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Estimation And Measurement of Correlation Coefficient Values for The Level of Erythroferrone and Liver Enzymes in Beta-Thalassemia Patients

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ABSTRACT

Background: Thalassemia is an inherited blood disorder of the blood cells It is described as a low level of hemoglobin and a decrease in the number of red blood cells from the normal range.

Objective: This study amied to evaluate the levels and find the correlation coefficient between erythroferrone and liver enzymes in the beta thalassemia patients.

Patients and methods: In this study, samples were taken from patients with beta-thalassemia (60 samples) and 30 samples from healthy people.

Results: The current study's findings demonstrated that both erythroferon and liver enzymes were highly elevated in patients with thalassemia (P>0.05), and they were also strongly associated.

Conclusions: This study found association between increased levels of erythroferon and severe anemia also association between increased levels of liver enzymes and severe accumulation of iron.

Keywords: Thalassemia, Erythroferrone, Liver enzymes.

INTRODUCTION

Thalassemia It is a hereditary blood disorder that results from a genetic mutation that deletes one or more of the globin genes responsible for producing globin chains, which leads to a deficiency or complete absence in the production of hemoglobin chains [(1)].

And that adult hemoglobin has four protein parts called polypeptide chains, and these chains are classified into two β chains and two α chains [(2)], so people with thalassemia have a deficiency or absence in the manufacture of both or one of the beta chains or alpha [(3)]. Thalassemia suffers from the accumulation of iron in various organs of the body, including the liver and spleen, as a result of their continuous receipt of blood. Therefore, the accumulation of iron leads to pallor, jaundice, and a high level of iron in the blood [(4, 5, 6)].

Erythroferrone is a protein hormone encoded in humans by the ERFE gene. Erythroferon is produced by red blood cells and works by inhibiting hepcidin in the liver, thus increasing the amount of iron available for the manufacture of hemoglobin [(7,8)]. The most important role of the hormone erythroferon is to regulate iron metabolism by affecting the substance hepcidin [(7)] in humans and mice, where it is produced in erythrocyte precursors that multiply when new red blood cells are made, as is the case after bleeding when there is a need for more iron [(9)]. Alkaline phosphatase is an enzyme that removes phosphate groups from nucleotides, alkaloids, and proteins by a process called Dephosphorylation [(10)]. The enzyme is found in all tissues of the body but is concentrated mainly in the liver, kidney, placenta, and Bones [(11)]. Alanine transaminase is one of the transaminase enzymes, also called glutamate pyruvate transaminase, that catalyzes the transfer of the amine group from alpha-keto-glutaric alanine and in reverse to give glutamate and pyruvate. This enzyme is used to detect liver damage due to its presence mainly in the liver and at lower levels in the pancreas, heart, and muscles [(12)].

The aspartate aminotransferase is also called glutamate oxaloacetate transaminase. The enzyme is found in the liver, as well as in red blood cells, kidneys, and brain tissue. The concentration of the enzyme increases in case of damage to any of these organs [(13)].

PATINENTS AND METHODS

This study was conducted at Al-Karama Teaching Hospital in Baghdad Governorate from December 2021 to March 2022, and the study included 60 samples of patients with major beta-thalassemia and 30 samples of healthy people whose ages ranged from 5 - 35 years, and the information about each person was recorded according to the research form.

Ethical consent:

Written informed consent was obtained from each patient to participate in the current study. The Central Scientific Research Ethics Committee at Tikrit University approved this research. This work was carried out in accordance with the World Medical Association (Declaration of Helsinki) Code of Ethics for studies involving humans.

Inclusion criteria: Patients with thalassemia from both genders are involved, and patients who agreed to participate in this study.

Exclusion criteria: persons less than 5 year or older than 35 year, and other chronic hemolytic anemia.

Sampling: Five milliliters of blood were collected from the patients included in the study, placed in gel tubes and left to clot for 20 minutes, then centrifuged for fifteen minutes at a speed of three thousand revolutions per minute to collect serum. The serum was then placed in three Eppendorf tubes and stored in a deep freezer at −20

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°C, samples were brought to room temperature again before these tests were performed.

Biochemical studies:

Erythroferrone ELISA Kit: This kit frome a Chinese (Fine test) company where the method of interacation between the antibody and the antigen was adopted**.**

ALT (Alanine aminotransferase) Kit
AST (Aspartate aminotransferase) Kit
ALP (Alkaline Phosphatase) Kit
These Kits from the Germany company (Roche)

Study enrollment procedures: Detailed information was recorded for all cases, including age, gender, weight, and others. The presence of thalassemia was confirmed through the medical history taken from the patients and the tests they performed, such as complete blood count and hemoglobin electrophoresis.

Statistical analysis

The well-known Statistical system SPSS was adopted to infer p-value and means ± standard error of the studied variables in the patient groups and compared them with the healthy groups.

RESULTS

Table (1) showed that there was a significant increase in the level of Erythroferrone, where its concentration reached 13.82 ± 1.06 compared to the control group's 6.97 ± 1.54. Also, table (1) showed a significant difference between the values of the levels of alkaline phosphatase and alanine aminotransferase for the amino group of patients compared to the healthy controls being higher in patients. While the results of the values of aspartate aminotransferase for the amino group showed no significant difference between patients and healthy being higher in patients.

Table (1): The level of erythroferon and liver enzymes

variable		Number	Means ± SD	P-Value
Erythroferrone	Patients	60	13.82 ± 1.60	0.0007
	Healthy	30	6.97 ± 1.54	
Alkaline Phosphatase	Patients	60	123.5 ± 23.7	0.0004
	Healthy	30	63.6 ± 15.9	
Alanine Amino-transferase ALT	Patients	60	15.87 ± 3.03	0.050
	Healthy	30	13.50 ± 2.33	
Aspartate Amino-transferase AST	Patients	60	22.6 ± 4.4	0.53
	Healthy	30	18.1 ± 3.0	

The values of the correlation coefficient were found between the measured variables, as Table 2 shows the r values among the variables measured in patients, while Table 3 shows the r values among the variables measured in healthy people.

Table (2): The correlation coefficient between the variables in patients

	Erythroferrone	ALP	ALT
ALP	0.324		
ALT	0.293	0.342	
AST	-0.052	0.075	0.195

Table (3): The correlation coefficient between the variables in healthy

	Erythroferrone	ALP	ALT
ALP	0.212		
ALT	0.135	0.275	
AST	0.070	0.237	0.662

DISCUSSION

Table (1) showed the number of samples, mean values, standard deviation, and p-values for patients and healthy subjects for the biochemical variables erythroferon, alkaline phosphatase, alanine aminotransferase of the amine group, aspartate aminotransferase of the amine group. It was noticed from the results that there was a significant increase in the level of Erythroferrone, the reason for the increase in erythroferrone may be due to the accumulation of high iron due to thalassemia, and this accumulation leads to an increase in the level of erythroferrone in the blood [(14)].

In response to severe anemia, increased production of erythropoietin secreted by the kidneys, a vital hormone that stimulates the bone marrow to produce red blood cells. This hormone increases erythroferrone levels. Erythroferrone acts directly on the liver cells to reduce hepcidin production, which leads to its decrease. Low levels of hepcidin and high erythroferrone allow the release of stored iron, in addition to increasing the absorption of dietary iron to produce red blood cells [(15)]. Also, it is noticed from this study that there was a significant increase in the levels of liver enzymes in beta-thalassemia patients . The results of the study are identical to what was obtained from a previous study [(16)].

These enzymes indicate the extent of liver damage due to pathological conditions, and these enzymes are an indicator to assess the extent of damage to hepatocytes [(17)] .Continuous blood transfusions for patients with beta-thalassemia can lead to repeated transmission of the hepatitis virus, which becomes chronic, leading to the release of liver enzymes from hepatocytes into the blood serum [(18)].

Also, it was found by **Nathan and Oski** [(19)] that iron deposition in very large quantities in the liver can lead to increase cirrhosis of the liver and raising the levels of liver enzymes.

The scientist **Kleinman** [(20)] stated that one of the side effects of iron accumulation is an increase in the secretion of liver enzymes, which are delivered to blood continuously [(20)].

Others attributed the increase in these enzymes to the damage in the hepatic visceral cells due to the infection of these cells by viral hepatitis [(21, 22)]. The cause of elevated liver enzymes in beta-thalassemia major patients is caused by hepatic cellular damage due to iron accumulation in the liver, which leads to fibrosis and necrosis of hepatocytes [(23, 24)].

CONCLUSIONS

We concluded from the current study that there was an increase in the levels of erythroferrone in patients with major beta-thalassemia, which may be due to severe anemia. This leads to high levels in the blood. Also, there is an increase in the liver enzymes alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in patients due to the accumulation of severe iron that leads to necrosis and cirrhosis of the liver and thus the rise of these enzymes.

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