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Blood Transfusion-mediated Viral Infections in Thalassemic Children in Bangladesh

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To assess the prevalence of transfusion-mediated viral infections in multi-transfused thalassemics in Bangladesh, forty-two thalassemic children (Male = 24, Female = 18) were recruited. All children were less than twelve years of age. Seromarkers for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) were studied in these children prior to initiate blood transfusion and after they had received an average of 17.0±1.0 blood transfusions over a ten-month study period. The HBV and HCV markers were significantly higher in post-transfused subjects as compared to their pre-transfusion levels (HBsAg: 19.0 vs. 7.1%, p = 0.021 and anti-HCV: 16.7 vs. 2.4%, p<0.001). None of the thalassemic children was positive for HIV before or after transfusion. The serum total bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels also were significantly higher (p = 0.001, <0.001, <0.001 and <0.001, respectively) in post-transfused patients. Thus, HBV and HCV infections are major problems in multi-transfused thalassemic children in Bangladesh.

Key words: Thalassemia, blood transfusion, transfusion-transmitted infections

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INTRODUCTION

Thalassemia is one of the most common genetic diseases in the world. It is a major health problem, brings much morbidity, early mortality and a great deal of misery for a family both financially and emotionally. World Health Organization (WHO) reported that there are about 3% beta thalassemia carrier and about 4% HbE/beta thalassemia carrier in Bangladesh. More than two thousand thalassemic children are born every year in Bangladesh (Ahmed *et al*., 2004).

The patients suffering from beta thalassemia major and HbE/beta thalassemia do not survive for more than 5 years without blood transfusion. Blood transfusion is usually administered every two to five weeks to maintain the pre-transfusion hemoglobin level of 9-10 g dL^{-1} (Yaish *et al*., 2005). Thalassemic children, who survive mainly on regular blood transfusions, are more prone to acquiring various transfusion-transmitted infections, such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) (Choudhury and Phadke, 2001; Mollah *et al*., 2003). A wide range of prevalence of transfusion-transmitted infections among multi-transfused thalassemic children has been reported in different countries (De *et al*., 1990; Amarapurkar *et al*., 1992; Jamal *et al*., 1998; Mollah *et al*., 2003).

In Bangladesh, the practice of standard pre-transfusion screening of blood and blood products is often lacking due to the lack of knowledge and available facilities. In addition, the risky blood transfusion from professional donors is a common practice (Directorate General of Health Services, Ministry of Health and Family Welfare, Government of Bangladesh, Dhaka, 2001). Screening of hepatitis B is routinely done while hepatitis C virus screening has recently been introduced but only on limited numbers of blood donors prior to transfusion (Khan *et al*., 1993). The HIV infection, on the other hand, is screened very rarely. Thus, there always remains a possibility of acquiring various viral infections in thalassemics through blood transfusion. But, there are no accurate data in this area of research in Bangladesh.

This study aims at determining the extent of transfusion-transmitted viral infections like HBV, HCV and HIV among blood transfused thalassemic children in Bangladesh. In addition, the levels of serum total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were examined in multi-transfused thalassemics to assess liver function.

MATERIALS AND METHODS

Subjects: The study was conducted at Dhaka Shishu Hospital Thalassemia Centre and Greenland Hospital, Dhaka, during November 2003-August 2004. Forty-two thalassemic children (Male = 24, Female = 18, age<12 years) admitted in the hospital from different parts of Bangladesh were recruited. Blood samples of these children were obtained twice: at the beginning of the study before blood transfusion schedule was initiated and at the end of the ten-month study period after they had received a number of transfusions.

Sample collection: With prior written consent from the parents of the children, 5.0 mL of venous blood was collected in both the time in a plain glass test-tube from each subject and was allowed to clot at room temperature. The samples were then centrifuged at 1,500 rpm for 5 min to collect serum in micro-centrifuge tubes and preserved at -70°C until analysis.

Serological tests: The collected sera were tested for serological markers for HBV (HBsAg: Hepatitis B surface antigen), HCV (anti-HCV: antibody to hepatitis C virus) and HIV (anti-HIV: antibody to human immunodeficiency virus-1 and-2) following the 3rd-generation enzyme-linked immunosorbent assay (ELISA) method (Biotec, UK). All positive cases were double-checked following the standard laboratory procedures.

Biochemical tests: The collected sera also were tested for total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). These tests were performed on an automatic clinical chemistry analyzer (Dimension RXL, Dade Behring, USA) using commercially available assay kits (TBIL Fex® reagent cartridge, Cat. No. DF67A, ALT Fex® reagent cartridge, Cat. No. DF43A, AST Fex® reagent cartridge, Cat. No. DF41A and ALP Fex® reagent cartridge, Cat. No. DF15A, Dade Behring Inc., USA). All tests were performed following the standard laboratory procedures and the instructions of the manufacturers.

Statistical analysis: Data were analyzed by Chi-Square test when appropriate, using SPSS/Windows version 10.5. $p<0.05$ was considered statistically significant.

RESULTS

Demographic characteristics of the thalassemic patients: The average age, weight and height of the enrolled thalassemics are shown in Table 1. Of the 42 thalassemics,

24 (57%) were male and 18 (43%) were female. They had received an average of 17.0±1.0 blood transfusions during the study period.

Seroprevalence of HBV, HCV and HIV in the thalassemic patients: The seroprevalence of HBV, HCV and HIV in the thalassemic patients is shown in Fig. 1. The HBV and HCV markers were significantly higher in post-transfused subjects as compared to their pre-transfusion levels (HBsAg: 19.0 vs. 7.1%, p = 0.021 and anti-HCV: 16.7 vs. 2.4%, p<0.001). Besides, 2.4% (1 out of 42) post-transfused thalassemics showed co-infection of HBV and HCV while none showed such co-infection prior to blood transfusion. None of the thalassemic children was positive for HIV before or after blood transfusion.

Table 1: Demographic characteristics of the thalassemic patients

Parameters	Pre-transfusion	Post-transfusion
Age (year)	5.67±0.50 (Range, 1.0-11.0)	6.62±0.50 (Range, 2.0-12.0)
Sex, No. (%)		
Male	24 (57)	24 (57)
Female	18 (43)	18 (43)
Weight (kg)	15.5±0.8	15.6±0.8
Height (cm)	101.8±2.9	102.1±3.0
No. of blood transfusion[Ø]	NT	17.0±1.0

Results are expressed as mean±SEM. NT: No transfusion and [Ø]during the study period

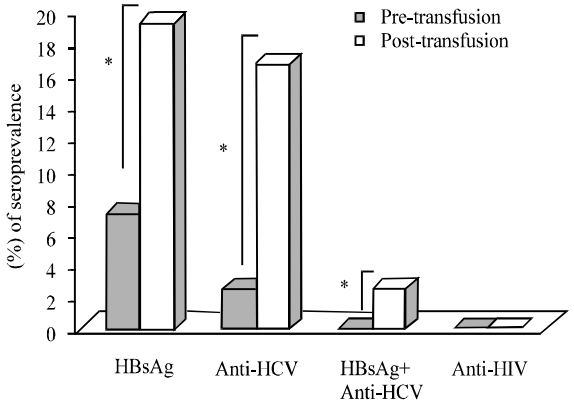

Fig. 1: Seroprevalence of viral infections (HBV, HCV and HIV) in the thalassemic children. HBsAg: Hepatitis B surface antigen, Anti-HCV: Antibody to hepatitis C virus and Anti-HIV: Antibody to human immunodeficiency virus-1 and-2. The HBV and HCV markers were significantly higher in post-transfused subjects as compared to their pre-transfusion levels (p = 0.021 and <0.001 respectively). Post-transfused thalassemics also showed significantly higher (p<0.001) prevalence rate of co-infection of HBV and HCV. p-value was calculated by Chi-Square test. *statistically significant

Table 2: Serum TBIL, ALT, AST and ALP levels of the thalassemic patients

Parameters	Pre-transfusion	Post-transfusion	p-value
TBIL (mg dL^{-1})	0.97±0.10	1.70±0.20	0.001
ALT (U L^{-1})	29.19±1.80	40.79±2.60	<0.001
AST (U L^{-1})	25.71±1.50	36.25±1.80	<0.001
ALP (U L^{-1})	173.80±13.0	278.45±12.5	<0.001

Results are expressed as mean±SEM. TBIL: Total bilirubin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and ALP: Alkaline phosphatase. p-value was calculated by Chi-Square test. p<0.05 was considered statistically significant

Serum TBIL, ALT, AST and ALP levels of the thalassemic patients: Table 2 shows the serum total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in thalassemic children. These biochemical parameters were increased significantly (p = 0.001, <0.001, <0.001 and <0.001 respectively) in post-transfused thalassemics as compared to their pre-transfusion levels.

DISCUSSION

Thalassemic patients are considered to be one of the high risk groups suffering from post-transfusion viral infections such as HBV, HCV and HIV (Amarapurkar *et al*., 1992; Choudhury and Phakde, 2001). In this study, the extent of these infections among multi-transfused thalassemic children in Bangladesh was determined. Thalassemic children are likely to be underweight (Mahachoklertwattana *et al*., 2003). As expected, all thalassemic children in this study also were under-weight before blood transfusion and even after they had received an average of 17.0±1.0 blood transfusions over the ten-month study period (BMI<18.5, as calculated from weight and height of the thalassemics) (Table 1).

The World Health Organization (WHO) ranked Bangladesh in the moderate to high-risk group of countries for HBV infection (Global distribution of hepatitis A, B and C, 2002), where the prevalence of HBV is 19-29% among professional donors and 2.4% among voluntary donors (Akbar *et al*., 1997; Khan *et al*., 1993). In this study, 19.0% of the post- transfused thalassemic children were HBsAg-positive (Fig. 1), which is higher than that (13.8% against 6.5% of non-transfused thalassemics) reported previously in Bangladesh (Mollah *et al*., 2003). The result also indicates that HBV seroprevalence in Bangladeshi multi-transfused thalassemics is much higher than that (2.4%) of Malaysia (Jamal *et al*., 1998), but slightly lower than that (22.1%) of Eastern India (De *et al*., 1990). The higher seroprevalence of HBV infection in Bangladesh might be due to poorly managed blood transfusion services and less awareness about HBV vaccination. About 7.1% of the

thalassemic patients were also found HBsAg-positive prior to blood transfusion (Fig. 1). They might have acquired the virus from infected mothers or in other ways, which need to be clarified.

The prevalence of HCV among the general population in Bangladesh ranges from 1.0-2.5% including 2.4% among professional donors (Dusheiko, 1999; Global distribution of hepatitis A, B and C, 2002; Khan *et al.*, 1993). In Bangladesh thalassemic children are dependent mostly on professional donors for blood transfusion (Khan *et al.*, 1993). In this study, only 2.4% of the thalassemic children showed anti-HCV positivity prior to blood transfusion and this anti-HCV positivity was significantly increased (16.7%) in thalassemics after a schedule of multiple blood transfusions (Fig. 1). In a previous study, Mollah *et al.* (2003) reported a similar seroprevalence of HCV in multi-transfused thalassemics in Bangladesh. The incidence of HCV infection in multi-transfused thalassemics in Bangladesh is thus very similar to that of Indian multi-transfused thalassemics (Amarapurkar *et al.*, 1992; Agarwal *et al.*, 1993).

The results of the study also indicate that post-transfused thalassemic children in Bangladesh acquired HCV much more often than HBV as compared to their pre-transfusion levels (~7 folds and ~2.7 folds, respectively, as calculated from Fig. 1). Similar results were reported previously by Mollah *et al.* (2003). Thus, HCV (besides HBV) also is a major problem in multi-transfused thalassemics in Bangladesh. The professional blood donors in Bangladesh harbor both HBV and HCV more often than voluntary counterparts (Khan *et al.*, 1993). The screening of hepatitis B is routinely performed while hepatitis C screening has only recently been introduced before transfusion. This might be the possible reason of higher seroprevalence of HCV than that of HBV in blood transfused thalassemics in Bangladesh.

About 2.4% (1 out of 42) of post-transfused thalassemic children also were found positive for co-infection of HBV and HCV (Fig. 1). Feitelson *et al.* (1994) reported co-infection of HBV and HCV in post-transfused children with beta-thalassemia. Co-infection of HBV and HCV has ominous implications in the pathogenesis of chronic viral hepatitis, leading to rapid progression towards cirrhosis of the liver (Hanley *et al.*, 1993). Thus, preventive measures such as early immunization, pre-transfusion screening of blood or blood products for HBV and HCV should be considered before transfusion.

In this study none of the thalassemic children was positive for HIV before or after blood transfusion (Fig. 1). This result is consistent with that of Mollah *et al.* (2003). The low seroprevalence of HIV could be related to the present low national incidence of the disease.

The serum total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels also were significantly increased ($p = 0.001$, <0.001, <0.001 and <0.001 respectively) in post-transfused patients as compared to their pre-transfusion levels (Table 2). The increased levels might be due to HBV and HCV infections (Agarwal *et al.*, 1993; Locasciulli *et al.*, 1993.) or iron overload in the liver, which is a common occurrence in transfusion dependent patients (Girelli *et al.*, 1998). However further study will be required to understand the actual cause.

Although the sample size is small in this study, the results clearly indicate that transfusion mediated viral infections (HBV and HCV) are significant problems for the multi-transfused thalassemic patients. Thus stricter pre-transfusion blood screening and mass vaccination are measures that merit attention.

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