



Original Article

Seroprevalence of HBV, HCV and HIV-1 and Correlation with Molecular Markers among Multi-Transfused Thalassemia Patients in Western India

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Abstract. Background: Multitransfused β -thalassemia major patients are always at high risk of having Transfusion Transmitted Infections (TTIs). This study was aimed to determine the seroprevalence of HBsAg, Anti-HIV-1/2, and Anti-HCV among these patients and to correlate the same with NAT testing.

Methods: A total of 196 patients with β -thalassemia were included in the study. Patients were screened for the presence of viral markers by third-generation ELISA test as well as for viral DNA/RNA by NAT test.

Results: Among 196 multi-transfused Beta-thalassemia patients, the seroprevalence of anti-HCV was very high 100 (51.1%), however, anti-HIV1/2 was 6 (3.1%), and HBsAg were 3 (1.5%). Surprisingly similar patterns were observed in the prevalence of molecular markers, as HCV-RNA were 66 (33.7%) of the patients along with HIV-1 RNA were 8 (4.1%), and HBV-DNA were 5 (2.5%) patients. Overall eight (4.1%) patients were found to have coinfections, where two were positive for HBsAg/anti-HCV by ELISA along with 3 (1.5%) were positive for HBV-DNA/HCV-RNA, 1 (0.5%) was positive for HIV-RNA/HBV-DNA, and 2 (1%) had coinfection of HIV-RNA/ HCV RNA by NAT testing

Conclusion: The prevalence of HCV infection among multi-transfused β -thalassemia patients is significantly higher than that of the HBV and HIV infections. This scenario should be controlled and monitored by doing regular follow-up testing schedules of such patients and also the administration of the booster dose of the HBV vaccine along with HCV treatment with antiviral DAAs.

Keywords: Blood transfusion; Hepatitis-B; Hepatitis-C; HIV-1; Beta-thalassemia; NAT test; and ELISA.

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Introduction. Thalassemia major patients have a high prevalence of transfusion-transmitted infections, mainly as a consequence of viral infections acquired through blood transfusion. Thalassemia is one of the common genetic conditions prevalent in India. It is estimated that there are about 65,000-67,000 Beta-

thalassemia patients in India, with around 9,000-10,000 cases added every year, with an estimated incidence of 2 per 1,000 births and a carrier frequency of 3-4%, it constitutes a significant health burden.¹

Thalassemia is a term for a group of disorders in which there are reduced levels of hemoglobin,

decreased red blood cell production, and anemia. Furthermore, severe anemia can cause serious, even life-threatening complications if left untreated.^{2,3} Therefore individuals diagnosed with beta-thalassemia undergo lifelong blood transfusion to maintain standard hemoglobin (Hb) levels for their health management.⁴ Hepatitis-B virus (HBV), Hepatitis-C virus (HCV) and Human immunodeficiency virus (HIV) are three most common chronic viral pathogens among multitransfused thalassemia major individuals⁴ as all these viruses can transmit through blood transfusion apart from other routes.

Coinfections of HIV/HCV, HIV/HBV, and HBV/HCV in thalassemia patients are associated with reduced survival.⁵ Therefore coinfection is common in people with high exposure to blood and blood products. The main concern with HIV/HCV coinfection is that it can lead to more severe liver diseases and increased the risk of progression to liver cancer, especially in immunocompromised thalassemic patients.⁶ There are several reports related to HIV/HCV coinfection among thalassemic patients; however there is no report related to HBV/HCV coinfection that can lead to liver cirrhosis and hepatocellular carcinoma (HCC), and no report related to HIV/HBV from different parts of India.^{7,8}

Blood units are screened with assays of steadily increasing sensitivity for Hepatitis-B surface antigen (HBsAg) since 1971, against HIV since 1989 and against HCV since 2001.⁹ Indeed, the risk of being infected by a contaminated blood unit today is orders of magnitude lower when compared to thirty years ago, due to continuous improvement and implementation of donor selection, sensitive screening tests. However, HCV is still a significant problem in patients with thalassemia.

Despite the enormous burden of thalassemia in India, a survey of blood transfusion practices noted that testing for transfusion-transmitted infections is unsatisfactory and poorly regulated in most blood banks, both private and government, throughout India.^{10,11} This results in a continuing risk of transmission of infectious agents in thalassemia children receiving multiple blood transfusions.

There is a scarcity of literature emphasizing the magnitude of transfusion-associated viral hepatitis in thalassemics in Western India. The need to explore the burden of transfusion-mediated infections in these patients cannot be overemphasized. Therefore this study was aimed to determine the prevalence of anti-HCV, HBsAg, and anti-HIV-1 and viral RNA/DNA positivity of HBV, HCV, and HIV-1 in thalassemic children. Moreover, we believe that this is the first work that shows the prevalence of HBV, HCV, and HIV-1 in thalassemia patients using both sero-molecular markers in Western India.

Materials and Methods. This study was approved by the Institutional Ethics Committee (IEC) and conducted in 2015. The study group included 196 beta-thalassemia Major children who are taking regular blood units for transfusion from Surat Raktadan Kendra & Research Centre (SRKRC). Blood samples of these patients were obtained from the serology Department of SRKRC prior to taking their informed consent having information like age, sex, address, history of the previous transfusions, total number of transfusions to date, the age when the first transfusion was given, etc.

Serum and Plasma samples. Blood sample of all the patients was collected in 2 ml EDTA and plain tubes; plasma and serum were separated by centrifugation at room temperature, labeled appropriately in two aliquots, and stored at -30°C in a deep freezer, till the tests for TTI serology as well as NAT was performed.

Serological assay. All the serum samples of 196 patients were screened for anti-HIV-1/2, HBsAg, and anti-HCV using third-generation ELISA kits. For anti-HCV, SD HCV ELISA 3.0 test system (Boi SD standard diagnosis Pvt. Ltd, India); for anti-HIV-1/2, Microlisa (J. Mitra & Co. Pvt. Ltd, India) and for HBsAg, SD HBV ELISA 3.0 test system (Boi SD standard diagnosis Pvt. Ltd, India) was used.

Nucleic Acid Amplification (NAT). All plasma samples of 196 patients were screened for research purposes for the viral genome of HBV, HCV, and HIV-1 with a commercially available RT-PCR kit (Altona Diagnostics GmbH, Germany). The PCR was performed on an ABI Prism 7500 Real-Time PCR System (Thermo Fisher, USA).

- i. *Extraction of the viral genome:* HBV-DNA, HCV-RNA, and HIV-1 RNA were extracted from plasma samples with the use of Chemagic Prepito-D automated nucleic acid extractor (PerkinElmer, USA), in combination with reagents/buffers of the Prepito Viral DNA/RNA Kit.
- ii. *Amplification of viral genome by Real-Time-PCR:* HBV-DNA and HIV and HCV-RNA were amplified by RealStar HBV PCR Kit 1.0, RealStar HCV RT-PCR Kit 1.0, and Real-Star HIV RT-PCR Kit 1.0 (Altona Diagnostics GmbH, Germany) as described in the manufacturer's protocol. The PCR was performed on an ABI Prism 7500 Real-Time PCR System (Thermo Fisher, USA).

Statistical software. The data were subjected to statistical analysis using SPSS version 10.0 software. Mean and standard deviations were computed. For discrete variables, the Chi-square test was applied to determine the association between two variables. A student's test was done to compare the mean of two

groups. A significant difference was accepted at $p = 0.05$.

Results. A total of 196 patients of thalassemia patients were included in this study. Amongst them, 133 (67.8%) were males, and 63 (32.14%) were females with the age group between five years to fifteen years (**Table 1**).

Hepatitis B Virus (HBV). Out of 196 multitransfused thalassemia patients, the prevalence of HBsAg positivity and HBV-DNA positivity were 1.5% (3/196) and 2.5% (5/196) respectively, in which three male patients were HbsAg positive and four male and one female patient were HBV-DNA positive (**Table 2A**). Every sample that positive for HBsAg was also positive for HBV-DNA, and there were two samples solely positive for HBV-DNA.

Hepatitis C Virus (HCV). Out of 196 multitransfused thalassemia patients, the prevalence of anti-HCV positivity and HCV-RNA positivity was 51% (100/196), and 33.7% (66/196) (**Table 2B**), in which 28 females and 72 males patients were anti-HCV positive and 13 females and 53 males patients were HIV-RNA positive. On the contrary, two samples that were positive for anti-HCV but found negative for HCV-RNA. In HCV seropositive samples, the positive rate of HCV-RNA was 64% (64/100).

Human Immunodeficiency Virus (HIV). Out of 196 multitransfused thalassemia patients, the prevalence of anti-HIV positivity and HIV-RNA positivity were 3.1% (6/196) and 4.1% (8/196), respectively (**Table 2C**), in which five males and one female patient were anti-HIV positive, and five males and three females were HIV-RNA positive. However, two anti-HIV positive samples were not found positive for HIV-RNA. On the other hand, two samples were solely positive for HIV-RNA.

Coinfections among multitransfused thalassemia patients. Amongst 196 multitransfused thalassemia patients, we found 2 (1.0%) coinfections cases of HBV and HCV by ELISA test. Furthermore there were 6

Table 1. Age and Sex wise distribution of multitransfused thalassemia patient.

Age group of patients	Total number of patients	Number of Male patients	Number of Female patients
<5 yrs	4	2	2
5-10 yrs	52	30	22
10-15 yrs	73	48	25
> 15 yrs	67	53	14
Total	196	133 (67.8 %)	63 (32.14 %)

Table 2. HBV, HCV and HIV infections among multitransfused thalassemia patients.

(A). HBV infection

Age group of patients	Total number of patients	HbsAg positive n (%)	HBV - DNA Positive n (%)
<5 yrs	4	0	0
5-10 yrs	52	1 (1.9)	2 (3.8)
10-15 yrs	73	1 (1.3)	2 (2.7)
> 15 yrs	67	1 (1.5)	1 (1.5)
Total	196	3 (1.5)	5 (2.5)

(B). HCV infection

Age group of patients	Total number of patients	Anti-HCV positive n (%)	HCV - RNA positive n (%)
<5 yrs	4	1 (25)	0
5-10 yrs	52	28 (53.8)	19 (36.5)
10-15 yrs	73	36 (49.3)	22 (30.1)
> 15 yrs	67	35 (52.2)	25 (37.3)
Total	196	100 (51.0)	66 (33.7)

(C). HIV infection

Age group of patients	Total number of patients	Anti-HIV positive n (%)	HIV - RNA Positive n (%)
<5 yrs	4	1 (25)	1 (25)
5-10 yrs	52	2 (3.8)	3 (5.7)
10-15 yrs	73	3 (4.1)	4 (5.4)
> 15 yrs	67	0	0
Total	196	6 (3.1)	8 (4.1)

P value for Chi $X^2 = 0.9$

(3.0%) cases of co-infections by NAT testing, in which 3 (1.5%) were HBV/HCV, 1 (0.5%) were HIV/HBV and 2 (1%) were HIV/HCV.

Discussion. The β -thalassemia is the most common inherited hemoglobin disorder in the Indian subcontinent, with an uneven distribution among the different endogenous populations. Furthermore, hemoglobinopathies cases are more in Gujarat compared to other Indian states. Earlier we have reported a high prevalence of β -thalassemia trait (BTT) and sickle cell trait (SCT) in South Gujarat.^{12,13,14}

Conventional treatment of patients suffering from β -thalassemia is based on adequate and safe blood transfusions and receiving regular iron-chelation therapy from early childhood, all of which improve the quality of life and survival of patients.¹⁵ On the other hand, blood transfusions expose the patients to the risk of acquiring transfusion-transmissible infections (TTIs). The possibility of acquiring TTIs is associated with the number of units transfused; therefore, the infection rate

of TTIs increases with age in subsequent years.¹⁶ Therefore, each blood transfusion/blood unit composes a chance for acquiring TTI, and as the number of blood transfusion increases, the higher the risk of exposure. Furthermore, since thalassemia patients need multiple transfusions, it is logical that the provision of blood may not be prompt in every visit in a single center. As a result, most of the thalassemia patients had received blood units from multiple hospitals or blood banks within or outside the state.

In the present study, seropositivity for TTIs was 57% (109/196). HIV seropositivity was 3.1%, HBV was 1.5%, and HCV was 51%. Different studies from all over India reported the highest TTIs transmission rates for HCV, ranging from 2.2-44%, followed by HBV ranging from 1.2-7.4% and HIV ranging from 0-9%.^{17,18,19}

The prevalence of anti-HCV in multiple-transfused patients is confirmed to be high.¹⁹⁻²¹ A three-year prospective study from India by Choudhury et al., 2001,¹⁹ observed that anti-HCV prevalence in the same number of thalassemia major patients was 23%, 30.7%, and 35.9% each year, respectively. The present study showed comparable results as described by Choudhury et al. 2001,¹⁹ and Mukherjee et al., 2017.²² Furthermore anti-HCV seropositivity in our patients (51%) was comparable among multitransfused patients in Jordan (40%),²³ Egypt (45% to 76%),²⁴ Iran (44.7%),²⁵ and Pakistan (51.3%).²⁶

In our study, the seropositivity of HBsAg among thalassemic children was 1.5%. Our result was comparable to a study in India, where the prevalence rate of HBsAg was ranged from 1.2-7.4%.²² However, the prevalence rate is still high compared to other Asian countries, such as Turkey and Malaysia, which reported lower HBsAg seroprevalence rates of 0.75% and 1%, respectively.^{27,28} Differences in the prevalence of TTIs amongst thalassemic could be related to geographical differences in the prevalence of the viral infections among blood donors, the nature of blood donors, whether replacement or voluntary and most important the nature of care individual thalassemics receives.

In India, it is mandatory to screen donated blood for anti-HIV 1 and 2 (since 1991), anti-HCV (since 2001), and HBsAg (along with malaria and syphilis) became mandatory since 2002. Since then, the risk has been limited to the blood units collected during the "window period". However, TTIs can still occur from blood units negative for the markers for these infections, as reported by different investigators and international studies.^{17,18} Since HBV and HCV are transmissible by the parenteral route and may be found not only in blood but also in other body fluids.

Nucleic acid testing (NAT) is widely recommended for the screening of the donor's blood. Makroo et al., 2008,²⁹ and our two recent studies; Mishra et al.,

2016,³⁰ and Ghosh et al., 2017A,³¹ have shown that blood units negative for HBsAg, anti-HIV, and anti-HCV have 1:1807, 1:15906 and 1:39761 NAT positivity rate and that the majority of the positivity was due to Hepatitis-B virus, underlining the need for HBV vaccination in thalassemic patients. Moreover, HBV infection can be prevented in these patients, as a very effective HBV vaccine is available; therefore, all patients who require multiple transfusions should be vaccinated right from the beginning.

In the present study, 77% of cases positive for anti-HCV were between 10 to 15 years of age; they have been receiving transfusions before 2001 when screening for anti-HCV became mandatory. There is a reduction in the development of anti-HCV post-2001, but it has not been eliminated. The causes of high anti-HCV prevalence may be due to donors being usually asymptomatic in early stages, despite being screened for anti-HCV, possibly due to missing early window period infections. Most of the cases of positive anti-HCV had received transfusions before anti-HCV become mandatory therefore patients have more chances of developing anti-HCV due to the cumulative increase in the number of transfusions.¹⁵

In the present study prevalence of HIV-RNA positive was higher (4.1%) while Anti-HIV positive was (3.15). Moreover, two samples were solely positive for HIV-RNA; it is due to immunosuppression with decreased production of antibodies, or the window period of a recent infection.^{17,18} Though two anti-HIV positive samples were not found positive for HIV-RNA, the primary purpose of anti-HIV screening tests is to risk factors for disease in large numbers of individuals, thereby initially anti-HIV positive samples should be verified by a second repeat testing.

In this study amongst 196 multi-transfused thalassemia patients, there were six cases of coinfections by NAT testing (two coinfections HIV-RNA/HCV-RNA, one coinfection HIV-RNA/HBV-DNA and three coinfections of HBV-DNA/HCV-RNA), wherein there were two coinfections cases of HBsAg/anti-HCV by ELISA. Coinfection is consequently common in people with high exposure to blood and blood products.³² The primary concern of coinfection is that it can lead to more severe liver diseases in multitransfused patients.⁶

NAT testing was introduced in the developed countries in the late 1990s and early 2000s and presently, around 33 countries in the world have implemented NAT for HIV;^{33,34} however, NAT is not as yet a mandatory screening test in India. We have implemented NAT testing at our center from April 2013; however, the implementation of NAT technology is still limited at different centers in India; this might contribute to the high infectious marker positivity observed in the present study. Blood screening using NAT can reduce the window periods of HIV, HBV and

HCV infections substantially.^{30,31,34,35} Various studies from India reported a combined NAT yield (NAT positive/Seronegative) for HIV, HBV and HCV is high as compared to that reported from other developed countries.^{29,36} These elevated yields of NAT suggest a higher prevalence of TTIs in India, highlighting the need for NAT in our country.

A vaccine for hepatitis-B is available and should be given to recipients, especially to multitransfused patients, before transfusion. Furthermore, a high prevalence of HCV in multi-transfused patients of Beta-thalassemia necessitates better methods of screening like NAT in the facilities. Periodic monitoring of multi-transfused subjects through hemovigilance should be made a part of the blood safety programs. Furthermore, HCV infection leads to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).³⁷ Until couple of years ago, the recommended therapy for HCV treatment consisted of chelation therapy along with pegylated-interferon alpha plus ribavirin, a therapy with significant side effects.³⁸ More recently, the use of Direct-acting Antiviral Agents (DAAs) gave a breakthrough and demonstrated to be appropriate in HCV management in patients with thalassemia disease for whom previous regimens gave restrictions.³⁸ DAAs has led to a real HCV eradication with negative viremia and sustained viral response between 90 and 98%.³⁹ Thus treatments with DAAs, with adequate iron chelation, and non-invasive monitoring liver status is recommended to prevent cirrhosis and HCC in thalassemia patients.⁴⁰ However it remains the big crisis of the costs of DAAs therapies, treatment regimens are very expensive, and this can limit their application, and lengthen the time for the global eradication of HCV.

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Transfusion Transmitted Infections mainly occur in patients who are dependent on blood transfusion like thalassemia major, sickle cell disease, chronic renal failure, etc. Such patients should be encouraged to stick to one center for their blood unit requirement for transfusion, although it is logical that the provision of blood may not be prompt in every visit. Therefore all sectors need to strict donor screening with a mandatory screening of blood products against the TTIs and control the quality of blood donors, along with the use of modern molecular biology techniques such as NAT for the screening of blood units, and bringing awareness in the community will surely help in reducing the problem statement. Furthermore, periodic screening of multi-transfused subjects through hemovigilance should be made a part of the blood safety programs.

Conclusions. Despite the standard procedures followed by blood banks to ensure blood safety, HBV, HCV, and HIV present a significant challenge in the management of thalassemia patients. There is still a severe risk for HCV infection. On the contrary, there is a minor risk for HBV infection in patients with thalassemia. Administering HBV vaccine along with HCV treatment with DAAs, in conjunction with adequate iron chelation, ensuring the immune status, and monitoring hepatitis markers might considerably minimize the incidence of viral hepatitis among them.

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