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Estimation of lipoxygenase activity and its relationship to some antioxidants in the sera of patients infected with hepatitis B virus.

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ABSTRACT— Hepatitis B virus infection is the main risk factor for hepatocellular carcinoma worldwide. Hepatitis B virus directly leads to carcinogenicity (cancer) through integration processes in the human genome. This study aimed to accurately characterize the activity of lipoxygenase in hepatitis B patients, ages (20-70) and its relationship to some antioxidants in the body and age. The study showed a significant increase in the activity of LOX enzyme in patients with hepatitis B virus compared to the control group. The concentration of GSH glutathione, as well as an increase in the concentration of Malone Dialdehyde, which is one of the products of oxidation of unsaturated fats, which is the basis for the enzyme LOX lipoxygenase.

KEYWORDS: Hepatitis B virus, Lipoxygenase, antioxidants, catalase, Superoxide dismutase

1. INTRODUCTION

The liver is the largest internal organ in the human body [1]. It performs many important functions, including that it is the source of all metabolic processes that process the nutrients the body needs, such as protein, glucose, vitamins and fats [1- 3]. The liver detoxifies and removes toxic substances from the body, including alcohol, ammonia and drugs [2], [3]. The liver can become infected with hepatitis B virus, the sign of the disease is jaundice with minor or accompanied clinical symptoms From mild symptoms such as jaundice, nausea and malaise to severe symptoms such as liver failure [4], [5] transmission of the virus occurs mainly through direct contact with blood, such as drug or intravenous drug use and blood or plasma transfusion [6], infections with hepatitis virus are considered (B) An epidemic, the World Health Organization (WHO) estimates its global prevalence at 3% of the population around the world [7].

Lipoxygenase is an enzyme that catalyzes the oxidation of unsaturated fatty acids to produce hydroperoxides [8]. Lipoxygenases are a class of non-heme protein-containing enzymes and are classified as oxidoreductases (redox enzymes) [9]. Lipoxygenase is found in plants, fungi, as well as in many types of bacteria [10], [11]. This enzyme was first discovered in 1928 by Hass and Bohn, who explained that the enzyme works to break down the carotenoid formula in soybeans. Ander and Hou found in 1932 that soybeans contain an enzyme that oxidizes unsaturated fats called lipoxidase. The scientist Sumner and Sumnerr noticed in 1940 that the enzyme lipoxidase is not identical to the carotene oxidase enzyme. The results were noted that the enzyme lipoxygenase when dissolved in unsaturated fat works on bleaching (color shortening) unlike carotene oxidase, the bleaching action is negligible, especially that the effect of Fat adding to the carotene bleaching rate is most likely due to oxidative stress [12].

An increase in the activity of lipoxygenase was found in the blood serum of patients with hepatitis B virus,

as well as an increase in the blood serum of women with breast cancer [13], as well as a high level in the sera of patients with vascular disease [14]. The lack of adequate studies of the enzyme lipoxygenase in humans, the difference in its activity from the normal levels is only evidence for the diagnosis of disease states.

Enzyme products play a large role in acute infection (inflammation), but may involve the breakdown of such infection [15]. References and research have described lipoxygenase and its relationship with inflammation [16], [17], cancer and vital blood vessels [11], [12], [18] and the role of eicosanoids in the treatment and prevention of diseases [19]. Lipoxygenase catalyzes the addition of oxygen to unsaturated fatty acids. Unsaturated fatty acids contain the active moiety of 1,4-cis,cis-pentadine to produce hydroperoxides, and the essential fatty acids linoleic acid, linolenic acid and arachidonic acid act as the basis materials for lipoxygenase [20].

The term antioxidant refers to any substance or compound that has antioxidative activity, which delays or inhibits the action of free radicals [21]. Antioxidants act to protect in several ways either by directly inhibiting the production of reactive oxygen species (ROS), preventing their proliferation or destroying them. Cells use a number of antioxidant mechanisms, and the properties of these systems vary from tissue to tissue, depending on their presence in the intracellular and extracellular milieu. Nutrition experts note that promoting an all-natural diet that contains most types of antioxidants can extend the life of an organism, improve its health, and reduce the signs of aging [22].

study design:
Experiments and measurements were carried out for patients with hepatitis B virus in Kirkuk governorate - Iraq for (90) samples of patients with hepatitis B virus, and they were matched by (50) samples of apparently healthy people as a control group.

The activity of lipoxygenase and a number of antioxidants (Superoxide dismutase SOD, catalase CAT, glutathione GSH, Malone didehyde MDA) was measured, and the relationship between the activity of lipoxygenase and antioxidants was studied.

2. Materials used and working methods

2.1 Sample collection
The study was conducted in the city of Kirkuk - Iraq for the period from 1/2/2020 to 30/6/2020 on a number of (90) patients with hepatitis B virus, whose ages ranged from (20-70) years old, and most of them were They visit the Liver and Gastroenterology Center in Kirkuk city at Azadi Teaching Hospital and Kirkuk General Hospital. Also, (50) samples were collected from apparently healthy people as a control group.

2.1.1 Estimation of lipoxygenase activity
The activity of lipoxygenase was calculated using the method [23], which includes the oxidation of the base material (linoleic acid) by adding two oxygen atoms to make the enzyme a catalyst, by measuring the increase in the absorbance resulting from the formation of paired dienes at the wavelength of 234 nm, during a period of five minutes, noting that the molar absorbance (ε) of the conjugated dienes is 25,000 molar-1. cm-1.

2.1.2 Estimation of the catalase enzyme activity
The principle of the method involves the reaction of meta-ammonium vanadate with hydrogen peroxide

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under acidic conditions, reducing vanadium (V) to (III). Hydrogen peroxide is a strong oxidizing agent that leads to the formation of a red-orange peroxovanadium complex, which absorbs at 452 nm [24].

2.1.3 Estimation of the activity of superoxide dismutase

The activity of superoxide dismutase was estimated using the Modified photochemical Nitroblue Tetrazolum (NBT) method. This method included the use of sodium cyanide as a peroxidase inhibitor. This method is based on estimating the activity of the enzyme SOD indirectly through the appearance of a change in the optical density of formalin formed by reduct of $O^{\cdot}_2$ For the dye nitrobutyrate (NBT), which in turn is generated by irradiation of blood serum [25] (as the decrease in the optical density of formazine indicates an increase in the activity of superoxide dismutase).

2.1.4 Estimation of the concentration of malon dialdehyde

The modified thiobarbituric acid reaction method used by researchers [26] was used to measure malondialdehyde, which is one of the final products of the fat super-oxidation process, and its level is an indicator for this process, as the measurement depends on the interaction between fat peroxides, especially malondialdehyde. With TBA in a pH dependent medium.

2.1.5 Estimation of the concentration of glutathione

The level of glutathione in serum was measured using the Ellman reagent method [27]

2.2 Statistical analysis

The data were analyzed according to a completely random design by analysis of variance (ANOVA) based on the statistical program SPSS (version 26) and the arithmetic means of groups were compared using Duncan Multiple Range test to show the differences between any two groups at a probability level ($P < 0.05$) and a coefficient was found Correlation coefficient to find the relationship between biochemical variables in the blood of people with hepatitis B virus and the control group.

3. Results and Discussion

3.1 Estimation of lipoxygenase activity UI/ml

The activity of lipoxygenase was calculated in the sera of patients with hepatitis B virus, using the method [23]. The results shown in Table (1) statistically indicated that there was a significant increase in the level of lipoxygenase activity at the probability level ($P \leq 0.001$). In the sera of patients infected with hepatitis B virus (0.273 ± 0.043 Ul/ml) compared to the level of its concentration in the blood serum of the control group (0.108 ± 0.030 Ul/ml), as the activity of lipoxygenase increases in patients infected with hepatitis B virus, as shown in Figure (1).

Table (1) Means ± standard error of LOX lipoxygenase activity in the blood serum of hepatitis B patients and the control group.

state	LOX activity Mean ± SD. (UI\ml)			P value
	All of case	Less than 45 years	More than 45 years	
Control	0.108 ± 0.030	0.1124 ± 0.02893	0.0931 ± .02056	0.02

No. of cases	**50**	**34**	**16**	
Patients	**0.273 ± 0.043**	**0.2665 ± 0.04633**	**0.2879 ± 0.03633**	**0.01**
No. of cases	**90**	**48**	**42**	
P value	0.001	0.001	0.001	

The results in Table (1) indicate an increase in the activity of lipoxygenase in the blood serum of patients with hepatitis B virus. These results are consistent with the study [28]. The Researcher [29] Where they indicated an increase in the concentration of the enzyme in patients with breast cancer, The researcher [30] also indicated an increase in the activity of lipoxygenase in the blood of patients with colon and prostate cancer. The researcher [31] noticed an increase in the effectiveness of lipoxygenase in the serum and blood tissues of patients with cardiovascular diseases. As explained by [32] an increase in the enzyme activity for patients with prostate cancer due to the increase in the formation of eicosanoid and leukotriene from increasing the digestion of fatty acids, which promote inflammation and increase the formation of cancerous tumors in different tissues of the body.

The results of the statistical analysis (variance) in Table (1) also showed an increase in the effectiveness of lipoxygenase lox in patients compared to the healthy ones, as the effectiveness was higher in the age group (more than 45 years) compared to the age group (less than 45 years), as shown in Figure (6), This result is consistent with the results indicated by the researcher [33] and in the absence of sources documenting the explanation of the reason for the increase in the activity of lipoxygenase with age, it is believed that the discrepancy in the activity of the enzyme between age groups is due to the destruction of unsaturated fatty acids Inside the body with age, as a result, it leads to the formation of metabolites of unsaturated fatty acids, which form biological regulatory compounds (such as eicosanoids and leukotrienes), which have been shown to have a role in many physiological disorders such as inflammation, arthritis and cancer cases. [34-36] as well as agrees with the study [28], which showed a high enzyme activity with an increase in the age of breast cancer patients.

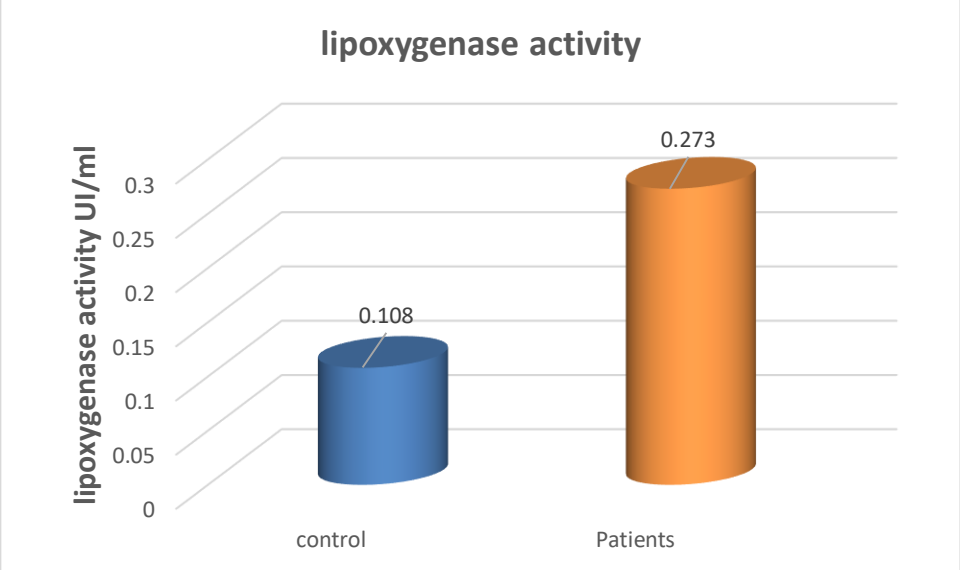

Figure (1) The activity of lipoxygenase in hepatitis virus patients compared to the control group.

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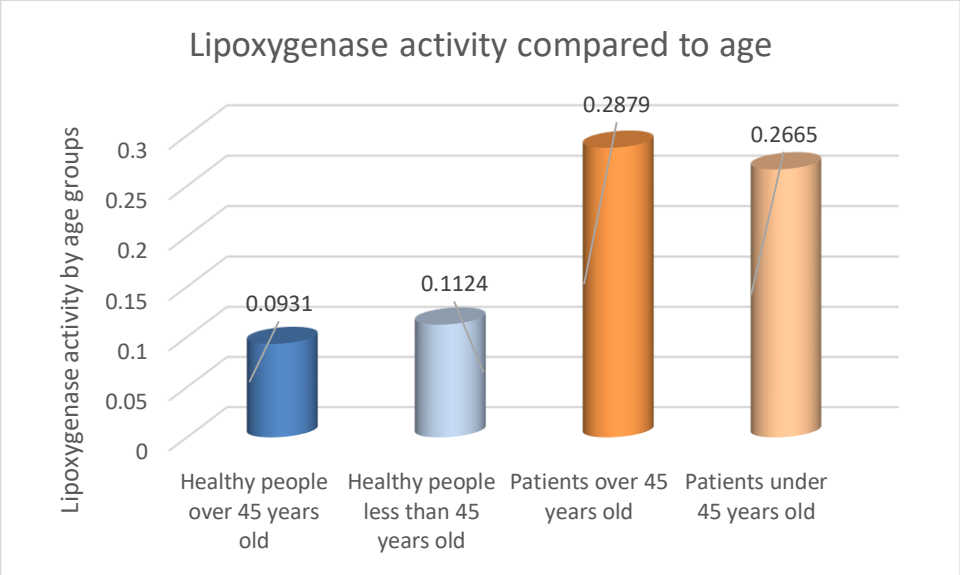

Figure (2): Relationship of lipoxygenase activity with age in hepatitis B patients and the control group.

The reason for the effectiveness of lipoxygenase can be due to exposure to external factors, genetic factors and the occurrence of infections, which leads to an increase in the response of the immune system by activating the immune system, which leads to the release of polyunsaturated fatty acids, including arachidonic acid, as these acids are considered the basis of lipoxygenase [37] and this as a result leads to increased digestion of unsaturated fatty acids, including arachidonic acid, linolenic and linoleic, which turns into multiple compounds, including eicosanoids [38] and leukotrienes, which are closely related to the lipoxygenase pathway, Leukotrienes are a bio-formation of Lipoxins which are a potent anti-inflammatory lipid mediator and have been registered as an important regulator in resolving and fighting Inflammation at the present time, [39] and has an important role in the development of inflammation and cancer, and the reason for the high activity of the enzyme may be due to the increase in the exudation of the cell membrane, which has a certain exudation towards chemicals In the case of inflammation and cancer, the membrane's exudation increases, and thus the enzyme leaves the cell, causing an increase in its concentration in the blood. It was found that inhibiting the activity of lipoxygenase prevents the spread of inflammation as well as the secondary growth of tumors and inflammation [40], [41].

3.2 Antioxidants Activity

Table No. (2) shows the mean ± standard error of the level of antioxidant activity (SOD, CAT, GSH, MDA) in the blood serum of patients infected with hepatitis B virus, a control group.

Type	No.	SOD activity Mean ± SD. (U/L)	CAT activity Mean ± SD. (U/L)	GSH activity Mean ± SD. (µmol/L)	MDA activity Mean ± SD (µmol/L)

Control	0	7.887 ± 1.708	56.728± 2.316	15.480 ± 5.300	5.317 ± 1.674
patients	0	6.009 ± 1.448	55.495 ± 2.083	9.967 ± 1.011	8.812 ± 4.846
P Value		0.001	0.02	0.001	0.001

The results shown in Table (2) statistically indicated that there was a significant decrease in the level of the antioxidant activity of superoxide dismutase (SOD) at a probability value (P≤0.001) in the sera of patients infected with hepatitis B virus type (7.887 ± 1.708 U) compared with the level of Its effectiveness in the blood serum of the control group is (U 7.887 ± 1.708), as its level decreases in patients infected with hepatitis B virus. Below is a chart showing the concentrations of SOD in patients and control group. As shown in figure (3).

The results shown in Table (2) statistically indicated that there was a significant decrease in the activity level of Catalase at the probability level (P≤0.02) in the sera of patients infected with hepatitis B virus type (55.495 ± 2.083 U/L) compared with its activity level in the serum of the group The control (56.728 ± 2.316 U/L) decreased enzyme activity in patients infected with hepatitis B virus, as shown in Figure (4).

The results shown in Table (2) statistically indicated that there was a significant decrease in the level of glutathione antioxidant activity at the probability level (P≤0.001) in the sera of patients infected with hepatitis B virus (9.967 ± 1.011 µmol/L) compared with its activity level in the blood serum. The control group (15.480 ± 5.300 µmol/L), its level decreased in patients infected with hepatitis B virus, as shown in Figure (5).

The results shown in Table (2) statistically indicated that there was a significant increase in the activity level of Malone didehyde at a probability value (P≤0.001) in the sera of patients infected with hepatitis B virus (8.812 ± 4.846 µmol/L) compared with its activity level in the serum of the group The control (µmol/L 5.317 ± 1.674) increased its concentration level in patients infected with hepatitis B virus. And as shown in the figure (6).

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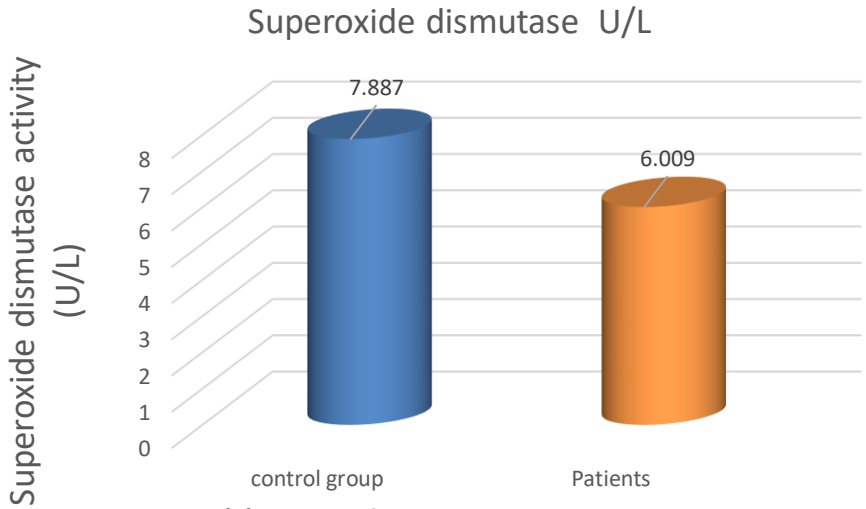

Figure (3) activity of superoxide dismutase in patients and control group

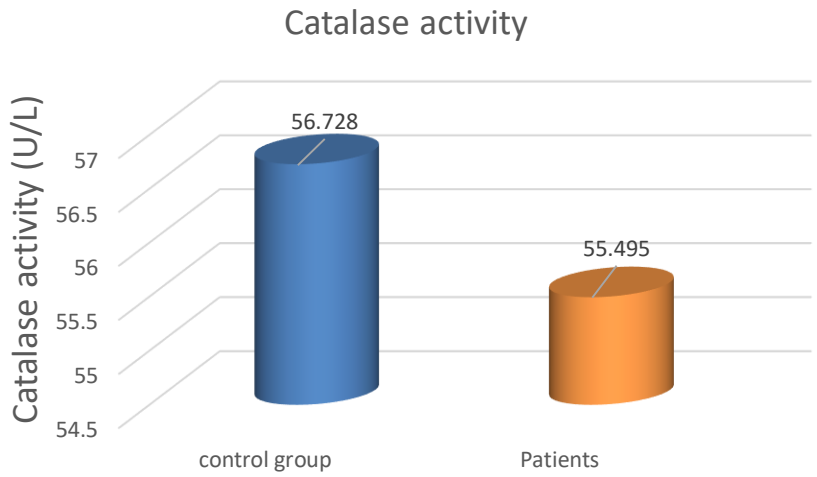

Figure (4) activity of catalase in patients and control group

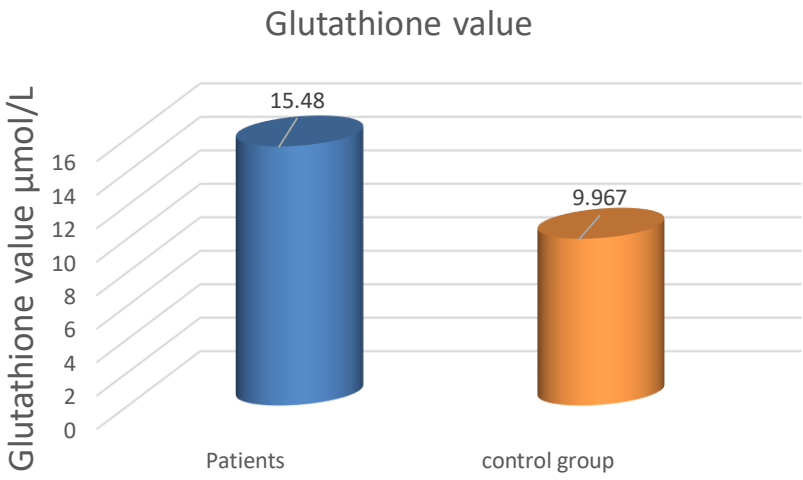

Figure (5) : Glutathione activity in patients and control group

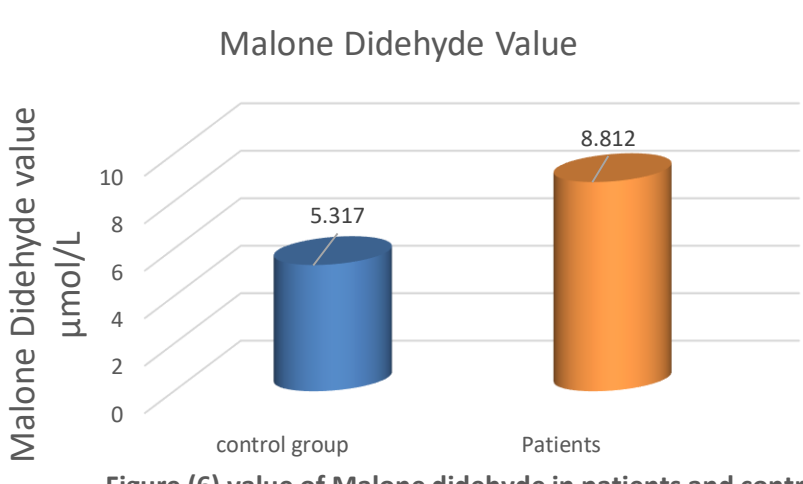

Figure (6) value of Malone didehyde in patients and control group

Figures (3), (4), (5), (6), levels of antioxidants (superoxide dismutase, catalase, glutathione and malondialdehyde) in hepatitis B patients and a control group.

The above results are in agreement with the study [42] which showed a decrease in the concentration of antioxidants (superoxide dismutase, catalase, and glutathione) and an increase in the concentration of malondialdehyde as it is one of the products of lipid peroxidation in inflammatory patients. Hepatitis B virus, and agrees with the study [43] in which he indicated that patients with hepatitis B virus have high oxidative stress and low levels of antioxidants, and treatment of these patients can be helped by adding certain antioxidants Alone or in combination with the treatment of hepatitis or liver cancer,

Also The above results agree with [44], which indicated an increase in the level of Malone dialdehyde and a decrease in the levels of antioxidants in patients with hepatitis C virus, It also agrees with the results reached by [45], which showed a decrease in the levels of antioxidants in children with acute viral hepatitis diseases, and consistent with [46]. It showed low levels of antioxidants in multiple stages of hepatitis B virus infection in Egypt.

Free radicals, which are constantly generated as byproducts of metabolism, catalyze oxidation of unsaturated fatty acids in membranes through a process called lipid peroxidation. Malondialdehyde (MDA) is a marker of oxidative stress and one of the end products of lipid oxidation. The level of MDA reflects the degree of lipid peroxidation.

An increase in free radicals leads to an increase in the production of MDA. [47], [48], [42] Fats, proteins, carbohydrates, and other cell components undergo oxidation, resulting in significant damage to cellular structures. The buildup of this damage is called oxidative stress [49]. Kupffer cells in the liver that are exposed to harmful reactions are the main effectors responsible for the formation of reactive oxygen species (ROS) affecting hepatic stellate cells (HSC) and hepatocytes [50]. Additionally, reactive oxygen species induce necrosis and apoptosis of hepatocytes, resulting in liver damage and progression to the final stage of liver disease [51]. In cirrhotic patients, stress-activated macrophages and neutrophils release high concentrations of oxidants that lead to oxidative stress. This, in turn, may damage DNA, proteins, and carbohydrates. Damage from lipid oxidation caused by toxic agents is known to result in liver injuries ranging from inflammation to necrosis in the event of continuous exposure to the agent and inadequate antioxidant systems [52], [53]. Cell damage from oxidative stress may lead to cirrhosis [54] The term "antioxidant" describes molecules that can stabilize or inactivate free radicals before they destroy cells [55]. Some antioxidant enzymes include superoxide dismutase, catalase and glutathione.

It is known that superoxide dismutase (SOD) is present in all aerobic cells and plays a role in defense against superoxide radicals formed as a result of aerobic reactions [56]. In biological systems, the enzyme SOD speeds up the reaction by about four times. SOD works with enzymes that destroy H2O2 such as catalase and glutathione reductase [57].

The catalase is one of the widespread enzymes in all breathing aerobic organisms and in cells that contain cytochromes. Its main function is to remove the toxic effects of hydrogen peroxide by displacing toxins complexes in hydrogen peroxide reactions. It also works to remove electrons that lead to the production of (O-2) [58], as the main reason for the decrease in the effectiveness of catalase is its consumption by oxidative compounds that break down the cell membranes, the most important of which is (malone didehyde), which is present in high levels in the blood of organisms exposed to oxidative stress. A negative relationship between its level and the activity level of the enzyme catalase [59]. The enzyme catalase (CAT) is found in peroxisomes or Oxidative particles play a role in the conversion of hydrogen peroxide into water and oxygen [60].

Glutathione (GSH) is the most important cellular redox and a major defender against oxidative stress. GSH is oxidized to glutathione disulfide (GSSG), which is actually two GSH molecules linked together at sulfur atoms [61]. GSH has been shown to have multiple roles in cell physiology, including, direct scavenging of reactive oxygen species (ROS), nitric oxide (NO) and its derivatives, RNS reactive nitrogen species, resulting in protection of the electron transport chain, DNA, lipids and proteins, and indirect neutralization. Toxin, S-gluta-thionylation of thiol protein groups, regulation of cell cycle progression and apoptosis.

From these considerations, it is clear that GSH cannot be considered merely as a free radical scavenger but has a heavy role in the network governing the choice between survival, necrosis, and apoptosis [62], [63] as well as in cell signaling [63], [64] and metabolism [61], [65]. Glutathione (GSH) plays a very important role in eliminating harmful reactive oxygen radicals and maintaining enzymatic activities. It stimulates the reduction of glutathione oxidized to glutathione. GSH, one of the most important intracellular antioxidant molecules, also has many physiological functions such as detoxification of xenobiotics, transport of amino

acids, maintenance of the reduced form of sulfhydryl groups, the role of coenzyme in some enzymatic reactions in addition to its role in the antioxidant defense system [65].

3.3 Correlation between lipoxygenase activity and antioxidant activity

Table No. (3) Correlation relationship between lipoxygenase and antioxidants (SOD, CAT.GSH, MDA)) for hepatitis B patients and the control group

Parameters	SOD	CAT	GSH	MDA
Patients	- 0.176*	0.264**	0.012	0.038
Control	0.048	- 0.108	0.301*	- 0.092

* ($P \leq 0.05$) , ** ($P \leq 0.001$)

The results showed, as in Table (3), that there was a negative correlation between lipoxygenase activity, SOD activity, and catalase CAT activity in patients, and there was a positive correlation between lipoxygenase activity and glutathione level in healthy subjects, and there were no correlations between lipoxygenase activity and the rest of the other variables in the table. And as shown in Figures (7), (8), (9), (10), (11), (12), (13) and (14).

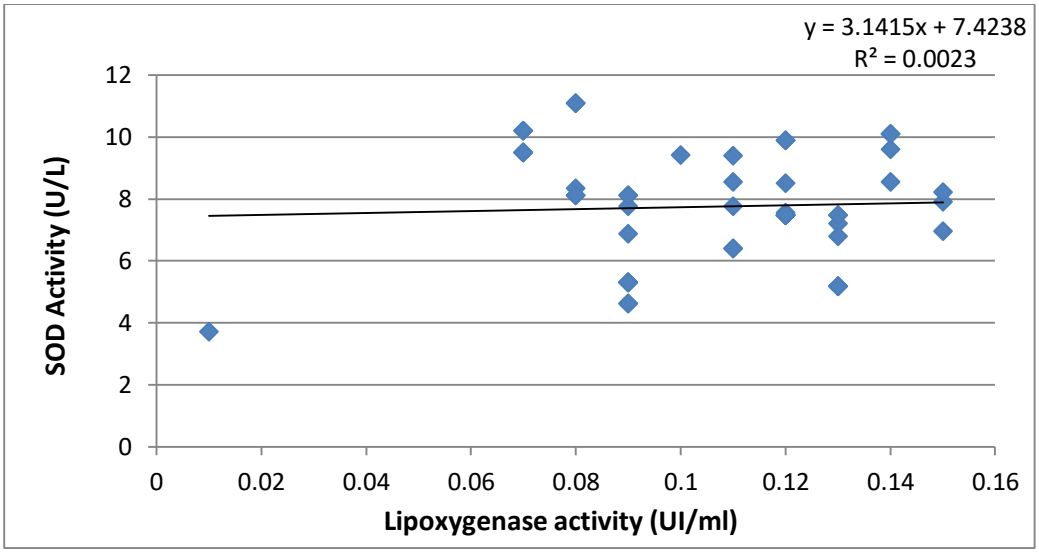

Figure (7) Correlation relationship between lipoxygenase and the effectiveness of SOD in the control group

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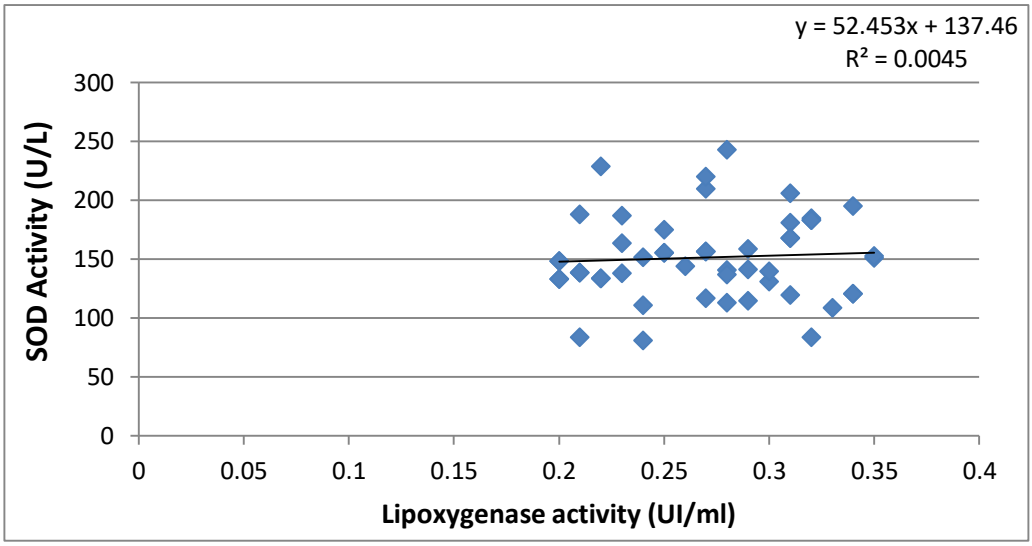

Figure (8) Correlation relationship between lipoxygenase activity and SOD activity in hepatitis B patients

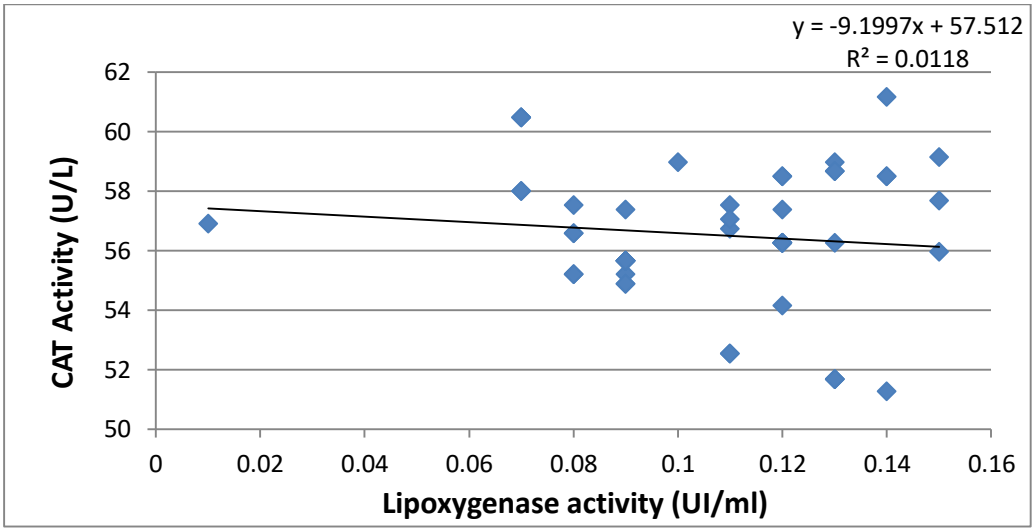

Figure (9) Correlation relationship between lipoxygenase activity and CAT activity in the control group

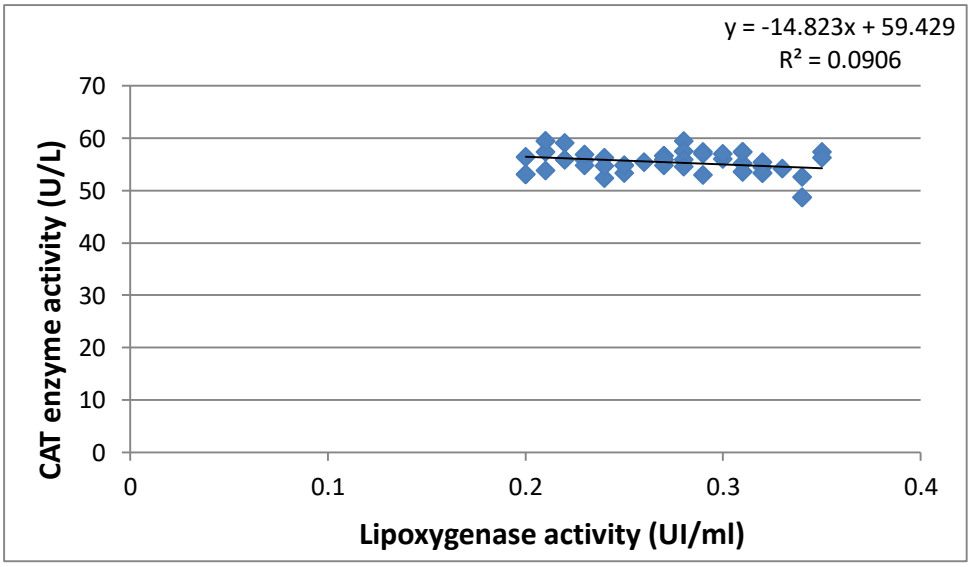

Figure (10) Correlation relationship between lipoxygenase activity and CAT activity in hepatitis B patients

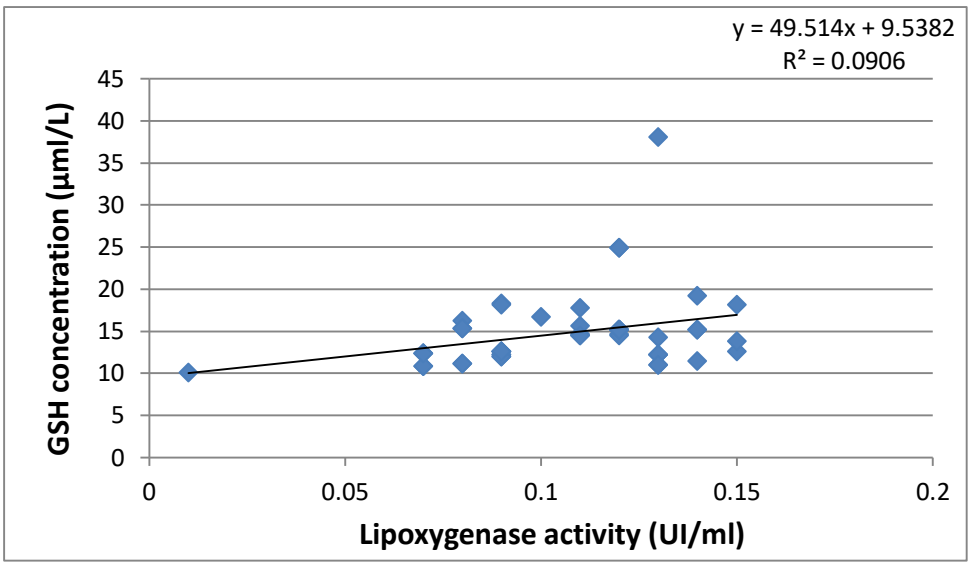

Figure (11) Correlation relationship between lipoxygenase activity and GSH level in the control group

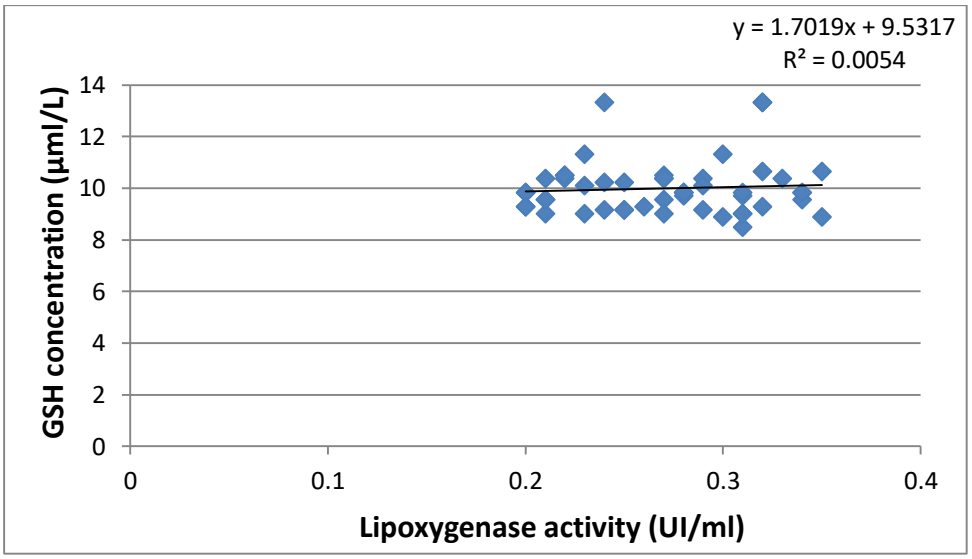

Figure (12) Correlation relationship between lipoxygenase activity and GSH level in hepatitis B patients

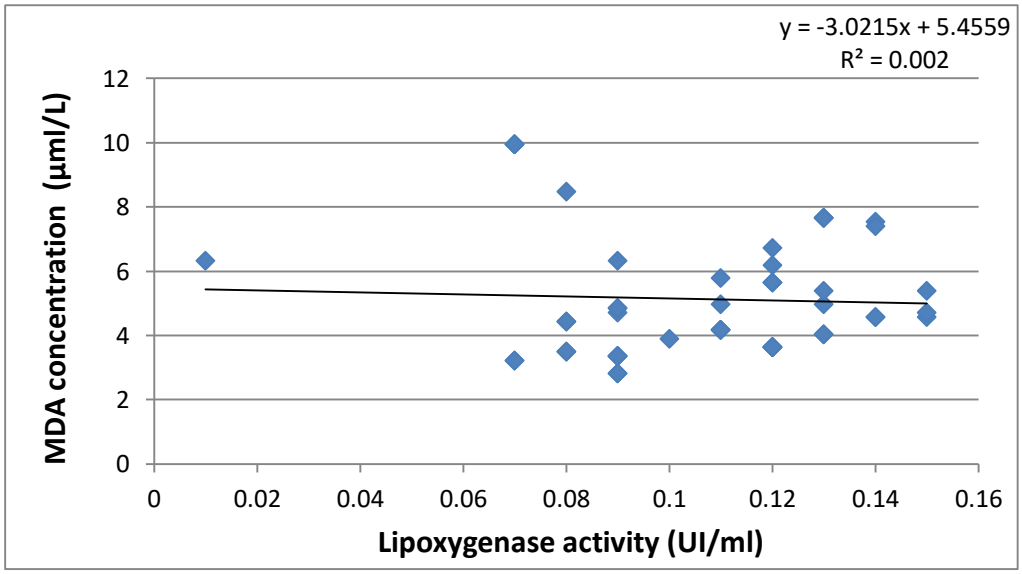

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Figure (13) Correlation relationship between lipoxygenase activity and the activity and level of MDA in the control group.

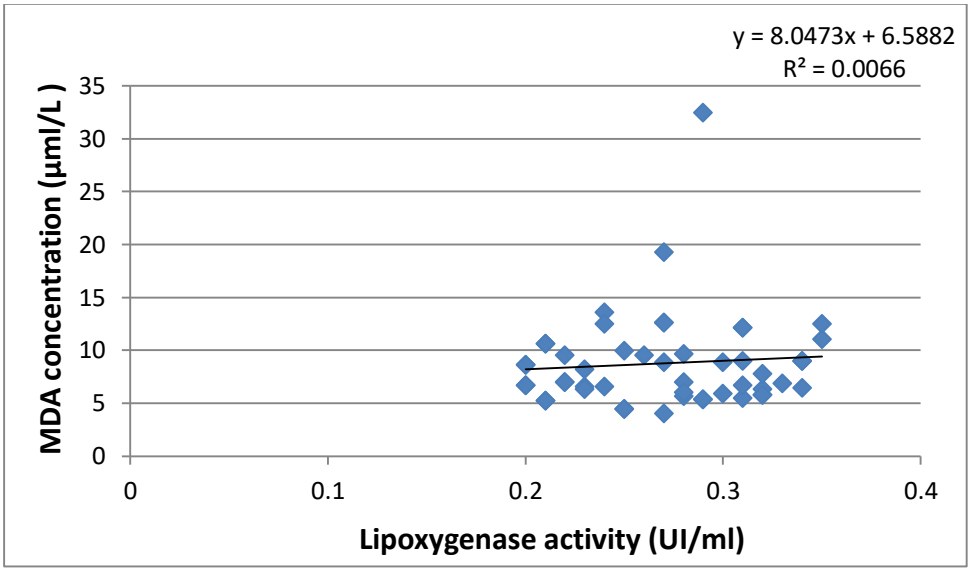

Figure (14) Correlation relationship between lipoxygenase activity and MDA activity and level in hepatitis B patients

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