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ORIGINAL ARTICLE

Current trends of seroprevalence of transfusion transmitted infections in Pakistani β-thalassaemic patients

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Abstract

Background: Though regular blood transfusion improves the survival, it carries the unavoidable risk of transfusion transmitted infections (TTI) in β-thalassaemic patients. Owing to the lack of uniformity in blood screening practices in Pakistan, TTI is still a major challenge. *Objectives:* To study the current trends of TTI in regularly transfused β-thalassaemics and their correlation with age, number of transfusions, hematological and biochemical markers. *Methods:* We carried out a prospective case-control study. 100 β-thalassemic patients and 200 healthy donors were recruited from June 2011 to June 2014. HCV antibodies, Hepatitis B surface antigen and human immunodeficiency virus antibodies (I & II) were evaluated. Complete blood counts, LFTs and serum ferritin were tested on all patients. *Results:* Mean age of patients and controls was 11.18±5.07 and 20.5±1.87 years respectively. In patients, 54% and 46% were males and females respectively. Anti-HCV antibody and HbsAg were positive in 27% versus 3% and 3% versus 2% in patients and controls respectively. None of the patients and controls was HIV reactive. Seropositivity of Anti-HCV was significantly higher in patients than that of controls (P<0.001). Anti-HCV positively correlated with age above 10 years, numbers of transfusions (≥150 units), high serum ferritin, elevated ALT and alkaline phosphatase (P<0.001). *Conclusions:* Over the decade, TTI magnitude has significantly reduced, but hepatitis C is still a main hazard. Further preventive measures including nucleic acid testing, voluntary donation and stringent donor selection will be required for reducing TTI in β-thalassaemics.

Keywords: Thalassaemia, Hepatitis B, Anti-HCV, HIV, TTI

INTRODUCTION

β-thalassaemia is an autosomal recessively inherited haemoglobinopathy which is highly prevalent in Pakistan.[1,2] An estimated 4000 to 9000 cases of β-thalassaemia major being added each year and a carrier frequency of 5 to 7% is seen in our part of the world.[3]

Blood transfusion support remains the treatment of choice due to financial constrains in our country. Regular blood transfusion obviously increases the life expectancy but has in turn culminated in the greatest susceptibility of blood-borne transfusion transmitted infections (TTI).[1,2] TTI commonly include HBV, HCV and HIV.[1,2,4] The probability of acquiring TTI is directly related with the infectious frequency in the healthy blood donor population.[5] One recent large study on stringently selected blood donors from Pakistan revealed a predominance of HCV infection, a declining trend in HBV and very negligible HIV infection.[6]

Blood safety is the major challenge in repeatedly transfused patients because of high prevalence of HCV and HBV in our population, relatively low percentage of volunteer donors and lack of standard screening in most of the blood banks in Pakistan.[6,7] However, the threat of spreading infection to transfusion recipients has considerably diminished owing to vigilant donor selection criteria and sensitive serological screening tools.[1,2] The greatest threat is the donation by seronegative donors during the infectious window period or with low level of viremia or undetectable mutant strains by the ELISA technique.[1,5]

HCV infection is globally prevalent, with an estimate of 170-200 million people affected.[4]

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Currently no protective vaccine is available against HCV; the provision of safe blood is the only protective measure.[2] Blood transfusion remains the main route of disease transmission in thalassemic patients, as routine screening for HCV was started in 1994 in Pakistan.[1] An anticipated 80% of thalassemics are HCV infected worldwide.[4] Inconsistency in HCV distribution has been reported in regional countries which include India, Iran, Saudi Arabia and Egypt at 25%, 18%, 63% and 69% respectively.[4] Although there is a varying trend globally, the prevalence is still high in developing countries.

HBV infection is also high (4.3%) in our country, but with early vaccination and regular pre-transfusion screening, gradual reduction has been achieved.[2,8,9] A diminishing trend of HBV is replaced by HCV as the most common TTI in these patients.[1] Earlier regional studies reported the seroprevalence of HBV and Anti-HCV as varying from 1.2 to 22% and 13 to 60% respectively in transfusion dependent thalassaemia patients.[1,10,11]

HIV prevalence in thalassemics is also variable in different countries. Fortunately, local studies have reported no positive case in our thalassemic patients previously.[1,12] In the neighboring country of India, a prevalence of 3% has been reported.[5]

The aim of our study is to study the current trends of prevalence of TTIs including HbsAg, Anti-HCV and HIV in transfusion dependent β-thalassaemics. A secondary objective is to ascertain possible correlation of TTI with the number of blood transfusions, haematological parameters and biochemical markers.

MATERIALS AND METHODS

Patients and controls

This is a prospective case-control study, from June 2011 to June 2014. One hundred patients with established β-thalassaemia major and two hundred healthy blood donors were enrolled in the study duration. Informed consent was obtained from parents/guardians in cases of minors and from patients ≥18 years of age.

All registered patients were on regular blood transfusion according to requirement. Patients who had received at least 15 transfusions were enrolled. Patients who did not give consent, have fewer transfusions (<15 transfusions), β-thalassaemia intermedia and compound heterozygous were excluded from the study. Ethical approval was granted by the Ethical and Research Committee of the Liaquat National Hospital, prior to enrolment.

Materials

Demographic data including age, gender, number of blood transfusions and medical history were recorded by thorough history from parents/guardians and from patients' medical records.

6ml of venous blood per patient was collected. Sera were separated and stored at 20ºC till the tests were performed. HbsAg, Anti HCV and HIV antibodies were determined by chemiluminescence method (Abbott Architect, USA). The samples were run in a batch of 20; at each time positive and negative controls were run simultaneously to validate the results. The cases were considered reactive when the sample absorbance/cut off ratio was >1.0 and negative if less than the defined value. Frequency of Anti-HCV was determined in two groups, age <10 years and >10 years, to determine the trends of seropositivity. Hematological parameters including hemoglobin/hematocrit, WBC and platelets were determined by Cell Dyne Ruby (Abbott, USA). Serum ferritin and liver function test were detected by HITACHI 912 (Japan) by photometric assay.

Data analysis

Data were entered and analysed using SPSS version 20. The results were expressed as mean ±SD for quantitative variables and qualitative variables are presented as frequency and percentages. Student's t test was applied for the comparison of means. Data were considered statistically significant at P value <0.05. Chi-square test was applied for correlation of Anti-HCV and HbsAg with maternal characteristics, hemoglobin/hematocrit, TLC, platelets, serum ferritin, serum bilirubin, liver enzymes and numbers of transfusions.

RESULTS

Out of 100 patients, 54 (54%) were males and 46 (46%) were females. The mean age of patients was 11.18±5.07 (range 2 to 23) years and of control was 20.5±1.87 (range 18 to 23) years.

The mean number of blood transfusion was 145.21±67.16 (22-351) units. 39% of patients were found to have received transfusion from a single institution/centre, the remaining 61% kept on changing the centre to seek for blood.

Seroprevalence of anti-HCV was 27% among patients compared with 3% in the healthy controls (P<0.001). In contrast, HbsAg reactivity was not statistically differently, being detected in 3% of patients compared with 2% in the control group. All the reactive cases of hepatitis B were

TABLE 1: Comparison between hepatitis C seropositive and seronegative thalassaemic patients

Evaluated parameters	Hepatitis C reactive Mean ± SD	Hepatitis C non reactive Mean ± SD	*P* value
Age (years)	15.92 ± 3.58	9.43 ± 4.37	<0.001
Hemoglobin (gm/dl)	8.34 ± 1.76	8.50 ± 1.55	0.65
Hematocrit (%)	23.98 ± 5.87	25.44 ± 4.33	0.17
Ferritin (µg/dl)	4805.59 ± 2165.02	3699.81 ± 2117.01	0.02
Total bilirubin (mg/dl)	2.07 ± 1.13	1.08 ± 0.54	<0.001
Direct bilirubin (mg/dl)	0.79 ± 0.83	0.41 ± 0.34	0.002
Indirect bilirubin (mg/dl)	1.42 ± 0.63	0.67 ± 0.38	< 0.001
ALT (u/l)	94.48 ± 37.67	48.04 ± 31.84	< 0.001
Alkaline phosphatase (u/l)	102.37 ± 58.29	52.42 ± 49.31	< 0.001
Numbers of transfusions	202.52 ± 52.37	124.01 ± 59.37	< 0.001

above 10 years of age and were not immunized against hepatitis B virus. None of the patients and controls was found to have HIV reactivity. The hematological and biochemical parameters of patients are shown in Table 1. Out of 27 patients with anti-HCV, 92.6% (25 patients) were in age group >10 years and only 7.4% (2 patients) were in <10 years of age group. The mean age of sero-positive and sero-negative patients were 15.92±3.58 and 9.43±4.37 years respectively (P<0.001). No statistical difference was established between male and female in relation to anti-HCV reactivity.

Anti-HCV positivity revealed significant positive correlation with increasing number of transfusions of >150 units of packed red cells and increased serum bilirubin levels (P<0.001). Serum ferritin, ALT and alkaline phosphatase were significantly higher in anti-HCV reactive versus non reactive patients (P<0.001).

There was no significant correlation (P>0.05) between HCV infection and hematological parameters (hemoglobin/hematocrit, TLC, platelets). HBV infection did not show statistical correlation with evaluated parameters.

DISCUSSION

β-thalassaemia major exemplified by decreased erythrocyte survival and increased iron burden causes diverse pathological expressions of primary and secondary complications. Prompt blood transfusion is the key treatment strategy in these patients. Unsafe blood transfusion practices pose challenges, particularly TTIs in the overall management plan.[13]

Formerly, TTI have always been a leading health problem in thalassaemic individuals.[10] The disease magnitude is significantly reduced in the last decade indicating progressive improvements in blood transfusion practices.[1] This is mainly due to effective implementation of strict donor selection and deferral criteria, and serological screening tools.[5]

HCV infection remains a major complication in multi-transfused thalassaemics in the last decade and has considerably contributed in the overall morbidity.[2,13] Its significance is more pronounced by the fact that it carries more risk of chronic liver disease in comparison with that of HBV infection.[2]

As illustrated in Table 2, the trend of seroprevalence for HCV infection has been declining continuously over the last two decades. In the present study, relatively high prevalence (27%) was detected in comparison with one prior local study (15%), reported from the Khyber Pakhtunkhwa province, Pakistan. This difference is attributed to the variation of age groups, transfusional and blood screening practices and receiving blood transfusions from unregistered blood banks where pretransfusion screening was not assured. It is reflected by the fact that only 39% of thalassaemics have received regular blood from our institute and the remaining, from other centres all over the city.

The HCV prevalence in thalassaemics was 25% in India (2012) and high in Egypt (2012) accounting for 40.5%.[14,15] Another regional study from Iran revealed a low prevalence (8%) in transfusion dependent thalassaemics.[16] A study reported from Australia revealed serological evidence of hepatitis C exposure in 41% of patients.[17] A relatively low prevalence was detected in Saudi thalassemics, being only 3.5%, in a study conducted in 2011.[18]

TABLE 2: Prevalence of transfusion transmitted infections in regional studies

Authors	Year	Hepatitis C reactive	Hepatitis B Reactive	HIV reactive
Bhatti FA, *et al*[11]	1995	60%	-	-
Mujeeb, *et al*[10]	1997	51%	-	-
Shamsi T, *et al*[26]	1998	46.1%	4.86%	0%
Hussain H, *et al*[27]	2008	41.7%		-
Riaz H, *et al*[12]	2011	43%	5.1%	0%
Ali I, *et al*[7]	2011	15%	-	-
Present study	2015	27%	3%	0%

The present study indicated that HCV positivity is not uncommon, particularly in the higher age group which is similar to a study from Iran.[19] The report by Ansari *et al* showed that 22% of thalassaemic patients above 10 years had HCV reactivity versus only 8.4% of patients <10 years of age.[1] The HCV seroprevalence is increasing with the escalation in the number of cumulative transfusions.[2] Significant positive correlation (P<0.001) was established with increased numbers of transfusion ≥150 units, indicating increased frequency due to more blood explosure.[15,20] It is in agreement with Shah *et al* who reported a prevalence of more than two folds for patients with above 50 units of cumulative blood transfusions.[21]

Our findings are similar to prior studies which also reported a significant relation between high serum ALT and HCV positivity.[22,23] Ameli M *et al* reported a mean ALT of 60.9 u/l in HCV positive versus 39.2 u/l in non-reactive patients (P=0.001).[22] In our study, a significant correlation was also established between anti-HCV and high serum ferritin. This finding is consistent with those reported from India and Iran.[19,23] In contrast to our results, one recent study from Egypt revealed significant association of anti-HCV with HbsAg infection.[15] This co-infectivity was attributed to a surprisingly high prevalence of HBV infection in Egypt thalassaemics in comparison with a low frequency in Pakistani thalassaemic patients.

Fortunately, HIV infection is not a problem in our state as reported previously and also currently no positive case is reported in this series.[1,12] However it is challenging in some countries where the HIV prevalence in the general population is high. The situation is alarming in India with a prevalence of 3% reported recently by Vidja.[5] A study from Brazil by Erich V *et al* indicated 17% positivity of HIV infection.[24]

To a great extent, HBV infection spread is reduced by pretransfusion screening and an early vaccination programme. In the present study only 3 out of 100 patients were HbsAg reactive versus 2% in healthy donors which is close to overall prevalence in our general population. When compared with earlier reports, our results are in concurrence with studies from Bangkok and India.[14,25] The study by Wanachiwanawin reported HbsAg reactivity in 2% of patients, and similar results were found by Jain *et al* who showed clearly that exposure rate to HBV infection has diminished drastically.[14,25] However HBV prevalence is high in some parts of the world such as Egypt where HBV infection was encountered in 29% of patients. This calls for urgent and crucial steps in the blood screening techniques and provision of vaccination in this high risk group.[15]

Appropriate serological screening tools are still yet not available in many blood banks in our country.[1] However, prevalence studies revealed that in last decade there have been significant reductions in the prevalence of TTI infections in our thalassaemic patients.

Lastly we acknowledge our study limitations, including the fact that serological statuses of patients prior to the start of transfusion were not determined. It may be the confounding factor of vertical transmission to patients owing to maternal positivity, although the disease positivity rate in the general population is low as compared with the patient population.

Conclusion

Over the last decade, TTI by HBV has been reduced, while HIV is not problematic in our country though HCV infection is still prevalent and a major health problem, partly due to the lack of proper screening and partly due to the lack of awareness, contributing to the disease spread. The effective implementation of stringent donor selection and improved serological

methodology, in parallel with carrying out nucleic acid amplification testing for donor screening are crucial for further effective reduction in the disease burden among the thalassaemics.

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