Common Transfusion-transmitted Infectious Agents among Thalassaemic Children in Bangladesh

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ABSTRACT

Transfusion-dependent children are more prone to acquiring various transfusion-transmitted infections (TTIs), such as hepatitis B (HBV), hepatitis C (HCV), HIV, and others. Since the magnitude of these infections among thalassaemic children in Bangladesh is not well-known, this study was conducted to assess the prevalence of TTIs among them (who received more than three blood transfusions) compared to their age- and sex-matched controls (non-thalassaemics and those who had never had a transfusion). Seromarkers for HBV, HCV, HDV, Treponema pallidum, and HIV were tested, and the results were analyzed using SPSS/Windows 10.5. Of 259 children studied, 152 (58.69%) were thalassaemic (mean age 6.8 ± 3.6 years), and 107 were controls (mean age 6.7 ± 3.53 years). The HBV and HCV-markers were found significantly more often among multi-transfused thalassaemic children than among the controls in terms of HBsAg (13.8% vs 6.5%, p<0.04), anti-HBc total (39.5% vs 9.4%, p<0.0001), and anti-HCV (12.5% vs 0.9%, p<0.0001). HBeAg did not differ (p=0.82) between the thalassaemics (9.52%) and the controls (14.28%), whereas anti-HBe differed (0% vs 57.14%, p<0.003). Neither the thalassaemics nor the controls were positive for HDV, HIV, or T. pallidum. Since more thalassaemic children acquired hepatitis B and C infections through multiple blood transfusions, it is recommended that the safe blood-transfusion programme be strengthened and mass vaccination against HBV (even who suffer from HCV) in Bangladesh be undertaken.

Key words: Blood transfusion; Thalassaemia; Hepatitis B virus; Hepatitis C virus; Hepatitis delta virus; Treponema pallidum; HIV; Bangladesh

INTRODUCTION

Children suffering from thalassaemia major survive mainly on regular blood transfusions, which may expose them to different transfusion-transmitted infections (TTIs), especially hepatitis B, hepatitis C, HIV, and Treponema pallidum (1). This is particularly true for developing countries like Bangladesh where standard pre-transfusion screening of blood and blood products are often lacking. Various studies among multi-transfused thalassaemic children in different countries demonstrated a wide range of prevalence of TTIs. The prevalence of HBV infection ranges from 0.53% to 45.0% (2-6) and of HCV from 14.0% to 40.5% (3-8) in thalassaemic children.

A significant proportion of the above-mentioned infectious agents is transmitted through blood transfusion. In Bangladesh, risky blood transfusion from professional donors is a common practice (9). That is why we initiated this study, since there are no accurate data in this area of research in Bangladesh, as in many other developing countries (10).

This study, the first of its kind in Bangladesh, determines the sero-prevalence of TTIs among...
thalassaemic children compared to age- and sex-matched controls (non-thalassaemics and those who had never received a transfusion). The findings are expected to help healthcare providers and policy-makers towards adopting more effective, sustainable and cost-effective strategies in the prevention and control of TTIs, particularly in countries such as Bangladesh.

MATERIALS AND METHODS

Study subjects

In total, 259 children were included in the study. Of them, 152 (58.69%) were thalassaemic, with another 107 controls of similar age and sex.

Sampling

The study subjects were recruited on Sunday, Tuesday, and Thursday of a week during June 2000-April 2001, totalling 120 days. The study subjects included all children, aged less than 15 years, suffering from thalassaemia and having received more than three blood transfusions at a date of six months prior to the day of sampling (since the incubation period for different TTIs may vary up to six months), who attended the Dhaka Medical College Hospital (DMCH) and the clinic of the Thalassaemic Society of Bangladesh. The thalassaemic children were identified based on: (a) history of progressive pallor/anaemia and blood transfusion, (b) physical examination revealing stunted growth with progressive hepato-splenomegaly, and (c) laboratory report of haemoglobin electrophoresis demonstrating thalassaemia. Controls were selected systematically, i.e. every 5th non-thalassaemic never-transfused child attending the paediatric outdoor of the DMCH with a minor illness, i.e. common cold, influenza, helminthiasis, tonsillitis, scabies, and otitis. Controls were matched by age and sex with the study children. We could enroll only 107 controls for the study within the timeframe of 10 months.

Collection and preservation of samples

With prior written consent from the parents of the children, three mL of venous blood was collected 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 five minutes to collect serum in micro-centrifuge tubes and preserved at -70 °C for further tests.

Prior ethical permission from the Bangladesh Medical Research Council was obtained for the use of human subjects and for the collection of blood.

Serological tests

The collected sera were tested at the Immunology Department of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) for the following serological markers: (a) HBV (HBsAg: hepatitis B surface antigen, anti-HBc total: antibodies IgM and IgG to hepatitis B core antigen), (b) HCV (anti-HCV: antibody to hepatitis C virus), (c) T. pallidum: antibody to T. pallidum, and (d) HIV (anti-HIV-1, anti-HIV-2 and anti-HIV-1 group O). All HBsAg-positive samples were tested for HBeAg (hepatitis B e-antigen) and anti-HBe (antibody to hepatitis B e-antigen) to assess the infectivity status and also anti-HDV (antibody to hepatitis D virus) to assess co-infection with the delta agent. All these tests, except for T. pallidum, were done using the third-generation enzyme-linked immunosorbent assay (ELISA) (DiaSorin, Italy). Antibodies against T. pallidum were tested by rapid plasma reagin (RPR), and the reacting antibody samples were confirmed by T. pallidum haemagglutination (TPHA) obtained from Shield, UK. HIV-markers were tested using ELISA (Vironostika HIV Uniform II) and, if found positive, were confirmed by line Immunoassay (Liatek HIV III). All tests were done following the standard laboratory procedures and the instructions of the manufacturers. All positive cases were re-checked. However, the third-generation ELISA has been proved to be sensitive enough to test for the seromarkers with the procedure we used (1,11).

Data management

Data were entered and analyzed, using SPSS/Windows 10.5, by chi-square tests (which include Pearson’s, likelihood ratio, and linear association), when appropriate. A p value of ≤0.05 was considered significant.

RESULTS

The mean (±SD) ages of thalassaemic children and healthy controls were, respectively, 6.8±3.6 (range 1.5-14.7) years and 6.7±3.53 (range 1.0-14.7) years. The male-to-female ratio was 1.6:1 and 1.5:1 respectively (Table 1).

Table 2 shows the findings of hepatotrophic viral seromarkers of the study subjects. These revealed significant differences for HBsAg (p<0.04), for anti-HBc total (p<0.0001), and for anti-HCV (p<0.0001). HBeAg did not differ between the thalassaemics and the controls (p=0.82), whereas anti-HBe differed (p<0.003) between the thalassaemics (0%) and the controls (57.14%).
Transfusion-transmitted infections among Bangladeshi thalassaemic children

69

of the subjects were positive for HDV, HIV, or T. pallidum (not shown in the table).

thalassaemic children may contribute to the prevalence of lower HBsAg (6). Furthermore, it may be speculated

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<th>Table 1. Distribution of study children by their age and sex</th>
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<td>Study subjects</td>
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<tr>
<td>Thalassaemic children (n=152)</td>
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<td>Control children (n=107)</td>
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<td>SD=Standard deviation</td>
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Furthermore, of the thalassaemic children who were either positive for HBsAg or anti-HCV (n=40), five (12.5%) had both HBV and HCV compared with none of the controls (data not shown).

<table>
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<th>Table 2. Results of serological tests* in thalassaemic and healthy children</th>
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<td>Serological test</td>
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<td></td>
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<tr>
<td>HBsAg</td>
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<td>Anti-HBc total</td>
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<td>Anti-HCV</td>
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<td>Anti-HBe</td>
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* None of the children tested for anti-HDV, RPR, TPHA, and anti-HIV was found positive
** Significance level of chi-square tests based on the difference in proportion of positivity to specific tests done

Blood transfusion still remains the mainstay of treatment for children with thalassaemia which presents them with an increased risk of TTIs (1). The World Health Organization ranked Bangladesh in the moderate to high-risk group of countries for HBV infection (12), where the prevalence of HBV is 19% to 29% among professional donors and 2.4% among voluntary donors (13,14).

In this study, 13.82% of the thalassaemic children were HBsAg-positive, which is higher than that reported from various other countries, where results ranged from 0.53% to 3.5% (4-6). However, findings of studies conducted in the early 1990s in India showed a much higher prevalence of HBsAg, ranging from 22% to 45% (2,3). These varied findings may be due to factors, such as the sensitivity of screening tests (1,15) and the status of the virus, e.g. the HBsAg test may be negative during the window phase, during the convalescence phase, and with very low levels of viremia (1,16), and the effectiveness of the screening programmes (6). In addition, active immunization against HBV among awareness about HBV vaccination. Of the controls, 6.52% were also HBsAg-positive. They may have acquired the virus vertically from infected mothers and in other ways, and 90% of these infants develop chronicity, thus giving rise to such a high prevalence (17).

We found anti-HBc total more often among the thalassaemic children than among the controls (p<0.0001), which is comparable with other reports (6,18). Positive anti-HBc total (IgM and IgG) indicates an HBV infection (19) and is the most valuable single serologic marker in diagnosing HBV infection even when HBsAg remains negative (20).

Among all HBsAg-positive children, HBeAg-positivity did not differ significantly between the thalassaemics and the controls (p=0.82). On the contrary, 57.12% of the controls developed anti-HBe antibody, but none from the thalassaemic group (p<0.003) did. The reason for this is not known. However, unlike in our study, Al-Shayeb et al. reported 3.5% HBcAg, and 22% anti-HBe-positivity among Arabian children suffering from hereditary haemolytic anaemia (6). Positive HBcAg indicates that the virus replicates, encodes, and infects liver-cells when it remains highly infectious (21). On the other hand, anti-HBe antibody indicates sero-
conversion, i.e. loss of HBeAg and conversion to anti-HBe-positivity (21), and appearance of anti-HBe is strong evidence that the patient will recover (17).

In our study, anti-HCV-positivity was observed to be significantly higher in the thalassaemics than in the controls (p<0.0001). Amarapurkar et al. and Agarwal et al. found HCV in 18% and 16.7%, respectively, of thalassaemic children (3,7). Karimi et al. reported anti-HCV-positivity in 15.7% of thalassaemic children as opposed to 0.59% of controls (4). Similarly, Kebudi et al. observed HCV-positivity in 14% of Turkish thalassaemic children (8). However, Jamal et al. and Al-Sheyyab et al. reported relatively higher anti-HCV-positivity (22.4% and 40.5% respectively) (5,6) in thalassaemic children. These differences in observations, again, could be related to the rigidity of individual pre-transfusion-screening protocols and the sensitivity of the screening tests employed (1,5-6). While the global prevalence of HCV remains 2-3% (11), among the general population in Bangladesh it ranges from 1% to 2.5% (12,22), including 2.4% among professional donors (14). In Bangladesh, thalassaemic children are dependent on repeated blood transfusions mostly from professional donors, who harbour both HBV and HCV more often than voluntary counterparts (14).

In developing countries, infections are acquired through: (i) transfusion of unscreened blood, (ii) mother-to-infant transmission, (iii) other parenteral exposure to blood/blood products, (iv) use of blood-contaminated implements for surgery or circumcision, (v) traditional scarification, ear-piercing, or tattooing, (vi) reuse of contaminated needles and syringes, and (vii) acupuncture (11). Considering the high prevalence and fatal consequences of HBV and HCV, it is essential to stop the transfusion of infected blood.

In this study, five (12.5%) of the thalassaemic children had HBV and HCV simultaneously. Feltelson et al. reported co-infection of HBV and HCV among thalassaemic children (23). Concomitant infection of both HBV and HCV has ominous implications in the pathogenesis of chronic viral hepatitis (24), leading to rapid progression towards cirrhosis of the liver (22). Therefore, preventive measures, especially HBV vaccination (to be given to all children, particularly thalassaemics and those suffering from HCV), and screening of blood/blood products, should be considered.

None of the children in our study was positive for HDV or HIV. Amarapurkar et al. reported an evidence of delta infection in 16.7% of HBsAg-positive Indian children (3). Different studies across the world have reported varied percentages of the prevalence of HIV/AIDS among multi-transfused thalassaemic children, ranging from zero to 2.9% (1,3-6,25-26). We do not know the exact cause of the low prevalence of HIV in Bangladesh, but perhaps, it could be related to the sociocultural context, high religious values, and rigid morale, which may also be the reasons preventing open promiscuity in our community. Although HIV infection is not a major problem in Bangladesh, the country’s strategic location and frequent travelling to neighbouring high HIV-prevalent countries make Bangladesh vulnerable to HIV/AIDS. Lastly, in our study, none of the children from either group was positive for T. pallidum. A review of the literature of the last 10 years also failed to reveal any evidence of such infection among thalassaemic children.

It can be concluded that a significantly higher sero-prevalence of both hepatitis B and C markers was observed among the multi-transfused thalassaemic children. In Bangladesh, since these children depend mostly on professional donors, there always remains a possibility of acquiring these infections through blood transfusions. Therefore, a multi-centre study should be conducted with a larger sample size from a wider geographic area to reach a firmer conclusion on the potential risks of TTIs among multi-transfused thalassaemic children.

Based on the findings of our study, it is recommended that the safe blood-transfusion programme be strengthened and mass vaccination against HBV (even who suffer from HCV) in Bangladesh be undertaken.

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