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Natural Anticoagulant Protein Levels in Patients With Beta-Thalassemia Major: A Case-Control Study

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Abstract

Background: β-thalassemia is a group of inherited blood disorders that affect the production of β-globin chains, leading to the reduction or absence of these chains. One of the complications observed in patients with β-thalassemia major (β-TM) is thrombosis, especially in those who receive frequent blood transfusions. This may be due to a decrease in the levels of the natural anticoagulants: protein C (PC), total protein S (PS), and antithrombin (AT).

Methods: In this case-control study, patients with β-TM, who had received at least 20 packed cell transfusions during their lifetime, were included. Patients with other underlying diseases like bleeding or thrombotic disorders were excluded. Totally, 118 patients with β-TM and 120 healthy individuals were included.

Results: The mean level of PC and AT was significantly lower in patients with β-TM (48.2 ± 65.4 and 57.42 ± 13.6, respectively) compared to the control group (97.1 ± 21.46 and 81.79 ± 14.3, respectively), with P value of 0.001 and 0.01, respectively. Although the difference was not statistically significant (P = 0.1), a similar trend was observed for total PS (61.12 ± 21.12 for patients versus 72.2 ± 35.2 for the control group). Of note, the decrease in PC, AT, and total PS levels compared to the control group was 50.36%, 27.5%, and 15.34%, respectively.

Conclusions: It seems that β-TM patients who receive prolonged blood transfusions frequently are at an increased risk of decreased in natural anticoagulants levels and therefore potentially are at risk of thrombosis.

Keywords: β-thalassemia major; Protein C; Protein S; Antithrombin; Thrombosis

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Introduction

β-thalassemia is a group of hereditary blood disorders caused by defects in the production of β-chains of hemoglobin (Hb), which results in either a reduced or absent production of β-chains. This imbalance between α and β chains leads to an excess of α chains and subsequently early destruction of the erythrocytes, resulting in anemia [1-3]. The estimated annual prevalence of symptomatic patients with β-thalassemia worldwide is 100,000 [3, 4]. In Iran, the estimated incidence of thalassemia is around 3.6%, making the country one of the regions with a high prevalence of thalassemia [2]. β-thalassemia can be classified into β-thalassemia major (β-TM, Cooley's anemia, Mediterranean anemia), β-thalassemia intermedia, and β-thalassemia minor (β-thalassemia trait, heterozygous β-thalassemia, β-thalassemia carrier) [1]. Patients with β-TM do not produce sufficient β-globin chain [1, 3], and as a result, they require regular transfusions of red blood cells (RBC) [2, 5]. This condition typically presents within the first 2 years of life [2]. Patients with β-TM usually are characterized by Hb levels less than 7 g/dL, mean corpuscular volume (MCV) of 50 to 70 fL, and mean corpuscular Hb (MCH) of 12 to 20 pg [1]. One of the complications observed in patients with β-thalassemia, particularly those with major and intermedia subtypes with regular blood transfusion, is thrombosis. Several studies have reported pulmonary embolism (PE), deep vein thrombosis (DVT), and portal vein thrombosis, have been observed in these patients [6, 7]. Various factors trigger throm-

Table 1. Coagulation Profile of Patients With β-Thalassemia Major in Comparison With Healthy Individuals

Parameters	Patients' mean parameter	Healthy subjects' mean parameter	P value	Normal range
PT (s)	19.5	14	0.001	10 - 14.5
APTT (s)	51.5	43	0.001	22 - 45
PC (%)	48.2 ± 65.4	97.1 ± 21.4	0.001	70 - 130
PS (%)	61.12 ± 21.1	72.2 ± 35.2	0.1	60 - 165
AT (%)	57.42 ± 13.6	81.79 ± 14.3	0.01	80 - 120

PT: prothrombin time; APTT: activated partial thrombin time; PC: protein C; PS: protein S; AT: antithrombin; s: second.

bosis in these patients, one of the most significant being the alteration in the level of the body's natural anticoagulants, such as protein C (PC), protein S (PS), and antithrombin (AT) [8, 9]. In general, under normal conditions and in healthy individuals, activated PC (APC) deactivates coagulation factors (F) Va and VIIIa by its proteolytic activity [10, 11]. PS is the body's natural anticoagulant that acts as a cofactor of APC and other coagulation factors [12] and AT, which is another natural anticoagulant with serine protease activity, inhibits coagulation factors such as thrombin, FXa, and with less extent, FIXa and FXIa [13]. Inflammation is a contributing factor that may result in a reduction in the natural anticoagulant of the human body, and it is better to take this into account in patients with thalassemia as thalassemia is correlated with the presence of inflammation [14-17]. Several studies have investigated the levels of these natural anticoagulants in patients with β-thalassemia and have found lower levels of them compared to the control group (healthy individuals) [2, 4, 5, 8, 14, 18]. However, some studies have reported conflicting results. Hadi et al reported that one of their patients with β-TM had a PC level of 140% [4]. In addition, although Eldor et al reported a significant decrease in PC and PS levels, but the levels of AT were within the normal reference range [19]. Due to the conflicting studies and the lack of large-scale studies in Iran, this study aimed to evaluate the level of PC, total PS, and AT in patients with β-TM who receive regular blood transfusion.

Materials and Methods

In this case-control study, 118 patients with β-TM (57 females and 61 males) and 120 healthy individuals (61 females and 59 males) as a control group were included. Patients with β-TM, who had received at least 20 packed cells during their lifetime, were included in the study. The samples were collected before pack cell transfusion during October 20, 2020, to July 20, 2021, from Southeast of Iran. The exclusion criteria comprised of patients who had other underlying diseases like coagulation factor deficiencies or a history of thrombosis or anticoagulant therapy. The evaluation of AT, PC, and total PS was performed using the STA®-Stachrom® AT III, STA®-Stachrom® PC, and STA®-Staclot® PS kits, respectively. Hb levels and platelet count were assessed using Sysmex kx_21 hematology analyzer, and activated partial thromboplastin time (APTT) and prothrombin time (PT) were analyzed using semi-automatic coagulation analyzer, STAGO- STart (Diagnostica Stago, France). Additionally, serum iron and ferritin were assessed using Hitachi 912 (Hitachi High Technologies, Tokyo, Japan). The normal ranges of all parameters are shown in Tables 1 and 2. Statistical analysis of data was conducted using SPSS software. To determine the normality of the data, either the Kolmogorov-Smirnov or Shapiro-Wilk test was performed. To compare the means between the two groups, the independent sample *t*-test and Mann-Whitney U test (if the data distribution was not normal) were used. A statistically significant difference was defined as $P < 0.05$.

Ethical compliance

All procedures performed in this study involving human participants were in accordance with the ethical standards of the responsible institution. This study was approved by the Ethics Committee of Babol University of Medical Sciences (IR.MUBABOL.HRI.REC.1399.112).

Results

The mean ages for male and female patients with β-TM were 17 and 18 year, respectively without statistically significant difference. The mean age of healthy individuals was 17.1 ±

Table 2. Laboratory Characterizations of Patients With β-Thalassemia Major in Comparison With Healthy Individuals

Parameters	Patients' mean parameter	Healthy subjects' mean parameter	P value	Normal range
Hb (g/dL)	9.5 ± 3.1	13.5 ± 4.1	0.01	12.5 - 17.5
Iron (μg/dL)	745.95 ± 32.2	52.12 ± 4.13	0.0001	35 - 150
Ferritin (ng/mL)	2145.10 ± 74.3	92.1 ± 4.58	0.0001	30 - 200
Platelet ($\times 10^9$/L)	621 ± 4	245 ± 1	0.0001	150 - 400

Hb: hemoglobin.

5.58. There was no statistically significant difference between patients and control groups (P = 0.33). The average number of blood transfusions per year for patients with β-TM was 15.3 ± 5.3 and this is why the mean serum iron level and ferritin level were significantly higher in patients with thalassemia major compared to healthy individuals. Additionally, the mean level of Hb in these patients (9.5 ± 3.1 g/dL) was lower compared to the control group (13.5 ± 4.1 g/dL; P = 0.01), with a decrease of 29.63% in patients.

Notably, results revealed that the mean level of PC was significantly lower in patients with β-TM (48.2±65.4%) compared to the control group (97.1±21.4%) with P value of 0.001. Similarly, the mean level of AT was significantly lower in thalassemia major patients (57.42±13.6%) compared to the control group (81.79±14.3% with P value of 0.01). Although a similar trend was observed for total PS, the difference was not statistically significant. In this setting, the average level of total PS was lower in patients with β-TM (61.12±21.1%) compared to the control group (72.2±35.2%) with P value of 0.1. In other words, the decrease in PC, AT, and total PS levels in comparison to the control group was 50.36%, 27.5%, and 15.34% respectively. The results of other laboratory findings and their comparison with healthy individuals are shown in Table 2.

Discussion

One of the important complications in patients with β-TM is thrombosis. An epidemiological investigation examined the occurrence of thrombotic events in both Iran and the Mediterranean areas, and the results indicated that among the 6,670 patients diagnosed with β-TM, a total of 0.9% experienced thrombotic events [20]. Additionally, the study conducted by Vassilopoulou et al revealed four cases of β-TM with ischemic stroke [21]. Furthermore, Karimi et al conducted a study on 40 patients with β-TM and stated that silent cerebral ischemia was detected in a total of 15 patients. (37.5%) [22]. Unlike the above studies, the study of Naithani et al found no evidence of thrombotic complications in patients with β-TM [8].

Through the conducted investigations, the cause of thrombosis was attributed to various factors. One of the most common of these factors is the reduction of the body's natural anticoagulants: PC, PS, and AT. In this context, our study found that the average level of PC was significantly lower in patients with β-TM compared to the control group (P = 0.001). Furthermore, the mean level of AT was also significantly lower in patients with β-TM than in the control group (P = 0.01). Similar results were observed regarding PS, but it was not statistically significant (P = 0.1). In this framework, in the study by Huang et al, which was performed on 129 patients with β-TM, β-thalassemia intermedia, α-thalassemia intermedia, and combined α-/β-thalassemia, the decrement of PC and PS was also observed in 95.3% and 77.5% of patients, respectively [14]. Similarly, in a study on 70 patients with β-TM, Hadi et al found significantly lower mean levels of PC (71.31%) and PS (34.3%) compared to healthy subjects (P < 0.001). Notably, one of the patients with β-TM had more than 140% of the normal amount of PC [4]. Moreover, Naithani et al similarly found lower levels of PC, PS, and AT in 26.2%, 28.6%, and 46.8% of patients with β-TM, respectively [8]. Although Eldor et al reported a significant decrease in both PC and PS levels, unlike the studies mentioned above, the levels of AT were within the normal reference range [19]. Singer et al found that most patients with thalassemia major had lower than normal levels of PC and PS [23]. Musumeci et al also observed low activity of PC and AT III in 94% and 55% of cases, respectively [24].

Another potential factor that may contribute to thrombosis in patients with thalassemia major is the microparticles (MPs). MPs are particles between 0.1 to 2 μm in length that form through the remodeling of the plasma membrane of all types of cells, such as circulating platelets, endothelial cells, and RBCs in response to cellular activation and apoptosis [25]. It has been reported that MPs may be one of the reasons for the decrease in the PC levels in patients with a β-TM. In this setting, MPs can be released from the cells during the blood components storage, and as these patients receive continuous transfusions of blood products, PC can bind to these MPs and phosphatidylserine, collectively increasing the removal of PC from circulation and a potential reduction of PC [26, 27]. An important point to note is that not only does PC bind to phosphatidylserine on MPs, but other vitamin K-dependent factors such as prothrombin, FVII, FIX, FX, and PS can also bind to phosphatidylserine on MPs [28].

Another cause of the reduction of these three proteins in patients with β-TM is liver damage due to hemosiderosis caused by repeated blood transfusions, as the liver is the site of the synthesis of these proteins [29]. In this context, the limitation of our study was the lack of conducting of liver function test (LFTs) assay as well as evaluating coagulation factors levels. Moreover, iron overload is associated with a state of chronic oxidative stress in patients with β-TM, resulting in the production of more reactive oxygen species in the RBCs and platelets of these patients than in healthy individuals. This phenomenon leads to platelet activation, consumption of PC and PS, and increased susceptibility to thrombotic complications [30].

It has been reported that non-transfusion-dependent thalassemia (NTND) patients have also a high risk of thrombosis (such as pulmonary hypertension, and silent cerebral ischemia). Additionally, it has been reported that PC, PS, and AT levels in these patients are lower than in healthy subjects [31-33]. Therefore, another limitation of our study pertained to not examining the aforementioned factors in NTDT patients and comparing them with our healthy and thalassemia major subjects.

The oxidation of globin subunits in RBCs is another reason for the reduction of these anticoagulant proteins in patients with thalassemia major. The oxidation of membrane proteins relies on iron and the generation of red-cell "senescence" antigens, such as phosphatidylserine [2, 34, 35]. Consequently, anticoagulant proteins such as PC may bind to phosphatidylserine or other negatively charged phospholipids that are abnormally present in the outer membrane of RBCs, resulting in a decrease of these anticoagulant proteins [25, 36]. Regular blood transfusions can reduce exposure to phosphatidylserine on the surface of RBCs, resulting in the prevention of thrombotic events [37]. Another cause of decreased PS and PC levels

is vitamin K deficiency, as these factors are vitamin K-dependent [4, 29]. In this setting, no measuring of vitamin K levels in our study is considered as another limitation of our study.

The note that should be taken into account is that thalassemia is correlated with inflammation. Multiple alterations related to inflammation, such as an augmentation in soluble thrombomodulin, can hinder the activation of PC and impede the process of coagulation. These alterations may potentially explain the notable reduction in PC activity observed in patients with β-TM [14, 38]. Regarding the changes of two other natural anticoagulants, PS and AT, there is little information in the literature review. Therefore, another cause of reduction in natural anticoagulants could be related to the inflammatory milieu in patients with β-TM [14].

Another issue that should be paid attention to is medication. However, certain medications, such as warfarin and heparin, possess the capability to induce alterations in the concentration of aforementioned natural anticoagulants within the human body [39, 40]. However, the participants involved in our investigation did not take the mentioned medications or any other pharmaceutical agents. Consequently, the utilization of drugs within our study does not constitute an intervening variable.

One of the causes of thrombosis in patients with thalassemia major is attributed to the presence of activated platelets in these patients [36]. Additionally, the expression of CD62p (p-selectin) and CD63 has been found to increase in the platelets of patients with thalassemia major, triggering the activation and aggregation of platelets [2, 41]. In this study, the mean platelet count in our patients was significantly higher than in healthy people, consistent with the results of the study of Huang et al [14]. However, Naithani et al reported thrombocytopenia in 33.3% of their patients with thalassemia major [8]. They attributed this finding to the hypothesis that most of their patients were on deferiprone [8]. Nonetheless, there is no definitive explanation for the mechanism of thrombocytopenia associated with deferiprone, and further research is needed to show the precise mechanism of deferiprone in the context of thrombocytopenia. Similarly, Marwaha et al reported thrombocytopenia with an incidence of 30.7% [42].

The frequent blood transfusions in patients with β-TM can have complications such as a severe increase in body iron, iron deposition in the organs, and subsequent organ failure [43, 44]. Iron deposition, along with circulating hemolysates, may also lead to parenchymal liver dysfunction, which can affect the production of coagulation factors. Nonetheless, some studies show that hepatic dysfunction due to hemosiderosis is a rare occurrence in children [1, 43, 45]. However, reduced levels of prothrombin and factors V, VII, and X have not only been observed in adults but also in children with β-TM, suggesting that prolonged PT and APTT are more likely related to thalassemia rather than parenchymal hepatic dysfunction caused by iron deposition [19, 43]. Nonetheless, prolonged PT and APTT may be associated with bleeding episodes. In this context, determining or discussing the primary cause of the prolonged PT and APTT in our patients is not clear without the assessment of coagulation protein levels. It is noteworthy that decreased procoagulant proteins are more likely to be balanced by decreased anticoagulant protein levels [43]. In this setting, assaying coagulation factors alongside anticoagulant proteins, which would have aided better interpretation of the results, was one limitation of our study, and we recommend the evaluation of coagulation factor levels in future studies.

In conclusion, it appears that patients with β-TM who receive frequent blood transfusions are at a higher risk of a decrease in the level of natural anticoagulant proteins such as PC, total PS, and AT and potentially are at risk of thrombosis. Therefore, the measurement of these three anticoagulant levels is important and could prevent the occurrence of thrombosis by appropriate interventions. Taken together, these findings, along with available data and data from future large-scale studies, could potentially lead to a better approach to managing the disease, particularly in the prevention of thrombotic events.

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Conflict of Interest

The authors declare no conflict of interest.

Informed Consent

Written informed consent was obtained from all participants or their parents for publication of the details of their medical cases before the study.

Author Contributions

AA had the idea for the article. AS, SoH, SaH, AD, BT, and MS performed the literature search. ST helped in providing samples. AA, MT, AD, SaH, BT, and MS conducted the required tests. AA, AS, SoH, AD, ST, BT, and MT drafted the work, and MS critically revised the work.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

References

1. Origa R. beta-Thalassemia. Genet Med. 2017;19(6):609-

619. doi pubmed
2. Hashemieh M. Thromboembolic events in severe thalassemia syndromes. Iranian Journal of Blood and Cancer. 2020;12(2):41-46.
3. Galanello R, Origa R. Beta-thalassemia. Orphanet Journal of Rare Diseases. 2010;5:1-15.
4. Hadi TK, Mohammad NS, Nooruldin SA. Protein C and protein S levels in beta-thalassemia major patients in Erbil, Kurdistan Region. Cell Mol Biol (Noisy-le-grand). 2020;66(5):25-28. pubmed
5. Sipahi T, Kara A, Kuybulu A, Egin Y, Akar N. Congenital thrombotic risk factors in beta-thalassemia. Clin Appl Thromb Hemost. 2009;15(5):581-584. doi pubmed
6. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, et al. Survival and complications in thalassemia. Ann N Y Acad Sci. 2005;1054:40-47. doi pubmed
7. Raz S, Koren A, Dan O, Levin C. Executive function and neural activation in adults with beta-thalassemia major: an event-related potentials study. Ann N Y Acad Sci. 2016;1386(1):16-29. doi pubmed
8. Naithani R, Chandra J, Narayan S, Sharma S, Singh V. Thalassemia major— on the verge of bleeding or thrombosis? Hematology. 2006;11(1):57-61. doi pubmed
9. Natesirinilkul R. Thromboembolism in beta-thalassemia disease, in Beta Thalassemia. 2019. IntechOpen.
10. Bouwens EA, Stavenuiter F, Mosnier LO. Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway. J Thromb Haemost. 2013;11(Suppl 101):242-253. doi pubmed pmc
11. Esmon CT. The protein C pathway. Chest. 2003;124(3 Suppl):26S-32S. doi pubmed
12. Hackeng TM, Sere KM, Tans G, Rosing J. Protein S stimulates inhibition of the tissue factor pathway by tissue factor pathway inhibitor. Proc Natl Acad Sci U S A. 2006;103(9):3106-3111. doi pubmed pmc
13. Hepner M, Karlaftis V. Antithrombin. Methods Mol Biol. 2013;992:355-364. doi pubmed
14. Huang Y, Long Y, Deng D, Liu Z, Liang H, Sun N, Xu Y, et al. Alterations of anticoagulant proteins and soluble endothelial protein C receptor in thalassemia patients of Chinese origin. Thromb Res. 2018;172:61-66. doi pubmed
15. Sarangi PP, Lee HW, Kim M. Activated protein C action in inflammation. Br J Haematol. 2010;148(6):817-833. doi pubmed pmc
16. Schlommer C, Brandtner A, Bachler M. Antithrombin and its role in host defense and inflammation. Int J Mol Sci. 2021;22(8):4283. doi pubmed pmc
17. Rigby AC, Grant MA. Protein S: a conduit between anticoagulation and inflammation. Crit Care Med. 2004;32(5 Suppl):S336-341. doi pubmed
18. Hassan TH, Elbehedy RM, Youssef DM, Amr GE. Protein C levels in beta-thalassemia major patients in the east Nile delta of Egypt. Hematol Oncol Stem Cell Ther. 2010;3(2):60-65. doi pubmed
19. Eldor A, Durst R, Hy-Am E, Goldfarb A, Gillis S, Rachmilewitz EA, Abramov A, et al. A chronic hypercoagulable state in patients with beta-thalassaemia major is already present in childhood. Br J Haematol. 1999;107(4):739-746. doi pubmed
20. Taher A, Isma'eel H, Mehio G, Bignamini D, Kattamis A, Rachmilewitz EA, Cappellini MD. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb Haemost. 2006;96(4):488-491. pubmed
21. Vassilopoulou S, Anagnostou E, Paraskevas G, Spengos K. Etiology and treatment of ischaemic stroke in patients with beta-thalassemia major. Eur J Neurol. 2011;18(12):1426-1428. doi pubmed
22. Karimi M, Toosi F, Haghpanah S, Pishdad P, Avazpour A, Rachmilewitz EA. The frequency of silent cerebral ischemia in patients with transfusion-dependent beta-thalassemia major. Ann Hematol. 2016;95(1):135-139. doi pubmed
23. Singer ST, Kuypers FA, Styles L, Vichinsky EP, Foote D, Rosenfeld H. Pulmonary hypertension in thalassemia: association with platelet activation and hypercoagulable state. Am J Hematol. 2006;81(9):670-675. doi pubmed
24. Musumeci S, Leonardi S, Di Dio R, Fischer A, Di Costa G. Protein C and antithrombin III in polytransfused thalassemic patients. Acta Haematol. 1987;77(1):30-33. doi pubmed
25. Youssry I, Soliman N, Ghamrawy M, Samy RM, Nasr A, Abdel Mohsen M, ElShahaat M, et al. Circulating microparticles and the risk of thromboembolic events in Egyptian beta thalassemia patients. Ann Hematol. 2017;96(4):597-603. doi pubmed
26. Spinella PC, et al. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. Critical Care. 2009;13(5):1-11.
27. Koshiar RL, Somajo S, Norstrom E, Dahlback B. Erythrocyte-derived microparticles supporting activated protein C-mediated regulation of blood coagulation. PLoS One. 2014;9(8):e104200. doi pubmed pmc
28. Owens AP, 3rd, Mackman N. Microparticles in hemostasis and thrombosis. Circ Res. 2011;108(10):1284-1297. doi pubmed pmc
29. Faraj SA. Hemostatic parameters in Thalassemia patients; a single institute experience. Journal of the Faculty of Medicine Baghdad. 2016;58(2):132-135.
30. Amer J, Fibach E. Chronic oxidative stress reduces the respiratory burst response of neutrophils from beta-thalassaemia patients. Br J Haematol. 2005;129(3):435-441. doi pubmed
31. Saliba AN, Taher AT. Morbidities in non-transfusion-dependent thalassemia. Ann N Y Acad Sci. 2016;1368(1):82-94. doi pubmed
32. Hashemieh M, Azarkeivan A, Sheibani K. A comparison of hemostatic changes in splenectomized and nonsplenectomized beta-thalassemia intermedia patients. J Pediatr Hematol Oncol. 2016;38(8):636-641. doi pubmed
33. Bhattacharyya M, Kannan M, Chaudhry VP, Mahapatra M, Pati H, Saxena R. Hypercoagulable state in five thalassemia intermedia patients. Clin Appl Thromb Hemost. 2007;13(4):422-427. doi pubmed
34. Cappellini MD, Motta I, Musallam KM, Taher AT.

Redefining thalassemia as a hypercoagulable state. Ann N Y Acad Sci. 2010;1202:231-236. doi pubmed
35. Kuypers FA, de Jong K. The role of phosphatidylserine in recognition and removal of erythrocytes. Cell Mol Biol (Noisy-le-grand). 2004;50(2):147-158. pubmed
36. De Franceschi L, Bertoldi M, Matte A, Santos Franco S, Pantaleo A, Ferru E, Turrini F. Oxidative stress and beta-thalassemic erythroid cells behind the molecular defect. Oxid Med Cell Longev. 2013;2013:985210. doi pubmed pmc
37. Asadov C, Alimirzoeva Z, Mammadova T, Aliyeva G, Gafarova S, Mammadov J. beta-Thalassemia intermedia: a comprehensive overview and novel approaches. Int J Hematol. 2018;108(1):5-21. doi pubmed
38. Loghmani H, Conway EM. Exploring traditional and nontraditional roles for thrombomodulin. Blood. 2018;132(2):148-158. doi pubmed
39. Lipe B, Ornstein DL. Deficiencies of natural anticoagulants, protein C, protein S, and antithrombin. Circulation. 2011;124(14):e365-368. doi pubmed
40. Khor B, Van Cott EM. Laboratory tests for antithrombin deficiency. Am J Hematol. 2010;85(12):947-950. doi pubmed
41. Taher AT, Musallam KM, Inati A. The hypercoagulable state in thalassemia intermedia. Hemoglobin. 2009;33(Suppl 1):S160-169. doi pubmed
42. Marwaha R, Hafis-Ibrahim C, Marwaha N. Hemostatic derangements and lupus anticoagulant in polytransfused patients with beta thalassemia. Pediatric Research. 1999;45(5):766-766.
43. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood. 2002;99(1):36-43. doi pubmed
44. Sirachainan N. Thalassemia and the hypercoagulable state. Thromb Res. 2013;132(6):637-641. doi pubmed
45. Maiti A, Chakraborti A, Chakraborty P, Mishra S. Subclinical haemorrhagic tendency exists in patients with beta-thalassaemia major in early childhood. Australas Med J. 2012;5(2):152-155. doi pubmed pmc