Evaluation of Protein S and Protein C Levels in B-Thalassemic Patients and Its Association with Blood Transfusion

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Abstract:

Background: Thalassemia is a chronic hemolytic anemia due to a partial or complete deficiency in the synthesis of α-globin chains or β-globin chains that compose of the major adult hemoglobin (α 2β 2). Protein C is a vitamin K-dependent serine protease and naturally occurring anticoagulant that plays a role in the regulation of hemostasis by inactivating factors Va and VIIIa in the coagulation cascade.Protein S is a non-enzymatic cofactor for activated protein C–mediated inactivation of factor VIIIa and Va. In addition, protein S may have activated protein C–independent anticoagulant activity. Aim of study:To evaluate protein S&C levels in thalassemic patients &its relation to blood transfusion .

Methods: the study included 80 subjects divided into two groups: Case group included 60 patients who were a known cases of transfusion dependent B-thalassemia and control group included 20 participants who were healthy and matched in age and gender with case group. Patients with other forms of hemolytic anemia like sickle thalassemia, who suffering from severe hepatic or cardiac dysfunction, those with other hemoglobinopathies, were excluded from the study. Plasma protein S and C Ag were calculated in both patient groups and in control group by ELISA.

Results: Plasma protein C was significantly lower in patients with splenectomy in comparison with patients without splenectomy. Protein C and S were significantly lower in patients with non-frequent than in patients with frequent blood transfusion. There was significant positive correlation between plasma protein C and protein S levels.

Conclusion: Patients with thalassemia major have lower level of protein C and protein S than non-thalassemic patient.Frequency of blood transfusion can affect level of protein C & protein S. Splenoctomized patients have lower levels of protein C and S than non-splenoctomized.

Introduction:

Thalassemia is a chronic hemolytic anemia due to a partial or complete deficiency in the synthesis of α-globin chains or β-globin chains that compose the major adult hemoglobin (α 2β 2). (1) It is caused by one or more mutations in the corresponding genes. The unpaired globin chains are unstable; they precipitate intracellularly, resulting in hemolysis, premature destruction of red blood cell [RBC] precursors in the bone marrow, and a short life-span of mature RBCs in the circulation. (13)

Beta-Thalassaemia is one of the commonest genetic disorders in the world. It occurs due to mutation in β-gene of autosomal chromosome11. (2)It is divided into three categories based on the zygosity of the beta-gene mutation, Beta-thalassemia minor, Beta-thalassemia major and beta-thalassemia

intermedia (3).

Protein C is a vitamin K-dependent serine protease and naturally occurring anticoagulant that plays a role in the regulation of hemostasis by inactivating factors Va and VIIIa in the coagulation cascade. (4)

Protein S is a non-enzymatic cofactor for activated protein C–mediated inactivation of factor VIIIa and Va. In addition, protein S may have activated protein C–independent anticoagulant activity by directly binding and inhibiting factor VIIIa in tenase complex, factor Va and factor Xa in the prothrombin's complex. (5)

Several etiologic factors may play a role in the pathogenesis of the hypercoagulable state in thalassemia such as platelet activation and increased circulating aggregates, shortened platelet survival, increased urinary excretion of thromboxane A2 and prostacyclin metabolites, decreased levels of naturally occurring anticoagulants such as protein C and protein S, and an elevated plasma level of thrombin-antithrombin complex have also been reported . (5)

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Patients, Materials and Methods:

It is a case control study,samples from 80 subjects aged between 2 – 19 years and divided into two groups:1-Case group: Included 60 patients who were a known cases of transfusion dependent B-thalassemia.The patients subclassified according to frequency of blood transfusion into frequent transfusion (37 patients) and non-frequent transfusion (23 patients), and also subclassified according to spleen status into splenoctomized (11 patients) and non-splenoctomized (49 patients).2-Control group: Included 20 participants who were healthy and matched in age and gender with case group.

Four ml of blood were withdrawn by venipuncture under aseptic technique from every patient (before receiving the blood transfusion) and every control. The total venous blood sample divided into two smaller samples as follows: 1- Two ml in EDTA tube for complete blood count, blood film and retic count. 2-(1.8 ml) of blood in (0.2 ml) of trisodiumcitrate with gentle mixing. plasma was obtained by centrifugation of blood at room temperature for15 minutes at 1000 g within 30 minutes of collection ,plasma divided into aliquots then stored at (-80°C) which is then used to measure protein s &protein c antigen by ELISA.

Normal pooled plasma was prepared by pooling of plasma from 20 normal healthy donors and then divided into aliquots each containing about 1ml stored at (-80°C).

Inclusion criteria& Exclusion criteria

The Inclusion criteria of patients are those with transfusion dependent B-thalassemia, Not receiving blood transfusion in the last 2 week.

The Exclusion criteria included the Patients suffering from severe hepatic or cardiac dysfunction; Patient with history of familial thrombophilia or use of anticoagulant therapy or antiplatelet drugs.

Statistical analysis

Data were summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables were expressed as mean and standard deviation (SD); whereas, categorical variables were expressed as number and percentage. Independent samples t-test was used to compare mean values between two groups, while Chi-square test was used to study associations between any two categorical variables; however, Yates correction was used instead when more than 20 % of cells have expected count less than 5 and Spearman correlation was used to assess correlation between quantitative variables. The level of significance was set at P value ≤0.05.

The results:

Hematological characteristics of thalassemia patients and control subjects are shown in table 1. The mean RBC, Hb, PCV and MCV were significantly lower in patients with thalassemia than control groups . However, the mean RDW was significantly higher in patients with thalassemia than control groups as shown in table 1.

Table 1: Hematological characteristics of thalassemia patients and control subjects

Characteristic	Patients group n = 60	Control group n = 20	P
RB C			
Mean ±SD	±0.43 2.99	±0.38 4.70	† 0.001>
Range	-4 1.7	-5.49 4.06	HS
Hb g/dl			
Mean ±SD	±0.87 7.66	±1.21 12.74	† 0.001>
Range	-9.4 5.16	-15.1 11	HS
% PCV			
Mean ±SD	±2.81 24.06	±3.59 38.80	† 0.001>
Range	-30 15.5	-47.1 32.8	HS
MCV fl			
Mean ±SD	±3.82 78.87	±6.54 81.99	† 0.011
Range	-88.2 70	-94.4 70.5	HS
% RDW			
Mean ±SD	24.31±3.23	±4.45 15.65	† 0.001>
Range	-34 16	-26.1 11.5	HS
Serum Ferritin			
Mean ±SD	±1298.56 3164.20		---
Range	-6532 803		

n: number of cases; **SD**: standard deviation; †: independent samples **t-test; HS**: Highly significant at **P ≤ 0.01**

As shown in table(2):

Mean plasma protein C of thalassemia patients was 70.71 ±23.72 and that of control group was 77.88 ±6.24; therefore, protein C was lower in thalassemia patients in comparison with control group; however, the difference was statistically insignificant (P = 0.187), as shown in table (2) .

Mean plasma protein S of thalassemia patients was 74.24 ±19.50 and that of control group was 81.58 ±8.24; therefore, protein S was lower in thalassemia patients in comparison with control group; however, the difference was statistically insignificant (P = 0.107), as shown in table (2)

Table 2: Comparison of plasma protein C and protein S between thalassemia patients and control subjects

Protein	Control group n = 20	Thalassemia group n = 60	P
Protein C			
Mean ±SD	±6.24 77.88	±23.72 70.71	† 0.187
Range	-91.30 60.19	24.01-150.00	NS
Protein S			
Mean ±SD	±8.24 81.58	±19.50 74.24	† 0.107
Range	-98.00 66.00	-128.00 38.00	NS

Mean plasma protein C was significantly higher in patients with frequent blood transfusion than in patients with non-frequent blood transfusion (P < 0.001), 84.35±17.26 versus 48.77 ±14.33, respectively, as shown in table(3). In addition, it was significantly higher in control subjects than in patients with non-frequent blood transfusion (P < 0.001), 77.88 ±6.24 versus 48.77 ±14.33, respectively, as shown in table (3), but, there was no significant difference between control subjects and patients with frequent blood transfusion (P = 0.110), 77.88 ±6.24 versus 84.35±17.26, respectively, as shown in table (3).

Besides, Mean plasma protein S was significantly higher in patients with frequent blood transfusion than in patients with non-frequent blood transfusion (P < 0.001), 84.64 ±16.54 versus 57.50 ±9.87, respectively, as shown in table (3). In addition, it was significantly higher in control subjects than in patients with non-frequent blood transfusion (P < 0.001), 81.58 ±8.24 versus 57.50 ±9.87, respectively, as shown in table (3), but, there was no significant difference between control subjects and patients with frequent blood transfusion (P = 0.404), 81.58 ±8.24 versus 84.64 ±16.54, respectively, as shown in table (3).

Table 3: Comparison of plasma protein C and S between patients with frequent blood transfusion and patients with non-frequent blood transfusion

Protein	Control n = 20	Blood transfusion	
		Frequent n = 37	Non-frequent n = 23
Protein C	±6.24 77.88	84.35±17.26	±14.33 48.77
P -value	Control vs frequent 0.110 NS	Control vs non-frequent 0.001 > HS	Frequent vs non-frequent 0.001 > HS
Protein S	±8.24 81.58	±16.54 84.64	±9.87 57.50
P -value	Control vs frequent0.404 NS	Control vs non-frequent 0.001 > HS	Frequent vs non-frequent 0.001 > HS

Discussion:

In the current study, Mean plasma protein C of thalassemic patients was non-significantly lower in comparison with control ,also mean plasma protein S of thalassemic patients was non-significantly lower in comparison with control group In contrary, different results observed in Hassan et al study at 2010, in which plasma levels of protein C and protein S were significantly lower in thalassemic patients than in the control group ,also they concluded that protein C plays a major role in the hypercoagulable state in thalassemic patients and necessary to raise the issue as to whether it would be cost-beneficial to recommend prophylactic antithrombotic therapy in high-risk thalassemic patients.(6)

In the same consistence, Rosnah and colleagues in their study that conducted in Malaysia in 2014, Their result showed that mean Protein C and protein S levels were significantly lower in thalassemia patients compared to age-matched normal control, concluded a significantly decreased Protein C and protein S in thalassemia patients which might suggests hypercoagulable state in thalassemia patients (7).

Furthermore, Study by Abosdera and colleagues in 2014 on 50 β thalassemia major patients revealed different findings in the form of significantly low level of Protein C and protein S among thalassemic patients in comparison to age- and sex-matched healthy controls included in the study, they concluded that significant changes in natural coagulation inhibition and fibrinolysis processes favoring thromboembolism can be detected in otherwise healthy thalassemic children.(8)

Different sample size contributed in each study can determined significance association, additionally, the cause behind the reduction of these factors can be attributed to fact that these proteins are very sensitive to even a mild degree of impairment of the synthetic function of the liver that not to be yet diagnosis, which is a common occurrence in thalassemia due to various causes as hepatic hemosiderosis, viral infections, and vitamin and protein deficiency (9), low Protein C, protein S level can be due to inherited deficiency of Protein C and protein S, in addition to increase in consumption. However, it is very unlikely because even the prevalence of more common congenital thrombophilic mutations like factor V Leiden and prothrombin G20210A mutations are not increase in thalassemia Patient.(10)

Additionally, protein C was significantly lower in patients with splenectomy in comparison with patients without splenectom ,while protein S was non-significantly reduced between patients with splenectomy than those without splenectomy .In comparison to other studies, an Egyptian study done by Hassan and colleagues in 2010, in which Fifty patients with β-thalassemia major (30 non-splenoctomized and 20 splenoctomized) and 20 healthy children as a control group were included, results obtained showed that Plasma levels of protein C and protein S were significantly lower in splenoctomized patients in comparison to those in control group .(6)

Different sample size can have considered as one of the causes of the difference between the studies. The greater re-duction in protein C and S levels in splenoctomized thalassemic patients might be due to procoagulant on the surface of RBCs and abnormal platelets that are not removed from circulation in the case of splenectomy, with the resultant consumption of protein C and protein S in an attempt to control the hypercoagulable state.(11)

In this study, mean protein C was significantly lower in patients with non-frequent blood transfusion than those with frequent transfusion . Besides, protein S also was significantly lower in patients with non-frequent blood transfusion .

In comparison to other studies, an Egyptian study done by Hassan and colleagues in 2010, found that thalassemic patients with frequent blood transfusions, whether splenoctomized or not, had higher levels of protein C than their peers with infrequent blood transfusion (80.8±3.8 vs. 70.25±10.4, P=0.05), while protein S was non-significantly decreased in those had non-frequent transfusion than those with frequent transfusion (81.3±2.5 vs. 71±8.5, P=0.1) (6). In Rosnah et al study in 2014, results showed a significantly lower mean of Protein C in splenoctomized patients [54.00(14.36)] and non-splenoctomized [54.84 (12.86)] than control [94.14 (16.34)]. However, the difference between splenoctomized and non-splenoctomized was not statistically significant.(15) The impact of frequency of blood transfusion on the hypercoagulable state in Eldor et al study in 2002, found that levels of protein C and protein S were lower in infrequently transfused patients than in frequently transfused ones (14,12)

Conclusion:

Patients with thalassemia major have lower level of protein C and protein S than non-thalassemic patient,Frequency of blood transfusion can affect level of protein C and protein S & Splenoctomized patients have lower levels of protein C and S than non-splenoctomized.

References:

1. Muncie HL, Jr., Campbell J. Alpha and beta thalassemia. American family physician. 2009;80(4):339-44.
2. Jalil T, Yousafzai YM, Rashid I, Ahmed S, Ali A, Fatima S, et al. Mutational Analysis Of Beta Thalassaemia By Multiplex Arms-Pcr In Khyber Pakhtunkhwa, Pakistan. 2019;31(1):98-103.
3. Rustam Z, Kamalia A, Hidayat R, Subroto F, Suryansyah A. Comparison of Fuzzy C-Means, Fuzzy Kernel C-Means, and Fuzzy Kernel Robust C-Means to Classify Thalassemia Data. Update. 2019 Jan 1;1:1.
4. Heit JA. Thrombophilia: clinical and laboratory assessment and management. Consultative Hemostasis and Thrombosis. 3rd ed. Philadelphia: Saunders Elsevier. 2013 Jan 1:205-39.
5. Giordano P, Del Vecchio GC, Altomare M, Coppola B, Schettini F, Lolascon A, De Mattia D. Resistance to activated protein C in thalassaemic patients: an underlying cause of thrombosis. European journal of haematology. 1998 Aug;61(2):123-7.
6. Sirachainan N, Thongsad J, Pakakasama S, Hongeng S, Chuansumrit A, Kadegasem P, Tirakanjana A, Archararit N, Sirireung S. Normalized coagulation markers and anticoagulation proteins in children with severe β-thalassemia disease after stem cell transplantation. Thrombosis research. 2012 Jun 1;129(6):765-70.
7. Petkov GH, Efremov GD, Efremov DG, Dimovski A, Tchaicarova P, Tchaicarov R, Rogina B, Agarwal S, Kutlar A, Kutlar F, Reese AL. β-Thalassemia in Bulgaria. Hemoglobin. 1990 Jan 1;14(1):25-33.
8. Rund D, Rachmilewitz E. β-Thalassemia. New England Journal of Medicine. 2005 Sep 15;353(11):1135-46.
9. Sipahi T, Kara A, Kuybulu A, Egin Y, Akar N. Congenital thrombotic risk factors in β-thalassemia. Clinical and Applied Thrombosis/ Hemostasis. 2009 Oct;15(5):581-4.
10. Wilairat P, Kittikalayawong A, Chaicharoen S. The thalassemic red cell membrane. The Southeast Asian journal of tropical medicine and public health. 1992;23:74-8.
11. Shebl S, El Sharkawy HM, El Fadaly NH. Haemostatic disorders in nonsplenectomized and splenectomized thalassaemic children.2019
12. Ruf A, Pick M, Deutsch V, Patscheke H, Goldfarb A, Rachmilewitz EA, Guillin MC, Eldor A. In-vivo platelet activation correlates with red cell anionic phospholipid exposure in patients with β-thalassaemia major. British journal of haematology. 1997 Jul;98(1):51-6.
13. 13.Vij R, Machado RFJC. Pulmonary complications of hemoglobinopathies. 2010;138(4):973-83.
14. Bain BJ, Lewis S/M. Preparation and staining method for blood and bone marrow films. In: Bain BJ, Bates I, Laffan M/A, Lewis SM, editors. Dacie and Lewis Practical Haematology. 12thed. Philadelphia: Churchill Livingstone; 2016.p27-29& P.50-58.
15. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood, The Journal of the American Society of Hematology. 2013 Jan 1;99(1):36-43.