

EVALUATION OF LACTOFERRIN , IL-6 AND SOME BIOCHEMICAL VARIABLES IN B -THALASSEMIA INFECTED PATIENTS

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Abstract

Background:- Thalassemia is a hereditary blood disorder caused by a lower than normal rate of hemoglobin in the body. It is also a type of severe anemia characterized by a deficiency in a specific chain of globin chains, as it leads to a decrease in the level of hemoglobin in red blood cells as a result of a loss or decrease in the production of one.

Objective: The current study was designed to evaluation of β -thalassemia infection effects on some biomarkers such as Lactoferrin -LTF, Interleukin 6 -IL-6 , Ferritin , Iron , Zinc -Zn .

Methods: The study was conducted on 40 patients infected with β - thalassemia and 20 healthy people (control group), who attended the general hospital of baghdad city from 20/4/2024-20/6/2024.

The results : The results showed a significant ($P<0.05$) rise in serum level of (LTF , IL-6 , Ferritin , Iron) and a significant decrease in concentration of (Zn) in patients compered healthy people. The research results also showed, through the area under the curve, that the sensitivity and specificity of the studied variables were of high values, which indicates that the variables can be considered an important predictive marker for diagnosing thalassemia patients.

Key words / β -Thalassemia , Lactoferrin , IL-6, Ferritin , Iron

Introduction

Thalassemia is a hereditary blood disorder caused by a lower than normal rate of hemoglobin in the body. It is also a type of severe anemia characterized by a deficiency in a specific chain of globin chains, as it leads to a decrease in the level of hemoglobin in red blood cells as a result of a loss or decrease in the production of one. or more than one polypeptide chain. Either the alpha or beta chain results in anemia, or what is known as anemia, as hemoglobin loses its main function of transporting oxygen to cells ⁽¹⁾. One of the main causes of the disease is the presence of a different group of mutations that may affect the polypeptide chain as a replacement or replacement. Deletion of one of the genes or one of the nucleotides of DNA, thus causing a problem in the formation of hemoglobin and the manufacture of ineffective red blood cells. The size of these pellets is small and they lack pigment and therefore do not perform their function, as they cannot carry sufficient oxygen for the body's needs ⁽²⁾.The clinical symptoms felt by a person with thalassemia, which is the main sign of severe anemia, as the patient becomes dependent on blood transfusions permanently to continue his life ⁽³⁾. Visible symptoms also include paleness of the face, repeated attacks of fever, malnutrition, and a gradual and visible enlargement of the abdomen as a result of enlargement of the liver and spleen. Growth is slow, a type of mental retardation appears, and heart disease, diabetes, and chronic liver and kidney disease appear ⁽⁴⁾.

Lactoferrin (LTF) is a glycoprotein with a molecular weight of 80 KD that belongs to the transferrin family, this multi-functional protein is the most important biostimulant in human milk and other external secretions such as saliva, tears, semen, and secretions. urine, and neutrophil granules ⁽⁵⁾, The concentration of lactoferrin is found in particular in breast milk and is linked to the

breastfeeding stage, as the rise level are found in colostrum, about 7 g/L, then it decreases in mature milk, 2-3 g/L ⁽⁶⁾.

Interleukin 6 is a type of interleukins, as it is a glycoprotein with a molecular weight of 21-26 kilodaltons and contains 28 amino acids ⁽⁷⁾, Interleukin 6 is a pro-inflammatory cytokine that is produced by various types of cells, including B lymphocytes, macrophages, mast cells, and other non-lymphocytes such as fibroblasts, endothelial cells, and lymphocytes. Keratinocytes and cancer cells ⁽⁸⁾.

Ferritin is one of the main proteins for iron storage because it has a large cavity that can accumulate large amounts of iron ⁽⁹⁾. Ferritin is a protein found in all cells of the body but is found in high concentrations in cells of the bone marrow, spleen, and liver. It therefore provides intracellular storage of bioavailable iron in a safe and accessible form. It protects cells from iron-mediated free radical formation and the toxicity that may result. about the Fenton reaction, the process of converting hydrogen peroxide produced by energy production in mitochondria into a highly toxic free hydroxyl radical ⁽¹⁰⁾.

Materials and Method

Blood Specimens Collection: Five ml of blood was collected from each patient infected with β . Thalassemia and healthy people .the sample was collected during period from 20/4/2024-20/6/2024 , a case-control study was designed for 40 patients (20 males and 20 females) with β – thalassemia and 20 healthy people . The current study was designed to evaluation of β . thalassemia infection effects on some biochemical such as Lactoferrin, Interlukin 6 , Ferritin , Iron , Zinc . The levels of ferritin, lactoferrin, and IL-6 in blood serum were estimated using a kit Cloud-clone corp prepared by the company American , As for the iron level, it was measured using a kit prepared from the Spanish company LiNEAR ⁽¹¹⁾, while the zinc level in the blood serum was estimated using a kit prepared from the American company Elabscience ⁽¹²⁾.

Statistical Analysis

The process of collecting data for the samples used for the study and analyzing them statistically was done using the (SPSS) system by extracting the arithmetic mean and standard deviation. The test was also used to analyze the differences between the main and secondary groups. Significant differences were chosen for these groups under a probability level of $P \leq 0.05$.

Results:

Evaluation of level Lactoferrin , Ferritin , Iron , Zinc , IL-6 in the two study groups:

Table (1) shows the (mean \pm S.D)of Biochemical parameter in the two study groups.

Parameters	Mean \pm SD		p-value
	Control	Patients	
Lactoferrin(ng/ml)	16.840 \pm 5.207	29.711 \pm 11.964	<0.0001*
Ferritin(ng/ml)	16.271 \pm 5.812	55.078 \pm 15.198	<0.0001*
Iron(μ g/dl)	267.623 \pm 78.937	936.902 \pm 278.424	<0.0001*
Zinc(μ mol/L)	0.740 \pm 0.069	0.492 \pm 0.092	<0.0001*
IL-6 (pg/ml)	91.579 \pm 23.627	152.332 \pm 43.807	<0.0001*

* $p \leq 0.05$

The results of the current research showed a significant elevated in the (Lactoferrin , IL-6, Ferritin ,Iron) and a significant decrease in the level of (Zinc) in the serum of patients compared healthy person . at its level, the probability of ($P \leq 0.05$). as in the following figures:

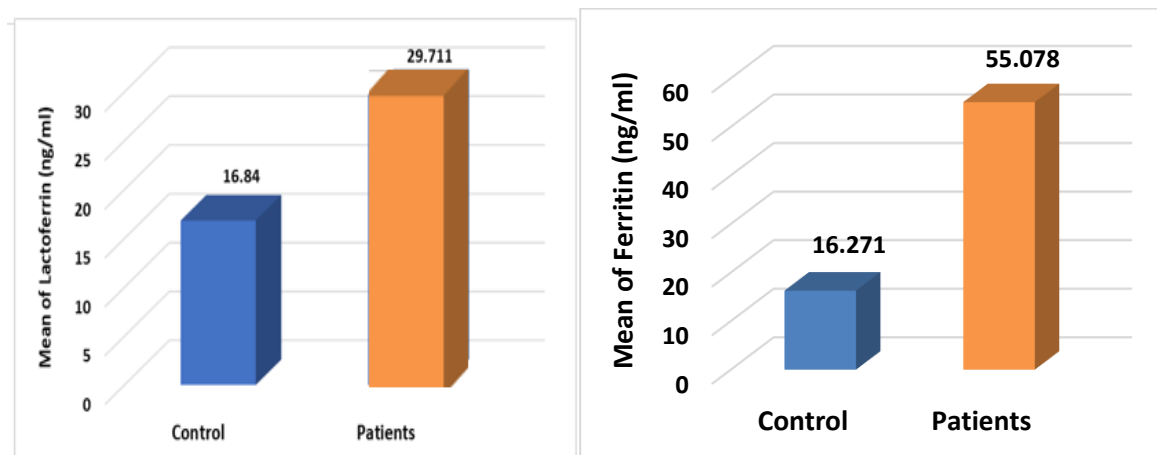


Figure (1): LTF level in the blood serum of both groups
 Figure (2): Ferritin level in the blood serum of both groups

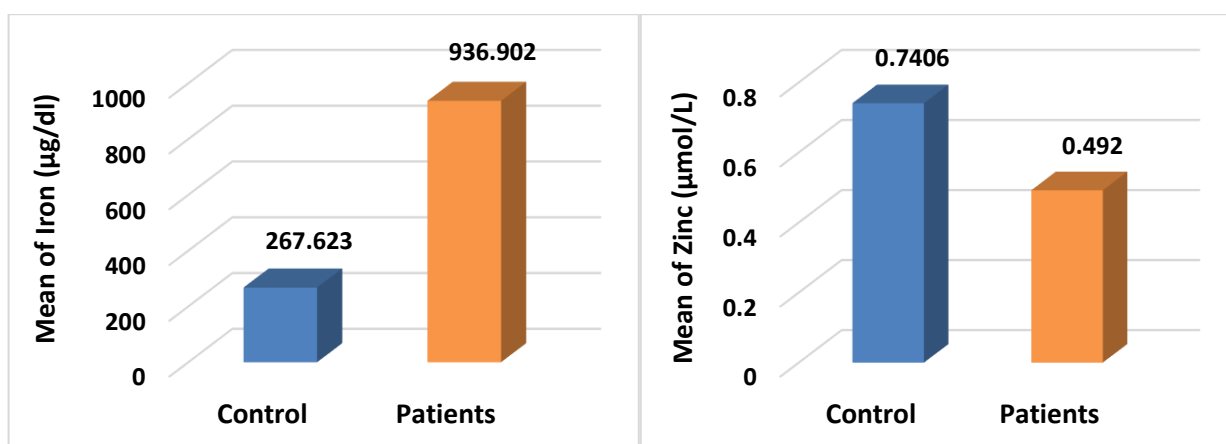


Figure (3): Iron level in the blood serum of both group
 Figure (4): Zn level in the blood serum of both groups

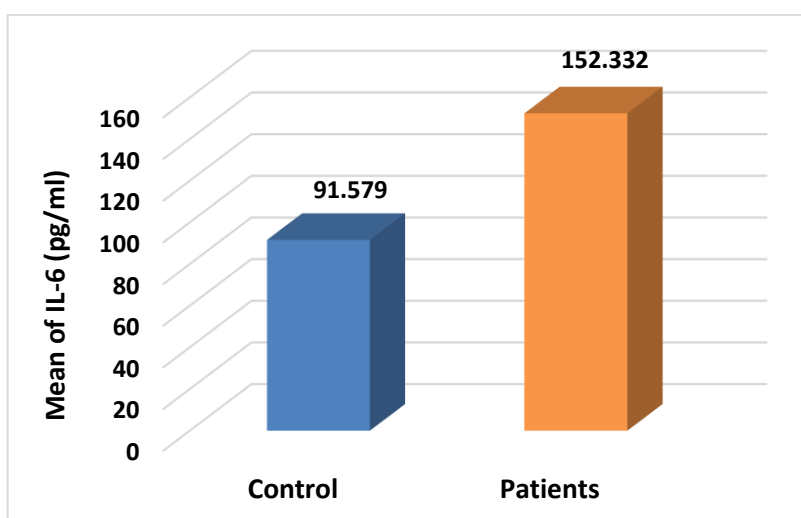


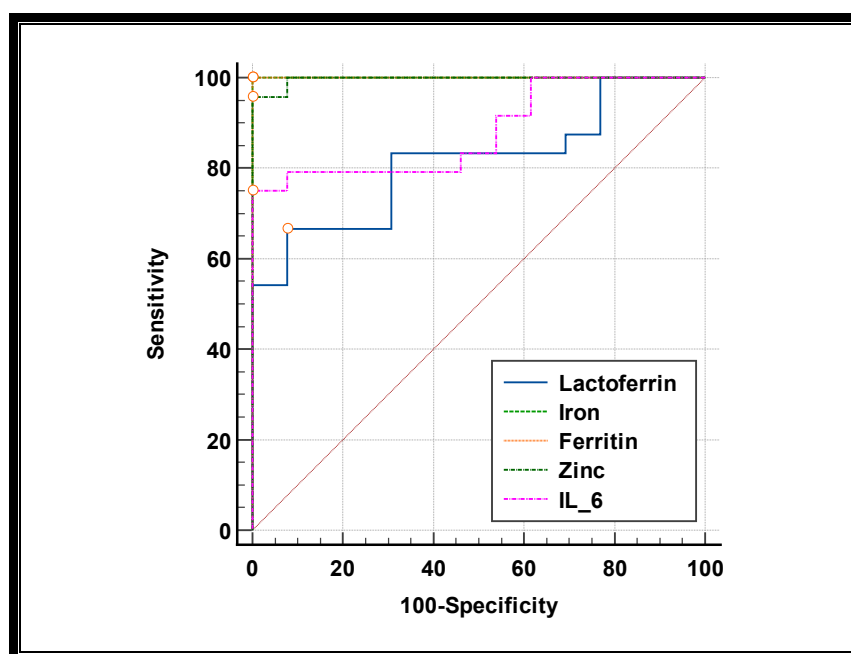
Figure (5): IL-6 level in the blood serum of both groups

Area under the ROC curve (AUC)

Comparing the results with ROC analysis (table 2, figure 6), variable analysis of Lactoferrin (AUC=0.892, $p<0.001$), Ferritin (AUC=1.000, $p<0.001$), Iron (AUC=1.000, $p<0.001$), IL-6 (AUC=0.914, $p<0.001$), Zn (AUC=0.996, $p<0.001$) were significant parameters in predicting the risk of β -thalassemia

Table(2) Predictive Values for Infected Individuals

Parameters	Cut off	Sensitivity %	Specificity %	Accuracy	AUC	P-value
Lactoferrin	>22.005	76.47	94.44	0.7092	0.892	<0.0001
Iron	>412.469	100.00	100.00	1.0000	1.000	<0.0001
Ferritin	>34.14	100.00	100.00	1.0000	1.000	<0.0001
Zinc	≤ 0.664	100.00	94.74	0.9474	0.996	<0.0001
IL_6	>121.707	75.00	100.00	0.7500	0.914	<0.0001

**Fig (6):-Shows the ROC of all parameter in all groups****Discussion**

The results of the current study showed that lactoferrin made progress in patients with thalassemia compared to healthy celebrities, and they did not look forward to the results, given that studies indicated that foods containing lactoferrin, one of the prominent stars, may be useful in getting rid of anemia caused by iron deficiency causes, as Lactoferrin, with which food is taken orally, restores iron to then, not only by facilitating iron absorption, but also by surgical treatment to get rid of anemia in chronic diseases, which are characterized by iron deficiency in physiological function⁽¹³⁾. Therefore, lactoferrin is a particularly promising alternative to the inorganic iron formulations currently used for the prevention and treatment of anemia. As a multifunctional immunomodulator, antioxidant agent and regulator of intestinal iron absorption, lactoferrin has already been shown to be effective and safe for the prevention and treatment of some anemias and chronic infections⁽¹⁴⁾. Therefore, the increase in the level of lactoferrin in our study may be explained by the repeated blood transfusions that lead to an increase in the level of iron, and thus to the maintenance of lactoferrin in its high or normal state, as it is one of the proteins associated with iron. Therefore, there is a need for more studies to know the relationship of the lactoferrin protein with patients people with thalassemia. on the other hand, it was found from the research results that the area

under the curve was 0.892, the sensitivity rate was 76.5%, and the specificity was 94.4. This indicates that the lactoferrin level will be a good tool in the future that will enable patients to diagnose thalassemia. Also the results of this study are consistent with the findings of Hossaini⁽¹⁵⁾ and Hasoon⁽¹⁶⁾ and Ghazala⁽¹⁷⁾, who showed that there is a significant elevated in the percentage of ferritin in the serum of thalassemia patients. The results of the current study are also consistent with Suman⁽¹⁸⁾, who indicated that the cause of increased ferritin in thalassemia patients is the result of ineffective erythropoiesis and multiple blood transfusions that lead to an increase in iron, While the body cannot secrete more than 1 mg of iron per day. This estimation of blood ferritin concentration is the most common test for estimating iron overload in beta-thalassemia patients. When the blood ferritin concentration reaches 1000 ng/mL commonly after a blood transfusion, it is generally taken as a landmark for initiating iron chelation therapy⁽¹⁹⁾. It has been found that patients with iron overload have high levels of ferritin, and therefore have a low number of red blood cells⁽²⁰⁾, and that any inflammatory condition can cause a high level of ferritin, which is why it is an important diagnostic marker for thalassemia patients⁽²¹⁻²²⁾. The study of Muhammad also showed that a severe increase in the level of ferritin in the blood leads to an increased risk of poor growth. Therefore, the high iron level may be due to increased absorption of iron by the digestive system in thalassemia patients⁽²⁴⁾. It has also been found that blood transfusion is a cause of an increase in iron, as well as its absorption from the digestive system and hemolysis in thalassemia patients, as excess iron deposits in the organs lead to several diseases, including heart disease, which is the main cause of death in thalassemia patients. Under normal circumstances, iron is transported in cells by responsive proteins⁽²⁵⁾. It was also noted from the research results that the area under the curve was 1.000 and the percentage of specificity and sensitivity were 100% for both ferritin and iron, respectively. This may be explained by the fact that iron and ferritin are important diagnostic variables that reveal the prediction of the severity of patients for patients with beta thalassemia.

On the other hand, it was observed that there was an rise in the level of interleukin 6 in the blood serum of patients with thalassemia compared to healthy people, and this is consistent with many studies⁽²⁶⁻²⁸⁾, which indicated that inflammatory cytokines, including interleukin 6, may be a risk factor for predicting the severity of thalassemia. (IL-6) is one of the inflammatory cytokines that participates in the inflammatory and immune response⁽²⁹⁾. An elevated in its levels was found in patients with beta thalassemia, and this may be due to a chronic inflammatory condition, as sulfated iron works to release interleukin 6, or the increase in interleukin 6 is attributed to excessive stimulation of macrophages, which contributes to iron metabolism⁽³⁰⁾. It was also noted from the research results that the area under the curve was 0.914 and the percentage of specificity and sensitivity were 100% , 75.0 % respectively for IL-6,. This may be explained by the fact that IL-6 are important diagnostic variables that reveal the prediction of the severity of patients for patients with beta thalassemia.

Zinc is one of the important minerals, as it was found that its decrease is linked to beta thalassemia, so the results of the study found a decrease in its concentration⁽³¹⁾. The result agree with⁽³²⁾ who showed a reduced in Zn in patients with thalassemia compered healthy people. so the cause of its deficiency may be due to high iron levels, as well as to multiple factors, including hyperuria and liver dysfunction, in addition to poor absorption, and it may form chelating compounds with iron, as they are removed through urine, so the elements can be measured. Trace in urine is likely to increase⁽³³⁾. It was also noted from the research results that the area under the curve was 0.996 and the percentage of specificity and sensitivity were 100 % , 94.7 % respectively for Zn,. From the results, low zinc may be of important diagnostic value for thalassemia patients.

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