Evaluation of Liver Enzymes, Interleukin-6 and Tumer Necrosis Factor-Alpha in Children Suffering from Thalassemia and Treated With Deferoxamine and Deferasirox Drug in Kirkuk City

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Abstract

Background and objective: Thalassemia It is a genetic disease caused by a disorder in the synthesis of the globin chain that leads to a low level of hemoglobin, and frequent blood transfusions lead to an overload of iron, which interferes with the metabolism and leads to tissue and organ damage. This study aimed to evaluate the effect of the iron chelator deferasirox deferoxamine on liver enzyme activities (aspartate transaminase, alanine transaminase), the level of the cytokines interleukin-6 and tumor necrosis factor-alpha in the serum of thalassemia patients.

Methods: The study was conducted in the Thalassemia Center at Azadi Teaching Hospital, Kirkuk , Iraq , from November 2021 to April 2022 . It included 90 Thalassemia patients (Group 1: 30 patients treated with deferrocirox , Group 2: 30 treated with deferoxamine , Group 3: 30 who were not treated with any iron chelator) and 20 healthy individuals (control group) and their ages were (1-6 years).

Results: Patients treated with deferoxamine had higher serum levels of AST, ALT, IL-6, TNF-α compared to deferoxamine-treated and untreated patients and the control group.

Conclusion: Our results in our current study showed that both the iron chelators deferasirox and deferoxamine affect liver enzymes and raise the levels of cytokines, although deferasirox was more effective than deferoxamine.

Keywords: Deferasirox , Deferoxamine , Liver enzymes IL-6, TNF-α

1. Introduction.

Thalassemia is a Greek word consisting of two syllables: Thalassa, which means sea, and aemia, which means blood. It is a genetic blood disease that is transmitted from parents to children and affects the formation of hemoglobin in the children's blood, causing severe anemia [1]. Thalassemia was first recognized clinically in 1925 when Thomas Cooley described the syndrome of anemia, splenomegaly, and bone abnormalities among Italian offspring [2]. This disease is also known as Mediterranean anemia due to its widespread prevalence in the Mediterranean regions, and it is a disease mainly caused by point mutations of the clobin genes, and it is one of the most common disorders in the world, and there are about 1.67% of the total population Carriers of Thalassemia, and this disease spreads in the Mediterranean coast, the Middle East and Southeast Asia [3].

Thalassemia affects both males and females [4]. This disease has become one of the most prevalent genetic diseases, causing huge public health problems in many regions of the world [5]. It is characterized by the absence or production of low amounts of hemoglobin, the oxygen-carrying protein in red blood cells. Studies have shown that the hemoglobin molecule consists of two alpha and beta chains. This disease results from a decrease or absence in the synthesis of these chains [6].

Thalassemia has been classified into two types, the first is alpha thalassemia [7] and the second is beta thalassemia, depending on the type of affected Clopin chain [8]. Blood transfusion is a major reason for the continuation of the lives of people with thalassemia, but serious and varied complications occur due to repeated blood transfusions, which may lead to hepatotoxicity due to excess iron deposition, and this iron is stored in the form of ferritin, which is harmful to a large extent, which leads to an imbalance in the functions of organs [9].

To prevent complications caused by iron, chelating drugs should be used, including iron chelators Deferoxamine, Deferipron and Deferasirox with repeated blood transfusions in anemia in beta thalassemia major and central [10]. The first iron chelate used was deferoxamine, which is given by injection (intravenously IV or subcutaneously SC) [11]. Therefore, due to the presence of several negative effects in this type of treatment, including reactions at the injection site and systemic reactions, it was necessary to use an oral chelator, deferiprone, the first iron chelator to be taken orally, to chelate the excess iron by binding to iron, which will be Excreted through urine [12].

Deferiprone leads to many adverse effects such as

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agranulocytosis due to myelotoxicity, stomach disorders, liver disorders, and joint pain [13]. In 2005, a new chelator was introduced that is more effective and has relatively fewer negative effects compared to deferiprone. Deferasirox is a new iron chelator that is taken orally for the treatment of iron overload in beta thalassemia major and middle. Digestive problems are one of the side effects of deferasirox, and symptoms include abdominal pain, nausea, vomiting, diarrhea, skin rashes, increased liver enzymes, metabolized by the liver and later excretion of faeces. [14].

Furthermore, β-thalassemia major and meso- are associated with several immune disorders due to defects in phagocytosis and lymphocyte functions and these changes result in iron overload, splenectomy, and repeated exposure to antigens through frequent blood transfusions. [15].

2. Patients and Methods.

This study was conducted in Azadi Teaching Hospital in Kirkuk Governorate, Iraq, from December 2021 to April 2022. This clinical study was conducted on patients with Thalassemia. Oral informed consent was obtained from the patient's family to enroll in this study.

3. Study design.

The study included 90 patients with beta-thalassemia, who were treated with frequent blood transfusions, deferasirox and deferoxamine. The patients were selected from referrals to the Thalassemia Center at Azadi Teaching Hospital. The patients were divided into three groups according to the type of treatment, the group:1 consisted of (30) transfusion-dependent patients, all receiving deferasirox (Exjade®) orally, iron chelate at a dose of 20 mg/kg per day, group 2: consisted of (30) patients Transfusion-dependent thalassemia receiving iron-chelating agent deferoxamine (Desferal®) at a dose of 20-40 mg/kg per day several times per week, Group 3: comprised of (30) transfusion-dependent beta-thalassemia patients who were not on treatment Iron chelators, aged 1-6 years regardless of gender, and all patients were diagnosed by a pediatrician. Patients were examined at the Thalassemia Center, which is a major center to serve Thalassemia patients, and the following symptoms showed that they had the disease, including pallor of the face, protrusion of the facial bone, protrusion of the upper incisors and neck ligaments from the frontal bones, as well as a family history of thalassemia.

2.2- Control group

The study included a group of healthy people, numbering 20 people, aged between 1-6 years, who were found to be uninfected after conducting laboratory tests on them by a specialist doctor and who had no family history of hereditary blood diseases.

2.3- Methods.

2.3.1- Liver function parameters.

Determination of Serum Aspartate Aminotransferase (AST)and Alanine Aminotransferase(ALT) was determined using Mindray BS-200 and was done according to the manufacturer's instructions in the AST,ALT Kit (IFCC method) without pyridoxal phosphate activation .

2.3.2- Immunological parameters.

Determination of serum human interleukin-6 and tumor necrosis factor-alpha levels by enzyme-linked immunosorbent assay (ELISA kit) performed according to the instructions of the manufacturer Elabscience (USA).

4. Statistical analysis

In this study, the results include mean ± SD and significant differences (P.Value) between groups examined by SPSS (Software Statistical package for social science) in conducting statistical analysis where ANOVA and news (f) were used to determine the presence or absence of significant differences. According to groups whose number is more than two groups, count the significant difference at the 0.01 probability leve.

5. Results and Discussion

3.1- Liver enzymes activity in the serum The results shown in Table (1) showed a significant increase ($p \leq 0.01$) in the activity of liver enzymes (AST) aspartate aminotransferase (ALT) and alanine aminotransferase (ALT) in the group of thalassemia patients treated with iron chelators deferoasirox (33.62 ± 8.39) (30.88 ± 8.45 and diferoxamine (72.65 ± 8.64) (63.22 ± 7.39) and those who did not abuse any chelators (22.69 ± 7.21)) (22.69 ± 7.21) compared with the control group (16.77 ± 3.81) (18.11 ± 5.13) units/liter. The results also showed that there was a significant increase among the group taking deferasirox treatment with deferoxamine compared with the non-users of iron chelators in patients with thalassemia.

Table (1) Measuring the activity of liver enzymes AST, ALT (Different letters mean there is a significant difference when ($p \leq 0.01$)Mean±SD

Groups Parameters				
	Control groups healthy	group:1	group:2	group:3
	20	30	30	30
AST (U/L)	16.77±3.81 d	33.62±8.39 b	72.65±8.64 a	23.10±5.60 c
ALT (U/L)	18.11±5.13 d	30.88±8.45 b	62.22±7.39 a	22.69±7.21 c

The results of the current study agreed with the findings of Choi et al. [16], who indicated that there was a significant increase in the activity of liver enzymes (AST) and (ALT) among patients taking deferasirox and deferoxamine treatments and non-users compared to the control group, and it was found that there was a significant increase among the drug users deferasirox and deferoxamine compared with patients who were not taking iron chelators.

Liver enzymes (AST) and ALT are two enzymes that belong to the family of transaminase enzymes. These enzymes are elevated due to liver disease. When certain types of liver cells are damaged, these enzymes leak into the bloodstream and can be measured as indicators of liver cell damage due to excess iron, causing Leads to elevated liver enzymes [17]. As excess iron is one of the disorders

that thalassemia patients suffer from, which causes chronic hepatitis, cirrhosis and liver cancer due to frequent blood transfusions that lead to the accumulation of excess iron, as the high level of liver enzymes in the blood ALT, AST are positively correlated With the high level of ferritin in the blood, which in turn depends on the excess iron in thalassemia patients [18].

The elevated levels of ALT and AST among thalassemia patients who permanently receive transfusions are due to excessive hemolysis, otherwise due to the need to synthesize peptide chains through the activity of these enzymes in the transport of amine groups and thus, are more common in many tissues of the body. Like the heart, liver, and kidneys, they are largely deposited due to excessive amounts of iron in the organs, which leads to cell death due to the formation of free radicals that attack all vital molecules such as lipids, proteins and cellular DNA [19].

In addition, deferasirox is effective in improving hepatic febrile disease, as a significant decrease in serum ferritin concentration was observed in thalassemia patients taking deferasirox, which leads to a decrease in ALT and AST due to the iron chelator deferasirox has a longer half-life as its effect lasts much longer After dosing [20]. In contrast to users of deferoxamine, which has a short half-life and also causes side effects upon injection, which leads to non-compliance with it properly [21].

3.2- level average Interleukin-6 and tumor necrosis factor-alpha

The results shown in Table (4-12) show a significant increase ($p \leq 0.01$) in the average levels of IL-6 and TNF-alpha in the groups of Thalassemia patients treated with iron chelators deferrosirox (3.60 ± 0.98) (5.78 ± 1.18) and diferoxamine (6.10 ± 1.21) (10.76 ± 2.24) and non-addicts to any chelator (4.47 ± 1.45) (7.04 ± 1.68) respectively compared to the control group (0.90 ± 0.38) (1.97 ± 0.61) (pg/ml) ,The deferoxamine group showed a significant increase in comparison with the group of patients taking deferrosirox and non-administering of iron chelator in patients with thalassemia.

Figure (2) average level of interleukin-6 necrosis factor alpha (Different letters mean there is a significant difference when ($p \leq 0.01$).

Groups Parameters	Mean±SD			
	Control group) healthy(	group:1	group:2	group:3
	20	30	30	30
IL-6 (pg/ml)	0.90 ± 0.38 d	3.60±0.98 c	6.10±1.21 a	4.47±1.45 b
TNF-α (pg/ml)	1.97 ± 0.61 d	5.78±1.18 c	10.76±2.24 a	7.04±1.68 b

The results of the current study were in agreement with the findings of Noori et al. [22], Sarhat et al. [23], as they indicated that there was a significant increase in patients taking deferoasirox and deferoxamine and non-administering of the two treatments compared to the control. It was found that there was a significant increase in the average concentration of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in patients taking deferoxamine iron chelator compared with patients taking deferoasirox and non-users.

Interleukin-6 (IL-6) is a cytokine involved in the systemic inflammatory response, is also involved in local tissue inflammation and promotes T helper cell differentiation into Th17 cells consisting of macrophages and T lymphocytes which plays an important role in the immune response [24], Tumor necrosis factor-alpha (TNF-α) is a cytokine with a large variety of properties involved in the activation of white blood cells and endothelial cells [22]. TNF performs many immune and inflammatory functions, and TNF-alpha cells produce macrophages and monocytes when stimulated by stimuli in the body. TNF is also produced by B and T lymphocytes in response to the stimulating antigen [25].

The current study indicated an increase in the levels of IL-6 and TNF-α in the serum of thalassemia patients compared to the healthy ones, as thalassemia patients suffer from a chronic inflammatory condition, as iron overload may play a major role in releasing IL-6 and TNF-α In thalassemia patients an elevated serum level of IL-6 is relevant to the pathophysiology in beta thalassemia patients, and it is likely that the increased production of IL-6 is due to overstimulation of macrophages which contributes to abnormalities in iron metabolism (27), The increase in TNF_α levels is a reason for activation of macrophages due to iron overload and stimulation of macrophages due to repeated transfusion treatment, which are erythrocyte progenitors, which contributes to the formation of inactive erythrocytes [26].

Repeated blood transfusions and iron chelator treatment improved thalassemia patients, but excess iron and its toxicity led to other health problems, as excess iron leads to increased oxidative stress and inflammation in thalassemia patients [27]. The resulting increase in NTBI has been associated with an increase in reactive oxygen species (ROS), which leads to ROS activation resulting in the production of pro-inflammatory cytokines such as TNF-α, and TNF-α causes direct injury to compromised cells, facilitating the activation of mononuclear cells and the production of cytokines such as interleukin- 6, as iron leads to the formation of free radicals, which is a major cause that leads to cell damage and death [28]. reported that deferasirox is an effective chelating agent in thalassemia major and significantly reduces IL-6 and TNF-α compared to the iron chelator deferoxamine [29]. These results can be explained by the inability of deferoxamine to consistently control LPI and NTBI levels due to its short plasma half-life, [30] compared to deferasirox, which has a longer half-life as its effect lasts much longer after doses. That the longer half-life of deferasirox iron chelate reduces inflammation by better controlling LPI and NTBI levels after chelation administration. The continued presence of iron chelate in plasma may help avoid iron overload, thus preventing iron-related morbidity and mortality [31].

However, it appears that poor compliance of deferoxamine-treated patients is the primary reason for elevated levels of these cytokines, because patients who

comply well with deferoxamine do not have a significant difference in serum IL-6 and TNF-α levels compared to deferasirox, So the benefits of good compliance with deferoxamine over poor compliance were not only lowering serum ferritin but also reducing an inflammatory marker that was elevated in patients with poor compliance, and this indicates that both iron chelators are effective in reducing these markers if children with thalassemia comply well with treatment [32].

6. Conclusion

The results of our current study showed that both deferasirox and deferoxamine iron chelators affect liver enzymes and raise their levels and elevate cytokine levels, although deferasirox was more effective than deferoxamine. The fact that children who take deferoxamine suffer from poor compliance as it causes a group of symptoms for the disease in addition to the fact that chelate has a short half-life in contrast to deferasirox, which is generally good as it is safe, no symptoms appear on patients while taking it, and it has a longer half-life than deferasirox. Therefore, it is recommended to use deferasirox orally.

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