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Original Research Article

Prevalence of hepatitis B and hepatitis C virus infection in repeatedly transfused thalassemics in a tertiary care hospital in eastern India

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ABSTRACT

Background: Patients of thalassemia who are conventionally treated by a regular transfusion regimen are at a risk of developing transfusion transmissible infections, including hepatitis. The present study was conducted to assess the prevalence of hepatitis B and hepatitis C virus infections among repeatedly transfused thalassemia patients.
Methods: A total of 207 patients of beta-thalassemia, who had received at least 10 transfusions were tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibody using enzyme linked immunosorbant assay (ELISA).
Results: The overall number of anti-hepatitis C antibody seropositive was 51 (24.6%) and that for hepatitis B surface antigen positives was 7 (3.38%). The prevalence rate for hepatitis B surface antigen was in agreement with average national values, but in case of anti-HCV antibodies the prevalence rate values were comparatively much higher.
Conclusions: Stringent measures need to be taken on urgent basis while screening blood for anti-hepatitis C antibody and hepatitis B surface antigen including inclusion of other sensitive assay like nucleic acid test (NAT) as well as better donor recruitment.

Keywords: HCV, HBV, Pretransfusion screening, Thalassemia

INTRODUCTION

Beta-thalassemia are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of haemoglobin resulting in variable phenotypes ranging from mild to severe anaemia. Worldwide, 15 million people have clinically apparent thalassemic disorders.

Reportedly, disorders worldwide, and people who carry thalassemia in India alone number approximately 30 million. Of the various thalassemia, beta-thalassemia major is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia.[1]

Treatment of thalassemia major mainly includes regular RBC transfusions, iron chelation and management of secondary complications of iron overload. The goals of transfusion therapy are correction of anaemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption. The decision to start transfusion in patients with confirmed diagnosis of thalassemia should be based on the presence of severe anaemia (Hb< 7 g/dl) for more than two weeks, excluding other contributory causes such as infections. This prevents growth impairment, organ damage and bone deformities, allowing normal activity and quality of life.[2,3]

If a regular transfusion program that maintains a minimum Hb concentration of 9.5 to 10.5 g/dL is initiated, growth and development tends to be normal up to 10 to 12 years. Although red cell transfusions are lifesavers for patients with thalassemia, they are responsible for a series of complications and expose the patients to a variety of risks. Apart from iron toxicity, hypersplenism, venous thrombosis and osteoporosis, these patients are confronted by new clinical challenges, particularly in the form of transfusion transmitted diseases, especially hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections.[4,5] Post-trans fusional viral hepatitis has significantly contributed to morbidity in thalassemia with increased risk for development of hepatocellular carcinoma.[4,6]

Hepatitis C virus is a hepatotrophic, single strand ribonucleic acid (RNA) virus of family Flaviviridae and genus hepacivirus. An estimated 180 million people worldwide are chronically infected with hepatitis C with the global prevalence being around 2% and 3 to 4 million persons getting newly infected each year. A large number of genotypes and subtypes have been identified among hepatitis C virus isolates from all over the world.[7] Of the six main groups of sequence variants, corresponding to types 1-6, genotype 3 is the most prevalent genotype in patients with chronic hepatitis C in North and Central India and is associated with significant hepatic steatosis and fibrosis.[8]

Several studies on voluntary or mixed donors have noted a prevalence of hepatitis C below 2% in India and 0.71% in West Bengal.[9] It should be remembered that HCV hepatitis is more threatening than HBV hepatitis due to a greater risk of chronic liver disease.[10] It is estimated that about 2 billion people have serological evidence of current or past HBV infection worldwide, of which more than 350 million have chronic HBV and 1.2 million die annually from chronic hepatitis, cirrhosis and hepatocellular carcinoma.[11] The global prevalence of HBV infection varies widely; and its endemicity ranges from high (≥8%) to intermediate (2-7%) and low (<2%).[12,13] In India, HBsAg prevalence among general population ranges from 2% to 8%, placing India in intermediate HBV endemicity zone and the number of HBV carriers is estimated to be 50 million. Compared to other parts of India, the distribution patterns of HBV genotypes/subgenotypes and mutants is characteristically distinct in eastern part, where in addition to HBV genotypes A and D, genotype C is also present in a comparable proportion.[14]

Fortunately, HBV infection can be, to a great extent, prevented by a pre-transfusion immunization, HCV infection has gained importance particularly as one of the major complications in multiple-transfused patients during the last decade. This is especially true for countries where HCV is more prevalent in general population and therefore also amongst blood donors. The prevalence rate of seropositivity increases with the number of transfusions.[15] However, since no vaccine is so far available against hepatitis C, the only effective protective measure against this virus is provision of HCV negative blood for transfusion in thalassemia patients.[16]

The aims of this study include to determine the prevalence of HCV and HBV seropositivity amongst multiple-transfused thalassemia major patients in our setup and to judge the effectiveness of our current screening system for detection of these viruses in donor blood samples prior to transfusion.

METHODS

Study sites and testing technique

The study was hospital-based conducted at department of biochemistry, Calcutta National medical college, Kolkata. Cases of Beta-thalassemia major, diagnosed by clinical features, high performance liquid chromatography (HPLC) and hematology based laboratory findings and receiving transfusion management at thalassemia clinic of the aforementioned institute, were randomly selected. This is an IEC approved study and informed consent was obtained from the individuals during collection of blood samples in this study. Known cases of beta-thalassemia major, who had been transfused, as a part of their management, at least 10 units of blood, irrespective of their age and sex were included in the study. Patients who had been transfused less than 10 units of blood as a part of their management and had history of jaundice or were HbsAg or anti-HCV Ab positive prior to starting transfusion, were not included in the study.

A detailed clinical account was entered in a proforma, taking into account with emphasis: age at time of study; age at diagnosis; frequency of transfusion; history of jaundice; enlargement of liver and spleen; haemoglobin level and hepatitis B immunization status; awareness about risk of developing transfusion transmitted hepatitis; whether transfused blood was always screened for hepatitis in the best of parents' knowledge and history of regular screening for hepatitis B and C infection.

About 5 ml of patient's blood sample was collected under aseptic precautions from the selected subjects by a clean venepuncture. The blood was allowed to clot. Serum was separated and stored at –200C till the test for hepatitis B surface antigen (HbsAg) and anti-HCV antibodies (anti-HCV Ab) were performed in a batch along with two negative and two positive controls, each in duplicate. Third generation ELISA kits (Erba Lisa Hepatitis B, Erba Lisa Hepatitis C, Transasia Bio-Medicals) were used for the estimations according to manufacturer instruction.

Statistical software

The statistical software using SPSS 16 was used for analysis of data.

The study was approved by the ethical committee for scientific research, Calcutta National Medical College, India.

RESULTS

A total of 207 patients of beta thalassemia major were included in this study and blood samples were collected between May and October 2011.Of the total number of candidates, 121 (58%) were males and 86 (42%) females, with a male: female ratio of 1.4:1. The age of thalassemia patients at time of diagnosis ranged from 6 months to 8 years (mean age being 2 years and 7 months). The age at the time of this study ranged between 2 years 3 months and 21 years (mean age of 6 yrs and 6 months).

The interval between successive transfusions varied between 7 days to 1 month (mean 15 days) in different patients. No patient had jaundice during the study or clinical evidence of viral hepatitis in the past. Hepatomegaly was observed in all the patients, and splenomegaly was noted in 123 (59%) cases; 6 patients had been splenectomised for hypersplenism. Interestingly all the patients had received transfusions from only one thalassemia management centre (Calcutta National Medical College). All the parents were aware of the risk of transfusion-transmitted hepatitis; they were all convinced that their child was being transfused with properly screened blood. The centre providing transfusion care to the patients in present study was providing only screened blood (for hepatitis B and C).

99% of the cases had received two doses of recombinant DNA vaccine against Hepatitis B after being diagnosed with thalassemia. Out of 207 cases, 51 thalassemics were anti-hepatitis C antibody seroreactive (24.64%). Of them 25 were male (20.7%) and 26 were female (30.2%). Out of the total thalassemic cases, 7 (3.38%) were HbsAg seroreactive, 5 were male (2.3%) and 2 were female (4.1%). Only a single male patient was detected to have co-infection with both the viruses. Mean age of positive cases (Anti-HCV antibody: 10.42 years and HbsAg: 8.3 years) was higher than the virus negative thalassemia cases (7.6 and 7.9 years respectively) (Table 1).

Table 1: Anti-HCV antibody and HbsAg status in multiple transfused thalassemia cases.

	Anti-HCV (+ ve)	Anti-HCV (-ve)	HbsAg (+ve)	HbsAg (-ve)	Co-infection
Total cases (207)	51 (24.64%)	156 (75.36%)	(3.38%)	200 (96.62%)	1
Mean age of cases (In years)	10.42	7.6 ($p<0.05$)	8.3 ($p<0.05$)	7.9 ($p<0.05$)	
Mean number of transfusion	25	17 ($p<0.05$)	32	12 ($p < 0.05$)	

DISCUSSION

Transfusion associated infections continue to be a big threat to the safety of blood supply, more so in the developing and underdeveloped countries.[17] The safety of blood transfusion is compromised in India due to its dependence on replacement donors, endemic hepatitis in this region, high cost of screening and a lack of funds and trained personnel. In this study, mean age was significantly ($p<0.05$) higher in patients with positive HCV antibody and HbsAg cases compared to negative subjects. The mean number of transfused units of packed red blood cells was significantly ($p<0.01$) higher in patients with positive HCV antibody and HbsAg cases compared to negative subjects implying that the risk of infection rises with each transfusion (Table 1).[18]

Present study showed a very high seroprevalance of anti HCV antibodies among the thalassemia patients compared to the national average value (2%) (Table 1). Studies conducted by Williams et al, have shown a seroprevalance of 11.1% for anti HCV antibodies in multiple transfused thalassemia major patients.[10] Positive seroprevalance in present study findings were higher in comparison to results of similar studies conducted in India and other countries.[19,20] This may be due to difference in assay system used to test for anti-HCV and differing donor selection criteria. Also, blood might be collected during the seronegative window period. The high seroprevalence in this study could have been because of improper clinical examination and screening of donors. Proper pre-donation screening of donors and rejecting volunteers with a history of viral hepatitis had shown to lower prevalence of post-transfusion hepatitis C infection as reported by Siddiqui et al.[21] Preference should be given to non-professional / non-remunerated donors. Also, high-risk donors with history of IV drug abuse, high-risk sexual behaviour need to be excluded. The present study showed HbsAg prevalence (3.38%) to be much lower than that of positive anti-HCV Ab (24.64%) among the thalassemics with seroprevalence values being similar to the national overall scenario. Seven children who were found HBsAg positive, were either non-vaccinated or had received only a single dose of vaccination before starting transfusion. Similar results were found in studies in Jordon and Iran with measured HBsAg prevalence being 3.5% and 1.5% respectively indicating vaccination against Hepatitis B decreases the incidence of HBsAg positivity.[22] The low incidence of HBsAg positivity could be due to development of good antibody titre after vaccination. Ideally, a minimum of two doses of vaccination should be given before starting

transfusion in a newly diagnosed thalassemia case. However, cases have been reported of post-vaccination infection from harbouring viruses of subtype ayw (genotype D) having surface (S)-gene mutations (G587A) in a region critical for reactivity to antibody to hepatitis B surface antigen.[23] In India, blood units have been screened with assays of steadily increasing sensitivity due to availability of antibodies against hepatitis B surface antigen (HBsAg) since 1971, against HIV since 1989 and against Hepatitis C virus since 2001[24]. Results of present study clearly indicate that there still exist lacunae in our present conventional procedures for screening of virus infected blood. The relative percentage of cases seropositive for both the viruses and especially for anti-HCV antibodies was higher for cases who had received more transfusions as compared to those who had received lesser units of blood (Table 2). We should use the most sensitive screening method with the least possible false negative results.

Table 2: Relation between number of total packed RBC units received and HCV and HBV seropositivity.

Number of transfusions	Number of individual	Number of positive cases	
		Anti-HCV Ab	HbsAg
10-20	74	9 (12.16%)	02 (2.7%)
21-30	88	26 (29.55%)	03 (3.4%)
30-40	33	10 (30.3 %)	02 (2.7%)
> 40	12	6 (50%)	00

Blood screening using the viral antigen and nucleic acid amplification tests (NAT) can reduce the window periods of HIV, hepatitis B virus and hepatitis C virus infections substantially.[25] Estimates of the risk of blood-borne infections are essential for monitoring the safety of blood supply and the impact of new screening tests. Blood transfusion, a lifesaving modality, can be made safer by the introduction of the NAT for screening of blood units for HIV, hepatitis B and hepatitis C viruses and it can be made cost-effective by Analysing multiple samples together.[26] Education regarding transfusion transmitted infections, including HCV, HBV and HIV infections, is of prime importance. Further, in all the centres taking care of thalassemia management, uniform strict criteria for donor selection should be adopted. A serious consideration should be given to history of jaundice and drug addiction, etc. in donors. While selecting donors, preference must be given to relatively younger donors without past history of jaundice.[27]

CONCLUSION

The patients should be encouraged to stick to one thalassemia management centre, although it is understandable that provision of blood may not be prompt in every visit. The voluntary blood donor service, can tremendously improve upon the "safe blood transfusion''. Since the prevalence of hepatitis is relatively high in our population, strict measures must be undertaken to reduce the spread of infections through awareness campaigns on war footing. We must mention some shortcomings of this study. As the sample size was small and the study was conducted in a small area of India, the findings of this study should not be extrapolated to the whole country population. To overcome these, further studies should be done in other parts of the country taking larger sample size.

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