 16 (13.45%) were confirmed HCV RNA-positive using qualitative PCR. The highest prevalence was noted in the 9–12 years age group. A strong correlation was observed between the number of transfusions and HCV positivity, with a 60% positivity rate in those who received more than 100 transfusions. Additional factors such as rural residence, splenectomy, and lack of chelation therapy were associated with higher HCV rates.

Conclusion: This study emphasizes the high burden of HCV among multi-transfused thalassemic children and advocates for routine NAT/PCR screening and continuous HCV surveillance in high-risk pediatric groups.

Keywords: Hepatitis C virus, β -thalassemia major, ELISA, qualitative PCR, Transfusion-transmitted infections, Pediatric seroprevalence.

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1. Introduction

Hepatitis C virus (HCV) is an RNA virus of the *Flaviviridae* family, primarily transmitted through blood and blood products. Globally, over 180 million individuals are chronically infected with HCV, and transfusion-dependent populations such as thalassemics are particularly vulnerable.^{1,2} Chronic HCV infection can lead to progressive liver damage including fibrosis, cirrhosis, and hepatocellular carcinoma.^{3,4}

Thalassemia, especially β -thalassemia major, is a hereditary disorder marked by reduced or absent synthesis of

β -globin chains. In India, the carrier rate is around 3–4%, affecting approximately 35–45 million people.^{5,6} Regular blood transfusion is the cornerstone of thalassemia management but significantly increases the risk of TTIs, including HCV, and complications like iron overload.^{7,8}

Indian studies report varying HCV prevalence rates among thalassemia patients, ranging between 7% and 25%.^{9,10} The World Health Organization recommends targeted surveillance for such high-risk groups.¹¹

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This study aims to evaluate the prevalence of HCV in multi-transfused thalassemic children at a tertiary care center in Vadodara using PCR-based confirmation.

2. Materials and Methods

This was a cross-sectional observational study conducted over six months, from February to July 2024, at tertiary care hospital in Vadodara, Gujarat. The study population comprised children diagnosed with β -thalassemia major who were receiving regular blood transfusions at the hospital's dedicated thalassemia daycare unit. A total of 119 patients were included, selected through purposive sampling.

Inclusion criteria required patients to have a confirmed diagnosis of β -thalassemia major and a documented history of receiving at least five blood transfusions. Children were excluded if they had never undergone transfusion, had incomplete demographic or clinical data, or suffered from pre-existing liver conditions unrelated to HCV infection.

Following informed consent from caregivers, demographic information—including age, sex, residence (urban or rural), and clinical history—was obtained via structured interviews. Detailed transfusion history, including the number and frequency of transfusions, presence of splenectomy, and iron chelation therapy status, was also recorded.

Blood samples were collected under aseptic conditions. Five milliliters of peripheral venous blood was drawn from each participant and transferred into sterile, plain tubes. The samples were centrifuged at 10,000 rpm for 5 minutes to separate serum, which was then aliquoted and stored at -20°C until further analysis.

Initial screening for anti-HCV antibodies was conducted using a third-generation Qualisa ELISA kit. The assay was read at 450 nm wavelength, and the cut-off value (COV) was calculated as the mean optical density (OD) of negative controls plus 0.3. Samples with OD values greater than the COV were classified as reactive and retested in duplicate to confirm the reactivity.

All ELISA-reactive samples underwent confirmatory testing using qualitative real-time PCR. RNA extraction was carried out using the silica column-based method, ensuring high purity and yield. The qualitative PCR assay targeted the conserved 5' untranslated region (5'UTR) of the HCV genome, employing fluorescence-based amplification techniques. Only those samples with detectable HCV RNA were considered confirmed positive, ensuring high specificity and diagnostic accuracy.

3. Results

Among 119 thalassemia children, 16 (13.45%) were PCR-confirmed as HCV-positive.

Table 1: Sex-wise distribution

Sex	Total Patients (n=119)	HCV Positive (n=16)	Percentage (%)
Male	70	9	12.86%
Female	49	7	14.29%

Table 2: Age-wise distribution

Age Group (Years)	Total Patients	HCV Positive	Percentage (%)
<2	5	0	0.00%
2–4	19	1	5.26%
5–8	31	3	9.68%
9–12	36	8	22.22%
13–18	28	4	14.29%

Table 3: HCV positivity by number of blood transfusions

No. of Transfusions	Total Patients	HCV Positive	Percentage (%)
0–25	52	1	1.92%
26–50	30	3	10.00%
51–75	19	4	21.05%
76–100	13	5	38.46%
>100	5	3	60.00%

Table 4: Residential background

Residence Type	Total Patients	HCV Positive	Percentage (%)
Urban	74	9	12.16%
Rural	45	7	15.56%

Table 5: Blood group distribution

Blood Group	Total Patients	HCV Positive	Percentage (%)
A	22	3	13.64%
B	37	5	13.51%
AB	14	2	14.29%
O	46	6	13.04%

Table 6: Splenectomy status

Splenectomy	Total Patients	HCV Positive	Percentage (%)
Yes	31	6	19.35%
No	88	10	11.36%

Table 7: Iron chelation therapy status

On Chelation Therapy	Total Patients	HCV Positive	Percentage (%)
Yes	97	11	11.34%
No	22	5	22.73%

4. Discussion

This study identified a 13.45% prevalence of HCV infection among multi-transfused thalassemic children, confirmed through qualitative PCR, underscoring the persistent threat of transfusion-transmitted infections in this vulnerable group. (Table 1) Notably, the highest infection rate was found in the 9–12 years age group (22.22%), likely due to cumulative transfusion exposure over time. In contrast, no cases were observed among children under two years of age, reinforcing the role of transfusion frequency and duration as key risk factors. (Table 2)

A direct relationship between the number of transfusions and HCV positivity was evident, with infection rates increasing progressively—from 1.92% in patients who received fewer than 25 transfusions to 60% in those who received more than 100. This highlights the ongoing risk of repeated transfusions, especially in the absence of molecular donor screening. (Table 3)

Additional demographic and clinical risk factors also influenced HCV prevalence. Children from rural areas demonstrated a slightly higher infection rate (15.56%) compared to their urban counterparts (12.16%), which may reflect disparities in healthcare quality and blood safety. Splenectomized children exhibited a higher HCV prevalence (19.35%) than non-splenectomized ones (11.36%), possibly due to increased transfusion requirements. (Table 4–Table 6) Similarly, those not receiving iron chelation therapy had a higher positivity rate (22.73%) than those on regular chelation (11.34%), suggesting that adherence to chelation therapy may be linked to better overall follow-up and infection control. Importantly, no statistically significant association was found between HCV prevalence and ABO blood group types, implying that blood group does not influence HCV susceptibility. (Table 7)

4.1. Compared with previous Indian studies, our prevalence rate (13.45%) is lower

Mahmoud et al. reported 24.64% in Egypt,¹⁴ Modi et al. observed 20.4% in Gujarat,¹⁵ Agrawal et al. found 24.0% in Rajasthan,¹⁶ and Vidja et al. documented 21.0% in Gujarat.¹³ Gorakshakar & Ghosh also highlighted that transfusion-transmitted infections, particularly HCV, remain the most common infectious complication among Indian thalassemics, with rates often exceeding 20% in earlier reports.¹² All of these studies relied solely on ELISA, which may have overestimated infection rates due to the inability to distinguish between past and active infections. In contrast, our study employed ELISA for initial screening and PCR for confirmation, ensuring that only active infections were counted. This explains the observed discrepancy, as some ELISA-reactive cases were not PCR-positive—likely representing false positives or past resolved infections. Thus, ELISA alone may overestimate prevalence, whereas PCR

provides a more accurate assessment of active infection burden.

These findings collectively emphasize the necessity of incorporating NAT/PCR-based HCV screening in routine practice, particularly for high-risk pediatric groups such as those with high transfusion burdens, rural backgrounds, splenectomy history, or inadequate chelation therapy. The study provides compelling evidence to support the upgrading of transfusion screening protocols and improving long-term care strategies for thalassemic children.

5. Conclusion

This study highlights a significant burden of hepatitis C virus (HCV) infection among multi-transfused thalassemic children, particularly those with higher transfusion exposure, rural residency, history of splenectomy, and lack of chelation therapy. The use of qualitative PCR for confirmation strengthens the reliability of the findings by accurately identifying active infections. Given the increasing prevalence with the number of transfusions, this study emphasizes the urgent need for comprehensive and routine screening protocols including NAT/PCR-based testing. Strengthening chelation therapy follow-up, improved post-splenectomy monitoring, and ensuring safe transfusion practices through voluntary repeat donations are crucial steps in minimizing the risk of HCV transmission in this vulnerable pediatric population. Key recommendations include:

1. Mandatory NAT/PCR donor screening.
2. Routine HCV monitoring in high-risk pediatric groups
3. Strengthening chelation therapy follow-up and post-splenectomy care.
4. Promoting voluntary repeat blood donations.

6. Ethical Considerations

Approval was obtained from the Institutional Ethics Committee of SBKS Medical Institute & Research Centre, Piparia, Vadodara with ref. no. SVIEC/ON/Medi/RP/Aug/25/6.

7. Source of Funding

None.

8. Conflict of Interest

None.

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