An enzymatic and biochemical study of patients with beta thalassemia

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Abstract---The current study aimed to study some biochemical variables for thalassemia patients. The study included 50 thalassemia patients in the Qadisiyah Governorate Center for Genetic Blood Diseases - Thalassemia during the period from October 2020 to January 2021. The average age of male patients was (15 to 35), The healthy subjects were about 35 samples of males, so the average values for patients It is as follows, evaluate the activity of the enzyme to the patients’ were (GPT) (40.622 ± 0.158), (GOT) values are (45.8626 ± 0.279), And the (ALP) values were (200.5468 ± 0.427). And the (GGT) values were (59.9672 ± 0.262), some of the enzymes Antioxidants evaluated for patients were as follows (CAT) is (0.2138 ± 0.003), and (MDA) were (5.4964 ± 0.024).The (SOD) values for patients were (1.037 ± 0.039), and the (GSH) Glutathione value for patients was (0.9966 ± 0.00666). The ferritin value for patients was (769.9766 ± 2.899), While the values of healthy males for the following enzymes were (GPT) (15.3826 ± 0.041), (GOT) were (22.0824 ± 0.08), (ALP) were (120.9844 ± 0.170)). And the (GGT) values were (33.1998 ± 0.075) some of the enzymes Antioxidants evaluated for healthy where the level of (CAT) in healthy was (0.621 ± 0.004), The (MDA) was (1.6156 ± 0.004), The (SOD) values were (2.179 ± 0.004), (GSH) Glutathione was (2.5696 ± 0.058).and the ferritin value was (45.0736 ± 0.134). The values of biochemical variables for thalassemia patients in our study showed significant differences compared with normal values (P < 0.05).We conclude from these results that the changes occurring in these biochemical parameters are due to liver and heart diseases due to the high level of iron in the blood.

Keywords---enzymatic, beta thalassemia, biochemical.
Introduction

Thalassemia is a blood disorder caused by a genetic mutation that leads to impaired production of the hemoglobin chain. Thalassemia patients usually have complications such as anemia, issues related to blood transfusion, hepatic or cardiac involvement, and psychosocial effects (Sardar et al., 2021). Mutations in the -globin gene are the most prevalent cause of genetic diseases in humans, with 350 -thalassemia mutations reported to date (De Sanctis et al., 2017). Its problems have a negative influence on afflicted persons’ quality of life, including negative consequences on their general health, school performance, mental health status, and physical, social, and psychological elements of their existence. (Ansari et al., 2014; Nashwan et al., 2018). There is a scarcity of information on the epidemiology of -thalassemia. According to more than ten-year-old data, -thalassemia carriers make up around 1.5 percent of the global population, and over 40,000 afflicted newborns are born each year, with half of them requiring blood transfusions. (Modell & Darlison, 2008). More than 90% of individuals with -thalassemia reside in a "belt" that stretches from Africa to Southern Europe and the Middle East, all the way to Southeast Asia, where thalassemia is most prevalent (Steinberg et al., 2009).

Types of thalassemia

Alpha Thalassemias

Chain genes are divided into four categories:

- Carriers who remain silent (Silent Thalassemia - one defective gene)
- α trait of thalassemia (two defective gene)
- Illness Hemoglobin H. (Three genes are faulty in hemoglobin H disease.)
- Severe hydrops fetalis - the four genes are faulty) Fetal hydrops [γ4] hemoglobin Bart's (Neslon, 1987).

Beta Thalassemias

These are hereditary blood illnesses caused by a deficiency in the production of the beta-globin chain, A variety of phenotypes exist, ranging from asymptomatic to severe anemia (Galanello & Origa, 2010). The buildup of alpha-globin chains that form identical tetramers is caused by a deficiency in the production of beta-globin chains. Homotetramers that are unstable, resulting in a reduction in hemoglobin and red blood cell formation. And these chains are deposited. (Gupta et al., 2011).

The three types of β- thalassemia

1- Silent beta thalassemia
The simplest kind of thalassemia, the beta type, is caused through asymmetric marriages and is difficult to differentiate from other types of anemia since the patient has no clinical symptoms (Galanello & Origa, 2010).

2- Beta -thalassemia minor
It is the mildest form of beta thalassemia caused by the inheritance of a non-beta gene. Patients with homozygous clobene deficiency, which results in a small reduction in clobene production, do not always require therapy for clinical symptoms. (Weathall & Clegg, 2008)
3- Beta-thalassemia intermedia
A disorder in which the absence of beta polypeptide in hemoglobin causes more severe anemia and substantial medical problems. In afflicted individuals, the disease is defined by the presence of two faulty genes while still manufacturing some beta-globin. The clinical severity of this condition varies depending on the degree and functional competency of beta-Globin (Hay & Weatherall, 2017).

4- Beta-thalassemia major
is the most difficult form of beta-thalassemia. It's caused by a lack of β-synthesis, which prevents considerable amounts of HbA, nemia occurs due to a large irregularity in the production of a chain of hemoglobin (alpha >> beta). Surplus unpaired alpha-globin chains precipitate inside RBCs, causing damage to RBC plasma membranes and intravascular hemolysis. Furthermore, early erythroid precursor death/apoptosis/ lysis /necrosis lowers the amount of RBCs even more. Severe anemia induces hypoxia, and the ensuing EPO causes bone marrow hyperplasia, which leads to extramedullary hematopoiesis (Cunningham, 2010). The newborn with thalassemia major looks to be healthy at labor. Because fetal hemoglobin (Hb F) predominates throughout pregnancy, this is the case. it is devoid of beta chains. As the newborn converts from gamma to beta globulin, anemia appears a few months after birth. The infant's growth is stunted, and he or she frequently has difficulties (because to poor oxygen absorption in the body with major anemia), fever episodes (due to severe sickness), sluggish bowel movement, and other digestive disorders. If left untreated, it can lead to the expansion of organs such as the spleen, liver, and heart, as well as the weakening and brittleness of bones. The disease leads to death before even the age of twenty (Hay & Weatherall, 2017). If treatment is neglected, it leads to Severe and chronic anemia, Delayed physical and mental development, especially in the bones of the head, and thinning of the bones, Liver and spleen enlargement dental problems, And weak general immunity. (Prakash et al., 2013).

**Ferritin**

Is an iron storage protein that stores iron in a non-toxic form, deposits it in a secure location, and transports it to where it is needed. The quantity of iron stored in the body, which is necessary for red blood cell synthesis, is directly proportional to ferritin levels in serum (Chrysohoou et al., 2006). There is too much iron if ferritin levels are high. Ferritin is also utilized as a marker for iron deficiency. Ferritin levels in males and females should be between 12–300 and 12–150 ng ml1, respectively (Farmaki et al., 2010). Patients with iron excess have a much higher amount of ferritin, which may assist distinguish thalassemia patients from those with iron deficiency, both of whom have a low red blood cell count. (Ashena et al., 2007).

**Methods and Materials**

**Blood samples**

Blood samples were taken from patients with thalassemia during their visit to the Center for Genetic Blood Diseases (Thalassemia) for the purpose of treatment, examination and care for them. 50 samples were taken from only males, their ages ranged from (15 to 35 years) and from where 5 ml of blood was withdrawn from them in the morning and conducted. They had blood tests, and blood serum
was obtained. The blood serum was obtained from them by placing them in clean, dry test tubes. Then it was placed in a centrifuge at 3000 rpm for ten minutes, after which it was

The separated serum was withdrawn and placed in Eppendorf tubes and kept frozen at(-20°C) until it is used to measure the chemical and immunological variables included in the current study. Blood was taken from healthy subjects (35) for comparison purposes as the control group.

**Methods of measuring some biochemical components and some enzymes:**
Liver function levels of alanine aminotransferase (ALT) were measured. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) Alkaline phosphatase and, (GGT) Glutamyl transferase. Using a Biolyzer ® 300 (Bakr et al., 2017).

**Oxidation indicators**
- Estimation of (MDA) in serum. Determination in blood serum (MDA) by using the modified method (Guidet & shah, 1989) By measuring the amount of (MDA), which is one of the main products of lipid peroxide. According to (Schemedes & Holmer, 1987).
- The activity of (GSH) enzyme was estimated using the colorimetric method (Burtis & Ashwood, 1999).
- (SOD) is measured according to its method Misra & Fridovich. At 37°C in the air (Misra & Fridovich, 1972).
- The enzyme activity (CAT) is measured (Aebi, 1974).
- And Ferritin iron stock level was determined by Vidas automatic tester and kits from the biomerieux supplier. The principle of operation of the device is to combine the Enzyme immunoassay sandwich method with final fluorescent detection (ELFA) final. (AL Ghanimi, 2016)

**Statistical Analysis**
The results were presented as the mean minus the standard error of the mean (SEM). To assess all groups' unpaired values, two-way analysis of variance (ANOVA2) and Newman-Keuls were used. P<0.05 was used to determine if differences were significant. The software was used for all statistical analyses (Spss Institute, Inc., USA).

**Result and Discussion**
The results showed that there were significant differences in liver enzymes between the values of patients and healthy subjects, according to the following;

**Liver function assessment**
1-GGT
The results of the statistical analysis showed a significant increase of (P<0.05) in GGT in patients compared to healthy people, where the level of GGT in healthy was (33.1998 ± 0.075) and in patients (59.9672 ± 0.262), and LSD was (0.82) between groups, figure (1).
Iron overload is the main cause of elevated liver enzymes. The severity of hepatitis is related to significant increase in iron overload in thalassemia patients (Filiz et al., 2016). Since the liver is the first organ to metabolize iron, it is the first organ to be damaged by excess iron. Liver enzymes are related to the extent of liver damage and increased levels of ferritin in the liver. Serum of thalassemia patients and iron overload as a result of repeated blood transfusions (Ameli et al., 2008) GGT located on the plasma membranes has been known to be one of the biomarkers of oxidative stress, as it has a protective effect in maintaining the levels of glutathione (GSH) (the antioxidant inside the cell), but it works to break it down outside the cell, so the generation of free radicals can lead to intracellular glutathione depletion and consequently enhanced GGT activity in the circulation (Park et al., 2015).

2-ALP
The results of the current study showed that there was an increase in significant differences at (P < 0.05) in ALP in patients compared to healthy people, where the level of ALP in healthy was (120.9844 ± 0.170) and in patients (200.5468 ± 0.427), and LSD was (1.08) between groups, figure (2)
One of the most important complications of thalassimia is osteoporosis. Serum ALP activity raised significantly in Thalassemic patients at the age of above 10 years. (Santraet al., 1999). Alkaline phosphatase enzyme, the results of the research (Muhammad et al., 2015) showed a significant increase in the activity of this enzyme. The reason for this may be due to the fact that most of the activity of this enzyme comes from bone tissue. Thalassemia patients suffer from the dissolution of this tissue, which leads to the leakage of this enzyme into the blood circulation, and then an increase in the activity of this enzyme (Samir et al., 2012). Liver alkaline phosphatase enzymes are found in the cell membranes of the hepatic sinuses and bile ducts, and their levels rise with blockage of the intrahepatic and extrahepatic bile ducts with sinusoidal obstruction. Liver cell damage status (College et al., 2010)

The ALP enzyme is present in the liver and bone marrow, and the bulk of the alkaline phosphate enzyme in the blood serum comes from these two tissues (bone marrow - liver) and is secreted into the blood circulation. Therefore, the high activity of this enzyme is often due to the pathological conditions of these two tissues or perhaps due to the effects of iron on liver cells, as increased iron concentrations may destroy liver cells, which leads to the transfer of this enzyme in large quantities to the bloodstream and thus to an increase in the effectiveness of this enzyme compared to healthy people. (Muhammad et al., 2013).

ALP is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines, or from leukocytes (Fishman, 1990). Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface (Moss, 1997; Schlaeger et al., 1982). Abnormal ALP levels may also be a sign of metastatic
cancer of the liver, lymphoma. In some of these situations ALP levels may be markedly elevated and the only sign of liver involvement. In these cases, liver ultrasound examination is extremel important when the patient history is not suggestive of disease, (Pratt & Kaplan, 2000; Gopal & Rosen, 2000)

3-GPT(AIL)
The results of the current study showed that there was an increase in significant differences at (P < 0.05) in GPT in patients compared to healthy people, where the level of GPT in healthy was (15.3826 ± 0.041) and in patients (40.622 ± 0.158), and LSD was (0.88) between groups, figure (3).

ALT is an enzyme found primarily in the liver but also in the heart and other tissues, it is more useful in diagnosing liver function than SGOT (Warnes et al., 1982). show the results of ALT activity this increase in ALT were generally transient and occurred more commonly in patients with hepatitis C (Cohi et al., 2000). ALT activity was elevated in all Thalassemic patients, which is due to the symptoms of liver damage (Dabrowska et al., 2001). As the major site of iron storage, the liver is a conspicuous victim of excess iron depositon (Bonkovsky, 1991). Transfusional iron overload occurs with severe conditions, conditions that fulfill this criteria include Thalassemia major (Capron et al., 1984), β-Thalassemia major is associated with varying degree of liver damage which cause the elevated plasma transaminases activities in those patients (Lucarelli et al., 1990; Poonknzhali et al., 1999).

Elevated levels of ALT are used largely as markers for liver damaged and impaired liver functions (Telfer et al., 2000). Previous studies reported high elevated levels of ALT among thalassemic patients in association with multiple blood transfusions (Galanello and Origa, 2010; Telfer et al., 2000 & Tienboon et al, 1996).
Among patients with chronic infection 5% - 20% have been reported to develop cirrhosis, thalassemia major, other liver diseases, clinically identified cirrhosis, contraindication for liver biopsy. The elevation of serum alanine aminotransferase (ALT) levels in prediction of severity of liver injury in patients with chronic hepatitis C is debated (Dobrowska et al., 2001). These results were agreement with other studies (Lucarelli et al., 1990)(Poonknzhali et al., 1999).

4-Aspartate aminotransferase (GOT)(AST)
The results of the current study showed that there was an increase in significant differences at (P < 0.05) in GOT in patients compared to healthy people, where the level of GOT in healthy was (22.0824 ± 0.08) and in patients (45.8626 ± 0.279), and LSD was (0.44) between groups, figure (4).

![GOT (U/L)](image)

**Fig (4) level of GOT between healthy and patients**

AST is an enzyme found primarily in the liver, heart, kidney, pancreas, and muscles,, decreased levels can be found in vitamin B deficiency (Liu & Olivieri, 1994) AST is widely distributed in the heart, liver, kidney and erythrocytes, and damage to any of these tissues may cause raised levels (Zilva & Pannall, 1984) show the result of GOT activity in serum Elevated activity of GOT in serum of patients compared to and contro. The effect of iron overload on heart cause congestive cardiomyopathy and other problems i.e. (pericarditis, restrictive cardiomyopathy, and angina without coronary artery disease (Liu & Olivieri, 1994). Liver fibrosis and cirrhosis are well known complications of thalassemia. Transaminases are expressed as multiplied by the upper level of the normal range to identify the role of iron overload in the natural history of liver fibrosis (Harmatz et al., 2000). These results are in agreement with other studies (Ansor & Kooloobandi, 2002), (Cunningham et al., 2001)
Antioxidants Profiles

1- MDA

The results of the current study showed that there was an increase in significant differences at (P < 0.05) in MDA in patients compared to healthy people, where the level of MDA in healthy was (1.6156 ± 0.004) and in patients (5.4964 ± 0.024), and LSD was (0.08) between groups, figure (5).

Fig (5) level of MDA between healthy and patients

Show the results of MDA in both patients groups and control. There was a high significant elevation in (MDA) in all Thalassemia patients. Platelet derived (MDA) may be a useful test of membrane damage in different patient with Thalassemia(Jewell and Marcus, 1984). Oxidative reaction in β-Thalassemia patients are involved in premature cell removal and anemia (Travazzi et al., 2001) The iron overload in β-Thalassemic patients generates oxygen free radicals and peroxidative tissue injury (Cighetti et al., 2001).

Free and total (MDA) levels were higher in the patients of Thalassemia major than in the other type. The results confirm the peroxidative status generate by iron overload in Thalassemia patients the rapid formation of marked a mounts of free (MDA) despite the chelation therapy in Thalassemia major patients (Cighetti et al., 2001). The generation of (ROS) is a steady-state cellular event in respiring cells. Their production can be grossly amplified in response to a variety of pathophysiological conditions. The release of hemoglobin during hemolysis and the subsequent therapeutic transfusion in some cases lead to systemic iron overloading that further potentiates the generation of ROS (Chan et al., 1999) resulting in peroxidative damage to membrane lipids and proteins (Hershko et al., 1998). The results of serum MDA levels in this study agreed the results obtained by Das who found that there was a statistically significant increase in the level of serum MDA in patients as compared to controls (Das et al., 2004) Our results
were also coincide with the result obtained by Cighetti who concluded that there was a marked amounts of free MDA produced due to iron overload in patients despite the chelating agents taken by them. In the TM patients serum ferritin levels showed a relationship with the free serum MDA levels in parallel manner, while the total MDA level correlated positively with the serum level of non-transferrin binding iron NTBI (Cighetti et al., 2002).

2- Catalase (CAT)

The results of the current study showed that there was an decrease in significant differences at (P < 0.05) in CAT in patients compared to healthy people, where the level of CAT in healthy was (0.621 ± 0.004) and in patients (0.2138 ± 0.003), and LSD was (0.041) between groups, figure (6).

![Fig (6) level of CAT between healthy and patients](image)

Human erythrocytes are normally rich in catalase (Hugo, 1974). Catalase can be inhibited by ascorbate, and sunlight under aerobic conditions and also by peroxidase (Kurata et al., 1993). The findings of this study (Al-Mudalal et al., 2005) indicate that a possible explanation for lower red cell catalase activity found in the more severe genotype of beta thalassemia is that the greater amount of hydrogen peroxide might produce direct toxic damage to catalase (Kirkman & Gaetani, 1984; Eaton et al., 1972). This study showed significantly lower red cell catalase activity (although higher than normal subjects) in patients with the more severe form of the disease expressed both as per g Hb and per ml red blood cells and that was similar to other study (Prasartkaew et al., 1986).

3- GST (Glutathione-s-Transferase)(GSH)

The results of the current study showed that there was an decrease in significant
differences at (P < 0.05) in GSH in patients compared to healthy people, where the level of GSH in healthy was (2.5696 ± 0.058) and in patients (0.9966 ± 0.00666), and LSD was (0.04) between groups, figure (7).

As GST enzyme is a biotransformation enzyme with many functions such as detoxification (Hayes et al., 1997).

4- Superoxide dismutase (SOD)

The results of the current study showed that there was a decrease in significant differences at (P < 0.05) in SOD in patients, compared to healthy people, where the level of SOD in healthy was (2.179 ± 0.004) and in patients (1.037 ± 0.039), and LSD was (0.11) between groups, figure (8).
Changes in the oxidant-antioxidant equilibrium in cells are caused by SOD, an intracellular enzyme. The role of enzymes is to catalyze chemical reactions. Ion free radicals, particularly $O_2$, are converted to $H_2O$ (Fukai & Ushio-Fukai, 2011). Because of the situation of iron excess (due to transfusions and poor erythropoiesis), massive free radicals accumulated in thalassaemia patients. Through the Fenton reaction, iron (Fe) can speed up the conversion of molecular oxygen to reactive oxygen radicals, superoxide, and hydroxyl groups. (Winterbourn, 1995; Shazia et al., 2012). According to the investigations, Patne et al. presented results in 2012 revealing that the levels of erythrocyte antioxidant enzymes, particularly SOD activity, fell significantly in transfusion-dependent patients (Patne et al., 2012).

Another study found a link between low antioxidant levels and the severity of pain and clinical presentation. (Kattamis et al., 2011). All of these studies imply that receiving a monthly blood transfusion lowers SOD levels. Other study centers, on the other hand, came up with different conclusions. Simsek and colleagues discovered that thalassemic patients had greater SOD levels than healthy controls and careers, but lower vitamin E levels. (Şimsek Orhon et al., 2005). According to other studies, there were no significant differences in SOD levels between healthy controls and thalassemic subjects. (Ghone et al., 2008). Antioxidant levels, particularly SOD, rise in a variety of situations, including an acute inflammatory phase, a state of shock, and exposure to high doses of pro-oxidants. The rise was linked to a compensatory mechanism aimed at breaking down free radicals produced by oxidative stress and lipid peroxidation. (Lü et al., 2010).

In an achronic clinical condition, deterioration was linked to the antioxidant system’s incapacity to adjust for excessive originators. The system was unable to counteract free radicals, which may have resulted in the breakdown of proteins (including enzymes) and cell membranes, lowering antioxidant enzyme numbers and function. (Willcox et al., 2004).

**Ferritin**

The results of the statistical analysis showed a significant increase of ($P<0.05$) in FER in a group of thalassemia patient grand beta type compared to the control group. If the average ferritin level in the control group was $(45.0736 \pm 0.134)$ and in patients $(769.9766 \pm 2.899)$, and LSD was $(4.96)$ between groups, As shown in the figure (9).
It shows the level of ferritin (ng/ml) in the blood serum of patients with beta thalassemia. Due to the low level of hemoglobin in thalassemia patients, as it reaches less than 8 g/dL, the use of blood transfusions to keep the hemoglobin level at a reasonable level (Mashaali et al., 2014). Therefore, the percentage of ferritin increases in patients with thalassemia major, which is attributed to the accumulation of ferritin as a result of frequent blood transfusions in patients, and ferritin represents the excess iron stores in the body (AlAthari and Mahdi, 2019). Ferritin binds with iron and is transported and stored in a non-toxic and soluble form (Mashaali et al., 2014). Iron and ferritin levels in the blood are positively correlated, which is why the ferritin concentration is used in the blood is usually used to account for iron overload in thalassemia patients (Porter and El-Beshlawy, 2011). Aziz and others (2009) explained that the increase in iron comes from increasing blood transfusions for the urgent need for them, and then the iron works.

Excess of free iron ions that stimulate oxidative stress that deplete the antioxidants in cell, which causes great damage to the organelles and cell membranes. A blood transfusion provides the body with about 1 mg of iron per ml of blood, which impairs the elimination of iron in the body in exchange for increasing the absorption of iron in the small intestine (Sinha ray et al., 2017). In addition to the increase in iron from continuous blood transfusion for thalassemia patients, iron also increases through its absorption in the digestive system, and its accumulation may exceed the body’s ability to detoxify ferritin (Asif et al, 2017). This was confirmed by a study (Jawad, 2016).

One of the secondary reasons for the increase in the amount of iron is the inability or efficiency of the medicines used to get rid of iron, the accumulated iron leads to an increase in oxidants and thus damage to the membranes and organelles of red blood cells in the stages of their formation (Pavlova et al., 2007).
was observed through the current results that the level of ferritin increased in beta thalassemia. Compared with the control group, these results agreed with (ElLehleh, 2017)

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