DOI: https://doi.org/10.54393/pbmj.v6i09.915

PAKISTAN BIOMEDICAL JOURNAL
https://www.pakistanbmj.com/journal/index.php/pbmj/index
Volume 6, Issue 9 (September 2023)

Original Article

Coagulation Abnormalities in Pediatric Patients with β-Thalassemia, An experience at a Tertiary Care Hospital

Ayesha Shabbir[1*], Saima Farhan[1], Attiq Ur Rehman[2], Neelam Mazhar[1], Sidra Hareem[1], and Iram Iqbal[3]

[1]Department of Hematology & Transfusion Medicine, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan
[2]The School of Allied Health Sciences, University of Child Health Sciences, The Children's Hospital. Lahore, Pakistan
[3]Department of Hematology & Transfusion Medicine, Lahore General Hospital, Pakistan

ARTICLE INFO

Key Words:
Coagulation, Thalassemia, Thromboembolic Events, Liver Enzymes, Coagulation Inhibitors

How to Cite:
Shabbir, A., Farhan, S., Ur Rehman, A. ., Mazhar, N., Hareem, S., & Iqbal, I. (2023). Coagulation Abnormalities in Pediatric Patients with β-Thalassemia, An experience at a Tertiary Care Hospital: Coagulation Abnormalities in Pediatric Patients. Pakistan BioMedical Journal, 6(09). https://doi.org/10.54393/pbmj.v6i09.915

***Corresponding Author:**
Ayesha Shabbir
Department of Hematology & Transfusion Medicine, University of Child Health Sciences, The Children's Hospital. Lahore, Pakistan
ashoo4678@gmail.com

Received Date: 7th August, 2023
Acceptance Date: 19th September, 2023
Published Date: 30th September, 2023

ABSTRACT

Life expectancy in thalassemia has markedly improved due to consistent blood transfer and amenability with iron chelation therapy, therefore this improvement is conditioned with various thromboembolic problems of this prolonged disorder including thromboembolic complaints. **Objective:** To determine coagulation abnormalities in beta (β) thalassemia major patients who have been multi transfused. **Methods:** This observational study was conducted at Department of Haematology & Transfusion Medicine, Children hospital & University of Child Health Sciences (CH&UCHS), Lahore, from October 2022 to January 2023. The study included 60 β-thalassemic patient, age less than 16 years whose samples were compared with upper and lower normal value as regards to Prothrombin Time (PT), Active Partial Thromboplastin Time (APTT), Protein S, Protein C, liver enzymes (Alanine Transaminase (ALT), Aspartate Transaminase (AST), Gamma-Glutamyl Transferase (GGT). The data were analyzed using IBM SPSS version 23.0. **Results:** When values were compared, natural clotting inhibitors (Protein S, Protein C) were remarkably reduced in β-thalassemic paeds patients ($p<0.001$).PT and APTT were prolonged in thalassemic children ($p>0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in β-thalassemic patients ($p < 0.001$ and $p < 0.001$ respectively) due to iron over load by multiple transfusions. **Conclusions:** Marked changes in coagulation inhibition supporting thrombotic tendency was observed in thalassemic children. There were reduced levels of protein C and protein S, independent of slightly prolonged PT and APTT and elevated levels of ALT, AST with normal GGT in thalassemic children.

INTRODUCTION

Thalassemia is inherited hemolytic condition initiated due to partial or complete defect of α or β globin chain production [1]. Basically, thalassemia is a heterogeneous group of genetic heritable disorders of hemoglobin synthesis, which is considered to be the most monogenic condition in the world [2]. It is a life-threatening situation which poses severe health and financial strains on patients and their families [3]. Almost 70,000 children with different types of thalassemia are born every year [4]. Clinically three main classes are transfusing ion dependent thalassemia major, non-transfusion dependent thalassemia intermedia with a moderate degree of anemia due to variety of genetic defects and thalassemia minor which is usually due to a carrier state for alpha or beta thalassemia and characterized by erythrocyte microcytosis and mild or no anemia [5]. β-thalassemia major is most common form. It results from mutations within beta (β) globin gene which effect β-globin chain production. β-thalassemia includes three kinds: Thalassemia Major (also called Cooley or Mediterranean Anemia), Thalassemia Intermedia and Thalassemia Minor also called β-thalassemia carrier or heterozygous β-

DOI: https://doi.org/10.54393/pbmj.v6i09.915

thalassemia [6]. Thalassemic patients already has a chronic subclinical hypercoagulable state. Hypercoagulability in thalassemic patients is understandable, it depends on different variables like closed renal and pulmonary vessels, high numbers of thromboembolic incidents, early atherosclerosis, compromised platelet accumulation, reduced platelet survival, and high urinary metabolites of thromboxane A2 (TXA2) and prostacyclin (PGI2) [7]. Red blood cell membrane abnormality, oxidative damage caused by free hemoglobin, lack of several proteins of clotting cascade, increased leukocyte activation are various factors that contribute to the disease' etiopathogenesis [8]. These problems should be a part of routine treatment procedures and follow up must be planned keeping in view these issues [9]. Different Researches shows that hypercoagulable state along with thromboembolic events in young patients but results were debatable and not comprehensive [10]. These patients are dependent on blood transfusion throughout their lives [1]. As prognosis is improving in these patients, degree of various severe problems is increasing too, TEE are one of such serious issues. Prolongation of PT and APTT, low level of natural anti-coagulants like protein C, protein S have been labelled in these patients, though the procedures implying in thrombotic affinity observed in some patients and hemorrhagic indicators have not been completely illuminated [11]. Limited literature is available on coagulation defects in paeds thalassemic patient, so this study is planned for the up gradation of knowledge to get them managed early to prevent complications. In this study we aimed to determine coagulation abnormalities in β-thalassemia patients who have been multi transfused.

METHODS

This observational study was conducted at Department of Haematology and Transfusion Medicine, Children Hospital and University of Child Health Sciences (CH&UCHS), Lahore from October 2022 to January 2023. The participants of this study included 60 children with β-thalassemia. The sample size was calculated from formula: Here,

$$n=[(\frac{Z\alpha}{2})]/d]^2\,[p(1-p)]$$

Multiple transfused thalassemia major patients with age <16 year were included in this study. The samples were properly labelled and collected in citrate vial and clot activator gel vial. Consecutive sampling method was used for the study. While children with preceding or current thromboembolic events, family history of clotting disorder, using anticoagulant and with any indication of liver failure or cardiomyopathy were excluded from the study. After acquiring informed consent, the blood sample was collected from multiple transfused thalassemia major patients in 3.2 % sodium citrate (blue top) vial for performing coagulation tests and in gel vial (yellow top) for performing liver function tests. Venous blood obtained in citrate vial was centrifuged and plasma was separated. Plasma was examined for Protein C, Protein S, PT and APTT in STA Compact Analyzer. Venous blood collected in gel vial was centrifuged and serum was separated. Liver function tests (AST, ALT, and GGT) was performed on serum in AU 480 Chemistry Analyzer. The results of β-thalassemic patients obtained were compared with upper normal values of variables. The data was entered and evaluated using IBM-SPSS V-23. Continuous variables such as age, PT, APTT, Protein C, Protein S, ALT, AST, GGT were described as Mean±SD, however categorical variables like gender was in form of frequencies and percentages. One sample t-test was applied to compare the means of upper or lower normal value and cases. A p-value of < 0.05 was considered statistically significant. Ethical approval was acquired from ethical committee of The School of Allied Health Sciences, University of Child Health Sciences, Lahore.

RESULTS

A total of 60 β-thalassemic children participated in the study. This study was completed in a duration of about 4 months (From October 2022 to January 2023) in the Department of Hematology and Transfusion, Children Hospital, Lahore. Baseline demographic features are summarized in Table 1.

Table 1: Baseline Demographics of subjects

Variables	Category	N (%)	Mean ± SD
Age (Year)			
Gender	Female	32(53.3)	7.94±3.36
	Male	28(46.7)	

SD: Standard Deviation. Categorical Variables are in form of N(%), while continuous variable is in form of Mean ± SD

The values of clotting inhibitors (protein S and protein C), and Liver Functional Enzymes is represented in Table 2. Considerably reduction in protein C and protein S were evident in thalassemic children.PT and APTT were slightly prolonged in thalassemic patients. The mean levels of ALT and AST were significantly higher but the mean level of GGT was not raised in thalassemic children.

Table2: One sample t -test Analysis

Variable	Upper/Lower Normal Value	Mean ± SD (Cases)	t-value	p- value	95 % CI
PT	14	14.29±2.17	1.034	0.305	0.27-0.85
APTT	34	36.30±6.45	3.15	<0.05	0.96-4.30
ALT	42	86.12±59.20	5.773	< 0.001	28.82-59.41
AST	37	81.85±58.36	5.953	< 0.001	29.77-59.93
GGT	30	24.95±10.34	3.782	< 0.001	7.72-2.38
Protein C	79	28.83± 8.44	47.066	< 0.001	52.35-47.99
Protein S	65	41.08±10.89	17.005	< 0.001	26.73-21.10

DOI: https://doi.org/10.54393/pbmj.v6i09.915

SD: Standard Deviation, CI: Confidence Interval, PT: Prothrombin time, APTT: Active Partial Thromboplastin Time, ALT: Alanine Transaminase, AST: Aspartate Transaminase, GGT: Gamma-Glutamyl Transferase. Lower Normal Values of PT, APTT, and Protein, Protein S are taken as reference while for ALT, AST&GGT upper normal value has been taken as reference values.

DISCUSSION

Hemostatic abnormalities are very common in thalassemic children. Protein C and Protein S play key role in controlling the clotting. If their level is not sufficient excessive clotting may occur, resulting blockage in the blood flow of veins or hardly arteries. In this research protein C and protein S levels were substantially decreased in cases as compared to normal values. These reduced levels cause activation of monocyte and endothelial cells along with platelet accumulation [12]. Hassan *et al* also reported decreased level of Protein C and Protein S in his study [13]. One reason for decreased levels of these proteins is sensitivity to minor grade of damage of liver's synthetic role that is common happening in thalassemic patients because of several issues such as infections, hepatic hemosiderosis and protein deficiency. Another reason for substantial decrease in Protein C was its great attraction to attach to Phosphatidylserine and other negatively charged phospholipids, that are oddly present in outer crust of thalassemic RBCs .These Proteins are also reduced in splenectomized patients because of the procoagulants on the surface of RBCs and irregular platelets that are not removed from blood circulation in situation of splenectomy, resulting in usage of Protein S and Protein C to control hypercoagulability [7]. So, Protein C and Protein S should be monitored in these patients during transfusion to prevent them from thrombotic complications in future. In this study liver enzymes (ALT and AST) were significantly elevated in thalassemic patients. Similar results were investigated in another study by Salama KM *et al*. [14]. The cause of elevated liver enzyme was iron overload due to numerous transfusions, liver infections (Hepatitis B and Hepatitis C). β-thalassemic patients generally undergo dissolution of tissues that results in leaking of ALT and AST in the blood circulation therefore increasing the action of enzyme [15]. Some studies have shown that moderate to high iron deposition in body along with elevated AST were the major danger factors for the Vitamin D deficiency in β-thalassemia patients [16]. Acute or Chronic Liver damage leads to elevated serum levels of ALT and AST [14]. In a study conducted by Esra Emaad Jabbar *et al*., GGT level was decreased in β-thalassemic patients same as in our study. GGT is present on plasma membranes and it is considered one of the indicator of oxidative stress because of its shielding effect in keeping the concentration of glutathione (GSH). It functions to breakdown it exterior to cell, so production of free radicals can result in Intracellular glutathione reduction and therefore increased GGT level in circulation [17, 18]. The level of GGT was not elevated in these patients in our study and this can be explained in terms that there are different stages of liver cirrhosis and their liver might be precirrhotic. PT ($p<=0.305$) and APTT ($p<0.05$) were prolonged. The borderline elongation of PT and APTT in our study are reduced than previous studies. Our findings are consistent with those found in other studies conducted by Naithani *et al* [3], Srevatsa *et al*., [19] and Faraj [20]. Prolonged PT and APTT may be caused by parenchymatous liver damage due to iron over load caused by multiple transfusions.

CONCLUSIONS

Marked changes in coagulation inhibition supporting thrombotic tendency was observed in thalassemic children. There was reduction in protein C and S levels, prolongation of PT and APTT and elevated levels of liver enzymes in thalassemic children due to deranged liver functions.

Authors Contribution

Conceptualization: SF
Methodology: AUR, NM
Formal Analysis: AS
Writing-review and editing: AS, AUR, SH, II

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Chhikara A, Sharma S, Chandra J, Nangia A. Thrombin activable fibrinolysis inhibitor in beta thalassemia. The Indian Journal of Pediatrics. 2017 Jan; 84: 25-30. doi: 10.1007/s12098-016-2208-x.

[2] Mohammad II and Al-Doski FS. Assessment of liver functions in thalassaemia. Tikrit Journal of Pharmaceutical Sciences. 2012 Sep; 8(1): 87-95. doi: 10.1016/j.hoc.2017.11.003.

[3] Naithani R, Chandra J, Narayan S, Sharma S, Singh V. Thalassemia major-on the verge of bleeding or thrombosis?. Hematology. 2006 Feb; 11(1): 57-61. doi: 10.1080/10245330500362087.

[4] Mettananda S and Higgs DR. Molecular basis and genetic modifiers of thalassemia. Hematology/Oncology Clinics. 2018 Apr; 32(2): 177-91.

[5] Shafique F, Ali S, Almansouri T, Van Eeden F, Shafi N,

DOI: https://doi.org/10.54393/pbmj.v6i09.915

Khalid M, et al. Thalassemia, a human blood disorder. Brazilian Journal of Biology. 2021 Sep; 83. doi: 10.1590/1519-6984.246062.

[6] Cecil RL, Goldman L, Schafer AI. Goldman's Cecil Medicine, Expert Consult Premium Edition—Enhanced Online Features and Print, Single Volume, 24: Goldman's Cecil Medicine. Elsevier Health Sciences; 2012.

[7] Kemahli S, Gürman C, Egin Y, Yildirmak Y, Sipahi T, Uysal Z, et al. Hypercoagulability in children with thalassemia major. Clinical and Applied Thrombosis/Hemostasis. 1997 Apr; 3(2): 129-32. doi: 10.1177/107602969700300211.

[8] Sirachainan N. Thalassemia and the hypercoagulable state. Thrombosis Research. 2013 Dec; 132(6): 637-41. doi: 10.1016/j.thromres.2013.09.029.

[9] Cappellini MD, Musallam KM, Poggiali E, Taher AT. Hypercoagulability in non-transfusion-dependent thalassemia. Blood Reviews. 2012 Apr; 26: S20-3. doi: 10.1016/S0268-960X(12)70007-3.

[10] Succar J, Musallam KM, Taher AT. Thalassemia and venous thromboembolism. Mediterranean Journal of Hematology and Infectious Diseases. 2011; 3(1). e2011025. doi: 10.4084/MJHID.2011.025.

[11] Abosdera MM, Almasry AE, Abdel-Moneim ES. Coagulation defects in thalassemic patients. Pediatrics & Neonatology. 2017 Oct; 58(5): 421-4. doi: 10.1016/j.pedneo.2016.07.009.

[12] Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood Reviews. 2008 Sep; 22(5): 283-92. doi: 10.1016/j.blre.2008.04.001.

[13] Hassan TH, Elbehedy RM, Youssef DM, Amr GE. Protein C levels in β-thalassemia major patients in the east Nile delta of Egypt. Hematology/Oncology and Stem Cell Therapy. 2010 Apr; 3(2): 60-5. doi: 10.1016/S1658-3876(10)50036-0.

[14] Salama KM, Ibrahim OM, Kaddah AM, Boseila S, Ismail LA, Hamid MM. Liver enzymes in children with beta-thalassemia major: correlation with iron overload and viral hepatitis. Open Access Macedonian Journal of Medical Sciences. 2015 Jun; 3(2): 287.

[15] Awadallah SM, Atoum MF, Nimer NA, Saleh SA. Ischemia modified albumin: An oxidative stress marker in β-thalassemia major. Clinica Chimica Acta. 2012 May; 413(9-10): 907-10. doi: 10.1016/j.cca.2012.01.037.

[16] Darvishi-Khezri H, Karami H, Naderisorki M, Zahedi M, Razavi A, Kosaryan M, Aliasgharian A. Moderate to severe liver siderosis and raised AST are independent risk factors for vitamin D insufficiency in β-thalassemia patients. Scientific Reports. 2020 Dec; 10(1): 21164. doi: 10.1038/s41598-020-78230-4.

[17] Buehring GC, Shen HM, Jensen HM, Jin DL, Hudes M, Block G. Exposure to bovine leukemia virus is associated with breast cancer: a case-control study. PloS One. 2015 Sep; 10(9): e0134304. doi: 10.1371/journal.pone.0134304.

[18] Jasim RH and Jabbar SS. Biochemical Study For Evaluation of some Clinical Parameters as Predictors of Myocardial Infarction in Patients With Type 2 Diabetes Mellitus Undergoing Elective Percutaneous Coronary Intervention. Journal of Kufa for Chemical Sciences. 2023 Jun; 2(10).

[19] Srevatsa K, Kangle RP, Jali SM. Role of Prothrombin time International normalized ratio and activated partial thromboplastin time in beta thalassemia major: A cross sectional study. Indian Journal of Pathology and Oncology. 2019 Apr; 6: 237-41. doi: 10.18231/j.ijpo.2019.046.

[20] Faraj SA. Hemostatic parameters in Thalassemia patients; a single institute experience. Journal of the Faculty of Medicine Baghdad. 2016 Jul; 58(2): 132-5. doi: 10.32007/jfacmedbagdad.582223.