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Study the Lipoxygenase Enzyme Activity on Patients With Thalassemia in Kirkuk Governorate

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Abstract: *This study was conducted at the Thalassemia Center of Azadi Educational Hospital, Kirkuk Health Directorate, Iraq, from June 1, 2024, to October 1, 2024. The study sample included 75 individuals, both males and females, aged between 4 and 67 years, divided into two groups: the first group comprised 50 thalassemia patients, while the control group included 25 healthy individuals. The results indicated a significant effect of thalassemia on the studied variables, as the disease led to increased levels of white blood cells, erythrocyte sedimentation rate, total iron-binding capacity, malondialdehyde, iron concentration, and lipoxygenase enzyme, recording values of 10.9 × 10^3 cells/mL, 25.36%, 321.84 µg/dL, 721.12 mmol/L, 2788.9 ng/dL, and 226.2 IU/L, respectively. In contrast, thalassemia reduced hemoglobin levels to 8.57 g/L. The gender category significantly affected both malondialdehyde and iron concentrations, while the age category significantly impacted white blood cell levels, total iron-binding capacity, serum iron, and lipoxygenase enzyme. Among the interactions, the most influential factor was found in the group of female patients over 30 years old, who recorded the highest significant concentrations of white blood cells and malondialdehyde, reaching 12.26 × 10^3 cells/mL and 1090 mmol/L. Conversely, female patients under 15 years showed the highest significant averages for the percentage of erythrocyte sedimentation rate and iron concentration, at 28% and 3270 ng/dL, respectively. Meanwhile, female patients aged 15-30 years exhibited the highest significant concentrations for total iron-binding capacity and lipoxygenase enzyme, at 361.8 µg/dL and 234 IU/L, respectively. Male patients aged 15-30 years recorded the lowest significant hemoglobin concentration, which was 7.33 g/L.*

Keyword: *Lipoxygenase, Thalassemia, Kirkuk.*

1. INTRODUCTION

Thalassemia is one of the most common groups of autosomal recessive genetic disorders globally. It is characterized by reduced or absent production of hemoglobin in red blood cells and chronic anemia of varying degrees of severity (Marengo-Rowe, 2007). The epidemiology of the different forms of thalassemia is not well understood. However, the disease is widely prevalent in regions extending from sub-Saharan Africa, through the Mediterranean, the Middle East, to the Indian subcontinent and East and Southeast Asia (Weatherall, 2018). Additionally, ongoing and recent population migrations mean that thalassemia can now be found in Northern and Western Europe and North America, making this disease a global health issue (Kattamis *et al*., 2020).

Thalassemia, a common single-gene hereditary disorder, mainly consists of alpha thalassemia and beta thalassemia (β-thal). Point mutations or deletions in the beta-globin gene cluster, located on human chromosome 11 at position p15.3, lead to β-thal by reducing or eliminating beta-globin synthesis (Taher *et al*., 2018). Hemolytic anemia is the primary pathological manifestation of beta thalassemia, occurring due to two reasons: early destruction of erythroid progenitor cells and a shortened lifespan of mature red blood cells in circulation

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(Fibach and Dana, 2019). Currently, beta thalassemia is mainly treated through blood transfusions, iron chelation, stimulation of fetal hemoglobin synthesis, bone marrow transplantation, and gene therapy (Ali *et al*., 2021).

Imbalance in the production of alpha globin chains compared to beta globin chains leads to the oxidation and deposition of alpha globin chains in the membranes of red blood cells, resulting in membrane destruction and hemolysis. The inhibition of alpha globin chain oxidation prevents the deposition of alpha globin chains through the binding of AHSP, as observed in several studies. Additionally, some antioxidant compounds exhibit similar effects (Che Yaacob *et al*., 2020).

Thalassemia patients experience iron accumulation because the body lacks an iron excretion system. Iron can act as an electron carrier and a cofactor for various processes, such as the Fenton reaction (Gattermann *et al*., 2021). These reactions often lead to the production of the hydroxyl radical, known as reactive oxygen species (ROS), which can cause oxidative stress and lipid peroxidation when their levels exceed those of antioxidants (Sousa *et al*., 2020). Excess iron in the body can damage organs, leading to conditions such as cardiac dysfunction, arrhythmias, cardiomyopathy, iron deposition, delayed sexual maturation, liver fibrosis, liver cancer, hepatitis, sexual dysfunction, infertility, and metabolic disorders. Therefore, it is crucial to manage the levels of bioavailable iron ions due to iron overload to prevent serious complications (Koonyosying *et al*., 2020).

Lipoxygenase (1.13.11.12 EC) is a class of non-heme iron oxidative enzymes expressed in various cell types, including endothelial, epithelial, and immune cells (Shahid *et al*., 2021). These enzymes catalyze the peroxidative oxidation of polyunsaturated fatty acids (PUFAs) that contain cis-cis double bonds by adding oxygen atoms to form corresponding hydroxy derivatives (Kuhn *et al*., 2015). Lipoxygenase enzymes are found in plants, fungi, bacteria, and mammals, participating in various biochemical and physiological processes (Shukla *et al*., 2020). LOX is primarily expressed in reticular, eosinophilic, epithelial, and connective tissue cells, with its main source being leukocytes, particularly neutrophils and monocytes (Vaezi *et al*., 2021).

The LOX enzymes are divided into five categories based on the site where the oxygen group enters, which are (5, 8, 11, 12, 15) (Vaezi *et al*., 2021). These forms participate in natural balance, inflammation, and pro-resolution response. There are two different types of isoenzymes - LOXs 12 based on tissue distribution differences: 12-ALOX is found in platelets and skin, while ALOX-12B is often found in skin and epidermis. Additionally, 15-LOX has two isoforms: 1-15-LOX is found in citrus, leukocytes, macrophages, epithelial cells, and

corneal cells, whereas 15-2-LOX is primarily found in skin, prostate, lung, and cornea (Wood *et al*., 2020).

The catalytic reaction of lipoxygenase (LOX) enzymes involves one-electron oxidation by an iron atom at the active site, converting ferric ion (Fe+3) to ferrous ion (Fe+2). The oxidation of the fatty acid substrate occurs by abstracting hydrogen from bis allylic methylene to yield a pentadienyl radical, which is then rearranged to provide a (1-cis, 3-trans-conjugated diene moiety) and introduce the specific oxygen at the pentadienyl radical to form a hydroperoxide radical. The hydroxyl radical intermediate is reduced to the corresponding anion, accompanied by the oxidation of iron to Fe+3 (Manda *et al*., 2020).

The current study aims to evaluate the activity of lipoxygenase and some other biochemical variables in thalassemia patients in Kirkuk Governorate.

2. MATERIALS AND METHODS

Study Sample: This study was conducted at the Thalassemia Center affiliated with Azadi Educational Hospital - Kirkuk Health Directorate, Iraq, from June 1, 2024, to October 1, 2024. The study sample included 75 individuals, both males and females, aged between 4 and 67 years, divided into two groups: the first group consisted of 50 thalassemia patients, and the control group included 25 healthy individuals.

Blood Collection: Five milliliters of blood were drawn using a butterfly needle through venipuncture and collected in glass tubes within a gel separation tube. The tubes were centrifuged for 10 minutes at 1500 RPM. The serum was separated from the cells, and the pale layer was removed. The serum was then collected for testing some targeted biochemical parameters in this study.

Studied Variables

White Blood Cell Count (WBC): The white blood cell count is performed using a hemocytometer. A specific volume of blood is diluted with Turk's Solution, which lyses red blood cells and stains the nuclei of white blood cells, facilitating their counting and differentiation. This dilution is crucial for reducing cell density, making individual cells easier to observe under a microscope. Blood is drawn to the 0.5 mark, then diluted with the solution up to the 11 mark. The diluted blood is mixed thoroughly before placing a drop on a microscope slide cover (Talaro, 2005).

Determination of Hemoglobin Concentration: To determine the concentration of hemoglobin, add 5 ml of Drabkin's solution, which has been dissolved in a small amount of distilled water (prepared from 1 g of sodium bicarbonate and 50 mg of potassium cyanide, with

the volume filled to 1 liter with distilled water), to a test tube. Then, add 0.02 ml of the collected blood, which has been preserved with the EDTA anticoagulant. Allow the mixture to stand for 10 minutes before measuring the absorbance using a spectrophotometer at a wavelength of 540 nm (Powers, 1989).

Measurement of Packed Cell Volume (PCV): For measuring Packed Cell Volume (PCV), a small amount of the anticoagulated blood is taken and placed in a PCV capillary tube, filling it two-thirds full. One end of the tube is sealed, and it is then placed in a microhematocrit centrifuge for 5 minutes at a speed of 5000 RPM. After centrifugation, the PCV is read using a hematocrit reader, which indicates the percentage of packed red blood cell volume relative to the total blood volume (Turgeon, 1990).

Determination of Total Iron Binding Capacity (T.I.B.C): The Total Iron Binding Capacity (T.I.B.C.) was determined by adding a sufficient amount of Fe3+ to saturate the iron-binding sites on apotransferrin. Excess Fe3+ was then removed through adsorption using basic magnesium carbonate powder. Following this, centrifugation was performed, and the remaining bound iron in the supernatant was measured using the direct method REF 92108 (Ferene) (TIETZ, 1999).

Estimation of Malondialdehyde Levels: The concentration of malondialdehyde in serum was estimated through the reaction between lipid peroxides (primarily malondialdehyde) and thiobarbituric acid (TBA). This reaction occurs in an acidic medium, producing a colored product, and the absorbance is measured at a wavelength of 532 nm (Mulish *et al*., 2002).

Determination of Iron Concentration in Blood Serum: The concentration of iron in serum was determined using a colorimetric method with ready-to-use analysis kits from the French company BIOLABO. This method involves separating ferric ions from the iron-carrying transferrin protein in an acidic medium, followed by reduction to ferrous ions. The ferrous ions then form a colored complex with Ferene, which exhibits absorbance at a wavelength of 600 nm; the intensity of the resulting color is proportional to the concentration of iron (Hennesy *et al*., 1984).

Determination of Lipoxygenase Activity in Serum: The activity of lipoxygenase (LOX) was measured following the method described by researchers Shastry & Rao (1975), which involves the oxidation of the substrate (linoleic acid) catalyzed by the enzyme to add two oxygen atoms. The increase in absorbance resulting from the formation of conjugated dienes was monitored at a wavelength of 234 nm for 5 minutes. The molar absorptivity (E) of the conjugated dienes is 25,000 $molar^{-1}$ cm^{-1}.

Statistical Analysis

After collecting the necessary data for the studied variables, it was entered into a computer and organized using Microsoft Office Excel. Statistical analysis and comparisons of the means of the variables among the studied groups and categories were conducted using Duncan's multiple range test with the Statistical Analysis System (SAS). Graphical representations were also created using Microsoft Office Excel (Al-Zubaidi and Al-Jubouri, 2022).

3. RESULTS

White Blood Cells (WBC): Figure (1) shows the individual effect of the study factors on white blood cell levels. A significant impact of thalassemia on the concentration of these cells is observed, with the patient group recording the highest concentration at 10.9 × 10^3 cells/mL, which is significantly different from the healthy group that recorded 3.07 × 10^3 cells/mL. As for the effect of gender, no significant impact was found on white blood cell levels within the study sample. However, a significant effect of age was noted, with the age group over 30 years recording the highest average of white blood cells at 9.75 × 10^3 cells/mL.

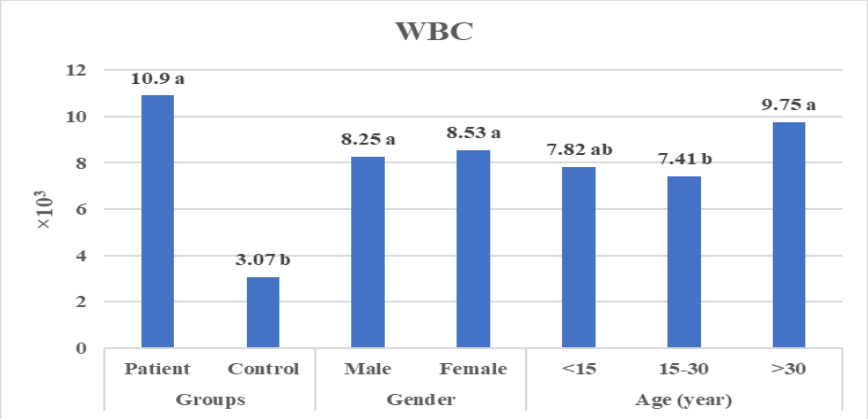

Figure (1): Individual Effects of Study Factors on White Blood Cell Concentration.

Table (1) shows the Interaction effects of the study factors on white blood cell concentration among the study sample, where significant effects are noted according to the results of the Duncan multiple range test at a significance level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the highest significant concentration of white blood cells at 10.99 × 10^3 cells/mL. In the interaction between the groups and age category, patients under 15 years recorded the highest significant concentration at 11.59 × 10^3 cells/mL. For the interaction between the patients' gender and age, the group of women over 30 years recorded the highest significant concentration of white blood cells at 10.4 × 10^3 cells/mL. The Interaction effect of the three factors under study showed that

female patients over 30 years recorded the highest significant concentration of white blood cells at 12.26 × 10^3 cells/mL.

Table (1): Interaction Effects of Study Factors on White Blood Cell Concentration.

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	11.92 a	9.78 ab	10.84 a	10.99 a
	Female	9.6 ab	9.23 ab	12.26 a	10.78 a
Control	Male	1.23 c	1.4 c	5.05 bc	2.37 b
	Female	4.5 bc	5.05 bc	1.1 c	4.04 b
Group × Age		<15	15-30	>30	-
Patients		11.59 a	9.5 a	11.55 a	-
Control		2.54 b	3.23 b	3.7 b	-
Gender × Age		<15	15-30	>30	
Male		8.36 ab	6.98 ab	9.19 ab	-
Female		6.2 b	7.83 ab	10.4 a	-

- Values followed by the same letter do not differ significantly from each other.

Hemoglobin (Hb): Fig. (2) shows the individual effect of the study factors on hemoglobin levels. A significant impact of thalassemia on the concentration of hemoglobin is observed, with the patient group recording the lowest concentration at 8.57 g/L, which is significantly different from the healthy group that recorded 13.33 g/L. Meanwhile, the effects of gender and age did not show a significant impact on hemoglobin levels within the study sample, and their results were similar.

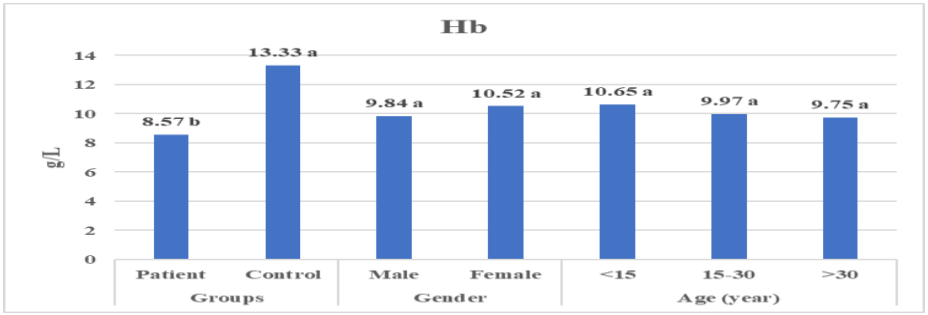

Figure (2): Individual Effects of Study Factors on Hemoglobin Concentration.

Table (2) shows the Interaction effects of the study factors on hemoglobin levels among the study sample, where significant effects are noted at a significance level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the lowest significant hemoglobin concentration at 8.09 g/L. In the interaction between the groups and age category, patients aged 15-30 years recorded the lowest significant concentration at 8.2 g/L. As for the interaction between patients' gender and age, the results

indicated no significant effect from the interaction between these two factors. The Interaction effect of the three factors under study showed that male patients aged 15-30 years recorded the lowest significant hemoglobin concentration at 7.33 g/L.

Table (2): Interaction Effects of Study Factors on Hemoglobin Concentration (g/L).

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	8.22 cd	7.33 d	8.56 bcd	8.09 b
	Female	8.5 bcd	9.08 bcd	9.6 a-d	9.28 b
Control	Male	14 a	14 a	12.5 abc	13.57 a
	Female	14 a	13 ab	11 a-d	13 a
Group × Age		<15	15-30	>30	-
Patients		8.26 b	8.2 b	9.08 b	-
Control		14 a	13.5 a	12.01 a	-
Gender × Age		<15	15-30	>30	-
Male		10.14 a	9.55 a	9.69 a	-
Female		12.17 a	10.38 a	9.83 a	-

- Values followed by the same letter do not differ significantly from each other.

Packed Cell Volume (PCV): Figure (3) shows the individual effect of the study factors on PCV levels. A significant impact of thalassemia on the percentage of packed cell volume is observed, with the patient group recording the highest percentage at 25.36%, which is significantly different from the healthy group that recorded 3.92%. Meanwhile, the effects of gender and age did not show a significant impact on the percentage of packed cell volume within the study sample, with values ranging between 16.83% and 19.62%.

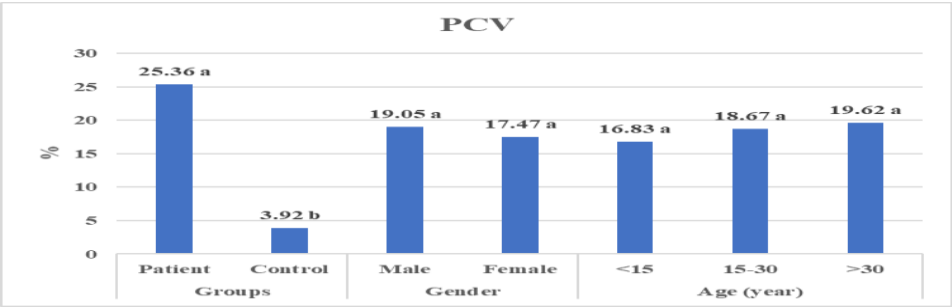

Figure (3): Individual Effects of Study Factors on Platelet Concentration.

Table (3) shows the Interaction effects of the study factors on the percentage of packed cell volume among the study sample, where significant effects are noted at a significance level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the highest significant average percentage of packed cell volume at 25.47%. In the interaction between the groups and age category, patients under 15 years

recorded the highest significant average at 27.14%. For the interaction between patients' gender and age, significant differences emerged, with women over 30 years recording the highest significant average percentage of packed cell volume at 21%. The Interaction effect of the three factors under study showed that female patients under 15 years recorded the highest significant average percentage of packed cell volume at 28%.

Table (3): Interaction Effects of Study Factors on Platelet Concentration (%).

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	27 a	26.75 a	22.6 a	25.47 a
	Female	28 a	24.75 a	25 a	25.2 a
Control	Male	1.67 b	8 b	8 b	5.29 b
	Female	3.5 b	1 b	1 b	2 b
Group × Age		<15	15-30	>30	-
Patients		27.14 a	25.75 a	23.8 a	-
Control		2.4 b	4.5 b	5.67 b	-
Gender × Age		<15	15-30	>30	-
Male		18.56 a	20.5 a	18.43 a	-
Female		11.67 c	16.83 b	21 a	-

- Values followed by the same letter do not differ significantly from each other.

Total Iron Binding Capacity (TIBC): TIBC measures the amount of iron that can be bound by proteins in the blood, particularly transferrin, which transports iron in the body. It is considered an indicator of the availability of iron in the body. If iron levels are low, TIBC is typically elevated. Figure (4) shows the individual effect of the study factors on total iron binding capacity levels. A significant impact of thalassemia on TIBC concentration is observed, with the patient group recording the highest average at 321.84 µg/dL, which is significantly different from the healthy group that recorded 89.33 µg/dL. Meanwhile, the effect of gender did not show a significant impact on total iron binding capacity within the study sample, while a significant effect of age was noted, with the age group between 15-30 years recording the highest average TIBC at 279.5 µg/dL.

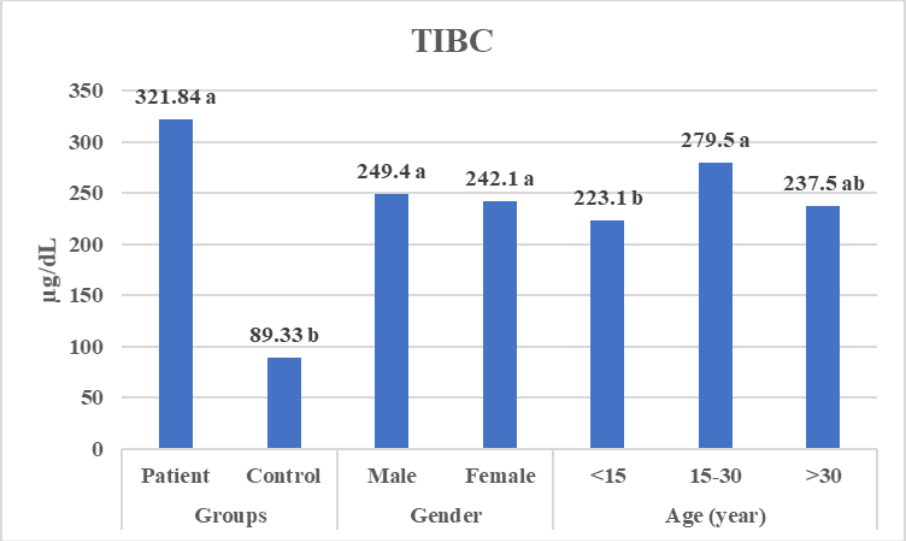

Figure (4): Individual Effects of Study Factors on Blood Iron Levels (TIBC).

Table (4) shows the Interaction effects of the study factors on total iron binding capacity levels among the study sample, where significant effects are noted at a significance level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the highest significant concentration of total iron binding capacity at 327.3 µg/dL. In the interaction between the groups and age category, patients aged 15-30 years recorded the highest significant concentration at 358.7 µg/dL. For the interaction between patients' gender and age, women aged 15-30 years recorded the highest significant concentration of total iron binding capacity at 291.5 µg/dL. The Interaction effect of the three factors under study showed that female patients aged 15-30 years recorded the highest significant concentration of total iron binding capacity at 361.8 µg/dL.

Table (4): Interaction Effects of Study Factors on Blood Iron Levels (TIBC) (µg/dL).

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	331.5 a	355.8 a	299.6 a	327.3 a
	Female	293 a	361.8 a	279.2 a	313.6 a
Control	Male	71 bc	91 bc	91 bc	82.43 b
	Female	91 bc	151 b	11 c	99 b
Group × Age		<15	15-30	>30	-
Patients		326 a	358.7 a	289.4 a	-
Control		79 c	121 b	64.33 c	-
Gender × Age		<15	15-30	>30	-
Male		244.7 a	267.5 a	240 ab	-

Female	158.3 b	291.5 a	234.5 ab	-

- Values followed by the same letter do not differ significantly from each other.

Malondialdehyde (MDA): Figure (5) shows the individual effect of study factors on MDA levels. It is observed that there is a significant effect of thalassemia on MDA concentration, where the patient group recorded the highest concentration of 721.12 mmole/L, significantly different from the healthy group which recorded 119.92 mmole/L. As for the effect of gender, the highest significant concentration for males was recorded at 538.1 mmole/L, while a significant effect of age was observed, with the age group over 30 years recording the highest MDA level of 605.5 mmole/L.

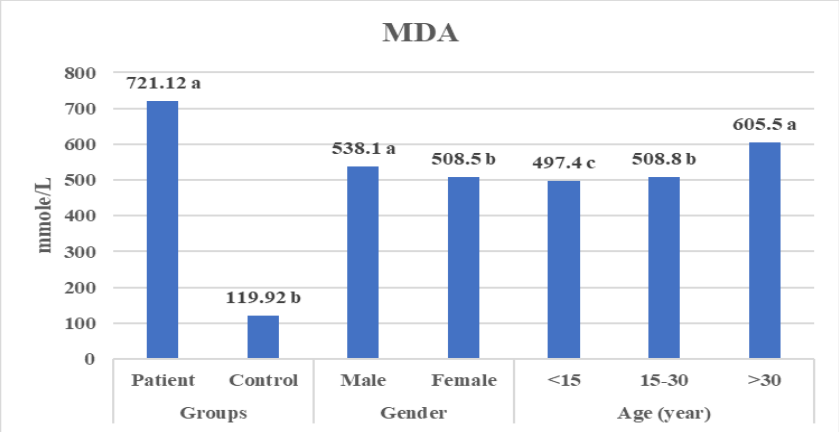

Figure (5): Individual effects of study factors on malondialdehyde (MDA) levels.

Table (5) shows the Interaction effects of study factors on MDA concentration in the study sample, where significant effects are observed at a probability level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the highest significant MDA concentration of 735.5 mmole/L. As for the interaction between groups and age category, the patient group over 30 years old recorded the highest significant concentration of 739.9 mmole/L. For the interaction between the gender of patients and their ages, the male patients over 30 years old recorded the highest significant MDA concentration of 650.6 mmole/L. The Interaction effect of the interaction among the three factors under study showed that female patients over 30 years old recorded the highest significant MDA concentration of 1090 mmole/L.

Table (5): Interaction effects of study factors on malondialdehyde levels (mmole/L).

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	636.8 b	739.8 ab	850.4 ab	735.5 a
	Female	1090 a	689.8 b	629.4 b	699.6 a
Control	Male	81 c	131 c	151 c	115.3 b

	Female	167.5 c	63 c	171 c	126.4 b
Group × Age		<15	15-30	>30	-
Patients		701.6 a	714.8 a	739.9 a	-
Control		115.6 b	97 b	157.7 b	-
Gender × Age		<15	15-30	>30	-
Male		451.6 c	536.8 b	650.6 a	-
Female		475 c	480.8	553 b	-

- Values followed by the same letter do not differ significantly from each other.

Iron Concentration (Fe): It measures the amount of available iron in the blood. This test shows the quantity of iron that is transported in the blood by transferrin. Figure (6) displays the individual effect of study factors on iron levels, where a significant effect of thalassemia on iron concentration is observed, with the patient group recording the highest concentration of 2788.9 ng/dL, significantly different from the healthy group which recorded 121.3 ng/dL. As for the effect of gender, it showed a significant impact on iron levels, with the male group recording the highest concentration of 1945.1 ng/dL, while a significant effect of age was also observed, with the age group over 30 years old recording the highest blood iron concentration of 2244.6 ng/dL.

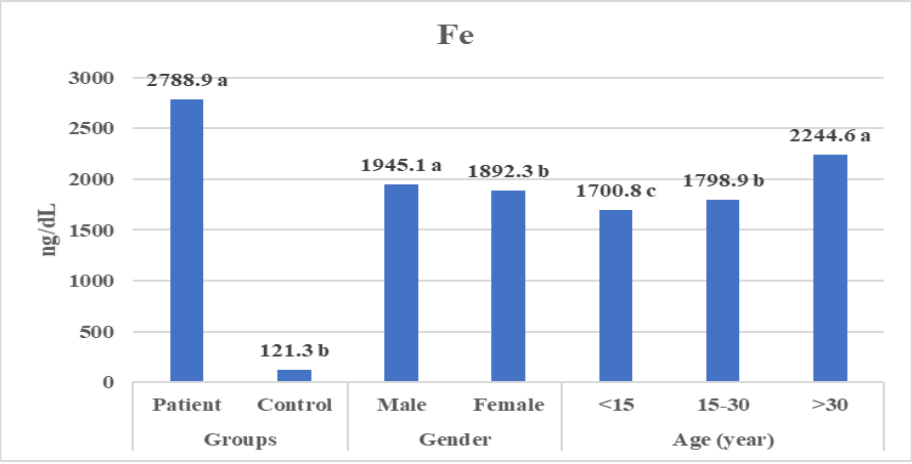

Figure (6): Individual effects of study factors on iron concentration.

Table (6) shows the Interaction effects of study factors on blood iron concentration in the study sample, where significant effects are observed at a probability level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the male patient group recorded the highest significant iron concentration of 2795.6 ng/dL. As for the interaction between groups and age category, the patient group over 30 years old recorded the highest significant concentration of 2881.8 ng/dL. For the interaction between the gender of patients and their ages, the female group over 30 years old recorded the highest significant iron concentration of 2328.8 ng/dL. The Interaction effect of the interaction among the three factors

under study showed that female patients under 15 years old recorded the highest significant iron concentration of 3270 ng/dL.

Table (6): Interaction effects of study factors on iron concentration (ng/dL).

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	2755.8 c	2603.3 d	2997.2 b	2795.6 a
	Female	3270 a	2671.5 d	2766.4 c	2778.8 a
Control	Male	125.3 e	131 e	110.5 e	122.7 b
	Female	114.5 e	113 e	141 e	119.2 b
Group × Age		<15	15-30	>30	-
Patients		2829.3 a	2637.4 b	2881.8 a	-
Control		121 c	122 c	120.7 c	-
Gender × Age		<15	15-30	>30	-
Male		1879 c	1779.2 d	2172.4 b	-
Female		1166.3 e	1818.7 c	2328.8 a	-

- Values followed by the same letter do not differ significantly from each other.

Lipoxygenase Enzyme (LOX): Figure (7) shows the individual effect of study factors on lipoxygenase enzyme levels, where a significant effect of thalassemia on the concentration of this enzyme is observed, with the patient group recording the highest concentration of 226.2 IU/L, significantly different from the healthy group which recorded 111.1 IU/L. As for the effect of gender, no significant impact on lipoxygenase enzyme levels was observed within the study sample, while a significant effect of age was noted, with the age group over 30 years old recording the highest lipoxygenase enzyme level of 204.2 IU/L.

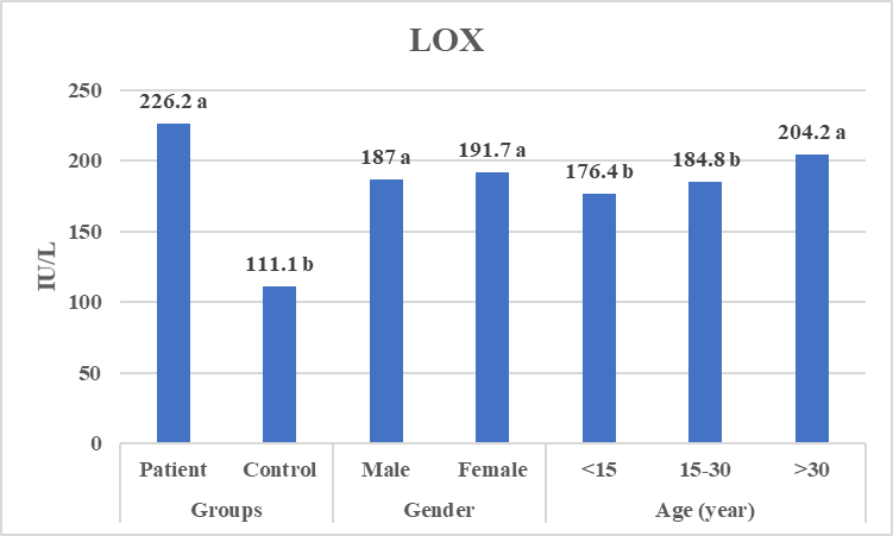

Figure (7): Individual effects of study factors on white blood cell concentration.

Table (7) shows the Interaction effects of study factors on lipoxygenase enzyme concentration in the study sample, where significant effects are observed at a probability level of 0.05. In the Interaction effect of the interaction between the study groups and gender, the female patient group recorded the highest significant concentration of lipoxygenase enzyme at 232 IU/L. As for the interaction between groups and age category, the patient group over 30 years old recorded the highest significant concentration of 231.7 IU/L. For the interaction between the gender of patients and their ages, the female group over 30 years old recorded the highest significant lipoxygenase enzyme concentration of 212 IU/L. The Interaction effect of the interaction among the three factors under study showed that female patients aged 15-30 years recorded the highest significant lipoxygenase enzyme concentration of 234 IU/L.

Table (7): Interaction effects of study factors on white blood cell concentration.

Group	Age / Gender	<15	15-30	>30	Group × Gender
Patients	Male	223.7 a	209.5 a	231.2 a	222.4 a
	Female	223 a	234 a	232.2 a	232 a
Control	Male	110 b	111 b	113 b	111.1 b
	Female	111 b	111 b	111 b	111 b
Group × Age		<15	15-30	>30	-
Patients		223.6 a	221.8 a	231.7 a	-
Control		110.4 b	111 b	112.3 b	-
Gender × Age		<15	15-30	>30	-
Male		185.8 b	176.7 b	197.4 ab	-
Female		148.3 c	193 ab	212 a	-

4. DISCUSSION

Figures (1-7) indicate a significant effect of thalassemia, along with the factors of age and gender, on the studied variables. Thalassemia led to increased levels of white blood cells, erythrocyte sedimentation rate, total iron-binding capacity, malondialdehyde, iron concentration, and lipoxygenase enzyme levels. Conversely, thalassemia reduced hemoglobin levels. Gender showed significant effects on both malondialdehyde and iron concentrations. Age category had significant effects on white blood cell levels, total iron-binding capacity, serum iron, and lipoxygenase enzyme levels. Looking at the three-way interactions presented

in Tables (1-7), it is evident that the most impactful group is female patients over 30 years old, who recorded the highest significant concentrations of white blood cells and malondialdehyde. Meanwhile, female patients under 15 years old showed the highest significant averages in the percentage of erythrocyte sedimentation rate and iron concentration. Female patients aged 15-30 years exhibited the highest significant concentrations for total iron-binding capacity and lipoxygenase enzyme. Male patients aged 15-30 years recorded the lowest significant hemoglobin concentration. These results are consistent with the findings of Azeez *et al*. (2016), who reported a high significant decrease in hemoglobin levels and packed cell volume, along with a significant increase in serum iron (ferritin) in a group of thalassemia patients compared to a healthy group. The results also align with Tawfeeq (2017), who noted no significant effect of gender on thalassemia, while children showed a significant prevalence of thalassemia compared to older age groups. It is noteworthy that many iron-containing enzymes, such as 12-lipoxygenase and cytochrome P450 oxidoreductase, are known for their promotional role in facilitating lipid oxidation, leading to ferroptosis (Stockwell, 2022). While maintaining appropriate iron levels is essential for normal physiological activities, excessive accumulation can lead to cellular ferroptosis (Li *et al*., 2020). The aforementioned evidence underscores the critical role of iron in mediating ferroptosis.

Lipid oxidation is the underlying root cause of ferroptosis, and since lipids constitute the vast majority of cell membranes, they are also primary targets for reactive oxygen species (ROS). This oxidation causes an imbalance in cellular oxidative balance, which in turn leads to oxidative damage and ferroptosis. Malondialdehyde and 4-hydroxynonenal are end products of lipid oxidation, which can be stimulated by enzymatic reactions. The specific mechanism by which enzymatic reactions cause lipid oxidation involves long-chain acyl-CoA synthase 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3), which first activate polyunsaturated fatty acids (PUFAs) for incorporation into cell membrane lipids (such as phosphatidylethanolamine, PE), forming a PUFA-PE compound. This PUFA-PE compound then undergoes catalytic reaction by lipoxygenases (LOXs) and cyclooxygenases (COXs), leading to lipid oxidation. It has been demonstrated that non-heme iron-containing dioxygenases, or LOXs, specifically target and oxidize polyunsaturated fatty acids, thereby enhancing lipid oxidation, ultimately promoting ferroptosis (Wang *et al*., 2021). It is expected that iron chelators taken by the thalassemia patients in the current study will inhibit platelet aggregation, as well as inhibit thromboxane A synthesis and the conversion of arachidonic acid into lipoxygenase-derived products (Barradas *et al*., 1989).

5. CONCLUSION

Thalassemia has led to increased levels of white blood cells, erythrocyte sedimentation rate, total iron binding capacity, malondialdehyde, iron concentration, and lipoxygenase enzyme. In contrast, thalassemia decreased hemoglobin levels. Gender had significant effects on both malondialdehyde and iron concentrations, while age group significantly affected white blood cell levels, total iron binding capacity, serum iron, and lipoxygenase enzyme. Among the three intersections, the group of female patients over 30 years recorded the highest significant concentrations of white blood cells and malondialdehyde. Meanwhile, female patients under 15 years had the highest significant average for the percentage of erythrocyte sedimentation rate and iron concentration. Female patients aged 15-30 recorded the highest significant concentrations for total iron binding capacity and lipoxygenase enzyme. Male patients aged 15-30 recorded the lowest significant concentration of hemoglobin.

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