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Effect of Beta-Thalassemia disease on insulin-like growth factor (IGF-1) and some antioxidants levels among patients in Tikrit City

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Abstract : The purpose of this study was to measure certain hematological parameters (Hb, PCV, and WBC counts), as well as the levels of malondialdehyde (MDA), glutathione (GSH), and insulin-like growth factor-1 (IFG-1). The study was carried out on blood samples from patients with thalassemia from the beginning of November 2021 to the end of March 2022. The study employed a total of (45) samples from the city of Tikrit city and (18) samples from a control group of healthy individuals. The age range for both groups was (15–17), The results showed that there were significant increase at ($P \leq 0.05$) in the levels of (Hb, PCV and WBCs count) in patients group which are (12.66±3.54 a) g/dl, (16.89±4.08 a) , (13.2±3.74 a) respectively and control group were (8.59±2.84 b) g/dl ,(10.24±3.52 b) and (11.37±3.66 b) respectively ,the results also showed a significance increase ($P \leq 0.05$) in levels of IGF-1 in patients group which are (7.832±2.33 a) ng/ml and (2.181±0.72 b) ng/ml in control group, the study revealed that there was a significance decrease($P \leq 0.05$) in levels of (MDA and GSH) in patients group which are (1.203±0.32 b)m mol/L ,((0.0186±0.007 b)m mol/L and (2.671±0. 512 a) m mol/L and (0.0402±0.010 a) m mol/L in control group respectively.

Key Words :Beta-thalassemia , IGF-1,Antioxidants ,Tikrit City

1.Introduction :

Lack of a chain's alpha or beta chains of hemoglobin, that are referred to as either alpha or beta thalassemia, results in the hematological hereditary disorder known as thalassemia [(1,2)]. Hemoglobin E (Hb E) or beta thalassemia (Hb E) is a kind of sickness that is prevalent throughout South East Asia, especially Thailand.. [(3,4)]. In this

disease, Insufficient beta globin chain synthesis results in large accumulations of chains of unpaired alpha globin [5,6].

The oxidation-damaged erythrocytes have a short lifespan in circulation because they are prematurely destroyed by phagocytic activity in the spleen. Reactive oxygen species may be produced by alpha chain aggregates, which may lead red blood cell senescence brought on by externalization and oxidative stress and phosphatidylserine release [7]. These pathological events draw attention to individuals with beta thalassemia/Hb E are observed to have significant anemia and splenomegaly. [7].

Delay in maturation and growth are two of beta-thalassemia major's most noticeable traits (BTM). Growth faltering starts after the age of four. It affects height, weight, and skeletal structure development. the next age, the pubertal growth spurt is significantly attenuated or eliminated, and the fusion of the growth plates is frequently postponed till the latter decade of lifes. There is a slowdown in growth that goes along with these modifications. Growth failure has a complex etiology that includes Hypogonadism, delayed puberty, hypothyroidism, and other endocrinopathies and deregulations of the GH-IGF1 axis) being the main contributors, factors include iron overload in multiple organs, chronic liver illness, dietary inadequacies, and incorrect chelating agent usage are all symptoms of chronic anemia and hypoxia. [8.9].

In many cells and tissues, insulin-like growth factor I (IGF-I) is a significant both a (GH)-stimulated somatic growth mediator and a GH-independent anabolic response mediator. It is created by several types of mesenchymal cell, mediates the majority of GH's physiological effects, and is a key factor in bone development, as well as increase in height, weight, and skeletal maturity [10,11].

One of the byproducts of the produced ROS is malondialdehyde (MDA), which is toxic and mutagenic and causes lipid peroxidation (PL) of the cell membrane. The biomarker MDA is used to test for oxidative stress. Research on oxidative stress has shown contradictory results. Studies on adults and children discovered a connection between SF and MDA levels in people with beta-thalassemia [12,13]. Gunarsih et al. and Al-Hakeim et al.[13], who did not discover a connection among SF and MDA, The results of the investigation by The link between NTBI and MDA was demonstrated by Cighetti et al.[14] .All cell types produce reduced glutathione (GSH), a vital endogenous antioxidant including erythrocytes, is membrane transporters such as the cystic fibrosis transmembrane conductance regulator (CFTR), the multidrug resistance-associated protein (MRP), and the organic anion transporting polypeptide

are strongly controlled depending on GSH production rate production and GSH export. [15].

2-Materials and Method :

2.1.Design of the study :

The research was carried out between early November 2021 and the end of March 2022, and it comprised Analyses of samples from (45) beta-thalassemia patients and (18) samples The patients from Tikrit city, who ranged in age from 15 to 27, were employed as the control group; they received blood transfusions and GH therapy.

2.2.Samples collection

Patients and healthy groups provided blood samples, which were subsequently used to assess the (Hb, PCV, and WBCs count) and Fresh non-hemolysis serum was stored in a deep freezer (- 20° C) after a 5 mL spun blood sample at 3000 rpm for 5–15 minutes in a macro centrifuge. Biochemical assays were performed on the serum, which was separated into three tubes: one for each parameter.

2.3.Hematological tests : This examination is one of the important tests and has great medical importance, as it helps in diagnosing many disease and included:

A-hB

B-PCV

C-WBCs count.

2.4. Determination of IGF-1 concentration in blood serum: IGF-1 concentration was measured by assay kit supplied by Shanghai Corporation of China for ELISA (enzyme-linked immunosorbent assay)

2.5.Determination of the concentration of malondialdehyde in blood serum : It was done using Thiobarbituric acid (TBA) reaction approach. One of the last phases of the oxidation process is MDA, which is measured by researchers using a modified approach, The interaction between lipids, particularly dialdehydes, and TBA in a medium that depends on the pH function makes the fat content and its level a good indication of this process.

2.6. Determination of glutathione concentration in blood serum: Serum glutathione level was measured using a modified Elllman reagent method.

3.Statistical analysis:

One-way analysis of variance (ANOVA) has been used to compare groups in the statistical analysis, and the Duncan multiple ranges test was employed to delineate significant differences, particularly across groups. The statistical analysis was carried out using the statistical software (SPSS). At the time when the statistical significance standard was created (P 0.05).

4.Results and Discussion :

4.1.levels of hemoglobin (Hb):

The results showed that SD±Mean of (Hb) levels in patients group were (12.66±3.54 a) g/dl and control group (8.59±2.84 b) g/dl as shown in Figure (1).In the present study levels of (Hb) was significantly increase (P≤0.05) comparison between the patient group and the control group.

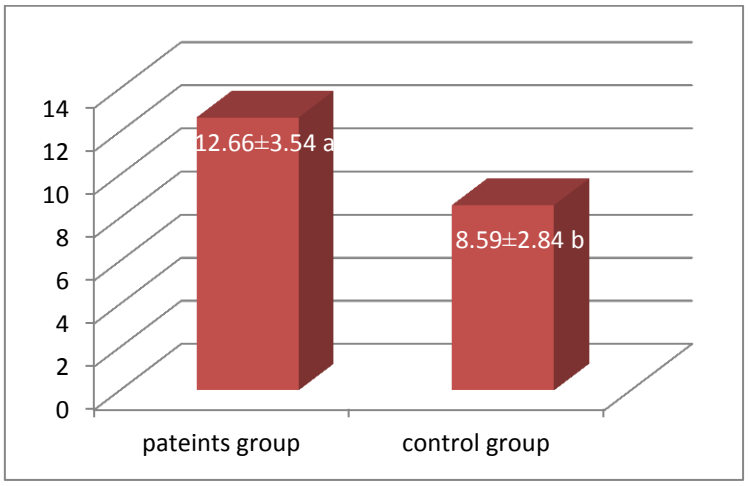

Figure (1) the concentrations of (hb) in patients group compared with control group by (g/dl)

***The different letters indicate significant differences at (0.05).**

4.2.levels of packed cell volume (PCV):

The results showed that SD±Mean of (PCV) levels in patients group were (16.89±4.08 a) and control group (10.24±3.52 b) as seen in Figure (2).In the present study levels of (PCV) was significantly increase (P≤0.05) in patients group when compared with control group.

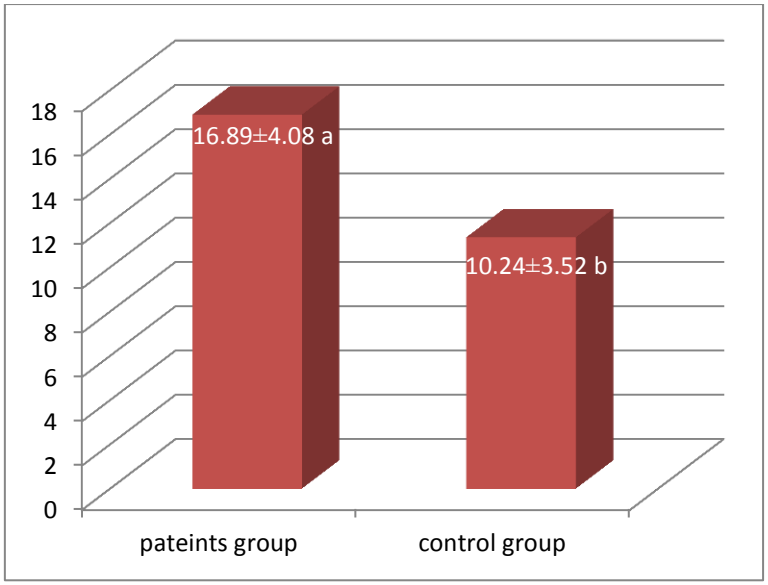

Figure (2) the levels of (PCV) in patients group compared with control group .

***The different letters indicate significant differences at (0.05).**

High amounts of hemoglobin (Fig.1) and PCV (Fig.2) might be caused by ineffective erythropoiesis, a distinguishing and important feature of thalassemia that results in anemia and severe bone marrow hyperplasia [16,17]. 60% to 80% of progenitors perish during the polychromatophilic stage as -globin chains accumulate in growing red blood cells as a result of a -globin deficit. [18]. Hepatosplenomegaly, increased skeletal system, extra-medullary hematopoietic masses and basal metabolism abnormalities of the face and skull, and weak bones are all results of the erythropoietin-driven proliferation of erythroid precursors and shorter red cell persistence [19,20]. Reduced hepcidin production enhances the body's absorption of iron from reserves is released by dietary iron [21,22]. The threshold needed to prevent inefficient erythropoiesis may be greater than the required quantity of hemoglobin to decrease anemic symptoms. [23].

4.3.Numbers of white blood cells (WBCs):

The results showed that SD±Mean of (WBCs) levels in patients group were (13.2±3.74 a) and control group (11.37±3.66 b) as shown in Figure (3).In the present study numbers of (WBCs) was significantly increase ($P \leq 0.05$) in patients group when compared with control group.

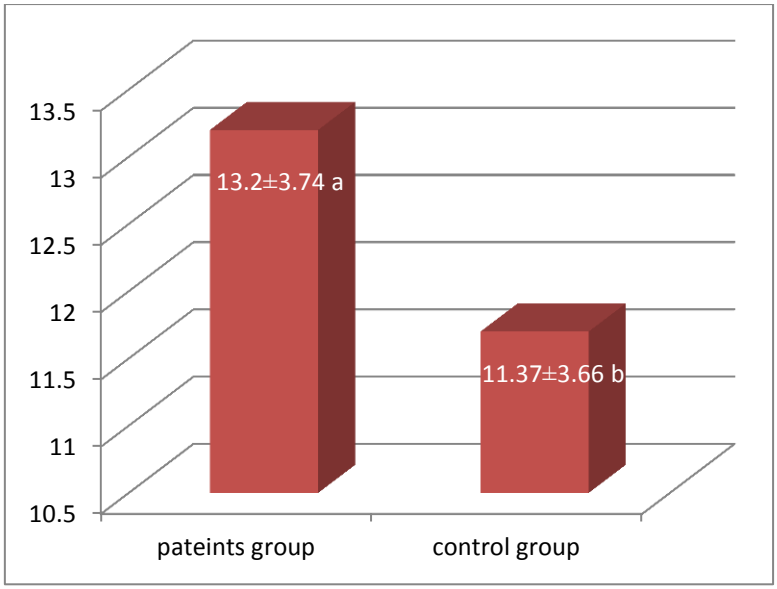

Figure (3) numbers of (WBCs) in patients group compared with control group(WBC $10^{\times 9}$ /L) .

***The different letters indicate significant differences at (0.05).**

The findings indicated that (WBCs) count (Fig. 3) may indicate that leukocytosis (high total WBC count) is more common in thalassemia patients and may be caused by an infectious agent or acute leukemia. Maintaining antioxidant regimens may be helpful in shielding people with thalassemia from further harmful effects of presentations since thalassemia patients are more susceptible to oxidation due to high iron. Hypersegmented neutrophils indicate a lack of vitamin B12. The blood in the peripheral circulation interferes with the red blood cell manufacturing process [(24)]. and it typically takes place as a result of the acute phase reaction in response to an infection and other inflammatory disorders, like Kawasaki illness.

4.4.levels of Insulin like growth factor-1 (IGF-1):

The results showed that SD±Mean of (IGF-1) levels in patients group were (7.832±2.33 a) ng/ml and control group (2.181±0.72 b) ng/ml as shown in Figure (4). levels of in the current research (IGF-1) was significantly increase ($P \leq 0.05$) in patients group compared to the control group.

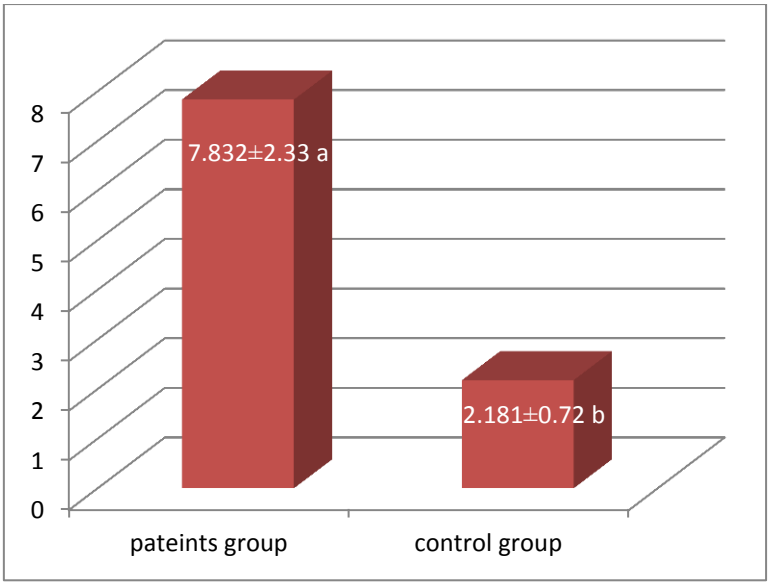

Figure (4) levels of (IGF-1) in patients group compared with control group.

***The different letters indicate significant differences at (0.05).**

These observations imply that thalassemic individuals exhibited some GH insensitivity, even if exogenous GH injection has enhanced growth rate and higher levels of IGF-1 in the blood in patients with BTM with normal GH production [(25,26)]. GH-binding protein in serum (GHBP)levels, however, were short thalassemic normal people without GH insufficiency, excluding a severe GH receptor deficiency (27) With a consistent finding of a positive association between vitamin D and IGF-I blood levels in healthy population-based cohorts individuals, Vitamin D has demonstrated to raise circulating IGF-I and IGFBP-3. [(28,29)].

Additionally, because of iron deposition, particularly in the second decade of life, hypoparathyroidism is one of the serious side effects of BTM. The prevalence ranges widely, from a very low 4% to a maximum of 27%. [(30)]. As demonstrated in vitro and in vivo investigations in people and animals both, IGF-I and PTH have complementary effects on bone, and part of the anabolic benefits are mediated by PTH through nearby IGF-I synthesis [(31)]. IGF-1 also increases collagen production and lowers collagen breakdown, which is crucial for preserving the proper amounts of bone matrix/ mass. Additionally, the role of osteoclasts is directly impacted by IGF-1 due to their expression of IGF-1 receptors [(32)]. Osteoporosis and demineralization are caused by inadequate GH-IGF-I secretion in patients of TM. [(33)].

Bone mineral density in thalassemic people is associated with both the auxanologic markers and the circulating levels of IGF-I. (HSDS, BMI, height, weight, and age). It

is hypothesized that improving mineralization and bone formation and preventing the onset of OP and subsequent patients with these fractures could be accomplished by Through rigorous dietary therapy, GH/IGF-I treatments with the calcium and/or vitamin D supplementation or both, the concentration of circulating IGF-I can be increased [34].

4.5.levels of Malondialdehyde (MDA):

The findings revealed that SD±Mean of (MDA) levels in patients group were (1.203±0.32 b)m mol/L and control group (2.671±0. 512 a)m mol/L as shown in Figure (5). Levels of (MDA) in the current study was significantly decrease (P≤0.05) in patients group when compared with control group

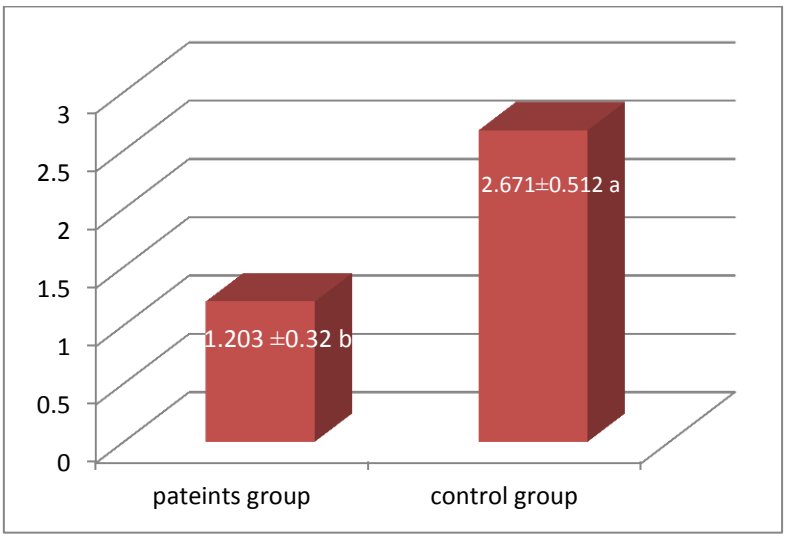

Figure (5) levels of (MDA) in patients group compared with control group by m mol/L.

***The different letters indicate significant differences at (0.05).**

The findings revealed (fig. 5) that patients had lower levels of MDA. The subject's plasma MDA levels were reduced to levels that were similar to those in patients who had taken antioxidants and iron chelation therapy for six to twelve months [35,36]. According to a research by Nasseri et al. [37], giving antioxidants to thalassemia patients lowers MDA levels. Therefore, regular antioxidant supplementation and iron chelation treatment were expected to have an impact on the plasma MDA levels found in this investigation.

An established sign of oxidative stress and cellular harm is malondialdehyde (MDA). Stress from oxidation occurs in a variety of disease situations, including thalassemia, chronic inflammation, and cancer. When oxidants and antioxidants are not balanced,

oxidative stress occurs. Cellular damage happens when oxidants outweigh antioxidants (i.e., when antioxidant activity is inadequate). ROS production also leads to cellular injury, DNA damage, and even cancer. [38].

4.6.levels of Glutathione (GSH):

The results showed that SD ± Mean of (GSH) levels in patients group were (0.0186±0.007 b) m mol/L and control group (0.0402±0.010 a) m mol/L as shown in Figure (6).In the present study levels of (GSH) was significantly decrease (P≤0.05) in patients group when compared with control group

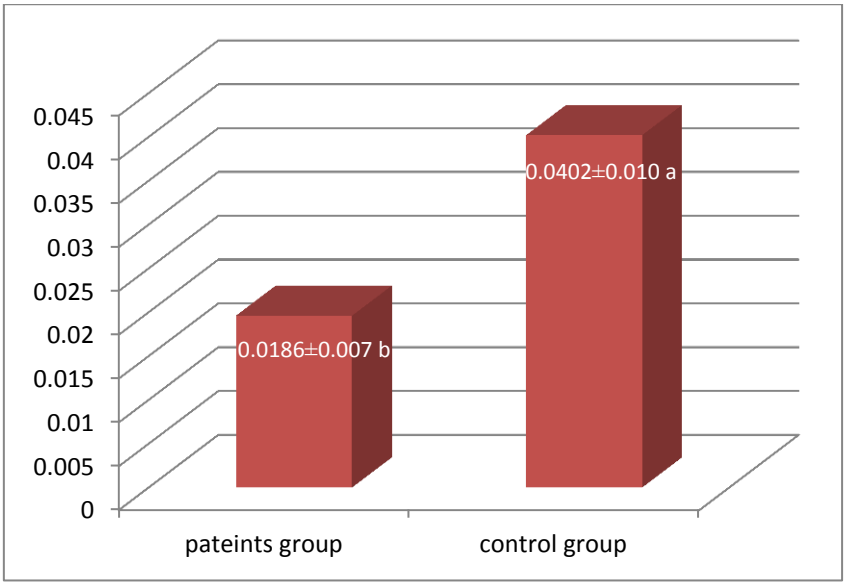

Figure (6) levels of (GSH) in patients group compared with control group by m mol/L.

***The different letters indicate significant differences at (0.05).**

One of the most significant intercellular reducing agents, reduced glutathione is extremely sensitive to oxidative stresses and serves a number of crucial roles, including the control of gene expression and defense against oxidative stress. [39]. [40].

The higher concentration of hydrogen peroxide may cause catalase to suffer direct toxic damage as a result, which might account for the reduced catalase activity reported in the more severe beta thalassemia genotype[41]. When there is significant oxidative stress, the concentration of this is significantly decreased. [42,43].

5.Conclusion : The findings of this study showed that there were significant differences in the hematological parameters measured in the study (Hb, PCV, and

WBC count) between patients and the control group, as well as high IGF-1 levels in the patient group and low levels of both MDA and GSH. These findings support the need for additional research to determine the relationship between the pathogenesis of thalassemia and levels of antioxidants and oxidative stress.

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