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The physiological and immunological assessments of roles of adipocytokines in blood transfusion dependent in β- thalassemia major.

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Abstract

Beta thalassemia major (βTM) represents the most severe form of beta-thalassemia that is recessively inherited. The study started from January 2021 to October 2021 by collecting blood samples from a seventy-five (75) transfusion-dependent β-thalassemia major. , 38 males and 37 females were registered at the center for thalassemia and inherited blood disorders, Shaheed Hemin teaching hospital, Sulaymaniyah, Kurdistan Iraq. Patients were divided into three groups. Each group included 25 patients whose ages ranged from (2 - 10), (11- 20) and (21 - 30) years, respectively. And 15 healthy subjects. Hematological parameters' ranges in βTM groups were mostly different from healthy controls group, where the present study showed a significant decrease ($P \leq 0.05$) in values Hb, RBCs, HCT, MCV, MCH, and MCHC, in the patient group, compared with the control group. Also, Activated immune responses were detected and indicated by elevated levels of serum Interleukin- 6 IL-6 and IL-1β in patients with βTM. Mean serum levels of adipokines plasminogen, and Hipcidin were significantly higher ($P \leq 0.01$) in βTM.

Keywords: Beta thalassemia major, Hematological parameters', Interleukin, adipokines.

Introduction

Beta-thalassemia major (βTM) is a hereditary disorder that is transmitted from disease-carrier parents to their children in an autosomal recessive manner. The major causative factor of this condition is the reduced synthesis of the hemoglobin beta chain due to a genetic defect in the beta-globin gene. This defect makes the body unable to produce sufficient amounts of these chains, thereby an imbalance of hemoglobin chains will lead to ineffective erythropoiesis and microcytic hypochromic anemia. This anemia starts in early childhood and continues throughout the whole life (Arab-Zozani *et al*., 2021; Mustafa, *et al*.,2020). By the use of complete blood picture indices, microcytic anemia can be recognized. Thalassemia major is distinguished by decreased Hb level (< 7 g/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean corpuscular Hb (MCH) > 12< 20 pg. Thalassemia intermedia is identified by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl, and MCH between 16 and 24 pg. Thalassemia minor is characterized by decreased both MCV and MCH and elevated Hb A2 levels (Galanello and Origa, 2010). Beta-thalassemia is characterized by the presence of many immunological defects, among which the increased production of some cytokines. It is known that (IL-6) is an important mediator of the pro-inflammatory agent. It has been demonstrated that plasma levels of IL-6 cytokines may be relevant in the pathophysiology of beta-thalassemia. During chronic anemia, the expression of receptors for pro-inflammatory cytokines is enhanced on the membrane of hematopoietic progenitor cells, and direct action of the cytokines on these cells could affect adversely the development and the number of erythroid progenitor cells. It has been demonstrated that elevated serum levels of TNF-α, and IL-1β in β-thal / Hb-E patients, and there is a positive correlation between these cytokine levels and the severity of disease (Kheansaard *et al*., 2011; Sterling *et al*., 2022; Abdulwahed,*et al*.,2020 ; Alkanaani, *et al*.,2020).

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Material and Methods

The study was started from January 2021 to November 2021 by a collection of blood samples from seventy-five (75) transfusion-dependent β-thalassemia majors with 15 healthy individuals as a control. Among the 75 patients, 38 males and 37 females with age groups ranging from (2-30) Years were registered at the Center for thalassemia and inherited blood disorders, Shaheed Hemin teaching hospital, Sulaymaniyah, Kurdistan Iraq.

Blood Collection

Collected through vein puncture and placed (4 ml) in ethylene-diamine-tetra-acetic acid (EDTA) tubes for Complete blood count tests, the rest were placed (3 ml) in anticoagulant-free test tubes and left for 15 minutes in water baths at 37 ºC.followed by centrifugation at 3000 rpm for 15 minutes and stored at -20°C after placed in Eppendorf tubes (1.5 ml) until special physiological, and immunological tests were performed.

Complete Blood Count (CBC)

Used 2 ml of EDTA blood samples from each subject. CBC was performed on all samples within two hours of sample collection. Seven (7) parameters were analyzed in this experiment and their values were automatically obtained by machine or Auto-analyzer.

Measurements of the level of the IL-6, IL-1β, Human Plasminogen Activator Inhibitor-1 (PAI-1), Hepcidin

The employed ELISA method in this study was the enzyme-linked immunoassay technology (sandwich method) used to determine the level of IL-6, IL-1β Human Plasminogen Activator Inhibitor-1 (PAI-1), Hepcidin using Sunlong kit/China follow the c company manufactures instructions.

Statistical Analysis

The SPSS statistical program was used to analyze the result between patients and control using the T.Test and at a probability level ($P \leq 0.5, 0.01$).

Results and Discussion

Measurement of some Physiological and immunological assessments of roles of adipocytokines in blood transfusion dependent in β- thalassemia major

Table 1. Hematological parameters in patients with beta thalassemia major. Results are expressed as Mean ± SD.

Hematological Parameters	Study Groups										
	Control n= 15		G1 (Age: 2-10) n= 25			G2 (Age: 11-20) n= 25			G3 (Age: 21-30) n= 25		
	Mean ± SD		Mean ± SD		P-value	Mean ± SD		P-value	Mean ± SD		P - value
	M (8)	F (7)	M (13)	F (12)		M (13)	F (12)		M (11)	F (14)	
Hemoglobin (g/dl)	14.6±0.26	13.8±0.3	≤8.6±0.25	8.9±0.26	<0.01	9.0±0.25	8.3±0.27	<0.01	9.3±0.28	8.8±024	<0.01
RBC (10^{12}/l)	4.67±0.18	4.6±0.19	3.2±0.14	3.0±0.15	<0.05	3.1±0.38	3.0±0.15	<0.05	3.2±0.15	3.1±0.13	<0.05
WBC (10^{3}/l)	8.6±3.3	8.5±3.5	6.5±2.6	8.1±2.7	NS	12.5±2.6	10.2±2.7	<0.05	19.2±2.8	21.9±2.5	<0.01
LYM (%)	47.77±3.8	47.47±4.0	47.98±3.0	46.25±3.1	NS	57.2±2.9	61.2±3.1	<0.01	64.3±3.2	66.6±2.9	<0.01

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MCV (fL)	84.7±4.2	85.2±2.5	71.2±3.1	70.0±2.2	<0.01	70.7±5.1	71.0±1.8	<0.01	65.1±1.6	68.7±5.0	<0.01
MCH (pg)	30.0±2.5	30.2±3.5	25.2±4.4	24.0±2.4	<0.01	23.8±5.4	22.9±2.4	<0.01	22.1±6.2	21.6±3.3	<0.01
MCHC (g/dL)	36.6±4.4	36.6±2.4	30.3±5.3	31.0±1.3	<0.01	27.5.8±2.3	28.8±2.6	<0.01	25.7±4.6	25.8±4.1	<0.01

Level of Hemoglobin (Hb) in Patients and Control Groups

The Results of Hb (g/dl) mean values in male and female subjects in the control group were 14.6±0.26 and 13.8±0.3 g/dl respectively. These values were significantly reduced ($P \leq 0.01$) in βTM patients of the three age groups to become 8.6±0.25 and 8.9±0.26 (Group 1), 9.0±0.25 and 8.3±0.27 (Group 2), 9.3±0.28 and 8.8±0.24 (Group 3) consecutively, with no significant difference between male and female patients in same groups (Table 1) (Figure 1).

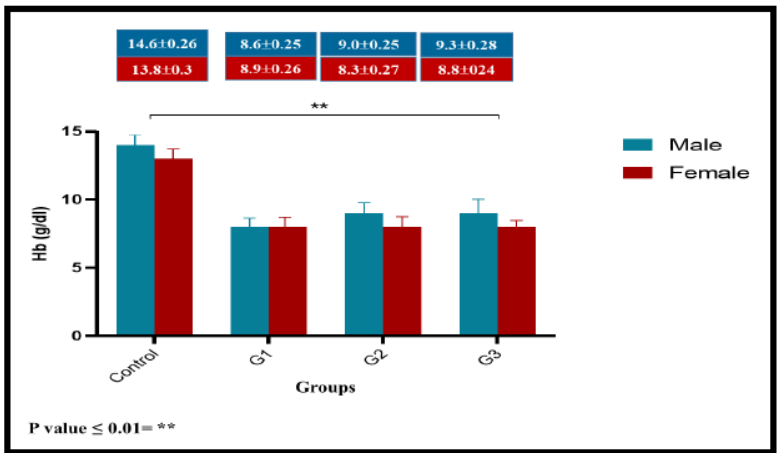

Figure 1. Hb levels in study groups.

Beta-thalassemia major (βTM) is the most severe form of beta-thalassemia inherited in an autosomal recessive condition. The disease is characterized by a defect in beta-globin chains either through deletion or mutation in the gene. The net result is a reduction in hemoglobin production (Yousafzai *et al*., 2019). The present results are in agreement with many previous studies that showed a marked reduction in hemoglobin concentration in patients with βTM (Yonus and Kashmoola, 2020).

Count of Red blood cells (RBCs) in Patients and Control Groups

The results were noticed for RBCs counts (1012/L), where there was a significant decrease ($P<0.05$) in the mean RBCs counts of male and female βTM patient groups (3.2±0.14 and 3.0±0.15 (Group 1), (3.1±0.38) and 3.0±0.15; Group 2) and (3.2±0.15 and 3.1±0.13; Group 3) compared with the control group (4.67±0.18 and 4.6±0.19). There was no statistically significant difference between male and female subjects in same groups (Table 1), (Figure 2).

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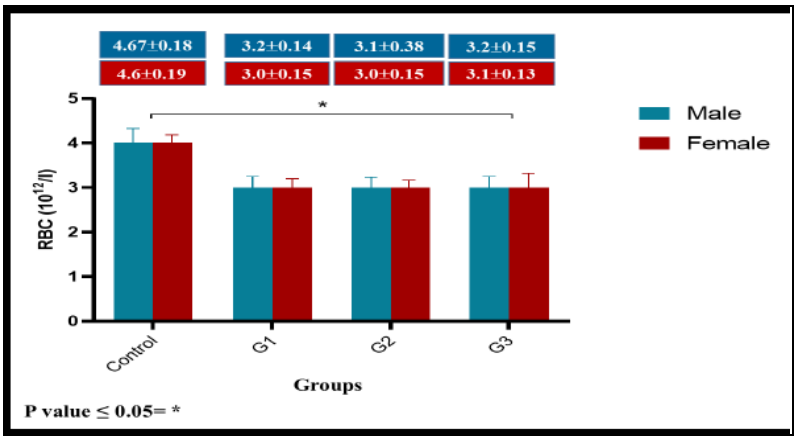

Figure 2 RBCs count in control and patients.

As a result of the defect in Hb synthesis in βTM, ineffective erythropoiesis can be developed leading to microcytic anemia (Mut *et al*., 2021). The red blood cells in these patients have an unbalanced synthesis of α- and β-globin chains. The consequence of the disequilibrium between α- and β-globin is the hemolysis of RBCs as well as apoptosis of erythroid precursors in the bone marrow and the spleen. This study showed that there is a remarkable decrease in red blood cell counts in all study groups. These findings are in agreement with many previous studies showing the decrease in red blood cell count in patients with βTM (Rivella, 2009; Rivella, 2012, Khawaji *et al*., 2020).

Level of lymphocyte (LYM) in Patients and Control Groups

Results of lymphocyte counts (109/L) in male and female subjects of the control group were (47.77±3.8) and (47.47±4.0) respectively. These results showed no significant (NS) change when compared with the βTM male and female patients of Group 1 (47.98±3.0 and 37.98±3.0). In groups 2 and 3, the counts of lymphocytes become significantly ($P \leq 0.01$) increased to become (57.2±2.9 and 61.2 ± 3.1) in group 2 and (64.3 ± 3.2 and 66.6 ± 2.9) in group 3 (Table 1), (Figure 3).

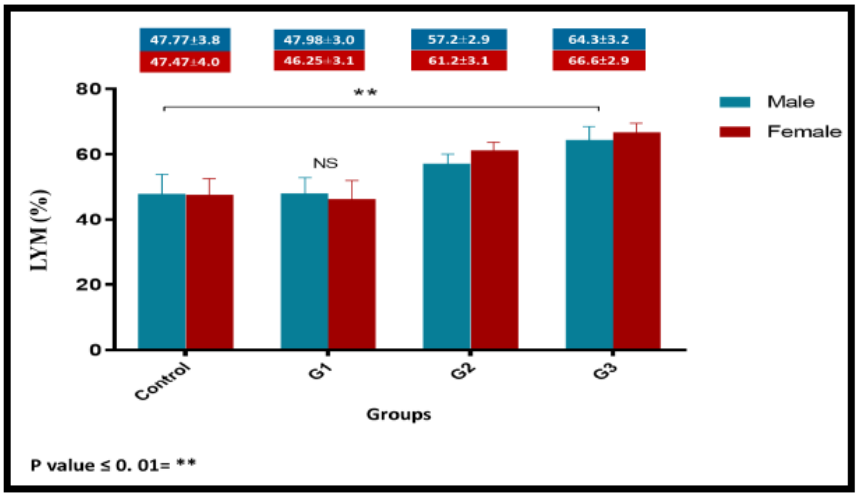

Figure 3. Lymphocytes count in control and patient groups.

Lymphocytosis is a characteristic of patients with βTM. The present study showed elevated lymphocytes count in adult patients. In agree with our findings in the study by Elsayh and coworkers (2016). They suggested that the high

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lymphocyte count is due to infectious complications caused by multiple blood transfusions in these patients as well as immune deficiency (Gluba-Brzózka *et al*., 2021).

Level of hematocrit (HCT) in Patients and Control Groups

The study obtained for HCT (%) in male and female subjects in the control group was 43.5±1.7 and 42.0±1.8 respectively. These values were significantly reduced ($P \leq 0.01$) in βTM patients of the three groups to become 23.7±1.3 and 24.5±1.3 (Group 1), 21.0±1.3 and 23.3±1.4 (Group 2), 25.1±1.2 and 26.0±1.4 consecutively(Table 1) and (Figure 4).

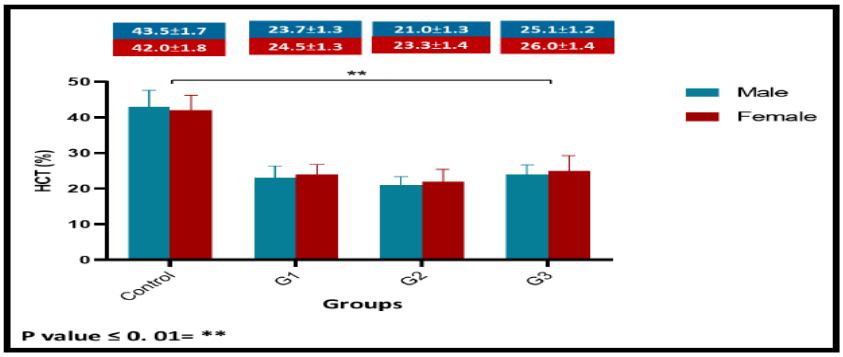

Figure 4. HCT (%) in control and patient groups.

The hematocrit is a measure of the percentage of RBCs in the body. Low hematocrit indicates that the patient is anemic and high hematocrit suggests polycythemia. We noticed a decrease in hematocrit in βTM patients' groups. The same finding was shown by Karim and coworkers (2016). They studied the hematological and biochemical status of βTM patients. The hematocrit levels were reduced in association with these patients' hemoglobin levels and RBC counts.

Level of mean corpuscular volume (MCV) in Patients and control groups

The mean MCV (fl) in male and female subjects in the control group were 84.7±4.2 and 85.2±2.5 respectively. These values were significantly reduced ($P \leq 0.01$) in βTM patients of all groups to become 71.2±3.1 and 70.0±2.2 (Group1), 70.7±5.1 and 71.0±1.8 (Group 2), and 65.1±1.6 and 68.7±5. (Group 3) consecutively when compared with the control group (Table 1) and (Figure 5).

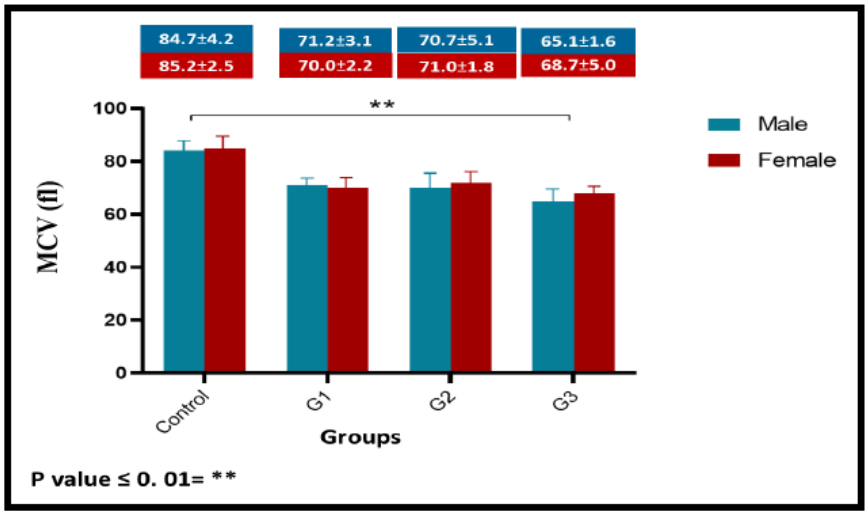

Figure 5. MCV values in control and patient groups.

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The mean corpuscular volume (MCV) is a specimen's average volume of red cells. Our findings showed that MCV is markedly reduced in βTM patients. These results are in agreement with several previous reports that demonstrated that MCV is decreased in βTM (Zhang *et al*., 2017; Yonus and Kashmoola, 2020).

Level of Mean corpuscular hemoglobin (MCH) in Patients and Control Groups

The same results were obtained for the mean values of MCH (pg), in male and female subjects in the control group which were 30.0±2.5.5 and 30.2±3.5 respectively.

These values were significantly reduced ($P \leq 0.01$) in all that groups of βTM patients to become 25.2±4.4 and 24.0±2.4 (Group1), 23.8±5.4 and 22.9±2.4 (Group2), and 22.1±6.2 and 21.6±3.3 (Group3) consecutively when compared with the control group (Table 1) and (Figure 6).

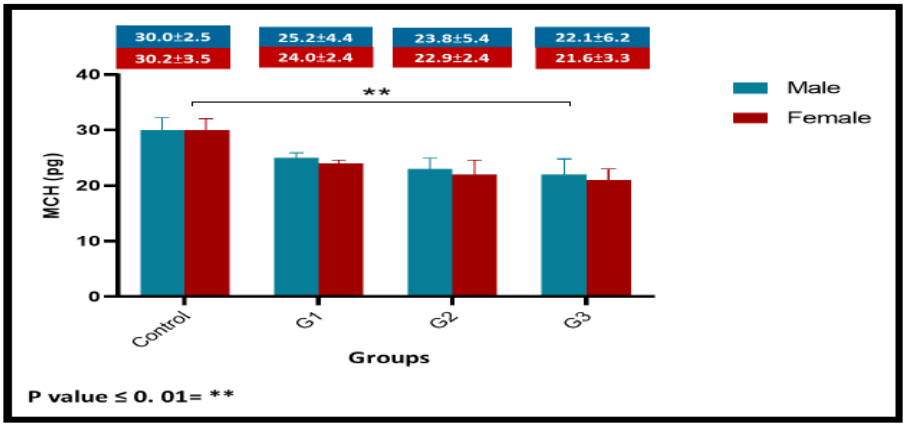

Figure 6. MCH values in control and patient groups

Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin in each of the RBCs. It has been shown that MCH is markedly reduced in patients with βTM (Brancaleoni *et al*., 2016). Our finding is in agreement with these studies where we observed a remarkable decrease in MCH values in patients with βTM. This may be attributed to the severe anemia and reduced Hb levels in these patients.

Level of Mean corpuscular hemoglobin concentration (MCHC) in Patients and Control Groups

The mean values of MCHC (g/dl) in male and female subjects of the control group were 36.6±4.4 and 36.6±2.4 respectively. These values showed a significant decrease ($P \leq 0.01$) in all patients of all groups of βTM patients where it was 30.3±5.3 and 31.0±1.3 (Group1), 27.5.8±2.3 and 28.8±2.6 (Group2), 25.7±4.6 and 25.8±4.1 (Group3) consecutively when compared with the control (Table 1) and (Figure 7).

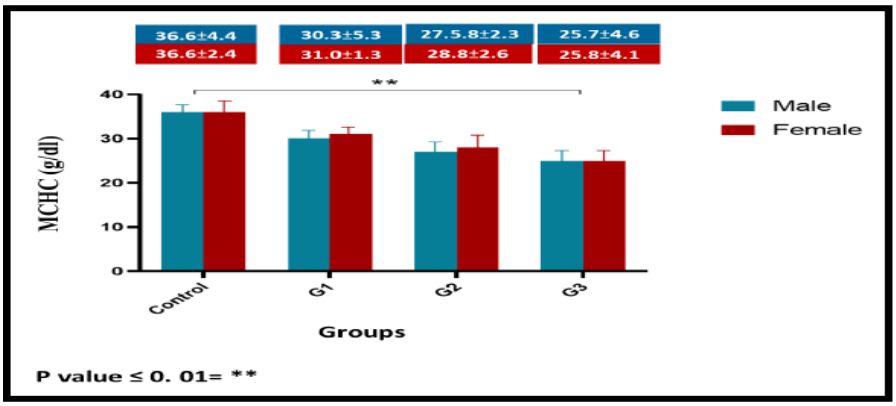

Figure 7. MCHC values in control and patient groups

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Mean corpuscular hemoglobin concentration (g/dl) is a measure of the average concentration of hemoglobin inside a single red blood cell. We found that the level of MCHC was markedly decreased in all patients with βTM when compared with the control. These results are in accordance with the finding of many previous studies where all hematological parameters such as Hb, HCT, and MCV were lower than the controls (Şimşek *et al*., 2005; Karim *et al*., 2016; Hu *et al*., 2021).

Estimation of IL-6 and and IL-1β in the control group and patients with beta-thalassemia major groups

Table 2. Levels of IL-6 and IL-1β in the control group and patients with beta-thalassemia major groups. Results are expressed as mean ± SD.

Variable	Study Groups										
	Control (n= 15)		G1 (n = 25)		P-value	G2 (n = 25)		P-value	G3 (n = 25)		P-value
	Male	Female	Male	Female		Male	Female		Male	Female	
IL-6	7.3±2.5	6.6±1.9	21.9±3.4	20.4±4.0	0.01	25.7±5.2	23.4±3.5	0.01	28.7±8.3	28.5±11.1	0.01
IL-1β	6.1±0.8	6.0±0.5	15.4±2.9	16.5±3.5	0.01	19.4±4.0	18.2±2.5	0.01	23.2±4.2	20.6±4.5	0.01

Level of interleukin-6 (IL-6) in Patients and Control Groups

The mean values of IL-6 (pg/ml) in male and female patients had increased high significant (P ≤ 0.01) in the three age groups compared with a control group to become 21.9±3.4 and 20.4±4.0 (Group 1), 25.7±5.2 and 23.4±3.5 (Group 2), 28.7±8.3 and 28.5±11.1 (Group 3), 7.3±2.5 and 6.6±1.9, respectively. Without no significant difference between male and female patients (Table 2) and (Figure 8).

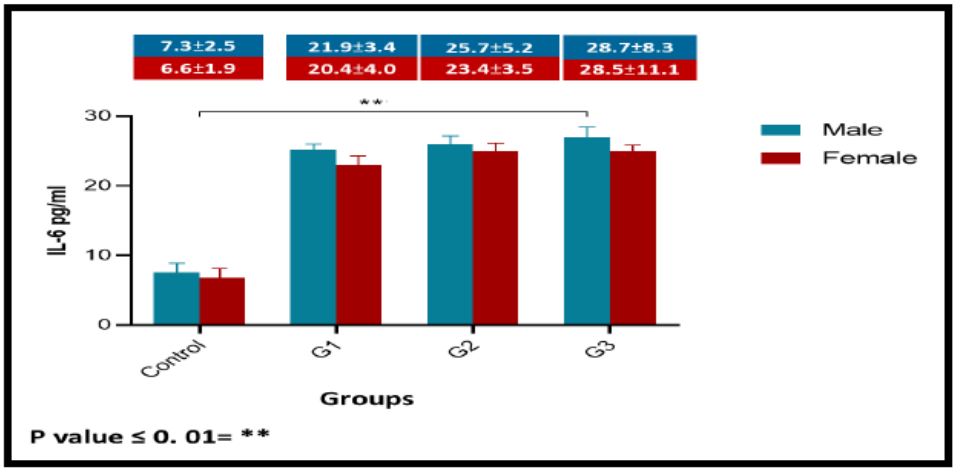

Figure 8. IL-6 values in control and patient groups.

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Beta-thalassemia is characterized by the presence of many immunological defects, among which the increased production of some cytokines. It is known that (IL-6) is an important mediator of the pro-inflammatory agent. It has been demonstrated that plasma levels of IL-6 cytokines may be relevant in the pathophysiology of beta-thalassemia. The present findings revealed markedly elevated levels of IL-6 in patients with beta-thalassemia major compared with the control. In accordance with these findings, many previous reports documented increased plasma IL-6 levels in patients with beta-thalassemia. The increased release of IL-6 might have contributed to disturbances in iron metabolism and is probably due to the overstimulation of macrophages (El-Rasheidy *et al*., 2016; Surchi and Ali, 2018; Mohammed *et al*., 2020).

Level of interleukin-1β (IL- 1β) in Patients and Control Groups

Likewise, the mean values of IL-1β (pg/ml) in male and female subjects in the control group were 6.1±0.8 and 6.0±0.5 respectively. These values were significantly increased (P ≤ 0. 01) in βTM patients of the three age groups to become 15.4±2.9 and 16.5±3.5 (Group 1), 19.4±4.0 and 18.2±2.5 (Group 2), 23.2±4.2 and 20.6±4.5 (Group 3) consecutively, with no significant difference between male and female patients (Table 2) and (Figure9).

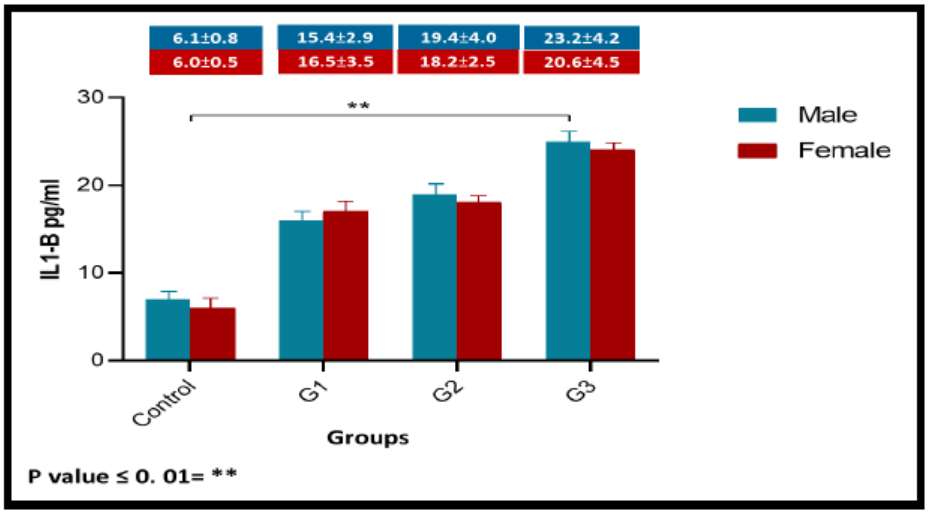

Figure 9. IL-1β values in control and patient groups.

Iron overload toxicity has been reported as the main cause of the immune abnormalities in β-TM, including defective phagocytic and chemotaxis activity of macrophages and neutrophils, reduced activity of natural killer cells, impaired lymphocytes responses to antigens, elevated immunoglobulin release and alterations in cytokines responses (Ricerca *et al*., 2009; Gharagozloo *et al*., 2009).

Table 3. Levels of Hepcidin, and Plasminogen in the control group and patientswith beta-thalassemia major groups. Results are expressed as Mean ± SD.

Variable	Study Groups										
	Control (n= 25)		G1 (n = 25)		P-Value	G2 (n = 25)		P-value	G3 (n = 25)		P-value
	Male	Female	Male	Female		Male	Female		Male	Female	

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Plasminogen	**57.5±5.7**	**64.0±4.5**	**58.2±15.0**	**61.4±7.6**	**NS**	**77.8±5.8**	**78.9±8.5**	**0.01**	**91.5±6.4**	**89.1±4.3**	**0.01**
Hepcidin	**12.9±0.6**	**14.9±2.4**	**18.6±4.0**	**19.9±3.4**	**0.05**	**22.4±2.2**	**23.8±2.3**	**0.01**	**28.2±2.8**	**27.6±2.8**	**0.01**

Level of Plasminogen Activator Inhibitor-1 in Patients and Control Groups

The mean values of Plasminogen (pg/ml) levels in male and female subjects in the control group were 57.5±5.7 and 64.0±4.5 respectively. These values were not significant in βTM patients of the G1 to become 58.2±15.0 and 61.4±7.6, and these values were significantly increased ($P \leq 0.05$ and $P \leq 0.01$) in βTM patients of the two age groups to become 77.8±5.8 and 78.9±8.5 (Group 2), 89.1±4.3 and 91.5±6.4 (Group 3) consecutively, with no significant difference between male and female patients (Table 3) and (Figure 10).

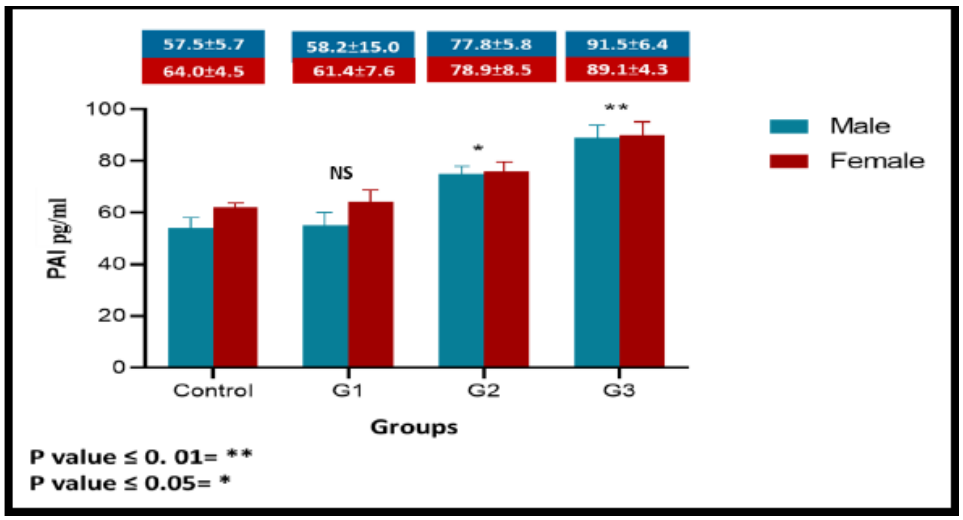

Figure 10: Plasminogen Activator Inhibitor-1 in Patients and Control Groups levels in control and patient groups.

As a consequence of iron overload, cardiac failure is developed in patients with βTM which represents a major cause of mortality in these patients. Additionally, these patients are more subjected to the pro-coagulant status which may lead to clinical thrombotic events. Numerous factors have been reported to contribute to the pathogenesis of this hypercoagulable state, including activation of platelet and deficiency of anticoagulants, and others (Kumar *et al*., 2017; Abdulrahman, et al.,2020).

The current study revealed an increase in the levels of plasminogen in patients with βTM. These findings are in line with previous studies indicating increased levels of PAI in βTM patients (Angchaisuksiri *et al*., 2007). Accordingly, evidence of chronic low-grade coagulation and platelet activation, low-grade inflammation, endothelial cell injury, defect in fibrinolysis, and decreased anticoagulants is observed in β-thalassemia patients. The increased risk of thrombosis is due to the elevated levels of PAI.

A further finding of the current study was the higher levels of PAI in group 3 (older patients). This observation can be explained as follow; older age patients may have multiple blood transfusions than younger patients which may cause iron overload and increase the risk of thrombosis. Consistency with our findings, several previous studies demonstrated that thalassemic patients receiving several blood transfusions are more subjected to thromboembolic events (Kumar *et al*., 2017).

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Level of Hepcidin in Patients and Control Groups

The results obtained with Hepcidin (ng/ml) levels in male and female subjects in the control group were 12.9±0.6 and 14.9±2.4 respectively. These values were significantly increased ($P \leq 0.05$ and $P \leq 0.01$) in βTM patients of the three age groups to become 18.6±4.0 and 19.9±3.4 (Group 1), 23.8±2.3 and 22.4±2.2 (Group 2), 27.6±2.8 and 28.2±2.8 (Group 3) consecutively, with no significant difference between male and female patients (Table 3) and (Figure 11).

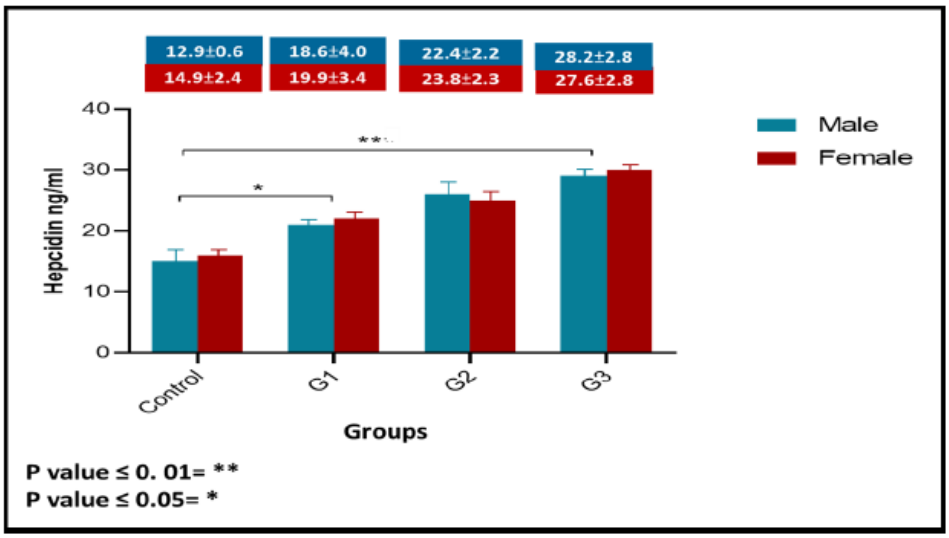

Figure 11. Hepcidin levels in control and patient groups.

Hepcidin is synthesized in the liver and is an important regulator of iron homeostasis. The release of hepcidin is upregulated by iron overload, inflammation, or as a response to infection (Grønlien *et al*., 2021).

Hepcidin control iron homeostasis through three mechanisms; 1) prevention of iron absorption from the duodenum, 2) inhibition of the release of iron from macrophages, and 3) influencing the movement of iron stores in hepatocytes.

Therefore, adeficiency of hepcidin, causes intestinal iron hyperabsorption and iron overload (Reichert *et al*., 2017).

In the present study, we found an increase in hepcidin levels in patients with βTM, and the level of the hormone is increased with age. Opposite to our finding, it has been reported that levels of hepcidin are low in βTM patients (Nemeth, 2013). The reduction in hepcidin levels in these patients is primarily due to decreased synthesis in the liver as well as anemia and iron overload.

Moreover, soluble factors produced by erythroid progenitors during ineffective erythropoiesis such as erythroferrone inhibits the synthesis of the hormone by the liver (Coffey and Ganz, 2018). The reason for the high levels of hepcidin in βTM patients in our study is the iron overload due to transfusion.

In transfusion-dependent thalassemias (TDT), in which regular RBCs transfusions are responsible for abnormal iron accumulation. In these patients, hepcidin levels fluctuate according to thesuppression of erythropoiesis by transfusions, with relatively high and low values immediately after and before red blood cell administration, respectively (Pasricha *et al*., 2013; Girelli and Busti, 2020).

References

1. Abdulrahman, M. A., Mustafa, H. A., Mustafa, M. A., AL-Samarraie, M. Q., & Ahmed, M. T. (2020). The diagnostic value of anti-mullerian hormone in female infertility evaluation. *Science*, *1*(3).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE08, 2022

2. Abdulwahed, A. M. H., Alkanaani, M. I., Alsamarrai, A. H., Hamad, M. A. M., Dakheel, A., & AL-Samarraie, M. Q. (2020). Determination of some visfatin hormone level and lipid profilein some breast cancer patients in Samarra city. *Annals of Tropical Medicine and Public Health*, *23*, 265-267.

3. Alkanaani, M. I., Rajab, E. R., Abdulwahed, A. M. H., Dabos, T., Alshammiri, B., Abdullah, S. N., & AL-Samarraie, M. Q. (2020). Visfatin hormone level and lipid profile in some hyperlipidemia patients in samarra city. *Biochem. Cell. Arch*, *20*(1), 1191-1193.

4. Angchaisuksiri, P.; Atichartakarn, V.; Aryurachai, K.; Archararit, N.; Chuncharunee, S.; Tiraganjana, A.; & Rattanasiri, S. (2007). Hemostatic and thrombotic markers in patients with hemoglobin E/β-thalassemia disease. *American journal of hematology*, *82*(11), 1001-1004.

5. Arab-Zozani, M.; Kheyrandish, S.; Rastgar, A.; & Miri-Moghaddam, E. (2021). A Systematic Review and Meta-Analysis of Stature Growth Complications in β-thalassemia Major Patients. *Annals of global health*, *87*(1).

6. Brancaleoni, V.; Di Pierro, E.; Motta, I.; & Cappellini, M. D. (2016). Laboratory diagnosis of thalassemia. *International Journal of laboratory hematology*, *38*, 32-40.

7. Coffey, R.; & Ganz, T. (2018). Erythroferrone: an erythroid regulator of hepcidin and iron metabolism. *Hemisphere*, *2*(2).

8. El-Rasheidy, F. H.; Essa, E. S.; Mahmoud, A. A.; & Nada, A. E. W. A. (2016). Elevated serum adiponectin is related to elevated serum ferritin and interleukin-6 in β-thalassemia major children. *Journal of Pediatric Endocrinology and Metabolism*, *29*(8), 953-958.

9. Elsayh, K. I.; Mohammed, W. S.; Zahran, A. M.; & Saad, K. (2016). Leukocytes apoptosis and adipocytokines in children with beta-thalassemia major. *Clinical and experimental medicine*, *16*(3), 345-350.

10. features among β-thalassemia major patients in Jazan region: A hospital-based study. *Journal of family medicine and primary care*, *9*(1), 412.

11. Galanello, R., & Origa, R. (2010). Beta-thalassemia. *Orphanet journal of rare diseases*, *5*(1), 1-15.

12. Gharagozloo, M.; Bagherpour, B.; Tahanian, M.; Oreizy, F.; Amirghofran, Z.; Sadeghi, H. M. M.; ... & Moayedi, B. (2009). Premature senescence of T lymphocytes from patients with β-thalassemia major. *Immunology letters*, *122*(1), 84-88.

13. Girelli, D.; & Busti, F. (2020). Replacing the suppressed hormone: toward better treatment for iron overload in β-thalassemia major? *Haematologica*, *105*(7), 1752.

14. Gluba-Brzózka, A., Franczyk, B., Rysz-Górzyńska, M., Rokicki, R., Koziarska-Rościszewska, M., & Rysz, J. (2021). Pathomechanisms of Immunological Disturbances in β-Thalassemia. *International Journal of Molecular Sciences*, *22*(18), 9677.

15. Grønlien, H. K.; Christoffersen, T. E.; Nystrand, C. F.; Garabet, L.; Syvertsen, T.; Moe, M. K.; ... & Jonassen, C. M. (2021). Cytokine and Gene Expression Profiling in Patients with HFE-Associated Hereditary Hemochromatosis according to Genetic Profile. *Acta Haematologica*, *144*(4), 446-457.

16. Hu, J.; Chen, X.; Li, X.; Xu, W.; & Fu, S. (2021). Comparison of Hematological Parameters and Critical Value Analysis in the Prevention and Control of Thalassemia in Hainan Li and Han Nationalities. *Open Journal of Blood Diseases*, *11*(2), 46-56.

17. Karim, M. F.; Ismail, M.; Hasan, A. M.; & Shekhar, H. U. (2016). Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. *International journal of hematology-oncology and stem cell research*, *10*(1), 7.

18. Karim, M. F.; Ismail, M.; Hasan, A. M.; & Shekhar, H. U. (2016). Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. *International journal of hematology-oncology and stem cell research*, *10*(1), 7.

19. Khawaji, M. M.; Hazzazi, A. A.; Ageeli, M. H.; Mawkili, Y. H.; Darbashi, A. H.; Kathiyah, A. M. A. A.; & Humedi, R. A. (2020). Clinical and hematological

20. Kheansaard, W.; Panichob, P.; Fucharoen, S.; & Tanyong, D. I. (2011). Cytokine-induced apoptosis of beta-thalassemia/hemoglobin E erythroid progenitor cells via a nitric oxide-mediated process in vitro. *Acta Haematologica*, *126*(4), 224-230.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE08, 2022

21. Kumar, A.; Batra, H. S.; Banerjee, M.; Bandyopadhyay, S.; Saha, T. K.; Misra, P.; & Ambade, V. (2017). PAI-1 study in thalassemia major patients receiving multiple blood transfusions. *Indian Journal of Clinical Biochemistry*, *32*(3), 343-346.

22. Mohammed, J. I.; Al-Bajari, S. A.; Akashe, M. A. A.; & Totanjee, A. N. (2020). Alterations in Antioxidants and Trace Element with Interleukin 6 Level in β Thalassemia Major Patients. *Medico-Legal Update*, *20*(1), 1410-1415.

23. Mustafa, A. A., Ali, A. H., Mustafa, M. A., & AL-Samarraie, M. Q. (2020). Physiological and hormonal study of women infertility. *Science Archives*, *1*(3), 160.

24. Mut, M. A.; Türkkan, E.; Dağ, H.; & Dursun, H. (2021). Evaluation of Transfusion-Related Hemodynamic Parameters in Patients with Beta-Thalassemia Major by Ambulatory Blood Pressure Monitoring Method. *Iberoamerican Journal of Medicine*, *3*(3), 187-195.

25. Nemeth, E. (2013). Hepcidin and β-thalassemia major. *Blood, The Journal of the American Society of Hematology*, *122*(1), 3-4.

26. Pasricha, S. R.; Frazer, D. M.; Bowden, D. K.; & Anderson, G. J. (2013). Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β-thalassemia major: a longitudinal study. *Blood, The Journal of the American Society of Hematology*, *122*(1), 124-133.

27. Ricerca, B. M.; Di Girolamo, A.; & Rund, D. (2009). Infections in thalassemia and hemoglobinopathies: focus on therapy-related complications. *Mediterranean journal of hematology and infectious diseases*, *1*(1).

28. Rivella, S. (2009). Ineffective erythropoiesis and thalassemias. *Current opinion in hematology*, *16*(3), 187-194.

29. Rivella, S. (2012). The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia. *Blood reviews*, *26*, S12-S15.

30. Şimşek Orhon, F. İ. L. İ. Z.; Öztürk, G.; KEMAHLI, S.; Erbaş, D.; & Hasanoğlu, A. (2005). Oxidant and antioxidant status in beta-thalassemia major patients. *Ankara Üniversitesi Tıp Fakültesi Mecmuası*, *58*(1), 34-38.

31. Sterling, J. K.; Baumann, B.; Foshe, S.; Voigt, A.; Guttha, S.; Alnemri, A., ... & Dunaief, J. L. (2022). Inflammatory adipose activates a nutritional immunity pathway leading to retinal dysfunction. *Cell Reports*, *39*(11), 110942.

32. Surchi, O.; & Ali, S. (2018). Biochemical Status of Beta-Thalassemia Major Patients in Erbil City. *Erbil Dental Journal (EDJ)*, *1*(1), 1-9.

33. Yonus, B. A.; & Kashmoola, M. A. (2020). Hematological parameters of β-Thalassemia Trait in Premarital Screening in Nineveh Province. *Ann Coll Med Mosul December*, *42*(2), 157-161.

34. Zhang, Y.; Li, Y.; Yu, L.; & Zhou, L. (2017). Association between serum resistin concentration and hypertension: A systematic review and meta-analysis. *Oncotarget*, *8*(25), 41529.