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Seroprevalence of Hepatitis B, Hepatitis C and Human Immunodeficiency Virus Among Multitransfused Thalassaemic Children in Dhaka, Bangladesh

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Abstract:

Background*: Thalassaemia is a congenital hemolytic disease caused by defective globin chain synthesis of haemoglobin and largely treated by repeated blood transfusions. Transfusion-transmitted infections still make a great challenge in the management of patients with thalassaemia major. The most important worldwide transfusion transmitted infections (TTI) are hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Despite concern about a possible increase in the incidence of these infections there are no recent data about the prevalence of HBV, HCV and HIV from Bangladesh.*

Objectives*: To evaluate the prevalence of hepatitis B, hepatitis C and human immunodeficiency virus in multi-transfused thalassaemia patients (MTP), to identify the possible risk factors and to evaluate the effect of compulsory screening of blood to prevent these infections.*

Methodology*: This cross-sectional study was conducted during 2011 to 2012 on 100 consecutive multi-transfused thalassaemic patients who were interviewed using a structured questionnaire and tested for serological markers of hepatitis B virus (HBsAg), hepatitis C virus (Anti-HCV) and human immunodeficiency virus (Anti-HIV 1+2).*

Results*: The overall prevalence of HCV, HBV, HIV and co-infection among (MTP) were 31%, 3%, 0% and 1%, respectively. Children who developed infection had a higher incidence of receiving transfusion from professional donors or unknown donors than the non-infected ones. Infected children had a higher frequency of receiving transfusions without screening and receiving more number of transfusions than their counterpart. Other non-transfusion related (NTR) risk factors such as surgical operation, dental procedures, needle stick injury were significantly higher in patients who acquired transfusion transmitted infections (TTI).*

Conclusions: *HCV infection was the most prevalent transfusion transmitted infection (TTI) among multi-transfused thalassaemia patients (MTP) and remains a major health problem for these patients. Children who received transfusion from professional donors and received unscreened blood had more chance of getting infection with transfusion transmitted infection.*

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Introduction:

Thalassaemia is a congenital hemolytic disease caused by defective globin synthesis, resulting in decreased quantity of globin chains. These chains are normally generated at identical rates, but in thalassaemia the excess globin chains from the non-mutated gene accumulate in the erythroid precursor cells. This leads to an excess of á-chains accumulation in â-thalassaemia. Decreased quantity of globin chains leads to reduced haemoglobin in red blood cells (RBC), decreased RBC production and anemia[1]. Almost 200 mutations in the â-globin gene have been described that may cause â thalassaemia,

and the majority of these are point mutations or small insertions or deletions of a few bases[2].

Homozygous carriers of â-globin gene defects suffer from severe anemia, which is being treated by repeated blood transfusion and therefore they are at increased risk for transfusion transmitted infections such as hepatitis B virus, hepatitis C virus and human immunodeficiency virus[3-4]. Preventing these infections is one of the most important goals of management of transfusion dependent thalassaemic children[5] Although the incidence of transfusion transmitted viral hepatitis has been reduced after the introduction of HBV vaccination and donor screening, thalassaemia patient may still develop viral hepatitis due to organism that could not be detected by current techniques of blood screening [6]. Moreover, the donors may have low viral load and donor may be in the window period, the time after infection to positive serology[7]. The window period are different for these three viral infections: 2-6 weeks for HBV[8], 2 weeks for HIV [9] and 6-10 weeks for HCV and occasionally up to 9 months[10].

Blood transfusion is safe in developed countries. Since the introduction of blood donor screening for HBV, HCV and HIV infections, the residual risk has been limited to blood unit collected during the 'window period'. To minimize even this residual risk, some national health authority has added determination of HBV-DNA and HCV-RNA by nucleic acid technology to the battery of screening tests [11]. The current risk of transfusion-transmitted viral infection is estimated to be less than 2.5 per 1 million donations in the United States, Canada and other European countries [12-13] .The situation differs in developing countries that have not yet incorporated the key requirements for modern blood transfusion system. Prevalence of these infections in multi-transfused patients is related to their prevalence in the community and is different from one country to another [14-15]. Risk of these infections transmitted to multi-transfused patients vary depending on the frequency of the infections in the donor population and screening test technology used for blood and blood products[16].

Hepatitis B virus is transmitted by blood or blood products or by sexual contact. In Bangladesh the prevalence is 5.8% [17]. The carrier rates of HBsAg varies worldwide from 0.1 to 0.2 % in Britain, USA and Scandinavia, to more than 3% in Greece and Southern Italy and even 10 to 15% in Africa and Far East [18]. Exposure to HBV can have variable results. Some patients are immune and have no clinical attack. In others, an acute attack develops, varying from anicteric to fulminant hepatitis. The risk of chronicity depends on the age of acquisition of infection and immune status of the host. The chance of chronicity is about 25-50 % when infected between 1 to 5 years, and 5-10% when infection occur in older children and adults[18].

After the discovery of hepatitis C virus (HCV) in 1989, it has proved to be the major cause of transfusion associated hepatitis in the world [19]. In a local study seroprevalence of hepatitis C was found 0.82% and 0.84% respectively in case of male and female healthy blood donors in Dhaka[20]. Over 75 % of HCV acute infections are asymptomatic and only around 25 % cases show mild nonspecific symptoms but mostly non-icteric. Up to 60 % of infections become chronic and present as chronic persistent hepatitis or chronic active hepatitis. About 20% of patients infected with HCV may develop cirrhosis in 10 -20 years. There is an association between HCV infection and carcinoma of liver. The incidence of hepato-cellular carcinoma is 1-4% per year in patients with cirrhosis[18].

In South Asia, the HIV epidemic situation is quite alarming. Bangladesh is surrounded by India and Myanmar and is nearer to Nepal, countries where the epidemic is severe [21]. Bangladesh is considered to be at risk for a large-scale HIV epidemic because of the presence of risk factors, such as poor access to medical care, relatively low level of HIV related knowledge and awareness of risk groups, illiteracy and a poor health care structure. The HIV prevalence in general population appears low (<0.2 %) and is estimated as <1% in all risk groups except for injecting drug users (IUD) (7%)[22].

Worldwide, from 0.3% to 5.7% of thalassaemia patients are hepatitis B surface antigen (HB_SAg) positive and from 4.4% to 85.4% are positive for anti-hepatitis C antibodies[16,23-25]. In a study from Bangladesh, 7% thalassaemic children were found to be HBsAg positive[26]. The prevalence of HBV chronic infection is higher in Asia and Southeast Asian countries, whereas HCV chronic infection is widespread throughout the world. The prevalence of HIV in thalassaemic children varies from 1.7% to 8.9%[27]. The aim of this study was to assess the

prevalence of the three major transfusion-transmitted infections HBV, HCV and HIV among thalassaemic patients.

Methodology:

This cross-sectional study was carried out on 100 multi-transfused thalassaemic children with β-thalassaemia and Eâ-thalassaemia from October 2011 to March 2012. Age of the cases were between 4 to 11 years. For this study, multi-transfusion was defined as cases who had been transfused 10 times or more. Study population included children who attended the out-patient department (OPD) of Pediatric Hematology & Oncology of BSMMU, Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU) and thalassaemia Center, Bangladesh Institute of Child Health (BICH) with the diagnoses of β-thalassaemia or, Hb Eβ-thalassaemia made by history, physical examination, complete blood count with PBF and Hb-electrophoresis. Patients suffering from pre existing liver diseases were excluded from the study.

Prior permission was taken for this study from the Institutional Review Board, BSMMU, Dhaka, Bangladesh. Keeping compliance with Helsinki Declaration, 1964 for Medical Research involving Human Subjects, the parents of the selected patients were informed verbally about the study design, the purpose of the study and their right to withdraw their children from the study at any time, for any reason and informed written consent was also obtained. Data were collected using a structured questionnaire containing all the variables of interest, which was pretested by few samples in OPD of pediatric haematology and oncology department of BSMMU.

After taking relevant history, patients were examined physically. Then five milliliter(ml) of venous blood was collected from ante-cubital vein or other visible veins with all aseptic precaution in the forearm by a syringe from each participating child. Blood samples were transferred to plastic tubes. Blood was allowed to clot for 10 minute and then centrifuged at 3000 rpm for separation of serum. Then samples were divided into four Eppendorf tubes, all samples were preserved at 2-8^0 C for temporary storage. For prolonged storage serum were stored at -20^{0}C, until analysis. All sera were tested for hepatitis B surface antigen (HBsAg), antibody to hepatitis C virus (Anti-HCV) and antibody to human immune deficiency virus I and II by ELISA method using kits manufactured by Immuno Diagnostic System, San Diego (HCV) and Bio test, Germany (anti-HIV & HBsAg), in the laboratory of Virology Department of BSMMU.

Data were analyzed using SPSS (Statistical Package for Social Sciences), version 11.5. The results were expressed as number and corresponding percentages for qualitative data and mean and standard deviation for quantitative data. The test statistics used to analyse the data were Chi-square (χ^2) or Fisher's Exact Test and Unpaired t-Test. Data presented on categorical scale were compared between groups using Chi-square (χ^2) or Fisher's Exact Probability Test, while the data presented on continuous scale were compared between groups using Unpaired t-Test. The level of significance was set at 0.05 and $p < 0.05$ was considered significant.

Results:

Out of 100 thalassaemic children, maximum number were found in the age group of 8-11 years, which was 43%, on the other hand 2nd highest category was 4-7 years, which was 34%. The lowest 23% of patients belonged to age group 12-15 years. The age and demographic characteristics of study populations is shown in table-I.

Out of 100 thalassaemia patient 27 has affected siblings by same disease in the family, 61 had no affected siblings,12 patients were the only child of the family. Mean age of diagnosis of thalassaemia was 1.5 ± 0.7 years and mean age of first transfusion was 1.6 ± 0.8 years among affected children. The average interval between two consecutive transfusions was 1.3 ± 0.4 months.

Eighty nine percent of the children received blood from voluntary donors, 11% from professional & unknown sources. Ninety percent of the children received transfusion from the institutional centers and the rest from private clinics. Forty four percent received transfusion from one center, 30% from two centers and 26% from multiple centers. In 89% cases, transfusion was given after screening of blood. The median years of transfusion and median numbers of transfusion received so far were 6.5 years and 52 respectively. More than three-fourth (77%) of the children were vaccinated against HBV.

Table-I

Demographic characteristics and transfusion transmitted infections

Demographic characteristics	Transfusion-transmitted infections		p-value
	Sero-positive (n = 33)	Sero-negative (n = 67)	
Age¶ (years)	9.9 ± 2.7	8.4 ± 3.4	0.390
Sex			
Male	21(63.6)	40(59.7)	0.704
Female	12(36.4)	27(40.3)	
Residence			
Urban	15(45.5)	24(35.8)	0.353
Rural	18(54.5)	43(64.2)	
Fathers' education			
Illiterate	3(9.1)	0(0.0)	
Primary	16(48.5)	32(47.8)	0.014
Secondary	7(21.2)	28(41.8)	
Graduate	7(21.2)	7(10.4)	
Fathers' occupation			
Service	8(24.2)	21(31.3)	
Farming	2(6.1)	4(6.0)	0.231
Business	4(12.1)	17(25.4)	
Others	19(57.6)	25(37.3)	
Monthly income (Taka)¶	12212 ± 6338	14358 ± 5324	0.079

Figures in the parentheses denote corresponding percentage.¶ Data were analysed using Student's t-Test and were presented as mean ± SD.# Data were analysed using Chi-square (χ2) Test.

Table-I shows that age and sex has no significant influence in the presence of transfusion-transmitted infections (p = 0.390 and p = 0.704). Residence, occupation & monthly income were also not found to be associated with transfusion transmitted infections (p = 0.353, p = 0.231 and p = 0.079 respectively). However, children who developed transfusion-transmitted infection belonged to relatively less educated fathers (p = 0.014) (Table I).

Table II

Prevalence of transfusion transmitted infections:

Transfusion transmitted infections	Frequency	Percentage
HB_sAg	03	3.0
Anti-HCV	31	31.0
Anti-HIV(I&II)	0	0.0

About one-third (31%) of the children demonstrated anti-HCV antibody in their blood and 3% were positive for HBsAg. None of the children exhibited HIVAb (Table II).

Table III shows that development of transfusion-transmitted infections was not found to be associated with interval between two consecutive transfusions (p = 0.819).Children with diagnosis of Tthalassaemia at older age was found to be significantly associated with infection (p=0.008).

Table III

Age at diagnosis, interval between two transfusion and transfusion transmitted infection.

Pertinent variables#	Prevalence of transfusion related infection		p-value
	Sero-positive (n = 33)	Sero-negative (n = 67)	
Thalassaemia diagnosed at age (years)	1.9 ± 0.9	1.5 ± 0.7	0.008
Interval between two consecutive transfusion (months)	1.2 ± 0.4	1.3 ± 0.4	0.819

Data were analysed using Student's t-Test and were presented as mean ± SEM.

Table IV

Prevalence of transfusion-transmitted infections and their risk factors

Risk Factors		Prevalence of transfusion transmitted infections		p-value
		Sero-positive (n = 33)	Seronegative (n = 67)	
Source of blood#	Voluntary	26(78.8)	63(94.0)	
	Professional or unknown	7(21.2)	4(6.0)	0.035
Transfusion received from#	Institutional centre	30(90.9)	60(89.6)	
	Private clinic	3(9.1)	7(10.4)	0.569
Transfusion received at#	One center	16(41.5)	28(41.8)	
	Two or more centers	17(58.5)	39(58.2)	0.398
Transfusion given#	After screening	26(78.8)	63(94.0)	0.028
	Without screening	7(21.2)	4(6.0)	
Years of transfusion¶		7.7 ± 2.7	6.7 ± 3.1	0.111
Vaccination against HBV#	Yes	28(84.8)	49(73.1)	
	No or incomplete	5(15.2)	18(26.9)	0.420

Figures in the parentheses denote corresponding percentage, ¶ Data were analysed using Student's t-Test and were presented as mean ± SD. # Data were analysed using Chi-square (χ^2) Test.

Children who developed infection had a higher incidence of receiving transfusion from professional or unknown donor than the non-infected ones (21.2% vs. 6%, p = 0.035). Infected children had a higher frequency of receiving transfusions without screening (21.2% vs. 6.0 %, p = 0.028). However institutional center, number of center, mean year of transfusion or vaccination status had no significant relation with development of infections (p=0.569, p=0.398, p=0.111 & p=0.420). (Table-IV).

Table V

Transfusion-transmitted infections and other non-transfusion related risk factors.

Other non-transfusion related	Prevalence of transfusion transmitted infections		p-value
	Sero-positive	Seronegative	
Risk factors.	(n = 33)	(n = 67)	
Surgical operation	17(51.5)	18(26.9)	0.015
Dental procedure	15(45.5)	12(17.9)	0.004
Ear, nose piercing	7(21.3)	11 (16.5)	0.476
Needle stick injury	7(21.2)	4(6.0)	0.022

Figures in the parentheses denote corresponding percentage

¶ Data were analysed using Student's t-Test and were presented as mean ± SD.

Data were analysed using Chi-square ($\div^2$) Test.

Thalassaemic children who received any surgical operation, dental procedure & having needle stick injury were significantly associated with development of transfusion transmitted infection (p=0.015, p=0.004 & p=0.022).Ear and nose piercing in thalassaemic children had no association with development of infection (p=0.476). (Table V).

Discussion:

Blood transfusion still remains the mainstay of treatment for children with thalassaemia. Due to need for repeated blood transfusion these children are at risk of acquiring several blood borne viral infections. Of these blood borne viral infections, hepatitis B and C and human immunodeficiency virus (HIV) infections are particularly important. These viruses are of great concern because of their prolonged viraemia and carrier or latent state. They also cause fatal, chronic and life-threatening disorders.

In the present study about one third (31%) of the patients were positive for anti-HCV Ab and 3% for HBsAg. Infection with HIV was absent (negative for anti-HIV Ab). In a similar study in 2003 at Dhaka there was 12.5% seropositivity of HCV in thalassaemic patient [28], which indicated that during the last one decade HCV infection has increased.

Like our findings high prevalence of HCV (31%), a low prevalence of HBsAg (3%) and no prevalence of HIV is observed elsewhere in the Asian countries. In the study on 755 multi-transfused thalassaemia major children at Shiraz, Iran showed that anti-HCV Ab, HBsAg and anti-HIV Ab were positive in 15.7%, 0.53% and 0% cases respectively[29]. Another study on 60 multi-transfused thalassaemic patients at Punjab, Pakistan, it was found that 35% were hepatitis C, 1.7% hepatitis B and no HIV positive patients[30].

In our study HIV infection was absent, this finding is similar with previous study carried out at Bangladesh [28], though the seroprevalence of HIV in thalassemic patients was 8.9% in neighboring country India [27]. Different studies across the world have reported varied percentages of the prevalence of HIV/AIDS among multi-transfused thalassaemic children. Perhaps low prevalence of HIV in Bangladesh is due to the sociocultural context, high religious values, and rigid morale, which may also be the reasons preventing open promiscuity in our community.

Our data of 31 % anti HCV seropositivity is similar to data from Kuwait [31], where study on 129 â-thalassaemia patients 33% and 7% cases were anti-HCV and HBsAg positive respectively. Our findings seem to be lower than those from other countries of the region such as in Pakistan[32], where seroprevalence of hepatitis C and hepatitis B were 43% and 5.1% respectively in 79 multi-transfused thalassaemia major patient and in Jordan, 40.5%, 3.5% positive for HCVAb and HBsAg respectively in their prevalence study over 143 multi-transfused patients[14]. However, the results are higher than those in Malaysia with 22.4% seropositivity for anti-HCV among 85 multi-transfused thalassaemic children[33], Iran with 15.7% seropositivity for anti-HCV among 466 subjects[29]. The wide variability among the prevalence reported in different studies is because of different sensitivity and specificity of the tests used, different HCV prevalence in the donor populations, different donor selection criteria and different blood screening techniques.

In our study subjects prevalence of Hepatitis B and C were significantly high in those children who had

previous surgery, dental procedure or needle stick injury. No statistically significant association was observed with ear, nose piercing and hepatitis. After dramatically improving the blood safety with new diagnostic tools, alternative routes of transmission, such as direct inoculation, can explain that in spite of blood donor screening, HBV and HCV has also been found in blood recipients, for instance, via contaminated needles, scalpels, or other pointed or sharp objects. People living in close community with HBV carriers can also become infected, probably via shared household devices, like shavers contaminated with blood[34].

Nosocomial patient-to-patient transmission of infection may occur by means of a contaminated colonoscope, via dialysis, or during surgery, including organ transplantation[35]. It should be considered that in this group of high-risk patients, risk of infection is not only limited to blood transfusion but nosocomial transmission may also play an important role in the infection transmission[36]. In our study population some of the children shared subcutaneous infusion pump for iron chelation and leucocyte depleted filter which might be the source of nosocomial infection. The high prevalence (31%) of Anti-HCV in our study in comparison to previous study[28] in Bangladesh of 12.5% Anti-HCV may be partly explained by nosocomial route of infection spread.

It has been reported that the prevalence of HCV antibody was directly related to the number of blood transfusions in multiple-transfused patients. Similarly, we found that the mean number of blood transfusions had statistically significant differences between anti-HCV and HBsAg positive patients (62±22) and anti-HCV& HBsAg negative patients (51±24) in the present study ($p < 0.05$).

Previous study carried out on 101 multi-transfused thalassaemic patients at Dhaka[26] shown only 16(15.84%) thalassaemic patients were vaccinated against Hepatitis B virus and 6(7%) thalassaemic patients were HBsAg positive. Whereas present study found 77% thalassaemic children were vaccinated against Hepatitis B virus. The decreasing trend of hepatitis B virus infection may be explained by increasing rate of vaccination due to public awareness about the disease and inclusion of hepatitis vaccine in expanded program on immunization (E.P.I) schedule. In case of hepatitis B, an effective vaccine is available, since no such vaccine is so far available against hepatitis C, the only effective protective measure against this virus is provision of HCV negative blood for transfusion. The children should be encouraged to take blood after proper screening from voluntary donor.

In this study, one (1.0 %) of the thalassaemic children had HBV and HCV simultaneously. There is a report of co-infection of HBV and HCV among thalassaemic children [37]. Concomitant infection of both HBV and HCV has ominous implications in the pathogenesis of chronic viral hepatitis [38], leading to rapid progression towards cirrhosis of the liver. Therefore, preventive measures, especially HBV vaccination (to be given to all children, particularly thalassaemia patients and those suffering from HCV), should be considered.

Conclusion:

From the study it may be concluded that HCV infection is the most prevalent transfusion transmitted infection (TTI) among multi-transfused thalassaemic children. Children who received transfusion form professional donor and received unscreened blood have more chance of getting infected with TTI.

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