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Some Hematological and Biochemical Complication in B-thalassemia Patients in Erbil-city

Research Project

Submitted to the department of biology in partial fulfillment of the requirements for the degree of BSc in Biology

By:

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Supervised by:

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DECLARATION

I declare that the Bachelor project research entitled: **Some Hematological and Biochemical Complication in B-thalassemia Patients in Erbil city** is our own original work, and hereby certify that unless stated, all work contained within this project research is our own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Signature:

Student: Name: Delan and Sumaya

Date: / /2024

SUPERVISOR CERTIFICATE

This project research has been written under our supervision and has been submitted for the award of the degree of Bachelor of Science in **Biology/Blood Physiology** with our approval as supervisor.

Signature Name: **Asst. Prof. Dr. Sarbaz I. Mohammed**

Date

I confirm that all requirements have been fulfilled.

Signature:

Name: **Mr. Mohammed Ali Saleem**

Head of the Department of Biology

Date:

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Delan & Sumaya

DEDICATION

To our lovely parents

And

To our sweet sisters

To our kindly brothers

LIST OF CONTENT

Some Hematological and Biochemical Complication in B-thalassemia Patients in Erbil-city

Delan ---------, Sumya ---------- and Sarbaz I. Mohammed

Abstract

Background: Thalassemias are a common cause of microcytic anemia and are due to impaired synthesis of the globin protein component of hemoglobin. Beta-thalassemia is an inherited disease with a wide phenotypic severity of the disease. The prevalence and carrier rates of β-thalassemia are relatively moderate in Iraq about 37.1/100 000.

Methods: 39 (16 males & 13 females) patients with B-thalassemia who underwent blood transfusion were randomly selected. Pre-blood transfusion risk factors were obtained from their medical records. Preoperative liver function tests, and blood CBC at 2 hours before blood transfusion and were compared with the non-thalassemic ones.

Result: while RBC ($p<0.001$), Hb ($P<0.001$), Hct ($p<0.001$) and platelet count in decreased significantly before blood transfusion. The level of GGT 115±7.992 & 109 (100.5-163.5) AST level (49.9±14.09 & 21(12.5-44.5), ALT Median (42.1±9.157 & 22(15-58), Total Bilirubin (1.75(1.388-2.985) & 0.3667±0.079), & 0.3667±0.079), and ALP 216 (85-362.8) & 157.1±30.34) was increased significantly,

Conclusion: We concluded that Liver function test, CBC values and leukocytes counts changes with severity of B-thalassemia disease.

Keyword: B-thalassemia, liver function test, anemia

1. Introduction

Thalassemias are a common cause of hypochromic microcytic anemia which arises from the reduced or absent synthesis of the globin chain of hemoglobin. Thalassemias are a quantitative defect of hemoglobin synthesis. This is in contrast with hemoglobinopathies, such as sickle cell disease, which are structural or qualitative defects of hemoglobin. **Beta-thalassemia** refers to an inherited mutation of the **beta**-globin gene, causing a reduced **beta**-globin chain of hemoglobin. The highest prevalence of **beta-thalassemia** mutations is in people of Mediterranean, Middle Eastern, and Asian descent. Over 200 different **thalassemia**-causing mutations have been identified in the **beta**-globin gene, leading to the disease's wide genotypic and phenotypic variability (Cao and Galanello, 2010, Hayder et al., 2023). The three classifications of **beta-thalassemia** are defined by their clinical and laboratory findings. **Beta-thalassemia** minor, also called carrier or trait, is the heterozygous state that is usually asymptomatic with mild anemia. Homozygosity or compound heterozygosity for **beta-thalassemia** mutations cause a more severe spectrum of anemias called **beta-thalassemia** intermedia and **beta-thalassemia major**. These two are distinguished clinically by transfusion dependence. **Beta-thalassemia major** requires routine transfusions, and intermedia does not (Khan et al., 2023).

Beta-thalassemia is an inherited disorder resulting from various mutations (over 200 disease-causing mutations have been identified) or, rarely, deletions of the **beta**-globin gene (HbB) on chromosome 11. These mutations are primarily point mutations that affect transcriptional control, translation, and splicing of the HbB gene and gene product (Cao and Galanello, 2010, Hayder et al., 2023).

The spectrum of disease severity is due to the bi-allelic inheritance of two copies of the **beta**-globin gene, one on each chromosome 11, as well as the heterogeneous pool of disease-causing mutations (Origa, 2017).

The frequency of **beta-thalassemia** mutations varies by regions of the world with the highest prevalence in the Mediterranean, the Middle-East, and Southeast and Central Asia. Approximately 68000 children are born with **beta-thalassemia**. Its prevalence is 80-90 million carriers, around 1.5% of the global population (Weatherall et al., 2010). The prevalence and carrier rates of β-thalassemia are relatively moderate in Iraq about 37.1/100 000 (Kattamis et al., 2020). The prevalence also parallels that of malaria as a proposed survival advantage provides the selective pressure for the high

carrier frequency in these populations. Gene drift and founder effects are other reasons why **thalassemia** is more frequent in the areas mentioned above (Frazer et al., 2012).

The pathogenesis of **beta-thalassemia** is two-fold. First, there is decreased hemoglobin synthesis causing anemia and an increase in HbF and HbA2 as there are decreased **beta** chains for HbA formation. Second, and of most pathologic significance in **beta-thalassemia major** and intermedia, the relative excess alpha chains form insoluble alpha chain inclusions that cause marked intramedullary hemolysis. This ineffective erythropoiesis leads to severe anemia and erythroid hyperplasia with bone marrow expansion and extramedullary hematopoiesis. The bone marrow expansion leads to bony deformities, characteristically of the facial bones which cause frontal bossing and maxillary protrusion. Biochemical signaling from marrow expansion involving the bone morphogenetic protein (BMP) pathway inhibits hepcidin production causing iron hyperabsorption (Khan et al., 2023). Inadequately treated patients and transfusion-dependent patients are at risk for end-organ damaging iron overload. Hepatosplenomegaly from extramedullary hematopoiesis and ongoing hemolysis also causes thrombocytopenia and hepatic dysfunction.

Patients with **beta-thalassemia major** (TM), if the diagnosis has not been determined prenatally, present between 6 and 24 months of age when hemoglobin production transitions from fetal (HbF) to adult (HbA). Severe anemia ensues and presents as feeding problems, irritability, failure to thrive, pallor, diarrhea, irritability, recurrent bouts of fever, and abdominal enlargement from hepatosplenomegaly. Untreated or undertreated infants, especially in resource-poor areas, will suffer from growth retardation, jaundice, brown pigmentation of the skin, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoietic sites, and skeletal deformities from bone marrow expansion. Frontal bossing, maxillary hypertrophy, and long bone deformities are common skeletal findings (Origa, 2017).

Clinical diagnosis of **beta-thalassemia major** (TM) is made in children less than 2 years of age that present with microcytic anemia, mild jaundice, and hepatosplenomegaly. The complete blood count (CBC) of a patient with **beta-thalassemia major** will show microcytic hypochromic anemia with Hb levels less than 7g/dl, the mean corpuscular volume between (MCV) 50 and 70 fl, and mean corpuscular Hb (MCH) between 12 and 20pg. **Beta- thalassemia** intermedia presents with values of Hb between 7 and 10 g/dl, MCV 50 to 80 fl, and MCH 16 to 24pg. In **beta-thalassemia** minor, the red cell number is often elevated, reduced MCV, MCH, and the

red cell distribution width (RDW) will typically show low elevations. The normal to mildly elevated RDW can help differentiate thalassemias from other microcytic hypochromic anemias, such as iron deficiency anemia and sideroblastic anemia where the RDW is typically very high. The peripheral blood smear will show microcytic hypochromic anemia with target cells, teardrop cells, and often coarse basophilic stippling. In severe forms of **beta-thalassemia**, there is anisopoikilocytosis with bizarre red cell morphology and numerous nucleated red blood cells (Origa, 2017, Khan et al., 2024).

Complete blood count (CBC) is often the first investigation in a suspected case of thalassemia. A CBC showing low hemoglobin and low MCV is the first indication of thalassemia, after ruling out iron deficiency as the cause of anemia. The calculation of the Mentzer index (mean corpuscular volume divided by red cell count) is useful. A Mentzer lower than 13 suggests that the patient has thalassemia, and an index of more than 13 suggests that the patient has anemia due to iron deficiency. A blood smear (also called peripheral smear and manual differential) is next, to assess additional red cell properties. Thalassemia can present with the following findings on the peripheral blood smear: Microcytic cells (low MCV), Hypochromic cells, Variation in size and shape (anisocytosis and poikilocytosis), Increased percentage of reticulocytes, Target cells, and Heinz bodies. Iron studies (serum iron, ferritin, unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), and percent saturation of transferrin) are also done to rule out iron deficiency anemia as the underlying cause (Singha et al., 2019).

Most β-thalassemia carriers have hypochromic microcytosis with mean corpuscular volume (MCV) < 80 fL and mean corpuscular hemoglobin (MCH) < 27 pg. These can be variable due to β-thalassemia mutations, genetic interaction between thalassemic genes, and blood cell counters. We have examined whether these indices are effective in screening of β-thalassemia in Thailand where thalassemia is prevalence and heterogeneous (Singha et al., 2019).

Patients with β-TM had significantly lower hemoglobin (Hb), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and non-high-density lipoprotein (non-HDL) and significantly higher very low-density lipoprotein (VLDL), triglycerides (TGs), LDL/HDL ratio, MDA, hs-CRP, total serum bilirubin (TSB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) than the control group ($p < 0.05$). A bivariate analysis revealed that TC has a significant positive association with Hb. The TG has a significant negative association with Hb and a positive association with serum ferritin. Furthermore, MDA, TG, TSB,

ALT and AST were significantly positively correlated with serum ferritin ($p < 0.05$)(Pennell et al., 2013, Khan et al., 2024)

Serum ferritin level (mean±SD) in thalassemic major patients in Group A (890±446.38 microgram/L) which is significantly higher above normal level. Serum bilirubin in thalassemic major patients (3.27±2.62 mg/dl) and in Group B (0.48±0.24 mg/dl), Serum ALT thalassemic major patients (53.06±34.0 U/L) and in without disease (16.70±4.81 U/L), AST thalassemic major patients (84.56±33.54 U/L) and in without disease (11.60±2.72 U/L) and ALP levels in Group A (422.42±226.99 IU/L) and in without disease (221.86±80.54 IU/L). All the values were significantly higher ($p<0.001$) in β-thalassemia patient than that of normal children (Brancaleoni et al., 2016, Jabbar et al., 2023).

The CBC and peripheral smear findings are non-specific. A diagnosis of **beta-thalassemia** requires hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) to demonstrate abnormal percentages of HbA, HbA2, and sometimes HbF. The general pattern of **beta-thalassemia** is a decreased HbA percentage and a mildly increased HbA2; less than 10% with variably increased HbF. A HbA2 above 10% suggests variant hemoglobin rather than **beta-thalassemia**. The magnitude of the HbA decrease depends on the genetic makeup of the affected individual. Patients with **beta** (+) alleles will have variably decreased HbA levels, and those that are homozygous **beta** (0) will produce no HbA. **Beta thalassemia** minor characteristically has increased HbA2 (4-8%) with variably normal-to-low elevations of HbF (Sultana et al., 2022). Complications are related to overstimulation of bone marrow, ineffective erythropoiesis, and iron overload from blood transfusions.

Iron accumulates in the heart, causing heart failure, which is the most common cause of death in **beta-thalassemia major** (TM) due to iron accumulation. The symptoms differ from nonanemic patients because of adaptation to chronic anemia. Usually, these patients have dyspnea, with resting tachycardia, low blood pressure, high ejection fraction, and high cardiac output (Pennell et al., 2013). Atrial fibrillation is another long-term complication of iron oxidative stress. According to several studies, it is more prevalent in TM patients than in the general population (Nomani et al., 2019). Iron also accumulates in endocrine glands causing hypothyroidism, hypoparathyroidism, adrenal insufficiency, diabetes mellitus, and hypogonadism (Kurtoglu et al., 2012).

The aim of this study is to evaluate the liver function parameters, and hematological features in beta-thalassemia patients and compare them with the control group consisted of Erbil patients.

2. Materials and Methods

2.1 Patients and sampling

This is a retrospective cross-sectional study of the patients who have undergone blood transfusion in the thalassemia center in Erbil city during November 2023 to February 2024. In this analysis, 39 patients (16 males and 13 females) and 20 (10 males and 10 females) without diseases were randomly chosen out of our statistical population. Mean age (21.26 ±1.729) year old. The analysis was performed based on a descriptive analytical method using the intended data collected from their medical records. The data regarding patient's age, gender, and past medical history including hepatic, biliary or hematologic disorders.

2.2 Blood samples collection

Blood samples were taken from peripheral veins by 5 ml vacutainer. two mL of blood samples was immediately transferred to ethylenediaminetetraacetate (EDTA) specimen bottles for measurement of hematological parameters, 3 ml put into clot activator tube for serum separation. Serum was separation by 3000 round per minute (rpm) centrifugation for 15 min.

Hematological measurements estimation of total leukocyte counts (WBCs), Eosinophil, hemoglobin (Hgb), haematocrit (Hct) means platelet volume (MPV), and platelets (PLT) variables were made using Hemolyzer 5'Analyzer

According to the laboratory data, the liver function parameters including ALT, AST, ALP, total and direct bilirubin level, and CBC for the pre-transfusion and at 2 hours before blood transfusion. Blood for biochemical measurement was taken on admission in all patients. biochemical activity was measured in blood samples by a colorimetric enzyme essay on the automatized Cobas c 501 system from Roche Diagnostics GmbH (Mannheim, Germany).

2.3 Statistical analyses

GraphPad Prism version 8.01 was applied for performing statistical analysis. unpaired t test, mean ± SE and Median used for analysis for present data.

3. Results & Discussion

3.1 The results of some haematological parameters are shown in Table 1&2.

The result indicates that RBC 3.68± 0.409 & 3.165±0.133, Hb level 8.45(7.85-9.9) & 9.2(8.2-9.9), Hct% 24.77± 0.743 & 24.31±0.697, MCV 74.85±1.75 & 77.58± 1.977 was decreases significantly in both sexes, when compared with healthy one. While RDW% ($p<0.01$) and platelet count ($p<0.001$) were increased significantly in male and females. This anemia may be due to poor management of transfusion treatment regimens, lack of adherence to international guidelines, and possibly failure to achieve a regular adherence to the treatment by patients. This is in agreement with the results of other studies in β-thalassemia children (Singha et al., 2019) who showed a decrease in Hb%, RBCs count and PCV% and using a combined MCV and MCH for β-thalassemia screening is highly recommended, especially in an area with high prevalence and heterogeneity of thalassemia like Thailand and other Southeast Asian countries and also (Yaghobi et al., 2017) who reported reduction in Hb values.

Which are in accordance with the outcomes of the study conducted by Fazlul Karim, et al. (2016). All hematological parameters including Hb, HCT, and RBC except RDW and PLts were found to be significantly lower than the controls, clinical data confirm that the decrease of the haemoglobin level is accompanied by a decrease in the number of erythrocytes and diminished values of their specific indexes. Iron overload starts another pathological mechanism leading to oxidative damage of erythrocyte membranes. The anemic presentation was reported in 78.6% of the patients as indicated by hemoglobin level (8.4 ± 1.4 g/dl) (Aldwaik et al., 2021**)**. Hyper-unstable beta globin's comprehend a group of beta globin mutants, which result in the production of Hb variants that are extremely unstable and precipitate before assembling with the alpha chains to produce the Hb tetramer. This results in ineffective erythropoiesis, which is exacerbated by the concomitant relative excess of the alpha chains (Cao and Galanello, 2010). Mean level of hemoglobin and red blood cells (RBCs) was significantly reduced incomprehension with control group and also Ayyash and Sirdah (2018) who showed that anemia associated significantly with B-thalassemia patients.

On the other hand, in the table (2) indicate that there are significantly increase in LYM% ($P<0.01$) in both sexes and monocyte percentage in men, while GRA% in men and women and GRA# in women were decreased significantly in comparison with healthy control, while total white blood cell counts increased but not significantly in present study, was shown in table 2. This elevation agrees with other studies by Jabbar et al., (2023) and Ayyash and Sirdah (2018) who showed elevation in WBCs counts in major B-thalassemia, the elevated leukocytes can be attributed to infection by hepatitis B and C and iron complication. Excess iron is extremely toxic to all body tissues, leading to significant morbidity and mortality among β-thalassemic patients as well as other iron-overload conditions where it causes serious and irreversible biological damage, such as cirrhosis, liver fibrosis, heart disease, and endocrine abnormalities (Origa 2017).

3.2 The results of the biochemical parameters were shown in Table 3.

In the current study, the level of GGT 115±7.992 & 109 (100.5-163.5) AST level (49.9±14.09 & 21(12.5-44.5), ALT Median (42.1±9.157 & 22(15-58), Total Bilirubin (1.75(1.388-2.985) & 0.3667±0.079), & 0.3667±0.079), and ALP 216 (85-362.8) & 157.1±30.34) was increased significantly, while serum Direct Bilirubin not changed in men and women respectively, in compression with healthy persons, shown in Table (3).

The results in our study were in accordance with another study conducted by Sharif et al., indicate that hepatic biochemical profile showed that the mean level of bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and bilirubin was significantly increased in BTM patients. This disarrangement of bilirubin is owing to peripheral hemolysis, that is quick to the point that it surpasses the liver ability to utilize the bilirubin, prompting increased bilirubin (Cappellini et al., 2018, Sharifi et al., 2020 and Jadhv 2023). In Aldwaik et al., (2021) study, liver dysfunction was manifested by the elevated levels of ALT and AST among our patients. This elevation was significantly correlated with an increase in serum iron levels. In addition to iron overload, elevation in the levels of liver enzymes in both the transfusion-dependent and transfusion-independent patients with β-thalassemia might be attributed to other factors such as viral infection (hepatitis B and C viruses), hepatic siderosis, bile obstruction, portal fibrosis, and even cirrhosis. Ayyash and Sirdah (2018) who showed that liver function parameters changed significantly in B-thalassemia patients and also significantly, higher AST ($P< 0.05$), ALT ($P< 0.001$), and ALP ($P< 0.001$) activities in beta-thalassemia patients were found in comparison to healthy individuals showed by Fazlul Karim, et al. (2016) and Srivastava et al., (2019). The b-Thalassemia major patients had significantly elevated liver enzymes ALT and AST, compared with the control group. This elevation agrees with other studies by Jabbar et al.,

the elevated liver enzymes can be attributed to iron deposition and infection by hepatitis B and C. Elevated serum transaminases in patients indicate massive cell membrane damage in tissues, especially in the liver Jabbar et al., (2023).

The concentration of SGOT and GPT levels were high, recorded among thalassemia patients in this study. This result is thus concurrent with the results of other previous studies. The increased SGOT and SGPT enzymes in thalassemia patients are due to liver cirrhosis and hypertrophy. The high level of SDB and STB, hyperbilirubinemia, can be attributed to the failure of the liver to metabolize the high bilirubin production due to the destruction of RBC in thalassemia patients by Banfa et al., (2022)

De et al., results revealed that AST and ALT changed in thalassemia patients. Many factors such as iron overload, liver injury due to oxidative stress might cause these changes. Oxidative drugs and iron supplements should be avoided for the patients of thalassemia receiving blood transfusions. It is suggested that appropriate iron chelators27 and antioxidant supplements improve antioxidant/oxidant balance in thalassemia patients. Liver enzymes should be measured and carefully monitored in every thalassemic child as hepatic dysfunction is inevitable in these children who receive multiple blood transfusions as main modality of treatment (De et al., 2019).

Table (1) Show RBC profile complications in major B-thalassemia

Parameters	males		P-Value	females		p-value
	Patient	healthy		Patient	healthy	
RBC (10*12/l)	3.68± 0.409	5.104± 0.2076	0.00	3.165±0.133	4.194±0.1761	0.001
HB (g/dl)	8.45(7.85-9.9)	14.23± 0.496	0.00	9.2(8.2-9.9)	11.4±0.3165	0.001
HCT (%)	24.77± 0.743	42.32± 1.15	0.00	24.31±0.697	38.67± 1.36	0.001
MCV (fl)	74.85±1.75	80.02± 3.35	0.05	77.58± 1.977	92.93± 3.35	0.001
MCH (pg)	29.15(27.38-29.8)	28.18± 1.33	N.S	28.6(27-29.3)	27.44± 0.767	N.S
MCHC(g/dl)	36.5(34.18-38.28)	35.25±0.450	NS	36(33.5-39.7)	35.78± 0.377	NS
RDW (%)	16.6±1.30	12.04± 0.240	0.01	15.08± 0.788	11.92± 0.788	0.001
PLt	434.5(273.8-500.8)	256.5±18.88	0.001	333 (261.5-462)	242.3±16.85	0.001

Table (2) Show Leukocytes profile complications in major B-thalassemia patients

Parameters	males		P-Value	females		p-value
	Patient	healthy		Patient	healthy	
WBC	9.233 ± 1.809	7.67 ± 0.5871	ns	8.041±0.5046	7.709±0.3704	ns
LYM#	2.9 ± 0.2703	2.56 ± 0.1439	ns	2.8 (2.3-4.1)	2.455±0.1436	ns
LYM%	42.6 (35.5-47.1)	32.77±1.292	0.01	43.42±2.904	32.77±1.294	0.01
MID#	0.6 (0.4-0.7)	0.47±0.0558	ns	0.6(0.4-0.9)	0.5±0.053	ns
MID%	7.9(7-8)	6.08±0.365	0.01	7.7(7.1-8.1)	6.7±0.612	ns
GRA#	4.6(2.6-6.3)	4.74±0.419	ns	4.4(3.47-4.9)	0.5±0.057	0.001
GRA%	49.7(41.7-56.7)	59.59±2.22	0.01	49(40.6-58.1)	60.64±1.653	0.001

Table (3) Show liver function profile complications in major B-thalassemia

Parameters	males		P-Value	females		p-value
	Patient	healthy		Patient	healthy	
GGT (U/L)	115±7.992	27.56±3.841	0.001	109 (100.5-163.5)	21(15-31.5)	0.001
GOT (U/L)	49.9±14.09	16(8-27)	0.05	21(12.5-44.5)	10(6-25)	0.05
GPT (U/L)	42.1±9.157	8(7.15-10)	0.001	22(15-58)	9(7.5-25.5)	0.05
TBIL (mg/dl)	1.75(1.388-2.985)	0.344±0.033	0.001	0.9(0.45-1.79)	0.3(0.2-0.4)	0.001
DBIL (mg/dl)	0.811±0.1577	0.2589±0.031	0.01	0.3667±0.079	0.2667±0.033	NS
ALP (U/L)	216 (85-362.8)	62.56±3.033	0.001	157.1±30.34	60.11±2.366	0.01

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