Original Article

Menace of Hepatitis C virus among multitransfused thalassemia patients in Balasore district of Odisha state in India

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Abstract

Context: Hepatitis C virus (HCV) is a potential cause of morbidity and mortality worldwide. It is most commonly transmitted through injecting drug use; the reuse or inadequate sterilization of medical equipment and the transfusion of unscreened blood products. Management of thalassemia requires long-term blood transfusion. Though it improves the overall survival, it carries a definite risk of infection which is expected to be higher in resource limited settings. **Aims:** To find the percentage of transfusion-transmitted infections (TTIs) in multitransfused patients of thalassemia in Eastern India. **Settings and Design:** The study was conducted to assess blood safety in rural population in India by measuring the percentage of TTIs including HCV in multitransfused thalassemia patients. **Methods and Materials:** One hundred and twenty three patients with major beta-thalassemia were enrolled in this study. The blood samples were tested using ELISA technique for all TTIs. HIV fourth generation kits, HbsAg, HCV third generation kits, malaria and syphilis, parbovirus IgM and parbovirus IgG kits, HEV Antigen and IgM antibody were used. **Statistical Analysis Used:** Proportions and means were calculated for categorical and continuous variables, respectively. Chi-square test was applied and *P* value of <0.05 was taken as significant. **Results:** The mean age of patients was 9.5 years ± 5.2 years. Among various TTIs, Hepatitis C and HIV was prevalent among 59.3% and 4.1% of the study participants, respectively. **Conclusions:** The causes of high prevalence of HCV may be due to donors being usually asymptomatic in early stages, despite being screened for HCV possibly due to missing early window period infections. The screening methodology of TTIs particularly HCV at the district and village level and consequent increased prevalence of HCV in multitransfused rustic population of India shows the extent of blood safety.

Keywords: Hepatitis C infection, multi-transfused, thalassemia, TTI Screening methods in district

Introduction

World Health Organization estimates that in 2015, 71 million persons were living with chronic hepatitis C Virus (HCV) infection worldwide and that 399,000 died from cirrhosis or hepatocellular carcinoma caused by HCV infection. In May 2016, the World Health Assembly endorsed the Global Health Sector Strategy (GHSS) on viral hepatitis, which proposes to eliminate viral hepatitis as a public health threat by 2030 (90% reduction in incidence and 65% reduction in mortality). Elimination of viral hepatitis as a public health threat requires 90% of those infected to be diagnosed and 80% of those diagnosed to be treated.[1] With 12 million HCV infected people living in India, viral hepatitis is now recognized as a serious public health problem.

TheHCVis a blood-borne virus. It is most commonly transmitted through injecting drug use through the sharing of injection equipment; the reuse or inadequate sterilization of medical equipment, especially syringes and needles in healthcare settings;

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and the transfusion of unscreened blood and blood products. Management of some conditions like thalassemia requires long-term blood transfusion. Although regular blood transfusion improves the overall survival of patients with β-thalassemia, it carries a definite risk of infection with blood-borne viruses. The risk of infection is expected to be higher in resource limited settings.

The study was conducted at All India Institute of Medial sciences, Bhubaneswar to assess blood safety in rural population in India by measuring the prevalence of transfusion transmitted infections (TTIs) including HCV in multitransfused thalassemia patients from Balasore district of Odisha state of India.

Subjects and Methods

This observational study was conducted over a period of 1 year during 2016--2017 at All India Institute of Medical Sciences (AIIMS), Bhubaneswar.

Study participants were recruited from approximately 1,000 thalassemia patients who were undergoing regular transfusions in the Balasore district of Odisha State. The thalassemia patients, who received >5 transfusions and transfused packed red blood cells (PRBCs) or whole blood were included in this study. Sociodemographic characteristics, that is, patients' age, sex and diagnosis, and transfusion history were recorded.

The blood samples were tested using ELISA technique for all TTIs. HIV fourth generation kits (Qualisa-HIV 1 and 2, Tulip Lot No. 46159), HbsAg (Qualisa---HBsAg, Tulip Lot No. 61251), HCV third generation kits (Qualisa–HCV, Tulip Lot No. 54264), malaria (Qualisa---Malaria, Tulip) and syphilis (Tulip Lot No. 82039), parbovirus IgM (NovaTec Lot No. 165-41) and parbovirus IgG (NovaTec Lot No 132-41) kits, HEV Antigen (Wantai Batch No 170402) and IgM antibody (Wantai Lot No EM20161201) were used.

Participant information sheet was provided to all the patients/their parents/guardians and assent/consent was obtained. Ethical approval was taken from Institutional Ethical Committee of AIIMS Bhubaneswar.

The data was entered in a Microsoft excel 2010 worksheet. Data were cleaned and analyzed using SPSS software version 20 (SPSS Inc., Chicago, IL). Proportions and means were calculated for categorical and continuous variables, respectively. Chi-square test was applied and *P* value of <0.05 was taken as significant.

Results

One hundred and twenty three patients with major beta-thalassemia were enrolled in this study. The mean age of patients was 9.5 years ± 5.2 years. 82 (66.7%) and 41 (33.3%) of participants were male and female, respectively. Maximum participants were in age group of 5--9 years [Table 1].

Among various TTIs, Hepatitis C and HIV was prevalent among 59.3% and 4.1% of the study participants, respectively. None of the study participants were test positive for HBsAg, parvovirus, syphilis, and malaria [Table 2].

The proportion of hepatitis C was higher among study participants of younger age and was significantly associated with age (*P* value <0.05) [Figure 1 and Table 3].

Discussion

Serological marker testing for TTI is compulsory in India. This study endeavors to evaluate the relevance and format of tests for TTIs particularly HCV and its prevalence in multitransfused rustic population of India. The thalassemia patients are taken as the sentinel population to learn of extent of blood safety at the district and village level considering the limitations such as donor base, and screening assays in estimating the prevalence of HCV.

In the present study, the HCV positivity was 59.3% whereas none of the study participant was positive for hepatitis B virus biomarkers. Similarly a study conducted in multi-transfused

Table 1: Sociodemographic distribution of thalassemia patients (*n*=123)

	Number	Percentage
Sex		
Male	82	66.7
Female	41	33.3
Age groups		
<5 Years	23	19.3
5-9 Years	42	35.3
10-14 Years	40	33.6
15 years and above	14	11.8

Table 2: Prevalence of various TTIs among thalassemia patients

TTIs	Number	Percentage
Hepatitis C		
Positive	73	59.3
Negative	50	40.7
HIV		
Positive	5	4.1
Negative	118	95.9
HBsAg		
Positive	0	0.0
Negative	123	100.0
Parvovirus		
Positive	0	0.0
Negative	123	100.0
Syphilis		
Positive	0	0.0
Negative	123	100.0
Malaria		
Positive	0	0.0
Negative	123	100.0

Table 3: Hepatitis C and its association with the sex and age of thalassemia patients

	Negative	Positive	*P*
Sex			0.897
Male	33	49	
Female	17	24	
Age groups			
<5 Years	5	18	0.005
5-9 Years	14	28	
10-14 Years	16	24	
15 years and above	11	3	

Egyptian thalassemic patients, insufficiency of serology testing resulting in up to 45.5% TTIs in thalassemia patients.[2] The high prevalence of TTIs particularly hepatitis C virus infection, reflects the unsafe practices or non-standard screening methodology.

In the present study, the assessment of blood safety was done by HCV testing of β-thalassaemic population of Baleshwar district of Odisha in India. Thalassemia and Sickle Cell Anaemia are two common genetically linked blood disorders in Orissa with former more prevalent in coastal districts including Balasore and the latter in western districts of the state.[3] Thalassemia patients from Balasore receive transfusions at various centres which differ in their infectious marker screening methodologies.

The elite connection of TTIs with transfusion in thalassaemics is conceivable as they require transfusion from their first year of birth, and furthermore on account of their young age other methods for procuring diseases could be excluded. This study emphasizes on the relevance of TTI testing methodologies for blood supplied to thalassaemics in district hospitals and rural India. The blood banks in India use reagents of diverse quality and sample groups are heterogeneous. The serology assays range from use rapid tests to third or fourth generation enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CIAs).[4-6] The prevalence of TTI in general population in India is estimated by the prevalence of TTI in donor population. The blood banks with component facility indicated a higher positivity of HIV (0.141); HCV (0.363) and HBV (0.969). Amongst the blood donor population including both voluntary and family/replacement donors the screening of replacement units, revealed a seropositivity of transfusion transmitted disease as 0.15% in HIV, 1.67% in hepatitis B surface antigen, 0.49% in hepatitis C virus. The seropositivity of transfusion transmitted disease in voluntary units was 0.08% in HIV, 0.24% in hepatitis B surface antigen, 0.001% in hepatitis C virus.[7] The prevalence of sero-positivity was more in replacement donors.

The typical blood screening assay for HCV is for detecting anti- HCV antibodies and was mandated in India in 2001. Antibodies remain in circulation for a very long time lasting years even after viral remission, hence antibody reactivity results suggest either a current HCV infection, a past infection or false positivity. The HCV antibodies can be detected only

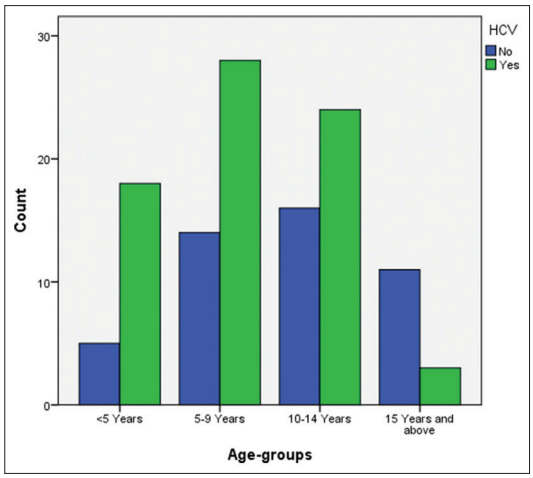

Figure 1: Bar diagram showing the age-group wise distribution of Hepatitis C positivity among study participants

after 65-70 days of window period (WP) and HCV has the longest period of non-detection of infectious units.[8] The delayed immune response means that the viral propagation proceeds unchecked and remains high during this interval. The third generation anti-HCV ELISA and CIA assays are known to have high false positivity. In a study from the United States only 21-34% of repeat reactives could be confirmed by a second reagent Western blot (WB) recombinant immune-blot assay (RIBA).[9] Amongst the Indian blood donors the initial reactivity using a combination assays of HCV antibody and core protein viral antigen which can reduce the WP by 21 days are not widely used because of cost.[10]

There is high prevalence of HCV in thalassaemics in all countries. Because of the unique profile of HCV with very long WP for antibody development it is critical to analyse the data for pre- and post-serology screening periods to gauge the impact of serology screening. The present study shows a prevalence of 59.3%. The high prevalence has been seen in many studies like the study by Hossein Norouzian *et al.*[11] (2016) showed a prevalence of 11.32%, Khalid Al –N *et al.*[12] (2016) with a prevalence of 41%, Ahmed Kamel M *et al.*[2] (2012) showed a prevalence of 40.5%, Sidhu M *et al.*[13] (2015) with a prevalence of 13.04%, Shrivastava M[14] (2015) showed a prevalence of 18.2%, Jain *et al.*[15] (2012) with a prevalence of 25%, Jaiswal *et al.*[16] (2001) with a prevalence of 21%.

The wide variability among the prevalence reported in different studies is because of different sensitivity and specificity of the tests used (third generation ELISA: 99% sensitive and 99.5% specific), different HCV prevalence in the donor populations, and differing donor selection criteria. This study shows the high risk of acquiring this infection.

The wide variance of the high prevalence of HCV has led to a consensus call in all the publications for improved pretransfusion screening, including the suggestion to adopt nucleic acid technology (NAT) testing.

The results by individual donation (ID) NAT testing, of 1 per 610 units being serology negative-NAT reactive is alarming. In spite of the fact that the cost for ID-NAT testing is viewed as excessively expensive for a medium development nation like India, the magnitude of TTIs will put a colossal cost load on the general public. The following studies highlight the current dilemma in India with the screened seronegative donations still posing a risk for TTIs.

Study	Sample size	NAT positive/seronegative
Makroo *et al.*[17]	12,224	8 NAT yield 1 in 1528 (0.065%)
Chatterjee *et al.*[18]	18,356	7 NAT yield 1 in 2622 (0.038%)
Agarwal *et al.*[19]	73,898	121 NAT yields 1 in 610

NAT is a highly sensitive and advanced technique which has reduced the window period of HCV to 1.34 days.

In spite of the mandated serology assays in India, the reports for all three viral TTIs indicate the inadequacy of serology-only screening in ensuring blood safety. The abundant literature available on postserology infections for HIV and HBV and limited information for HCV definitely point to the necessity of rethinking about the sufficiency of mandated serology tests alone. Indeed a majority of the publications recommend the use of NAT testing. By targeting the very early event in viral propagation through the detection of viral genomes which are the source of infection as NAT tests provide the shortest WP. The most sensitive NAT tests amplify the nucleic acids ever a million folds to detect the presence of very low number of copies of viral nucleic acid (NA). The current technologies are so fine-tuned for NA isolation, amplification, and detection that the WP from the day of presence of virus in blood to the day of detection depends on the virus doubling time.

The true value of diagnostics depends on assays that are early predictors of infection offering maximum blood safety. However, through breach in "safe blood transfusion" thalassaemics are confronted by new and more severe health challenges of TTIs contributing to increased mortality, and this can be extrapolated to general recipients as well. It can be undoubtedly stated that through the current serology screening practices, TTIs for HIV-1, HCV, and HBV are proceeding unchecked. Despite official positions, the approach of exclusive volunteer nonremunerated safe donors and vigilant donor deferral is not an immediately implementable way to curtail this increase.

From a blood supply point of view, family donors are a motivated population that significantly contributes to the Indian blood supply and cannot be deferred without due consideration of the evidence. Transforming the donor base to voluntary repeat donors is a sociological change which will take a long time to accomplish. For the immediate, implementable testing practice for maximum safety is the solution. Although the argument of unaffordability of ID-NAT testing has been the premise for pooled testing, the residual risk of the resulting TTIs places a serious burden on the health care, finances, society and human life. True health care costs applicable to Indian scenario have not been available.

These observations are a wake-up call for providing additional layer of security to national blood supply through NAT testing.

The causes of high prevalence of HCV may be due to HCV donors being usually asymptomatic in early stages, majority of donors become HCV positive despite being screened for HCV possibly due to missing early window period infections, therefore awareness about screening should be increased so that more patients are diagnosed early.

These data, however, cannot be extrapolated to the community as blood donors and pregnant women represent a selected and healthy population sample.

The reason of this wide range can be due to difference in type and sensitivity of testes, the prevalence of HCV in the relevant population, and the time of screening. The countries with a higher HCV prevalence in general population had a higher prevalence rate among thalassemia patients too.

There is an indisputable need for a shift towards preventive, continuous, and comprehensive care for multiransfused patients with HCV infection in order to improve their overall health status and to diminish the health burden posed by HCV. Increasing efforts should be directed towards linking multiransfused patients with HCV infection to primary care physicians (PCPs) especially at district levels where speciality and superspeciality services are minimal or nonexistent, so as to reduce the transmission of HCV.

PCPs especially at district levels can play a critical role in the initial steps in the care cascade for patients with HCV infection specifically as it relates to their role in screening, diagnosis, and referral to care.

The most important role of the PCP is in the broad execution of screening recommendations that will enable more patients to be diagnosed and subsequently treated. In addition, with the simplicity of current HCV regimens, many PCPs who see many patients with HCV can appropriately provide treatment for these patients because this would likely result in fewer patients lost to referral follow-up. This would benefit the communities and the PCP would be critical to expanding access to HCV treatment for the large number of individuals infected with HCV.[20]

Conclusions

Despite the mandatory testing of hepatitis C since 2001, the screening methodologies at district levels have failed to screen window period donors resulting in high hepatitis C positivity in multi-transfused thalassemia patients. Therefore to conclude

assess to better screening techniques is required to reduce the burden of disease.

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Conflicts of interest

There are no conflicts of interest.

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