

Changes in Coagulation Status in Patients with β -Thalassemia in Iraq: A Case-Control Study

Hussein Abdalzehra Wadaha, Hassan Dede Meshay, Mohammed Haseeb Khamees¹

Department of Hematology, Ministry of Health, Aldiwanah Teaching Hospital, Aldiwanah, ¹Department of Hematology, Ministry of Health, Baghdad Medical Office, Al-Imamein al Kadhimaen Medical City, Baghdad, Iraq

Abstract

Background: The pathogenesis β -thalassemia is characterized by anemia resulting from reduced β -globin synthesis with low hemoglobin A (HbA) production and higher production of hemoglobin A2 (HbA2) and fetal hemoglobin (HbF). However, thromboembolic events have been recently identified in patients with β -thalassemia due to hypercoagulable state. **Objectives:** The aim of this study was to evaluate the levels of some coagulation markers in Iraqi patients with β -thalassemia major and β -thalassemia intermedia. **Materials and Methods:** The study included three groups. The first group included patients with β -thalassemia major ($n = 39$), the second group included patients with β -thalassemia intermedia ($n = 11$), and third group included 20 apparently healthy control subjects. Each of prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin III (ATIII), and thrombin-antithrombin III (TAT) complex were measured according to the standard protocols. **Results:** Both PT and APTT in β -thalassemia major and β -thalassemia intermedia were higher than that of the control group with a highly significant difference ($P = 0.002$), whereas no significant differences were observed between patients with β -thalassemia major and β -thalassemia intermedia. Mean ATIII level was highest in the control group followed by β -thalassemia intermedia and then by β -thalassemia major groups ($P < 0.001$), whereas mean TAT complex was highest in thalassemia major group followed by β -thalassemia intermedia and then by control groups ($P < 0.001$). **Conclusions:** Prolonged PT and APTT in patients with β -thalassemia together with the reduction in the level of anticoagulant factor (ATII) suggest a role for liver impairment; however, the significantly higher TAT complex is suggestive of ongoing activation of coagulation cascade in patients with β -thalassemia.

Keywords: β -thalassemia, coagulation, Iraq

INTRODUCTION

Thalassemia disorders are caused by one or more of hundreds of mutations in the genes encoding for α - and β -globin chains of the hemoglobin.^[1] These mutations are inherited in an autosomal recessive pattern.^[2] The defect is quantitative and leads to complete or partial reduction in the synthesis of corresponding proteins. The hemoglobin molecule contains a tetramer of 2 alpha chains and 2 beta chains ($\alpha_2\beta_2$) and when there is a reduction in the synthesis of one chain, the other unpaired chains are unstable and will precipitate leading to hemolytic anemia. In addition, there is apoptosis of red blood cell precursors in the bone marrow and reduced red cell life span in the circulation.^[3]

In β -thalassemia, the inherited genetic defect in the β -globin gene results in reduced synthesis of the β -globin

chain.^[4] The disease is characterized by tremendous variability in both the genotypic makeup and the phenotypic presentation because of the extremely high number of mutations, approximately 200, affecting the β -globin gene.^[5] Epidemiologically speaking, the frequency of mutations involving the β -globin gene varies among different regions of the world and the highest prevalence and incidence rates are encountered in people originating from Asian descent, Middle East, and Mediterranean

Address for correspondence: Dr. Mohammed Haseeb Khamees, Ministry of Health, Baghdad Medical Office, Al-Imamein al Kadhimaen Medical City, Baghdad, Iraq.
E-mail: mohamed.haseeb1975@yahoo.com

Submission: 15-Jul-2021 **Accepted:** 02-Nov-2021 **Published:** 30-Jun-2022

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Wadaha HA, Meshay HD, Khamees MH. Changes in coagulation status in patients with β -thalassemia in Iraq: A case-control study. Med J Babylon 2022;19:157-61.

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_53_21

regions. It is estimated that approximately 68,000 children carry the disease at birth. Globally, the overall prevalence rate is approximately 1.5% indicating that approximately 80–90 million people carry the β gene mutation.^[6] However, a high prevalence rate has been reported in creation regions such as the people of Cyprus descent in whom the prevalence rate is estimated at 12%–15%.^[7]

In people of Arab descent, the prevalence rate varies among countries. The carrier rate among Arab countries varies from 1% to 11%. The number of mutations in different Arab countries is also variable and may range from 10 mutations in Saudi Arabia to 44 mutations in the UAE.^[8] In Iraq, “the prevalence of thalassemia had increased from 33.5/100,000 in 2010 to 37.1/100,000 in 2015, while the incidence rate had decreased from 72.4/100,000 live births to 34.6/100,000 live births between 2010 and 2015”.^[9]

The pathogenesis of the disease is characterized by two main events. The first is the anemia resulting from reduced β -globin synthesis with low hemoglobin A (HbA) production and higher production of hemoglobin A2 (HbA2) and fetal hemoglobin (HbF). The second is the precipitation of excess α chain leading to red blood cell (RBC) destruction and hemolysis. On the contrary, erythroid hyperplasia will cause bony deformities and extramedullary hematopoiesis will lead to hepatosplenomegaly. Repeated blood transfusion in severe anemia will lead to iron overload and its related complications.^[10]

From a clinical point of view, the disease may be classified into transfusion-dependent and nontransfusion-dependent categories; however, hematologists prefer to classify the disease into β -thalassemia major, β -thalassemia intermedia, and β -thalassemia trait.^[11] The diagnosis of β -thalassemia major is often done with the first 2 years of life in children with jaundice, microcytic anemia, and hepatosplenomegaly. Complete blood count will provide a way to characterize patients into β -thalassemia major, intermedia, or trait based on the hemoglobin level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), RBC count, and red cell distribution width (RDW) values.^[12] Serum ferritin measurement will provide a tool to estimate the state of iron overload. Blood transfusion and adequate iron chelation have resulted in expanding the life expectancy in patients with β -thalassemia major; however, a number of complications have been described. Thromboembolic events have been recently identified in patients with β -thalassemia due to hypercoagulable state.^[13,14]

In this study, we planned to evaluate the levels of coagulation markers, prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin III (ATIII), and thrombin-antithrombin complex (TAT) in Iraqi patients with β -thalassemia major and β -thalassemia intermedia as well as in a subset of apparently healthy control subjects.

MATERIALS AND METHODS

Study design

This study was planned to be a case-control study. The study started on June 15, 2020 and ended on April 3, 2021. The study included three major groups. The first group included patients with β -thalassemia major ($n = 39$), the second group included patients with β -thalassemia intermedia ($n = 11$), and the third group included 20 apparently healthy control subjects. Those patients were attending Al-Imamein al Kadhimaiein Medical City, Baghdad, Iraq.

Data collection

When either patients or control members met the inclusion criteria, blood sample was withdrawn soon as possible. Age and gender in addition to laboratory investigations were the main variables included in this study. From each participant, 5 mL of venous blood was withdrawn and transferred into an ethylenediamine tetraacetic acid (EDTA) tube and the complete blood count was done using “Abbott Cell dyne 3700”. Blood sample for purpose of PT and PTT was transferred into a blue cap tube (vacuum tube) with sodium citrate (32.06 mg/mL, final concentration of 3.8%) with a 9:1 ratio volume. Tests of coagulation were done using Sysmex CA 500 machine. ATIII plasma level was assessed using immune-turbidimetric assay (LIATEST AT III, Diagnostica Stago, Paris, France), whereas TAT complex was quantitatively measured using an enzyme-linked immunosorbent assay (ELISA) (Enzygnost, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

Ethical consideration

The ethical approval for the study was issued by the ethical approval consideration of the Al-Karkh Health Directorate, the formal representative of the Iraqi Ministry of Health. Verbal consent was obtained from each participant after a full illustration of the aim and the procedures of the study.

Statistical analysis

Data were transferred into two statistical software programs: the Statistical Package for Social Sciences (SPSS) version 16.0 (IBM, Chicago, Illinois) and Microsoft Office Excel 2007. Qualitative data were expressed as number and percentage, whereas quantitative data were expressed as mean, standard deviation, and range. Chi-square test was used to study the difference in proportion among groups. One-way analysis of variance (ANOVA) and *post hoc* least significant difference (LSD) test was used to study differences in means among groups. The level of significance was chosen at $P \leq 0.05$, whereas the level of high significance was chosen at $P \leq 0.01$.

RESULTS

Comparison of mean age and frequency distribution according to gender among β -thalassemia major, β -thalassemia, and control groups is shown in Table 1. This study included 20 control subjects with a mean age of 21.05 ± 9.84 years and an age range of 4–40 years. The mean age of patients with β -thalassemia major was 16.18 ± 8.25 years and that of patients with β -thalassemia intermedia was 17.09 ± 11.09 years. The age range of β -thalassemia major group was from 2.5 to 38 years and that of β -thalassemia intermedia group was 4–40 years. No significant difference was observed in mean age among study groups ($P = 0.159$). Control group included 9 (45.0%) men and 11 (55.0%) women, β -thalassemia major group included 3 (27.3%) men and 8 (72.7%) women, and β -thalassemia intermedia group included 18 (46.2%) men and 21 (53.8%) women. No significant difference was observed in the frequency distribution of patients and control subjects according to gender among study groups ($P = 0.522$).

The comparison of complete count parameters among β -thalassemia major, β -thalassemia, and control groups is shown in Table 2. Mean RBC, Hb, MCV, and MCH were significantly lower in patients with β -thalassemia than in control subjects ($P < 0.001$). There were minor differences in these means between patients with β -thalassemia major and patients with β -thalassemia intermedia; however, these differences did not reach statistical significance in most of the situations ($P > 0.05$). No significant difference was observed in mean white blood cells (WBC) count and mean platelet count among study groups ($P > 0.05$). Serum ferritin levels were higher in patients with β -thalassemia than those with control group in a highly significant manner ($P < 0.001$) and the level was highest among patients with β -thalassemia major.

The comparison of coagulation markers among β -thalassemia major, β -thalassemia intermedia, and control groups is shown in Table 3. Both PT and APTT means of β -thalassemia major and β -thalassemia intermedia were higher than that of control group in a highly significant manner ($P = 0.002$) and there was no significant difference in these means between patients with β -thalassemia major

and β -thalassemia intermedia ($P > 0.05$). Mean ATIII level was highest in control group followed by β -thalassemia intermedia and then by β -thalassemia major groups ($P < 0.001$), whereas mean TAT complex was highest in thalassemia major group followed by β -thalassemia intermedia and then by control groups ($P < 0.001$).

DISCUSSION

This study included β -thalassemia patients with an age range of 4–40 years and a mean of approximately 17 years; therefore, a substantial proportion of patients with β -thalassemia may reach the fourth decade of life and the extension of life expectancy of β -thalassemia patients may be related medical intervention in the form of blood transfusion and chelation therapy which have greatly increased the life of patients with β -thalassemia major. This observation is consistent with previous reports.^[15,16]

The lack of significant difference in mean hemoglobin, RBC count, MCV, and MCH between β -thalassemia major and β -thalassemia intermedia groups in this study suggests the well-accomplished goal of anemia correction using repeated blood transfusion in patients with β -thalassemia major; thus, the mean serum ferritin in patients with β -thalassemia major was significantly higher than those with β -thalassemia intermedia and this is in consistent with previous observation.^[17]

In this study, PT and APTT of patients with β -thalassemia were significantly lower than in the control group. Similar observation was made by previous authors.^[18–20] We also observed no significant variation in platelet count and this is consistent with the observation made by Maiti *et al.*^[18] The prolongation in PT and APTT may be due to some mild deficiency in a number of coagulation factors as suggested by previous authors.^[20,21] Other authors have suggested chronic activation of coagulation cascade as a response to repeated blood transfusion.^[18] In addition, in our study we observed a significantly lower level of ATIII in patients with β -thalassemia and even the level is lower in β -thalassemia major subset and this observation is consistent with the observation of previous authors.^[13,14]

Table 1: Comparison of mean age and frequency distribution according to gender among β -thalassemia major, β -thalassemia, and control groups

Characteristic	Control $n = 20$	β -thalassemia major $n = 39$	β -thalassemia intermedia $n = 11$	P
Age (years)				
Mean $\pm$ SD	21.05 ± 9.84	16.18 ± 8.25	17.09 ± 11.09	0.159 O
Range	4–40	2.5–38	4–40	NS
Gender, n (%)				
Male	9 (45.0%)	3 (27.3%)	18 (46.2%)	0.522 C
Female	11 (55.0%)	8 (72.7%)	21 (53.8%)	NS

n = number of cases, SD = standard deviation, O = one-way ANOVA, C = chi-square test, NS = not significant at $P > 0.05$

Table 2: Comparison of complete count parameters and serum ferritin among β -thalassemia major, β -thalassemia intermedia, and control groups

Characteristic	Control <i>n</i> = 20	β -thalassemia major <i>n</i> = 39	β -thalassemia intermedia <i>n</i> = 11	<i>P</i>
RBC $\times 10^{12}/L$				
Mean $\pm$ SD	4.86 $\pm$ 0.42 A	2.95 $\pm$ 0.49 C	3.05 $\pm$ 0.46 B	<0.001 O HS
Range	3.9–5.5	1.4–4.28	2.5–4.2	
Hb (g/dL)				
Mean $\pm$ SD	13.59 $\pm$ 1.29 A	7.26 $\pm$ 1.07 B	6.90 $\pm$ 1.16 B	<0.001 O HS
Range	12–16	4–9.4	4–8.1	
MCV (fL)				
Mean $\pm$ SD	84.65 $\pm$ 4.63 A	72.00 $\pm$ 10.67 B	70.28 $\pm$ 5.74 B	<0.001 O HS
Range	76–91	28.7–87	59.6–77	
MCH (pg)				
Mean $\pm$ SD	27.56 $\pm$ 2.14 A	24.15 $\pm$ 3.17 B	22.91 $\pm$ 2.96 B	<0.001 O HS
Range	24–31	14.5–28.6	18.3–27.4	
WBC $\times 10^9/L$				
Mean $\pm$ SD	6.95 $\pm$ 1.60 A	6.51 $\pm$ 1.49 A	6.29 $\pm$ 2.13 A	0.497 O NS
Range	4.5–10	3.5–10.2	3.1–9.4	
Platelet $\times 10^9/L$				
Mean $\pm$ SD	271.25 $\pm$ 74.45 A	291.38 $\pm$ 119.79 A	331.91 $\pm$ 189.99 A	0.425 O NS
Range	157–412	120–550	124–767	
Serum ferritin (ng/mL)				
Median (IQR)	58.50 (77.50) B	1975.00 (1233.00) A	1800.00 (1581.00) B	<0.001 K NS
Range	17–278	465–4164	196–3630	

n = number of cases, SD: standard deviation, RBC = red blood cell count, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, WBC = white blood cell, O = one-way ANOVA, K = Kruskal–Wallis test, NS = not significant at $P > 0.05$, HS = highly significant at $P \leq 0.01$, capital letters A, B, and C were used to indicate the level of significance following *post hoc* LSD test after one-way ANOVA or Dunn's test following Kruskal–Wallis test so that similar letters indicate no significant difference, whereas different letters indicate significant difference

Moreover, we observed significantly higher levels of TAT complex among patients with β -thalassemia and the level being higher in patients with β -thalassemia major subgroup and this observation is also in accordance with previous reports.^[19,22] Thromboembolic events have been described in patients with β -thalassemia and have been attributed to hypercoagulable state.^[13] The suggested reasons for such complication are the repeated blood transfusion and splenectomy.^[13,23,24] Liver decomposition due to viral hepatitis and hemosiderosis was also suggested to cause the reduced synthesis of these anticoagulant factors.^[19,25] A number of authors suggested increased anticoagulant consumption in patients with β -thalassemia as a result of long-term asymptomatic activation of coagulation cascade as inferred from higher TAT complexes concentrations seen among patients with β -thalassemia as compared to patients with the control group.^[19,25]

CONCLUSION

Prolonged PT and APTT in patients with β -thalassemia together with a reduction in the level of anticoagulant factor (ATII) suggest a role for liver impairment; however, the significantly higher TAT complex is suggestive of ongoing activation of coagulation cascade in patients with β -thalassemia.

Acknowledgement

We wish to express our deepest thanks to all patients with β -thalassemia participating in this study for their kind acceptance to be enrolled in this academic work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Table 3: Comparison of coagulation markers among β -thalassemia major, β -thalassemia intermedia, and control groups

Characteristic	Control <i>n</i> = 20	β -thalassemia major <i>n</i> = 39	β -thalassemia intermedia <i>n</i> = 11	<i>P</i>
PT (s)				
Mean $\pm$ SD	11.30 $\pm$ 1.72 B	12.95 $\pm$ 1.61 A	12.73 $\pm$ 1.79 A	0.002 O HS
Range	9–15	9–16	10–16	
APTT (s)				
Mean $\pm$ SD	30.75 $\pm$ 2.81 B	36.62 $\pm$ 4.71 A	35.36 $\pm$ 3.56 A	<0.001 O NS
Range	26–35	25–47	29–41	
ATIII (ng/mL)				
Mean $\pm$ SD	11.74 $\pm$ 4.07 A	7.55 $\pm$ 1.88 C	8.20 $\pm$ 2.78 B	<0.001 O HS
Range	6.6–22.6	4.5–13.6	5.6–15.6	
TAT complex (ng/mL)				
Mean $\pm$ SD	2.51 $\pm$ 0.72 B	5.12 $\pm$ 1.56 A	4.64 $\pm$ 1.74 B	<0.001 O HS
Range	1.6–4.1	1.9–8.2	2.3–7.2	

n = number of cases, SD = standard deviation, PT = Prothrombin time, APTT = activated partial thromboplastin time, ATIII = antithrombin III, TAT = thrombin-antithrombin III complex, O = one-way ANOVA, NS = not significant at $P > 0.05$, HS = highly significant at $P \leq 0.01$, capital letters A, B, and C were used to indicate the level of significance following *post hoc* LSD test after one-way ANOVA so that similar letters indicate no significant difference, whereas different letters indicate significant difference

REFERENCES

- Rivella S. β -thalassemias: Paradigmatic diseases for scientific discoveries and development of innovative therapies. *Haematologica* 2015;100:418-30.
- Christopher AF, Kumari A, Chaudhary S, Hora S, Ali Z, Agrawal SC. Unique pattern of mutations in β -thalassemia patients in western Uttar Pradesh. *Indian J Hum Genet* 2013;19:207-12.
- Needs T, Gonzalez-Mosquera LF, Lynch DT. Beta thalassemia. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2021.
- Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis* 2010;5:11.
- Nienhuis AW, Nathan DG. Pathophysiology and clinical manifestations of the β -thalassemias. *Cold Spring Harb Perspect Med* 2012;2:a011726.
- Origa R. β -Thalassemia. *Genet Med* 2017;19:609-19.
- Kountouris P, Kousiappa I, Papasavva T, Christopoulos G, Pavlou E, Petrou M, *et al.* The molecular spectrum and distribution of haemoglobinopathies in Cyprus: A 20-year retrospective study. *Sci Rep* 2016;6:26371.
- Hamamy HA, Al-Allawi NA. Epidemiological profile of common haemoglobinopathies in Arab countries. *J Community Genet* 2013;4:147-67.
- Kadhim KA, Baldawi KH, Lami FH. Prevalence, incidence, trend, and complications of thalassemia in Iraq. *Hemoglobin* 2017;41:164-8.
- Fibach E, Rachmilewitz EA. Pathophysiology and treatment of patients with beta-thalassemia: An update. *F1000Res* 2017;6:2156.
- Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. *Vox Sang* 2015;108:1-10.
- Lee YK, Kim HJ, Lee K, Park SH, Song SH, Seong MW, *et al.* Recent progress in laboratory diagnosis of thalassemia and hemoglobinopathy: A study by the Korean red blood cell disorder working party of the Korean Society of Hematology. *Blood Res* 2019;54:17-22.
- Abd El Mabood S, Fahmy DM, Akef A, El Sallab S. Protein C and anti-thrombin-III deficiency in children with beta-thalassemia. *J Hematol* 2018;7:62-8.
- Huang Y, Long Y, Deng D, Liu Z, Liang H, Sun N, *et al.* Alterations of anticoagulant proteins and soluble endothelial protein C receptor in thalassemia patients of Chinese origin. *Thromb Res* 2018;172:61-6.
- Karimi M, Emadmarvasti V, Hoseini J, Shoja L. Major causes of hospital admission in beta thalassemia major patients in southern Iran. *Iran J Pediatr* 2011;21:509-13.
- Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pourzahedgilani N, Bouzari N, Habibzadeh M, *et al.* Metabolic and endocrinologic complications in beta-thalassemia major: A multicenter study in Tehran. *BMC Endocr Disord* 2003;3:4.
- Mishra AK, Tiwari A. Iron overload in beta thalassaemia major and intermedia patients. *Maedica (Bucur)* 2013;8:328-32.
- Maiti A, Chakraborty A, Chakraborty P, Mishra S. Subclinical haemorrhagic tendency exists in patients with β -thalassaemia major in early childhood. *Australas Med J* 2012;5:152-5.
- Chhikara A, Sharma S, Chandra J, Nangia A. Thrombin activable fibrinolysis inhibitor in beta thalassemia. *Indian J Pediatr* 2017;84:25-30.
- McFadyen J, Butler J, Malan E, Tran H. Causes and clinical significance of prolonged activated partial thromboplastin times in thalassaemia major. *Ann Hematol* 2014;93:867-8.
- Andrew M, Manno M, Karparkin M. Demonstration of Kallikrein-like protease activity in nonactivated plasma of patients with Cooley's anemia. *Blood* 1983;61:232-7.
- Angchaisuksiri P, Atichartakarn V, Aryurachai K, Archararit N, Chuncharunee S, Tiraganjana A, *et al.* Hemostatic and thrombotic markers in patients with hemoglobin E/beta-thalassemia disease. *Am J Hematol* 2007;82:1001-4.
- Succar J, Musallam KM, Taher AT. Thalassemia and venous thromboembolism. *Mediterr J Hematol Infect Dis* 2011;3:e2011025.
- Cappellini MD, Musallam KM, Poggiali E, Taher AT. Hypercoagulability in non-transfusion-dependent thalassemia. *Blood Rev* 2012;26:S20-3.
- 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;3:60-5.