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Original Article

Sero-Molecular Detection of Transfusion Transmissible Infections Among Thalassemic Patients in Pakistan

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ABSTRACT

Objective: To assess the frequency of molecular markers along with serological markers of Hepatitis B virus, Hepatitis C virus and Human Immunodeficiency Virus among multi-transfused beta-thalassemia patients.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Transfusion, Rawalpindi Pakistan, from Apr to Jul 2020.
Methodology: A total of 105 beta-thalassemia patients were included in this study. Demographic information and frequency of transfusion was noted. Serological markers were detected using chemiluminescence microparticle immunoassay while molecular markers were identified using real-time polymerase chain reaction.
Results: Among 105 beta-thalassemia patients, 61(58.1%) were males and 44(41.9%) were females. The age range was from 2-34 years with a mean age of 11.8±6.4 years. Seropositivity and Nucleic Acid Testing reactivity was observed in 29(27.6%) and 16(15.2%) patients respectively. The frequency of hepatitis C Virus (HCV) antibodies and Ribonucleic Acid (RNA) was highest among infected patients while none of the patient was found to be reactive for anti-HIV and HIV-RNA. Viremia was detected in 12(11.4%) out of 29 seropositive patients and in 4(3.8%) out of 76 seronegative patients.
Conclusion: Hepatitis C Virus was the most prevalent in beta-thalassemia patients followed by Hepatitis B Virus and Human Immunodeficiency Virus. The serology should be augmented with NAT to detect viremia in seronegative cases as well as seropositive cases. The NAT will also help in early identification and treatment of infected patients and improve quality of life of these patients.

Keywords: Beta-Thalassemia, Nucleic acid amplification technique, Transfusion-transmitted infection.

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INTRODUCTION

Thalassemia is a mono-gene quantitative hemoglobinopathy which results in either decreased or defective production of alpha or beta globin chains. The most common type is Thalassemia Major in which lifelong regular blood transfusion is mandatory for survival and if left untreated patients usually die in the first or second decade of life.[1] Frequent blood transfusions result in the development of allo-antibodies which leads to destruction of red blood cells consequently manifesting as chronic anemia and expansion of bone marrow.[2] Globally, approximately 1.5% of the population is thalassemia carrier with the highest prevalence being observed in Southeast Asia, Africa and Mediterranean countries.[3] In Pakistan, an estimated 5% carrier rate makes beta-thalassemia a significant health problem with 10, 0000 cases being reported each year and adding to the already overburdened transfusion services in the country.[4] About one fourth of all 2.7 million blood donations were utilized for thalassemia patients per anum.[5] However, blood transfusion remains risky in transfusion-dependent patients due to absorptive iron overload and blood borne viral infections including Hepatitis-B virus (HBV), Human Immunodeficiency Virus (HIV) and Hepatitis-C virus (HCV). Therefore, screening of whole blood and blood products is an optimal preventive method recommended by World Health Organization (WHO).[6] In addition to the complications of the disease itself, co-infections of HBV/HCV, HIV/HBV and HIV/HCV further reduce life expectancy. The acquisition of transfusion associated infections is proportional to the prevalence of these viral infections in the general population and the problem is compounded by the use of low-sensitivity Enzyme-linked Immunosorbent Assay (ELISA) or rapid testing kits to detect these infections.[7] Even with good testing platforms there is an issue of window period of infections which can only be detected on Nucleic Acid Testing (NAT). To date, serological screening was only performed on thala-

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ssemia patients due to resource-constraints. Therefore, this was the first study in which was NAT employed in addition to serological testing to reveal the true burden of transfusion transmitted infections (TTIs) in Beta Thalassemia Major patients in Pakistan.

METHODOLOGY

The cross sectional study was conducted at Armed Forces Institute of Transfusion Rawalpindi Pakistan, from April to July 2020 after approval from Institutional Ethics Committee (Certificate no AFIT-ERC-20-008). The sample size was calculated using Raosoft sample size calculator with previous prevalence of Transfusion Transmissible Infections (TTIs) in thalassemia patients in Pakistani population being 36.5%.[4]

Inclusion Criteria: Beta-thalassemia major patientsof either gender who were being transfused red cell concentrates (RCC) at Armed Forces Institute of Transfusion (AFIT), Rawalpindi were included.

Exclusion Criteria: The patients whose parents were not available for consent or unwilling to take part in the study were excluded from the study.

The patients' demographic information such as age, gender, frequency of transfusion and weight were noted through direct interview. A written consent was obtained from all patients and parents/guardians in case of children and non-probability convenience sampling technique was used. A 3mL of blood sample was collected before transfusion. The separated serum was screened for serological evidence of HbsAg, anti-HCV and anti-HIV-1/2+p24 by chemiluminescence microparticle immunoassay (CLIA) (Architect SR i2000 Abbott Diagnostics, USA) whereas plasma was subjected to analysis of molecular markers including HBV-DNA, HCV and HIV-RNA by multiplex Real-Time Polymerase Chain Reaction (Roche, MPX Cobas 6800). Positive and negative controls were run to validate the serology and molecular screening results.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 23. Qualitative variables were presented as frequency and percentage whereas, mean and standard deviation was calculated for quantitative variables. Chi-square test was performed to find the association between gender, frequency of transfusion and age groups. The *p*-value less than or equal to 0.05 was considered as significant.

RESULTS

Among 105 Beta-thalassemia patients, there were 61(58.1%) males and 44(41.9%) females. The age of the patients was 11.8±6.4 years ranging from 2 to 34 years. Seropositivity and Nucleic Acid Testing (NAT) reactivity was observed in 29(27.6%) and 16(15.2%) patients respectively. The frequency Hepatitis C Virus (HCV) antibodies and Ribonucleic Acid (RNA)was highest among infected patients while none of the patient was found to be reactive for anti-HIV and HIV-RNA. Viremia was detected in 12(11.4%) out of 29 seropositive patients and in 4(3.8%) out of 76 sero-negative patients. Distribution frequency of serology and NAT reactive cases among beta-thalassemia major patients shown in Figure.

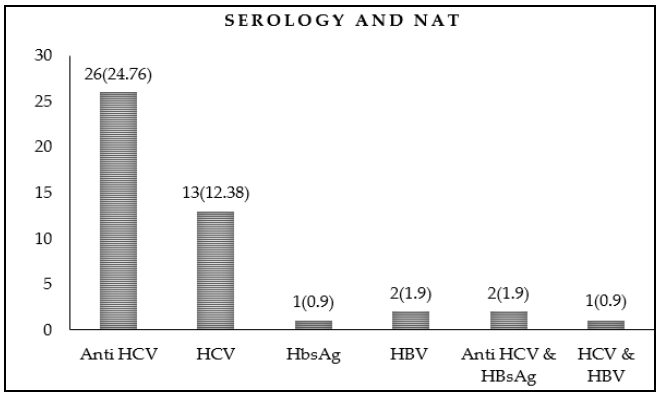

Figure-1: Distribution Frequency of Serology and NAT (Nucleic Acid Testing) Reactive cases among Beta-Thalassemia Major Patients (n=105)

Out of 105 multi-transfused patients, 29(27.6%) were found to be seropositive by CLIA (Chemiluminescence immunoassay) technique while frequency detected by RT-PCR assay was 16(15.2%). The number of HCV infected patients was higher than HBV infected patients detected using both techniques while none of the patient was found to be sero-reactive and NAT-reactive for HIV 1/2. Characteristics of sero-NAT reactive patients are shown in Table-I.

Males had higher seropositive rate and NAT reactivity as compared to females. Patients were categorized into two groups on the basis of frequency of transfusion. Seropositivity rate was slightly higher in the patients having twice a month frequency of transfusion as compared to patients with once in a month frequency of transfusion. Regarding association of TTIs with age, increase in seropositivity was observed with 10.4% patients found in >15years of age group. RT-PCR method had shown its impact in

Table-I: Characteristics of Serology and Nucleic Acid Testing (NAT) Reactive Multi-Transfused Beta-Thalassemia Patients (n=105)

Variables	Categories	Seropositive n(%)	Seronegative n(%)	*p*-value
Gender	Male (61)	16(15.2)	45(42.8)	0.001
	Female (44)	13(12.3)	31(29.5)	
	Total (105)	29 (27.6)	76(72.3)	
Frequency of Transfusion (days)	≥30(57)	11(10.4)	46(43.8)	0.107
	<30(48)	18(17.2)	30(28.5)	
	Total (105)	29(27.6)	76(72.3)	
Age groups (years)	<5 (17)	02(1.9)	15(14.2)	0.417
	5-10(32)	07(6.6)	25(23.8)	
	11-15 (31)	09(8.5)	22(20.9)	
	>15 (25)	11(10.4)	14(13.3)	
	Total (105)	29(27.6)	76(72.3)	
Variables	Categories	NAT-Positive	NAT Negative	*p*-value
Gender	Male(61)	09(8.5)	52(49.5)	0.001
	Female(44)	07(6.6)	37(35.2)	
	Total(105)	16(15.2)	89(84.7)	
Frequency of Transfusion (days)	≥30(57)	08 (7.6)	49(46.6)	0.199
	<30(48)	08(7.6)	40(38.1)	
	Total(105)	16(15.2)	89(84.8)	
Age groups (years)	<5(17)	00(00)	17(16.1)	0.112
	5-10(32)	04(3.8)	28(42.8)	
	11-15(31)	06(5.7)	25(23.8)	
	>15(25)	06(5.7)	19(18.0)	
	Total(105)	16(15.2)	89(84.8)	

terms of revealing viremia among thalassemia patients. Out of 29 seropositive cases, NAT reactivity was detected only in 12 patients (11.4%) while among 76 seronegative cases, 4 cases (3.8%) were found to be NAT-reactive as shown in Table - II.

Table-II: Results of NAT testing on Seropositive and Seronegative Beta-Thalassemia Patients (n=105)

Thalassemia Patients	n(%)
Seropositive	29(27.6)
NAT Reactive	12(11.4)
Seronegative	76(72.3)
NAT Reactive	04(3.8)

CLIA technique detected 2(1.9%) co-infection cases of HBV/HCV but only one of this case was found to be co-infected by NAT testing.

DISCUSSION

The findings of our study illustrated that the most common TTI was HCV which corroborates with the previous findings though different rate of prevalence have been observed due to geographical variations. In the present study illustrated overall 27.6% rate of seropositivity with maximum number of cases HCV, followed by HBV and none for HIV. Males were predominantly infected with HCV. The high frequency of HCV was also demonstrated among thalassemia patients in a local study conducted at five different thalassemia centers of Pakistan located in Islamabad, Karachi and Rawalpindi.[8] In comparison to other Asian countries, studies from Western and Eastern India reported high HCV seropositivity rate among thalassemia patients.[9,10] Also, consistent findings were revealed in a study conducted by Hossain *et al*. from Bangladesh.[11] Similar to our study design, study from Western India reported 57% seropositivity rate for TTIs which is in sharp contrast to our findings. However, HCV infection was found to be the most common among other TTIs.[10] The reported prevalence of HCV from Iran is low, which is in disagreement with current rate.[12] Similarly, high prevalence of HCV infection among thalassemic was shown in a study from Iraq.[13] Comparable to our findings, a study from Egypt reported 20.7% prevalence rate of HCV infection.[14] In contrast to the current frequency, anti-HCV seropositivity remained alarming in more than one-third of the thalassemic patients in Taiwan.[15] The current sero-prevalence of HCV (27.6%) among beta-thalassemia patients was very high as compared to general Pakistani population (6.2%).[16] The most likely reason for increased risk of HCV infection could be frequent exposure to blood units, lack of effective donor screening and voluntary remunerated blood donations, use of low-quality screening kits,

transmission from asymptomatic donor carriers as molecular screening is limited, unavailability of HCV vaccine and frequent hospitalization of thalassemia patients.[17]

In our study, the low frequency of HBV infection as compared to HCV among thalassemic patients might be due to availability of vaccine which is 80-100% effective in those recipients who avail complete vaccine series thereby reducing the HBV infection.[18] Improved HBV vaccination status also contributed to similar low prevalence rate of HBV infection among thalassemic in various studies from Bangladesh, India and Iran. However, results of study conducted in Sri Lanka contradict with our findings which revealed 6.4% cases of HBV infection.[19]

In the present research, all the thalassemic patients were negative for anti-HIV 1/2 and HIV RNA thus constituted a prevalence rate of zero percent. This might be due to concentrated prevalence of HIV in high-risk individuals such as commercial male and female sex workers, injecting drug users and trans-gender sex workers which are not a part of general donor community. Nonetheless, it warrants hemovigilance and surveillance as this can spill over in donor population in future.[20]

The present study showed a mean age of thalassemia patients as 11.8 years which was comparable with the findings of previous multi-center study conducted in Pakistan which showed a mean age of 10.1 years.[8] A study by Moshary *et al*. and Harfouche *et al*. showed similar mean age of 11.5 years respectively.[21,22]

In Pakistan, various blood centers employ poorly calibrated and validated rapid screening kits thus viral acquisition following blood transfusion remains a major concern, therefore, the high prevalence of HCV among thalassemic implies the use of low-quality screening kits which remains a leading cause of transfusion associated infections among our population.[23]

LIMITATIONS OF STUDY

First, it was based on data collected from a single transfusion center and second was the sample size due to which extrapolation of current findings to general population would remain inconclusive.

CONCLUSION

Hepatitis C virus was the most prevalent in beta-thalassemia patients followed by hepatitis B virus and human immunodeficiency virus. The serology should be augmented with NAT to detect viremia in seronegative as well as seropositive cases.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MAR & MAN: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AJA & EG: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SKN & MN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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