ORIGINAL ARTICLE

ANTITHROMBIN III LEVEL IN PATIENTS OF β-THALASSEMIA: A CROSS-SECTIONAL STUDY

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Background: Thalassemia is a genetic disorder associated with reduced production rate of either a single or more globin chains in the haemoglobin. Thalassemia is divided into two main types, alpha (α) thalassemia and beta (β) thalassemia. The aim of this study was to determine antithrombin III level in diagnosed patients of beta thalassemia major. **Methods:** This cross-sectional study was conducted at Sheikh Zayed Hospital Lahore from Jan 2014 to Jan 2015. The sample size was 100 including 50 healthy controls and 50 patients with β thalassemia major. Antithrombin III assay was done with Chromogenic determination of Antithrombin (AT) on an auto analyzer. **Results:** Mean age splenectomized group was 9.1±2.2 years and that for non-splenectomized and control group was 6.5±2.9. The mean antithrombin III level for the splenectomized group was 66.0±8.9% and that for non-splenectomized and control group was 78.6±9.6 and 91.9±4.7 respectively. The overall mean antithrombin III level for cases was 75.8±10.7. The differences for antithrombin III level were significant (p<0.001). The splenectomized group also had significantly lower antithrombin III level as compared to the non-splenectomized group (p=0.001). **Conclusion:** Antithrombin III level is significantly affected in thalassemia. A considerable difference was observed in antithrombin III levels of splenectomized and non-splenectomized patients.

Keywords: Antithrombin, Thalassemia, Splenectomized, Normality

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INTRODUCTION

Thalassemia is a genetic disorder associated with reduced production rate of either a single or more globin chains in the haemoglobin.[1] On the basis of molecular pathology, thalassemia is mainly divided into two main types namely α-thalassemia and β-thalassemia.[2] The β-thalassemia is categorized into three main types which are β-thalassemia minor, intermedia and major.[3] Thalassemia major also referred to as Cooley's anaemia is clinically a homozygous manifestation of β-thalassemia. This disorder is quite severe and stems from the inheritance of two alleles belonging to β-thalassemia on the copies of chromosome-11.[4–6] One of the more serious concerns with regards to public health authorities is the increase in the occurrence of thalassemia among the worldwide population. It is expected that thalassemia disorders will affect around 900,000 births worldwide in the coming 20 years.[7] This considerable growth in frequency of thalassemic disorders is mostly related to thalassemia major, which is starting to become one of the most commonly occurring haemoglobin disorders. Sixty percent population in a number of the Southeast Asian region suffer from thalassemic disorders.[8] The estimated worldwide carriers (having mutant globin alleles) exceed 270 million leading to severe forms haemoglobinopathy and thalassemia. In Pakistan, prevalence rate of thalassemia carriers is around 4.06%. However, the situation is not similar in every ethnicity of the country with a comparatively higher prevalence rate in Pathans (7.9%) compared to 3.26% in Panjabis.[9] It must also be noted that in Pakistan, a family with a thalassemia major patient has a 31% prevalence rate of the disorder in that family.[9,10]

Antithrombin-III glycoprotein contains 432 amino acids and is responsible for inactivation of numerous coagulation system enzymes.[11] Antithrombin-II is regarded as plasma cofactor and along with heparin, it provides interference with fibrinogen and thrombin interaction.[12]

The natural inhibitors of coagulation include Antithrombin III, Protein C and tissue factor. Each of these agents plays crucial roles in the control of coagulation. Not only do they assist in maintaining coagulation but also attenuate inflammatory response during sepsis. The presence of acceptable levels of these anticoagulants is important. Low levels of these agents are one of the main reasons for thrombotic events in thalassemic patients.[13]

Factors Xa and IXa, and thrombin is targeted by antithrombin.[14] Thrombin-antithrombin (TAT) inactivation rate rises, in presence of heparin, around 2,000 to 4,000 times.[15] Factor Xa inhibition by antithrombin is around 500–1,000 times in presence of heparin. On the other hand, enhancement in the inhibition of antithrombin-factor IXa is nearly 1 million times when physiological calcium levels are normal and heparin is present.[16] It is important to note

that inhibition of thrombin relies on ternary complex formation between heparin, thrombin and antithrombin III. Antithrombin inhibits serine protease (serpin), which is why its structure has similarities to a number of plasma protease inhibitors like pain cofactor II, alpha 1-antichymotrypsin and alpha 2-antiplasmin.[17] Plasmin and kallikrein, which are involved in coagulation of blood, are also inactivated by the inhibitor. Inactivation of protease is a result of protease trapping in the equimolar complex having AT. As a result of protease enzymes attack of the reactive bond, they are entrapped in the inactive antithrombin protease complexes.[18]

The incidence of AT deficiency has been estimated somewhere around 1:2,000 to 1:5,000 in the normal population. The first family recorded to suffer from an inherited deficiency of antithrombin dates back to 1965.[19] Inherited antithrombin deficiency has been classified into two types namely type I and type II on the basis of immunochemical as well as function antithrombin analyses.[20] It is highly essential that antithrombin activity in the body does not fall below 70% of its routine operational capacity for inhibiting blood coagulation proteases. In both type I and type II AT deficiencies, the functional level of AT falls below 50% of its routine operational capacity.[21,22] This study was conducted to determine the antithrombin III level in diagnosed patients of beta thalassemia major.

MATERIAL AND METHODS

This cross-sectional study was conducted at Sheikh Zayed Hospital Lahore from 3rd Jan 2014 to 3rd Jan 2015, after approval from ethics committee. Informed consent was obtained from patients and controls. Data was collected after obtaining the results of investigations including CBC, PT, APTT and Antithrombin III. Antithrombin levels in splenectomized and non-splenectomized thalassemic patients were compare with the antithrombin levels of healthy individuals (control group). The data was analyzed using SPSS-18.

Known β-thalassemia major patients and healthy controls between 2 years to 12 years were included. Known cases of coagulation abnormality, patients with history of intake of oral or intravenous anticoagulant drugs like warfarin, epinephrine, heparin, aspirin, clopidogrel etc., patients with carcinoma of liver/hepatic failure or any other malignancy, patients with pulmonary embolism and patients with recent infection were excluded.

This study was based on a collection of primary data from test results of patients and healthy individuals. Moreover, the selected subjects are also divided into three main groups. Group 1 having healthy controls, group 2 having splenectomized thalassemic patients and group 3 having non-splenectomized thalassemic patients. Fifty healthy controls and 50 patients with β thalassemia major were included in the study. Antithrombin III assay was done with chromogenic determination of AT on an auto analyzer.

RESULTS

This study was conducted with 100 subjects, divided into two groups of 50 cases and 50 controls. The cases were further divided into two subgroups: A having 11 splenectomized patients and B having 39 non-splenectomized patients.

Normality of data was tested for all variables in cases and controls, and it was noted that the data for all variables were deviating from normality ($p<0.05$) (Table-1). Moreover, the normality was tested for three groups and the data was normally distributed in all three groups except Splenectomized (Table-2).

Mean age of splenectomized group was 9.1±2.2 years and that for non-splenectomized and control group were 6.5±2.9 and 6.5±2.9 respectively. The mean age of cases was 7.1±2.9 years. The difference of age between cases and controls was insignificant with ($p=0.257$) (Table-2).

The mean antithrombin III level for the splenectomized group was 66.0±8.9% and that for non-splenectomized and control group were 78.6±9.6 and 91.9±4.7 respectively. The overall mean antithrombin III level for cases was 75.8±10.7. The difference for antithrombin III level between cases and controls was significant ($p<0.001$) (Table-3).

The difference antithrombin III among the groups was significant ($p<0.001$). It was noted that both non-splenectomized and splenectomized groups had significantly lower antithrombin III level as compared to control group ($p<0.001$). The splenectomized group had significantly lower antithrombin III level as compared to non-splenectomized group ($p=0.001$) (Table-4).

Table-1 Test of normality for data in two groups

	Shapiro-Wilk		
Group	**Statistic**	**df**	***p***
Age			
Cases (all variables)	0.950	50	0.033
Control	0.949	50	0.031
Antithrombin III level			
Cases	0.954	50	0.048
Control	0.931	50	0.006
Antithrombin III level			
Control	0.931	50	0.006
Splenectomized	0.959	11	0.761
Non Splenectomized	0.942	39	0.045

Table-2: Mean Age for Three Groups and Mean Antithrombin III Level

	Age			
Groups	**Mean±SD**	**Median**	**Q_1**	**Q_3**
Splenectomized	9.1±2.2	9.0	8.0	11.0
Non Splenectomized	6.5±2.9	6.0	4.0	9.0
Cases overall	7.1±2.9	7.0	4.0	9.0
Controls	6.5±2.9	6.0	4.0	8.0
Mann Whitney U=1086.5, z= -1.13, p=0.257				

Table-3: Antithrombin III level for children in three groups and comparison of cases with controls

Groups	Antithrombin III level			
	Mean±SD	Median	Q_1	Q_3
Splenectomized	66.0±8.9	65.0	59.5	74.0
Non Splenectomized	78.6±9.6	80.0	72.0	86.5
Cases overall	75.8±10.7	76.5	68.0	86.0
Controls	91.9±4.7	91.0	88.5	96.5
Mann Whitney U=181.0, z= -7.37, p<0.001				

Table-4: Comparison for antithrombin III level for children among three groups

Groups	N	Mean Rank	Kruskal Wallis ANOVA		
			Chi-square	df	*p*
Control	50	71.88	58.86	2	<0.001
Splenectomized	11	12.73			
Non-Splenectomized	39	33.74			

DISCUSSION

The mean antithrombin III level for controls and cases was significant (p<0.001). The splenectomized group had significantly lower antithrombin III level as compared to the non-splenectomized group (p=0.001). The non-splenectomized group had significantly lower antithrombin III level as compared to control (p=0.001). The splenectomized group had significantly lower antithrombin III level as compared to control (p=0.001). Our observations were in line with other studies.[22–24]

The patients of thalassemia intermedia especially when they are splenectomised are also prone to thrombotic events due to decreased levels of anticoagulants as observed in present study.[25]

Apart from significant decrease in anithrombin III level in thalassemic patients this study is also showing significant increase in the platelets count of the patients which are splecnectomized which is further aggravating the risk of thrombosis. Same findings were observed in other studies.[22–24] If the patients of thalassemia are regularly evaluated and monitored for Anithrombin III levels, they can be saved from thrombosis and their quality of life can be improved by timely actions and using accurate anticoagulants.

CONCLUSION

A considerable difference was observed in antithrombin III levels of splenectomized and non-splenectomized patients. Thus antithrombin III levels not only decline in thalassemia but they decrease further when thalassemic patients undergo splenectomy. This study is a step in the right direction for attaining more insight into the problem in order to prevent thrombotic events in thalassemic patients.

REFERENCES

1. Hoffbrand AV, Catovsky D, Green AR. Haemoglobin and the inherited disorders of globin synthesis. In: Wetherall DJ, (Ed). Postgraduate Hemtology. 5th ed. USA: Blackwell; 2011.p. 88.
2. Hofmann R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P, Heslop LEE, (Eds). Hematology: Basic Principies and Practice. Philadelphia: Elsevier; 2009.
3. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ 2001;79:704–12.
4. Tripodi A, Cappellini MD, Chantarangkul V, Padovan L, Fasulo MR, Marcon A, *et al*. Hypercoagulability in splenectomized thalassemic patients detected by whole-blood thromboelastometry, but not by thrombin generation in platelet-poor plasma. Haematologica 2009;94:1520–7.
5. Yasmeen H, Toma S, Killeen N, Hasnain S, Foroni L. The molecular characterization of Beta globin gene in thalassemia patients reveals rare and a novel mutations in Pakistani population. Eur J Med Genet 2016;59(8):355–62.
6. Dussiot M, Maciel TT, Fricot A, Chartier C, Negre O, Veiga J, *et al*. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. Nat Med 2014;20:398–407.
7. Collen D, Schetz J, de Cock, F, Holmer, E, Verstraete M. Metabolism of antithrombin III (heparin cofactor) in man: effects of venous thrombosis and of heparin administration. Eur J Clin Invest 1977;7(1):27–35.
8. Sirachainan N, Thongsad J, Pakakasama S, Hongeng S, Chuansumrit A, Kadegasem P, *et al*. Normalized coagulation markers and anticoagulation proteins in children with severe ß-thalassemia sease after stem cell transplantation. Thromb Res 2012;129(6):765–70.
9. Turk B, Brieditis I, Bock SC, Olson ST, Björk I. The oligosaccharide side chain on Asn-135 of α-antithrombin, absent in β-antithrombin, decreases the heparin affinity of the inhibitor by affecting the heparin-induced conformational change. Biochem 1997;36(22):6682–91.
10. Yang X, Alexander KP, Chen AY, Roe MT, Brindis, RG, Rao SV, *et al*. The implications of blood transfusions for patients with non-ST-segment elevation acute coronary syndromes: results from the CRUSADE National Quality Improvement Initiative. J Am Coll Cardiol 2005;46(8):1490–5.
11. Buller HR, Ten Cate JW. Acquired antithrombin III deficiency: laboratory diagnosis, incidence, clinical implications, and treatment with antithrombin III concentrate. Am J Med 1989;87(3B):44–8S.
12. Inthorn D, Hoffmann JN, Hartl WH, Mühlbayer D, Jochum M. Antithrombin III supplementation in severe sepsis: beneficial effects on organ dysfunction. Shock 1997;8:328–34.
13. Chang, W, Wardell, MR, Lomas DA, Carrell RW. Probing serpin reactive-loop conformations by proteolytic cleavage. Biochem J 1996;314:647–53.
14. Lane DA, Kunz G, Olds RJ, Thein SL. Molecular genetics of antithrombin deficiency. Blood Rev 1996;10(2):59–74.
15. Maher CA, Olds T, Williams MT, Lane AE. Self-reported quality of life in adolescents with cerebral palsy. Phys Occup Ther Pediatr 2008;28(1):41–57.
16. Sas G. Hereditary antithrombin III deficiency: biochemical aspects. Haematologia (Budap) 1984;17(1):81–6.
17. Hameed A, Hussain A, Fayyaz T, Tayyab M, Ahmad J. Chronic liver disease assessment of antithrombin III level. Professional Med J 2002;9:100–5.
18. Shoaib M, Shamsi T, Naz A. Arteriovenous thrombosis in chronic renal patients receiving renal replacement therapy. J Coll Physicians Surg Pak 2008;18:418–23.
19. Olds RJ, Lane DA, Chowdhury V, De Stefano V, Leone G, Thein SL. Complete nucleotide sequence of the antithrombin gene: evidence for homologous recombination causing thrombophilia. Biochem 1993;32:4216–24.
20. Rahmani MTH, Tayyab M, Tasneem T. Plasma antithrombin III levels in Pakistani women with pre-eclampsia. J Ayub Med Coll Abbottabad 1999;11(1):20–3.
21. Sheikh MA, Memon I, Ghori RA. Frequency of anemia in patients with systemic lupus erythromatosis at tertiary care hospital. J Pak Med Assoc 2010;60:822–5.

22. Eldor A, Durst R, Hy-Am E, Goldfarb A, Gillis S, Rachmilewitz EA, *et al*. A chronic hypercoagulable state in patients with beta-thalassaemia major is already present in childhood. Br J Haematol 1999;107:739–46.
23. Shebl SS, el-Sharkwy H, el-Fadaly N. Haemostatic disorders in non splenectomized and splenectomized thalassaemia children. East Mediterr Health J 1999;5:1171–7.
24. Yashar VBB, Barenholtz Y, Hy-Am E, Rachmilewitz EA, Eldor A. Phosphatidylserine in the outer leaflet of red blood cells from β-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. Am J Hematol 1993;44:63–5.
25. Cappellini MD, Coppola R, Robbiolo L, Graziadei G, Valentini M, Ariens RAS, *et al*. Procoagulant activity of erythrocytes in thalassemia intermedia. Blood 1996;88(Suppl 1):38a.

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