Original Article

β-Thalassemia

Anti- Phospholipid Antibodies in Multiple Blood Transfusion Dependent β-Thalassemia Major Patients at Hyderabad

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ABSTRACT

Objective: The study aimed to observe the anti-phospholipid antibodies in multiple blood transfusion dependent β-thalassemia patients at the Hyderabad.

Study Design: Observational / cross sectional study.

Place and Duration of Study: This study was conducted at pathology and pediatric department, Liaquat University of Medical & Health Sciences, Jamshoro, from June 2013 to May 2015.

Material and Method: A total of 121 patients were enrolled. Blood samples were collected in citrated tube. APTT was performed on Sysmex (CA 500) while Lupus anticoagulant and Anticardiolipin antibodies were performed on commercial kits.

Results: Of 121 patients 81(66.9%) patients were positive for anti-phospholipid antibody while 40(33.1%) were negative. Lupus anticoagulant were present in 69 (57%) and were not detected in 52 (43%). Cardiolipin antibodies were found positive in 45 (37.2%) while negative in 76 (62.8%) cases.

Conclusion: Anti-phospholipid is common in patients with beta thalassemia major. APLs are considered as a common acquired autoimmune hyper coagulation state for thrombotic risk factor.

Key words: Anti-phospholipid antibodies, anticardiolipin antibodies, Lupus anticoagulant, β-Thalassemia

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INTRODUCTION

Beta- (β) thalassemia is an inherited blood disorder caused by a defect in the synthesis of β-globin chain.[1,2] Prevalence of beta thalassemia has been reported 16% in Cyprus, up to 14% in Thailand, India, Pakistan, Bangladesh, China and 0.9% & 0.1 in African blacks and Europe respectively.[3] Transfusion of red blood cells and iron chelation therapy has immensely improved the quality of life of thalassemia major patients[4] however; these patients may develop complications including thrombosis pulmonary hypertension and thrombo embolic events.[5] The estimation of risk in patients of thalassaemia regarding hemostatic anomalies is thought to be resulting from hepatic dysfunction, chronic platelet activation, red cell membrane alteration and activation of intrinsic coagulation system. However further studies are required to clearly understand the risk factors.[6,7] Studies have reported the presence anti-phospholipid antibodies such as (APA), lupus anticoagulant (LA) and anti-cardiolipid antibody (ACA) in beta thalassemia major.[8] Antiphospholipid syndrome (APS) is an autoimmune disease in which antibodies such as lupus anticoagulant (LA), anticardiolipin antibodies (ACL) and anti-β2-glycoprotein-1 (anti-β2-GP1) are present and hence causing arterial and venous thromboembolism. Patients with repeated pregnancy morbidity and/or thromboembolic complications are distinguished by the presence of these antibodies. They are also associated with fetal loss, thrombocytopenia and neurological manifestations and has influence on multiple pathways including coagulation and complement pathways.[9,10] APLs with its associated clinical features got increasingly significant recognition from the past decade in various pediatric diseases. In the neonatal period APLs may be present as a result of vertical transmission resulting in disturbance of intricate balance between coagulation and anti-coagulation factor including reduced concentration of naturally occurring anticoagulant proteins and increased levels of factor VIII and von Willebrand factor with less active fibrinolysis that leads to the prothrombotic state. Numerous congenital and acquired risk factors such as coagulation inhibitors, genetic mutations like polymorphism of methylene tetrahydrofolatereductase

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(MTHFR) gene, maternal eclampsia, traumatic delivery, hyperchromocyteinemia, infections, dehydration, complex inherited heart diseases and catheter placement in the newborn may lead to neonatal thrombosis[10-12] The hypercoagulable state in beta thalassemia major patients is due the presence of reduced naturally occurring anticoagulation proteins such as The natural coagulation inhibitors protein C, protein S and anti thrombin.[13] Thrombocytopenia is a frequent finding in patients with APS, and balancing the need for anticoagulation when faced with significant thrombocytopenia can be a considerable challenge for clinicians managing such patients. This study will help us to identify the presence of anti-phospholipid antibodies in beta thalassemia patients so the new for approach for screening of such patients and treatment could prevent from the coagulation abnormalities.

MATERIALS AND METHODS

This Cross sectional study was conducted at Department of Pathology and Pediatric, Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro and thalassemia centers from June 2013 to 2015. A total of 121 subjects were selected for this study after an informed consent from the patient or next of kin and fulfilling the inclusion criteria. IRB approval was reserved for this study from institutional ethics committee. Two blood samples were collected from each individual, Ethylene diamine tetra acetic acid (EDTA) and citrate 3.8%. Full blood count (FBC)and activated partial thromboplastin time (aPTT) was performed on sysmex XE2100 and sysmexCA 500 automated coagulation analyzer respectively. Anti-phospholipid antibody :Lupus anticoagulant and anticardiolipin antibodies were determined by commercially available kits. Statistical Package for Social Science (SPSS) software version 22.0 was used for data analysis. Descriptive statistics was applied for qualitative variables such as gender anticardiolipin antibody, lupus anticoagulant and antiphospholipid antibodies. Effect modifier was controlled through stratification of age, gender and number of transfusions by applying chi squire test, $p \leq 0.05$ was considered as significant.

RESULTS

A total of 121 patients of beta thalassemia major with mean age 9.21±2.5; (range 3-13 years) of them most of the patients were the age group of 11-13 (57%).Sixty two were male (51.2%) while 59 (48.2) (male /female: 1.05:1). Lupus anticoagulant, cardiolipin antibody and anti-phospholipid antibodies were positive in 69(57%), 45(37.2%) and 81(66.9%) patients respectively. Antibodies were negative LA 52(43%), cardiolipin antibody 76 (62.8%) and anti-phospholipid antibodies 40(33.1%) (Table1). Minimum number of transfusion were 20 and maximum were 370 and mean transfusion were 132.17±62.173 (Table 2). Patients age and gender with anti-phospholipid antibodies were found to be non-significant i.e. (0.063), (0.082) while transfusion were found to be highly significant i.e. (0.001) as shown in table 5.

Table No.1: Frequency of lupus anticoagulant, cardiolipin antibody and anti phospholipid antibodies

Types of antibodies test	Positive	Negative
Lupus anticoagulant (n=121)	69(57%)	52(43%)
cardiolipin antibody (n=121)	45(37.2%)	76(62.8%)
anti-phospholipid antibodies (n=121)	81(66.9%)	40(33.1%)

Table No. 2. Anti-phospholipid antibodies status and number of transfusion

Number of Transfusion	Negative	Positive	Total
4-100	35	15	50
101-200	5	53	58
201-370	0	13	13

Table No.3: Anti-phospholipid antibodies status with various age groups

Age Groups	Negative	Positive	Total
3-6	4	7	11
7-10	19	22	41
11-13	17	52	69

Table No.4: Anti-phospholipid antibodies status in male and female

Gender	Negative	Positive	Total
Female	24	35	59
Male	16	46	62

Table No.5: Statistical significance in antiphos-pholipid antibody and various age groups, gender and no, of transfusions

	Age group	Gender	No, of Transfusions
p-value	0.06	0.08	0.0001

DISCUSSION

It is recommended to test the LA after discontinuation of antithrombotic therapy as screening for LA is performed using two phospholipid dependent coagulation tests.[14-18] Patients who are on oral anticoagulants show prolonged clotting time, mixing and confirmatory tests. Hence may lead to false positive results.[9] However ACL and anti β2-GP1 can be detected by using enzyme-linked immunosorbent assay (ELISA) techniques. Standardization of these assays is

not well established and variations may exist among batches.[9] This study revealed 57% incidence of LA in patients of thalassemia major.[19]

Anticardiolipin antibodies were positive in 37.2% of the thalassemia major patients. There was a 2 GP1 ACA in 13% of the cases of a-thalassemia. LA positive patients manifested higher numbers of transfusions and mean age as compared to LA negative patients but still the comparison was not proven significant statistically. A correlation of LA with multiple transfusions were observed.[8] Our study also showed no evidence of thrombosis in the patient having LA. One international study has mentioned the same findings. Two studies have reported the incident of intracranial bleeding and cerebral infarction associated with LA.[8]

The present study is of value for the blood transfusion dependent thalassemia patients as it will improve the health in future. The main limitation of the present study is small sample size, particular race and ethnicity of particular geographical area; hence results should be interpreted cautiously for other settings.

CONCLUSION

The present study shows increase frequency of lupus anti-coagulant and anti cardiolipin antibodies in multiple blood transfusion dependent β-thalassemias major demonstrating the presence of in comparison to normal individuals. The blood transfusions requirements were higher in patients with positive anti phospholipid antibody as compared with negative.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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