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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и http://www.nlm.nih.gov/bsd/uniform_requirements.html В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректур авторам не высылаются, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of 3 centimeters width, and 1.5 spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - 12 (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned
Requirements are not Assigned to be Reviewed.**

ავტორთა საქურაღებოლ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დაიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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EVALUATION OF PROTEIN C AND S IN B-THALASSEMIA MAJOR

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Abstract.

Background: Beta-thalassemia major is a genetic disease characterized by formation of little or no beta-globin chain, leading to premature death of red blood cells and hence to ineffective erythropoiesis. Aim of this study to evaluate Protein C and Protein S in patient with beta-thalassemia major and its correlation with haemoglobin, serum ferritin, D.dimer, prothrombin time and liver enzymes. **Method:** Study is a case control, for patients with beta-thalassemia major at Ibn Al-Atheer Hospital in Nineveh Province in Iraq during a period from July 2022 to November 2022. A total of (70) patients diagnosed as β -thalassemia major, from 5 to 40 years old presented at thalassemia center. A total of (30) normal persons, age and sex matched to the patients. Complete blood count, Protein C, Protein S, Pro-thrombin time, Ferritin, D.dimer, Aspartate aminotransferase, Alanine aminotransferase, done for all patients and control.

Result: Protein C and protein S were significantly lower in patients with β -thalassemia major in comparison to control. Prothrombin time was significantly prolonged in patients with β -thalassemia major. D.dimer was significantly increase in β -thalassemia major than control. Protein C and protein S level were significantly higher in cases with frequent blood transfusion than in those with non-frequent patient. Prothrombin time and D.dimer also significantly elevated in patients with non-frequent transfusion.

Conclusion: These findings suggest that patients with β -thalassemia major may be at a higher risk for coagulation abnormalities and should be closely monitored. Further research is needed to better understand the relationship between β -thalassemia major and coagulation parameters.

Key words. Thalassemia, hemoglobin, protein C, protein S, D.dimer.

Introduction.

Thalassemia is an inherited disorder of autosomal recessive gene disorder caused by impaired synthesis of one or more globin chains. The impairment alters production of hemoglobin (Hb) [1]. Normal adult haemoglobin molecules are made of chains called alpha and beta chains that can be affected by mutations. In thalassemia, the production of either the alpha or beta chains are reduced, resulting in either alpha-thalassemia or beta-thalassemia [2]. Beta thalassemias (β thalassemias) are a group of inherited blood disorders. They are forms of thalassemia caused by reduced or absent synthesis of the beta chains of hemoglobin that result in variable outcomes ranging from severe anemia to clinically asymptomatic individuals [3]. Ineffective bone marrow erythropoiesis and excessive red blood cell hemolysis together account for the anemia [4].

Protein C and protein S are glycoproteins, predominantly synthesized in the liver, that are important components of the natural anticoagulant system in the body. They are Vitamin K

dependent and serve as essential components in the maintenance of physiologic hemostasis [5,6]. Deficiency of protein C and protein S results in the loss of these natural anticoagulant properties, thereby resulting in unchecked thrombin generation, leading to thromboembolism [7]. Protein C and S deficiencies can be due to inherited gene mutations or due to acquired causes. The majority of the inherited forms are due to missense mutations (60% - 70%) followed by smaller percentages (1% - 15%) of nonsense mutations, splice site mutations, large deletions, small deletions/duplications/insertions, and point mutations [8,9]. Protein C and protein S are primarily synthesized in the liver. Protein S is also synthesized by platelets, endothelial cells, osteoblasts, vascular smooth muscle cells, and circulates in plasma [10]. Protein C is activated by the thrombin-thrombomodulin complex, to form activated protein C (APC) on the surface of the vascular endothelial cells. Once protein C is activated, free protein S in the plasma serves as a cofactor along with phospholipids and calcium, to inactivate factor V and factor VIIIa at specific polypeptide arginine cleavage sites. This results in impaired prothrombin activation, thereby exerting their anti-coagulant action by reducing thrombin generation. About 60% to 70% of protein S is in a bound form, non-covalently attached to C4-binding protein (CBP). This protein S-CBP complex enhances the cleavage of activated factor Va, but not as effectively as free protein S. Protein S also enhances the effects of APC in fibrinolysis and also exerts APC independent effects as well by direct inhibition of the tenase & prothrombinase complex, acting as an important cofactor to tissue factor pathway inhibitor (TFPI) during the inactivation of activated factor X and further inhibiting thrombin generation [11-14]. To evaluate the level of protein C and S in β -thalassemia patients. To correlate the levels of protein C and S with clinical findings, complication and severity of the disease. To correlate the levels of protein C and S with other laboratory parameters.

Materials and Methods.

This case control study was done at Ibn Al-Atheer Hospital in Mosul city in Iraq during a period between July 2022 and November 2022. A total of (70) patients diagnosed as β -thalassemia major, from (5 to 40) years old present at thalassemia center in Nineveh province.

The inclusion criteria were above 5 years of age, diagnosed as β -thalassemia major by clinical, hematological (CBP, HPLC) and some of them molecular.

The exclusion criteria: Patients with hepatitis B and hepatitis C were excluded. Comorbidity conditions known to alter hemostasis, recent infection.

Ethical consideration: This study was approved by an official permission by the ethics committee at Iraqi board medical specialities commission and by the directorate of health in Mosul. The study was explained to all patients and control.

Six ml of venous blood were collected by venipuncture method from every patient, (2 ml) of blood was placed in EDTA tube for checking of complete blood count (CBC), and (2 ml) was placed in sodium citrate tube for determination of protein C, protein S, D-dimer, and prothrombin time (PT), and (2 ml) put in plain tube was allowed to clot at room temperature for about half hour then putting in centrifuge at 2000 rpm for 15 minutes to separate serum and used for serum ferritin and biochemical analysis. CBC from EDTA container by using electronic haematology analyzer.

All investigations performed according to standard procedure recommended by the manufactures of each kit.

1. Prothrombin Time (PT) using BIO-TP kit from BIOLABO.
2. Protein C and Protein S using ELISA kit from AESKULISA, reading by BioTEK 800TSI instrument.

Statistical analysis:

The data collected during the study were summarized in sheets of Microsoft Excel 2007. The statistical analysis performed by using IBM-SPSS 20. The normality of these data tested by Shapiro-Wilk test, and the non-parametric tests were decided to be chosen. Frequencies, medians, minimum, maximum, 25th quartile and 75th quartile were calculated. The Mann-Whitney U Test used to calculate the difference between the two independent groups. The Spearman's correlation coefficient was used to investigate the relationship between study parameters. The "r" is correlation coefficient, values close to 1 indicate strong correlation between two variables and those close to zero indicate poor correlation. P-value ≤ 0.05 considered as significant.

Results.

The study sample include 100 subjects (70 beta thalassemia major patients and 30 healthy control subjects. Mean age at diagnosis/month was 11 months with range from 6-14 month and age of starting transfusion month was 11 months with range from 6-15 month. Palpable spleen found in 17.14% of the cases while the splenectomy rate reached 28.57%. Thalassaemic faces found in 19(27.14%) of the cases. The other complications of thalassemia distributed as; diabetic 11.43%, cardiac 2.85%, and thyroid 4.28%. Blood transfusion frequency (patients on regular blood transfusion each 14-21 days while (infrequent) patient whom don't take blood on regular schedule) showed in 87.14% of cases (Table 1).

Table 1. Clinical data in patient with β -thalassemia major.

Clinical data		Mean	Range
Mean age at diagnosis/month		11	6-14
Age of starting transfusion/ month		11	6-15
		Number	Percentage
Palpable spleen		12	17.14 %
Splenectomy		20	28.57%
Thalassaemic faces		19	27.14%
Chelation therapy		55	78.57%
Other complications of thalassemia	Diabetic	8	11.43%
	Cardiac	2	2.85%
	Thyroid	3	4.28%
Frequent transfusion (regular)		61	87.14%
Infrequent transfusion (irregular)		9	12.86 %

Hematological and biochemical parameters of cases were demonstrated in table 2.

Coagulation parameters of cases were showed in table 3. The medians of protein C, protein S, Prothrombin time PT, and D. dimer were (82) %, (92.5) %, (15) second, and (440) ng/ml, respectively.

The comparison of coagulation parameters between cases and controls was showed in table 4. The median of both protein C and S showed statistically significant lower levels among cases (82) % and (92.5) %, respectively than those among the controls respectively (94) % and (99) % with (p=0.0001). Prothrombin time was significantly prolonged among cases in comparison to control with (P=0.001). Moreover, median level of D. dimer among cases (440) ng/ml was significantly higher (p=0.0001) than that among controls (190) ng/ml.

Median level of protein C among those with frequent blood transfusion was (83.0) % which was higher than that among those with non-frequent transfusion (50) % in a statistically significant finding (p=0.0001). Medians of protein S were (94) % and (60) % among frequent and non-frequent transfusion; the difference was statistically significant at (p=0.0001). Prothrombin time and D. dimer among frequent (14.8) second and (420) ng/ml respectively, were significantly lower than those among non-frequent (17.2) second and (812) ng/ml respectively at (p=0.001) and (p=0.008) (Table 5).

Comparison of coagulation parameters between those with regular chelating or irregular chelating among cases was showed in table (6). Medians of protein C and S among those with regular chelating (86) % and (95) % respectively were significantly higher (p=0.0001) than those with irregular chelating (59) % and (77) % respectively. Median prothrombin time level among cases with regular chelating was (14.8) second and among those with irregular chelating was (16.4) second; the difference was statistically significant at (p=0.014).

Median of protein C among cases with splenectomy was (69) % and among cases with no splenectomy was (86); the difference was statistically significant (p=0.001). Median of prothrombin time showed statistically significant difference (0.004) between cases with splenectomy (15.55) second and cases without splenectomy (14.65) second. Although medians of protein S and D.Dimer among those with splenectomy were higher than those without splenectomy, the differences were statistically insignificant (Table 7).

Correlations of protein C with Hb, Ferritin <1000, and Ferritin ≥ 1000 among cases were statistically insignificant. While the level of protein C was indirectly and moderately correlated with Pt and D.Dimer in significant statistical associations (p=0.001) and (p=0.0001) respectively (Table 8).

Furthermore, protein S was insignificantly correlated with Hb, Ferritin <1000, and Ferritin ≥ 1000 . The significant indirect weak correlation was found between protein S with Pt (r= -0.235, p=0.05) and between protein S with D.Dimer (r= -0.349, p=0.003) (Table 8).

Correlation of protein C and S with GPT and GOT was demonstrated in table 9. The correlations of both proteins were indirect in statically significant associations.

Table 2. Hematological and biochemical parameters in patients with β -thalassemia major:

Parameters	Median	Minimum	Maximum	25 th Quartile	75 th Quartile
Hb (g/dl)	8.2	6.2	10.1	7.4	8.8
Hct (%)	24	5.3	29.9	21.37	25.77
RBC (x10 ¹² /L)	3.04	2.22	79.9	2.8	3.31
MCV (fL)	77.9	64.6	98	74.35	80.92
WBC (x10 ⁹ /L)	9.85	3.9	16.7	6.25	13.92
Granulocytes (x10 ⁹ /L)	4.75	1.9	9.62	3.3	6.22
Lymphocytes(x10 ⁹ /L)	3.4	1.4	11.8	2.2	7.2
Platelet (x10 ⁹ /L)	379	162	1094	287.75	579.25
ferritin (ng/ml)	1785	696	3716	1103.75	2401.5
GPT (U/L)	34	12	166	27	46.5
GOT (U/L)	38.5	13	185	30.75	52.5

Table 3. Coagulation parameters of patients with β -Thalassemia major:

Parameters	Median	Minimum	Maximum	25 th Quartile	75 th Quartile
Protein C (%)	82	31	113	68.25	95
Protein S (%)	92.5	52	142	83.5	99
PT (second)	15	13.2	17.9	13.9	16.1
D.dimer(ng/ml)	440	150	1100	180	612

Table 4. Comparison of coagulation parameters between patients and controls.

	Cases (n=70) Median (25 th Q, 75 th Q) Min.-Max.	Controls (n=30) Median (25 th Q, 75 th Q) Min.-Max.	p-value *
Protein C (%)	82 (68.25, 95) 31-113	94 (88.75,104) 79, 135	0.0001
Protein S (%)	92.5 (85.5,105) 52, 142	99 (95.75,117) 85, 141	0.0001
PT (second)	15 (13.9, 16.13) 13.2, 13.9	13.15 (13.1, 13.2) 13.1, 13.4	0.001
D. dimer (ng/ml)	440 (180,612.5) 150,1100	190 (178.75,212.5) 160, 370	0.0001

*Mann-Whitney U

Table 5. Comparison of coagulation parameters between those with frequent & non frequent transfusion among patients with β -Thalassemia major:

	Cases with frequent transfusion (n=61) Median (25 th Q, 75 th Q) Min.-Max.	Cases with non-frequent transfusion (n=9) Median (25 th Q, 75 th Q) Min.-Max.	p-value *
Protein C (%)	83 (73.5, 95.5) 59, 113	50 (41,59) 31, 84	0.0001
Protein S (%)	94 (89,105) 77, 142	60 (55.5,74) 52, 90	0.0001
PT (second)	14.8 (13.8,15.6) 13.2,17.6	17.2 (15.35,17.55) 14.2, 17.9	0.001
D.dimer (ng/ml)	420 180,600) 150,1100	812 (465,975) 170,1012	0.008

*Mann-Whitney U

Table 6. Comparison of coagulation parameters between those with regular and irregular chelating among patients with β -Thalassemia major.

	Cases with regular chelating (n=55) Median (25 th Q, 75 th Q) Min.-Max.	Cases with irregular chelating (n=15) Median(25 th Q, 75 th Q) Min.-Max.	p-value *
Protein C (%)	86 (74,96) 60, 113	59 (46, 81) 31, 110	0.0001
Protein S (%)	95 (89, 105) 77, 142	77 (58, 90) 52, 106	0.0001
PT (second)	14.8 (13.8,15.6) 13.2, 17.6	16.4 (14.3,17.4) 13.4,17.9	0.014
D.dimer (ng/ml)	420 (180,600) 150,1100	713 (180,960) 160,1012	0.065

*Mann-Whitney U

Table 7. Comparison of coagulation parameters between those with splenectomy and without splenectomy among patients with β -Thalassemia major.

	Cases with splenectomy (n=20) Median (25 th Q,75 th Q) Min.-Max.	Cases without splenectomy (n=50) Median (25 th Q,75 th Q) Min.-Max.	p-value *
Protein C (%)	69 (61.25,79) 31, 99	86 (76.75,97.75) 44,113	0.001
Protein S (%)	94.5 (81.75,104.75) 52, 142	92 (86.75,105) 53, 142	0.969
PT (second)	15.55 (14.87,16.7) 13.9, 17.9	14.65 (13.8,15.6) 13.2, 17.7	0.004
D.dimer (ng/ml)	552.5 (335, 778.25) 160, 1000	413.5 (180, 602.5) 150, 1100	0.082

*Mann-Whitney U

Table 8. Correlation of protein C and protein S with Hb and coagulation parameters.

Spearman Correlation	Value	Asymp. Std. Error^a	Approx. Tb	p-value	
Protein C (%)	Hb (g/dl)	0.173	0.131	1.449	0.152c
	Ferritin <1000(ng/ml)	0.409	0.247	1.554	0.146c
	Ferritin \geq 1000 (ng/ml)	-0.065	0.138	-0.482	0.632c
	PT (second)	-0.399	0.116	-3.592	0.001c
	D.dimer (ng/ml)	-0.433	0.114	-3.959	0.000c
Protein S (%)	Hb (g/dl)	0.173	0.129	1.448	0.152c
	Ferritin <1000 (ng/ml)	0.482	0.216	1.905	0.081c
	Ferritin \geq 1000 (ng/ml)	-0.035	0.136	-0.254	0.801c
	PT (second)	-0.235	0.121	-1.993	0.050c
	D.dimer (ng/ml)	-0.349	0.121	-3.075	0.003c

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Table 9. Correlation of protein C and S with GPT and GOT.

Spearman Correlation	Value	Asymp. Std. Error^a	Approx. T^b	p-value	
Protein C (%)	GPT (U/L)	-0.330	0.119	-2.882	0.005c
	GOT (U/L)	-0.399	0.111	-3.589	0.001c
Protein S (%)	GPT (U/L)	-0.301	0.122	-2.607	0.011c
	GOT (U/L)	-0.352	0.114	-3.097	0.003c

Discussion.

Current therapeutic approaches have substantially prolonged life expectancy in patients with thalassemia. A consequence of this is the appearance of new complications. Venous thromboembolic events, such as pulmonary embolism, deep venous thrombosis and portal vein thrombosis, have been observed. Several alterations that indicate a state of activation of the hemostatic mechanisms have been described in thalassemia major. Normally Protein C and protein S work together to inhibit the coagulation cascade [15]. This study depends on comparison of the level of protein C, protein S, D-dimer, prothrombin time PT, S. ferritin and liver function test in β -thalassemia patients and control group.

There is 70 β -thalassemia major patient, and 30 control age and sex matched healthy persons. The age of β -thalassemia major patients and control with interval from 5–40 year, include 49 male (48.49 %), and 51 female (51.51%), Most frequent age interval was at 15-20 years, were 26; 14 males and 12 females which near to Tareefa Kakakhan Hadi et al. [16], the mean age for β -thalassemia major patients was (19) years old.

The median age at diagnosis was 11 months range from 6 to 14 month similar to previous study with median of age at diagnosis was 11 months range from 4 month to 3 years [17]. Also, near to study by previous study, 2016 in Basrah was at 10.9 months [18]. The median age of starting transfusion was 11 months, range from 6 to 15 months near to median age of starting transfusion by previous study was 11.5 month [19].

Median level of protein C among those with frequent blood transfusion is higher than that among those with non-frequent transfusion in a statistically significant finding ($p=0.0001$) is near to finding of previous study which was median of protein C with frequent blood transfusion (72.36) % and with non-frequent one was (58.7) % [20].

About level of protein S, the difference in the median level between frequent and non-frequent transfusion was statistically significant at ($p=0.0001$) which near to significant. Median of D-Dimer among frequent blood transfusion was significantly lower than those among non-frequent at P.value ($p=0.008$) which was also significant by previous study in which D. Dimer was 230 ng/ml with frequent transfusion and 325 with non-frequent one.

This was explained by the higher plasma levels of D-dimer and lower levels of naturally occurring anticoagulant proteins among non-frequent patients with thalassemia than in regularly transfused patients with thalassemia major. As an additional or alternative mechanism of hypercoagulability in infrequently transfused patients, the role of thalassemic red cells was considered because protein C is adsorbed to the abnormal red blood cell membrane of thalassemics, it is plausibly accepted to be lower in those patients who are not on a transfusion program because most of their RBCs are of thalassemic nature capable of adsorbing protein C compared to those frequently transfused patients whose blood contains less of thalassemic red cells and more on normal RBCs [21].

Patients with regular blood transfusions for 2-3 weeks have a protective effect and have a relatively low anti-thrombin lowering effect. because abnormal liver function, low-level

natural anticoagulant proteins (such as protein C) found in the study are possible because protein C, protein S are synthesized in the liver, the defect is very sensitive to the liver and even mild [21].

Median of protein C among cases with splenectomy was significantly low in comparison to non splenectomized cases with ($p=0.001$), that near to study by previous study in which splenectomized cases with level of protein C (63.4%) and non-splenectomized with median level of protein C (74.5%) with p.value (0.033) [21].

The reduction in protein C levels was significantly higher in splenectomized thalassemic patients rather than non-splenectomized ones. Consistent with study by Musumeci et al. [22] reported that the lowest values of protein C were found in older splenectomized patients. Also, these results are consistent with Shirahata et al in his study on 48 patients with β -thalassemia/hemoglobin E disease [23]. In contrast to our results, Shebl et al found that there was no significant difference in protein C and protein S levels between splenectomized and non-splenectomized patients with β -thalassemia major [24]. The same was also reported by Cappellini et al in patients with thalassemia intermedia [25]. The greater reduction in protein C and S levels in splenectomized thalassemic patients might be due to procoagulants 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 [25,26].

The difference in the median of D. Dimer among cases with splenectomy and those without splenectomy which is statically insignificant. Dimer was 300ng/ml in non-splenectomized and 512 ng/ml with splenectomized cases with insignificant P.value 0.1455. These finding could be due to small sample size for both groups of patients [27-29].

Correlation of protein C and S with GPT and GOT was indirect and weak in statistically significant associations. In this study, serum liver enzymes levels which reflect the function of the liver were moderately elevated. Therefore, the impairment of liver function alone may not explain the significantly low levels of protein C and protein S found in the current study. liver impairment was not the only cause of the reduction in natural anticoagulant proteins in β -thalassemia patients. There may be another explanation for the significant decrease in proteins and maybe this type of protein binds to phosphatidylserine, or other negatively charged phospholipids, abnormally exist in the external membrane of the thalassemic erythrocytes as previously discussed [30,31].

Conclusion.

Protein C and protein S were significantly lower in patients with β -thalassemia major in comparison to control. Prothrombin time was significantly prolonged in patients with β -thalassemia major. D-dimer was significantly increase in β -thalassemia major than control. Protein C and protein S level were significantly higher in cases with frequent blood transfusion than in those with non -frequent patient. Prothrombin time and D. dimer also significantly elevated in patients with non-frequent transfusion. In cases with irregular chelation their coagulation parameters,

protein C and S significantly abnormal. In splenectomized cases, protein C level was significantly lower than cases without splenectomy. Prothrombin time and D. dimer were significantly higher in splenectomized cases than other whom not splenectomized. Protein C and protein S were significantly low in cases with elevated liver enzymes.

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