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Analytical Study of Enzymatic Antioxidants (GPX1, CAT) in Non-Alcoholic Fatty Liver Patients, Relationship to Age and Gender

Havin Adel Qadir [1,*], Omar Salih Hassan [1]

[1] Department of Chemistry, College of Education for Pure Sciences, University of Tikrit, Tikrit, Iraq.
*Corresponding author : ha230020pep@st.tu.edu.iq

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Abstract

Enzymatic antioxidants glutathione peroxidase (GPX1) and catalase (CAT) in patients with non-alcoholic fatty liver (NAFLD) reduce the effects of oxidative agents by converting them into water molecules, this study aims to evaluate the accuracy and sensitivity of the analytical method in detecting very low concentrations of GPX1 and CAT and the relationship to NAFLD. The study included males and females with a ratio of 44:46 and after examining them with ultrasound, they were divided into three groups, including the healthy group, the group of patients with light accumulated fat, and the group of patients with medium to severe accumulated. GPX1 and CAT were evaluated by the immunoassay method, lipid levels, fasting sugar, liver enzymes were measured with Cobas 6000 technology. Body mass index (BMI) was calculated by the ratio of weight (kg) to height square in meter (m^2). Quantitative results for the lowest concentrations of GPX1 and CAT showed that they were highly accurate, sensitive and repeatable. The statistical significance was high and acceptable for high concentrations in patients for fasting sugar, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and BMI especially in women, either GPX, CAT, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG) especially in males, but CAT was not statistically significant ($p > 0.05$). The results confirmed that the analytical method was highly accurate, and the biomarkers were relevant and influential in NAFLD, the effect of the enzymatic antioxidants GPX1 was more than the CAT that did not show it.

1. Introduction:

The most common disease among the majority of the world's population is non-alcoholic fatty liver disease (NAFLD), a global health issue that has led to the spread of obesity. When fatty foods are consumed in large quantities the accumulation and decomposition of lipids in tissues and lipid cells increases, which contributes to increasing the release of free fatty acids and the formation, and then accumulation of triglycerides in the liver. These fats activate oxidative damage while causing dysfunction in the functions of both mitochondria and antioxidants [1].

The process of disruption in metabolism was associated with increased hyperlipidemia, insulin resistance and high blood pressure and II diabetes, and it was found that insulin resistance is one of the main components that link NAFLD disease to type II diabetes. Therefore, the incidence of type II diabetes increases in these patients [2]. Previous studies have classified the degree of fat accumulation in adipose tissue in NAFLD patients, which was found to range from simple to moderate and severe liver fat [3]. Beyond metabolic disorder, there are other factors that led to the emergence of NAFLD disease, such as the age and sex of the individual and heredity [4].

In the process of fat accumulation, a state of imbalance occurs during the comparison of the amount of production of reactive oxygen types (ROS) with antioxidants, which requires

a balance between them to avoid the formation of reactive oxygen species (ROS) in the mitochondria and subsequent oxidative stress process [5]. Enzymatic antioxidants play a vital role in restricting reactive oxygen and its types and maintain cell health [6]; therefore, high amounts of reactive oxygen and its types versus low antioxidant activity cause progressive liver damage by activating inflammation and destroying cells.

Previous research indicates that the level of glutathione peroxidase) GPX1 (and catalase) CAT (enzymatic antioxidants is low in NAFLD patients, due to their ability to counteract oxidative stress, but continuing this process leads to a gradual decrease in their activity [7].

Hydrogen peroxide is one of the most important types of reactive oxygen that is dissolves in water, and has the ability to change fats in tissues and also change the structure of biological molecules. Therefore, the role of the enzyme CAT comes in getting rid of it by decomposing it into a molecule of oxygen with two molecules of water. Similarly, the enzyme GPX1 converts it into water only. In addition, gets rid of lipid peroxides by converting them into corresponding alcohols [8], [9].

This study had two primary goals. First, we aimed to evaluate the analytical performance using the characteristics of accuracy and sensitivity in detecting very low concentrations of the level of enzymatic antioxidant indicators in NAFLD patients. Second, to determine if there is a relationship between different lipid degrees with enzymatic antioxidants in males and females in non-alcoholic fatty liver disease. This research paves the way for future studies and opens many questions, and innovation of ways and Trials related to the use of enzymatic antioxidants as a means of early and potential detection of NAFLD patients.

2. Materials and Methods:

2.1 Study Design:

The study was conducted in Kirkuk Governorate from Iraq, after obtaining approval from the Kirkuk Health Department, blood samples were collected from one of the government hospitals / educational Azadi. The study ran from November 2024 to February 2025.

2.2 Exception to the Study:

Before collecting blood samples from the participants in this study, participants were carefully screened. Individuals who use alcohol, pregnant and lactating women and those with chronic diseases were excluded: autoimmunity, congestive and coronary heart, cancer, hypothyroidism, vitamin deficiency, thalassemia, osteoporosis, arthritis, viral liver, very excessive body weight, mental illness.

2.3 Study Participants:

The study included individuals with fat accumulation on the liver, patients with type II diabetes and hypertensive patients, while the healthy group consisted of individuals who did not suffer from any diseases.

2.4 Data Collection:

The participants in this study were selected and diagnosed from among those visiting the hospital for the purpose of examination and treatment, and the diagnosis was made by ultrasound machine (Voluson E6; E12497; America) by specialized doctors. After interviewing them and obtaining their consent, demographic data (age, sex, family history of the disease) and clinical symptoms were collected, and most importantly the daily dietary pattern used and physical activity, and then blood was drawn from them.

Samples (n=90) were collected from males and females aged between 30-70 years, and were divided based on the degree of liver fat apparent during their diagnosis ultrasound into three groups,

A. Group of healthy people (n=29), do not suffer from any known diseases.

B. Group simple fatty liver (n=29), 12 of them some of whom suffer from diabetes or hypertension disease or both.

C. Group moderate to severe liver fat (n=32), 22 of them some suffer from diabetes or hypertension disease or both.

2.5 Sample Processing and Measurement Procedures:

Approximately 5 ml of blood was drawn from participants who had fasted overnight for at least 10 hours. After 20 minutes, the blood clotted at room temperature, and then the blood samples were transferred to centrifuge tubes for separating serum from red blood cells, and then treated with a centrifuge at a speed of 6000 rpm (Hettich Rotofix 32 A; Model No. 1206; Serial No. 0043761-05; Germany) for five minutes, and after completion, the serum was saved in Eppendorf tubes with a size of 1.5 ml and was frozen to -20 °C to the time of the start of the analysis procedures.

GPX1 and CAT test kits were purchased from company Sunlong biotech in China (No.: SL3451Hu , SL2050Hu ; sensitivity: 0.1 pmol.mL^{-1}, 0.01 ng.mL^{-1}); coefficient of variation between tests (CV% $<$ 10%), and the measurements were made by enzyme-linked immunosorbent assay ELISA (Microplate washer-incubator; Paramedical-PKL; SN: 591808002; Italian).

Liver enzyme test kits were purchased (ALT: 20764957 ; AST: 20764949) and lipid indicators (TC2: 03039773190 ; TG: 20767107 ; HDL-C4: 07528566) and glucose (GLUC3: 04404483) from (Roche Diagnostic ; Germany), and the measurements were made with the cobas 6000 technique (Hitachi High technologies corporation; SN:14E8-06 ; Part no.727-0189 ; Japan).

2.6 Calculation of the Accuracy, Sensitivity and Repeatability of the Analytical Device:

The accuracy of the analytical method is evaluated by its ability to detect very low concentrations, and this involved taking multiple readings of a sample with a very low concentration to obtain reliable results.

Five samples of the standard low-concentration solution were prepared for both GPX1 and CAT, and then the absorbance of each sample was read five times (n=5) at a wavelength of 450 nm over three consecutive days (n=25). The accuracy and repeatability were calculated through the relative standard deviation RSD % of the total reading of the samples with low concentrations, and then the sensitivity was determined by calculating the detection limit (LOD) and the quantitative estimation limit (LOQ) using the following relations,

$$LOD = \frac{SD}{s} \times 3.3$$

$$LOQ = \frac{SD}{s} \times 10$$

Where s represents the slope of the standard curve derived from a series of solutions with concentrations different, and SD represents the standard deviation of the low-concentration standard sample readings [10].

Standard Dilute Solutions Preparation:

To prepare the standard dilute solutions of concentrations different, a serial dilution method was followed across five numbered tubes, 20 μ L of the standard diluent is added to each tube then add to,

1. Tube 1, 40 μL of the concentrated standard solution is added only to the first tube and mixed thoroughly.
2. Tube 2, 40 μL is transferred from the first tube to the second tube and mixed well.
3. Tube 3, 20 μL is transferred from the second tube to the third tube and mixed.
4. Tube 4, 20 μL is transferred from the third tube to the fourth tube and mixed.
5. Tube 5, 20 μL is transferred from the fourth tube to the fifth tube and mixed.

This serial dilution process produces a series of standard concentrations,

1. For GPX1, 48, 32, 16, 8, and 4 units.
2. For CAT, the concentrations are 3.6, 2.4, 1.2, 0.6, and 0.3 units.

These diluted solutions are then placed into plastic microwell plates, where their absorbance is measured (Zahrat AL-Rawan; China).

2.7 Enzymatic Antioxidant Detection:

The detection method used by enzyme-linked immunosorbent assay (ELISA) includes moderately acidic conditions. where the enzymatic antibodies in the serum interact with antibodies in polystyrene wells that include 96-wells. Subsequently, then reagents are added consisting of antibodies associated with an enzyme, leading to a reaction occurs accompanied by a blue complex formation. After adding the stop solution, the color changes to dark yellow, and the blue complex is called 3,3,5,5-tetramethylbenzidinediimin [11].

2.7.1 Enzyme Glutathione Peroxidase (GPX1):

In order to obtain the titration curve, a series of standard dilute solutions of GPX1 were prepared. This was done by diluting a concentrated standard solution of it at a concentration of (72 pmol . mL^{-1}) with a standard diluent solution, utilizing the law of solution dilution. This process yielded the following concentrations: 4, 8, 16, 32, 48 $pmol.mL^{-1}$.

Then these concentrations are placed in a mold (ELISA plate) consisting of 96-wells, and after that, about 40 μL of the sample dilution solution is added to each well, and then about 10 μL of the sample serum is added, and the mold is covered with transparent adhesive. The process of incubating the mixture begins for half an hour at a temperature of 37 °C, and then to ensure the removal of non-related materials, we wash the wells five times with a diluted washing solution with distilled water. In addition to each well, except the well dedicated to the blank solution HRP-Conjugate about 50 μL and start the process of incubation for half an hour with a temperature of 37C°. In addition to each well, about 50 μL of chromogen A and B were added, and the colors of the solutions were changed from colorless to bright blue and then incubated for 15 minutes at 37 C°. 50 μL of stop solution is added to each well, and the colors of the solutions were observed to change from blue to yellow.

Absorbance is measured by the plate reader at a wavelength of 450 nm (microplate reader; Paramedical-PKL; SN:-572002014; Italian).

2.7.2 Enzyme Catalase (CAT):

The practical method used to detect CAT is the same as that used to measure GPX1 but in different concentrations. For the purpose of obtaining the standard curve of CAT, a series of dilute standard solutions of different concentrations of CAT were prepared by diluting a concentrated standard solution of it at a concentration of (5.4 ng. mL^{-1}) with a standard diluent solution, utilizing the law of solution dilution.

This process yielded the following concentrations: 0.3, 0.6, 1.2, 2.4 , 3.6 $ng.mL^{-1}$.

2.8 Diagnosis of Routine Indicators:

The routine indicators in this study were measured using the Cobas 6000 device, and this system operates principle of "electrochemiluminescence", a chemical reaction between two compounds: one with high glow and the other oxidizable. For this reaction to happen, the reaction needs to be stimulated (chemically or electrically) to excite the electrons. Once arousal, they quickly return to their lifeless state (low energy), emitting visible light that can usually be measured by a fluorescent detector and spectrometer, and this phenomenon is called "Chemiluminescence" [12]. The device system includes chemical and immunological analysis with a high level of analytical efficiency for routine indicators as stated in previous studies [13].

2.9 Statistical Analysis:

To visually simplify the direction of the data, use program Excel 2024 to analyze data as percentages and column analysis. Quantitative data were expressed as arithmetic averages and standard deviations and to compare them in each group. The Minitab program (ver.17) and one-way ANOVA analysis were used, and Dunkin' test to find out the difference and similarity between individual group, with statistical significance being important at the level of probability ($p < 0.05$). Spearman's correlation was used to examine the bilateral relationships of variables measured within the level of statistical significance ($p < 0.05$) to valuation susceptibility to NAFLD disease.

3. Results:

The results of this research were derived from the master's thesis entitled " Evaluation of the level of Malone dialdehyde and some enzymatic antioxidants in the serum of patients with non-alcoholic fatty liver disease".

Table 1 presents the total number of males and females in each group divided in this study, in addition to the ratio of males to females in each group on the one hand, and the total number of males and females in all groups participating in the study. Table 2 presents the number of males to females

Table 1. Demonstrates the percentage of male and female participation in the patient and healthy groups.

Groups	No. Males	No. Females	Ratio of M. to F.
Con	13	16	1:1.23
D1	17	12	1:0.71
D2	14	18	1:1.29
Total	44	46	1:1.05

Con: represents the healthy group, D1: represents the group of patients with mild fatty liver, and D2 : represents patients with moderate to severe fatty liver , Ratio of F. to M. :- Ratio of females to males.

suffering from type II diabetes or high blood pressure or both in individual groups. Table 3 presents the data of the standard

Table 2. Chronic diseases in patients with non-alcoholic fatty liver disease.

Diseases	No. males to females		
	Con	D1	D2
	29	29	32
Blood pressure	0:0	6:3	7:3
Type 2 diabetes	0:0	0:1	1:2
Type 2 diabetes & blood pressure	0:0	1:1	5:4

Con: Healthy group, D1: Light mental accumulation set, D2: Moderate to severe mental accumulation group.

low-concentration solution of GPX1 (4 $pmol.mL^{-1}$) and CAT (0.3 $ng.mL^{-1}$), along with their calculated mean and standard deviation. This table involve the characteristics of accuracy, sensitivity and repeatability that represent the limits of detection (LOD), quantification (LOQ) and standard relative error (RSD%) based on the slope values of the standard curve (GPX1: 0.046, CAT: 0.6436). The linear ratio R^2 (coefficient of determination) was included for the calibration curve of standard solutions of different concentrations and showed a high value of 99 %. Table 4 presents data for three groups categorized by sex. The data are represented by a confidence interval level of 95% and in the form of mean and standard deviation at the level of statistical significance (p) less than (0.05). It also this table involve measurements for the indicators of enzymatic antioxidants, liver enzymes, fasting sugar, lipids, and body mass index, below is a detailed breakdown of each indicator,

• **Glutathione peroxidase (GPX1)**:

I Showed statistically significant and the result is acceptable, demonstrating its effective role in NAFLD disease and

Table 3. Analytical properties for the detection of enzymatic antioxidants.

Enzymatic antioxidants	Five readings for low concentration				
Catalase (CAT)	0.3116	0.3116	0.3115	0.3115	0.3116
Glutathione peroxides (GPX1)	0.2280	0.2281	0.2281	0.2281	0.2280
Summary of the results of the above readings					
Analytical criteria					
	Mean	SD	Slop	R^2	RS D%	LOD	LOQ
Catalase (CAT) ($ng.mL^{-1}$)	0.3116	6.32×10^{-5}	0.046	0.9993	0.01	3.2×10^{-4}	9.8×10^{-4}
Glutathione peroxides (GPX1) ($pmol.mL^{-1}$)	0.2280	6.32×10^{-5}	0.6436	0.9958	0.02	1.0×10^{-3}	1.0×10^{-2}

Mean:- Mean readings of low concentration , SD :- Standard deviation of readings , RSD% :- Standard relative error, LOD :- Limit of detection , LOQ :- Limit of quantification, R^2 :- Coefficient of determination.

the highest arithmetic average was in healthy males.

Concentration Trends:

Figure 1 presents the higher concentration of GPX1 in patients compared to healthy individuals, with this trend being more noticeable in males than in females. As for the Figure 2 it shows that when comparing males it increased in D1 group by 9% then gradually decreased in D2 group by 1.2%, while in females it decreased in D1 group by 28% and gradually increased in D2 group by 3.3%. Finally, from Table 6 it is clear that GPX1 has a very strong and positive relationship with Catalase (CAT).

• **Catalase (CAT):** It's result showed no statistically significant acceptable, indicating that it had no effect on NAFLD disease and the highest arithmetic mean was in males of the D1 group.

Concentration Trends:

Figure 2 presents the higher concentration of CAT in patients compared to healthy individuals, with this trend being more noticeable in males than in females. As for the Figure 2 it shows that when comparing males it increased in D1 group by 39% and in D2 group by 12%, while in females it decreased in D1 group by 21% and then increased in D2 group by 1.7%.

• **Total cholesterol (TC):** it had high statistical significance and the result was very acceptable, demonstrating its significant effect on NAFLD disease and the highest arithmetic mean was in women in the D2 group.

Concentration Trends:

Figure 1 presents the higher concentration of TC in patients compared to healthy individuals, with this trend being more noticeable in females than in males. As for the Figure 2 it shows that when comparing males it increased in D1 group by 25% and in D2 group by 19%, while in females it decreased in D1 group by 27% and increased in D2 group by 25%.

• **Triglycerides (TG):** Statistical significance was important for TG and the result is acceptable, demonstrating its effect on NAFLD disease and the highest arithmetic mean was in women in the D2 group.

Concentration Trends:

Figure 1 presents the higher concentration of TG in patients compared to healthy individuals, with this trend being more noticeable in males than in females. As for the Figure 2 that it shows when comparing males, it increased in D1 group by 34% and in D2 group by 49%, while in females it decreased in D1 group by 18% and increased in D2 group by 26%.

Finally, from Table 6 it is clear that it is positively and strongly associated with TC and AST, Alanine Aminotransferase (ALT) , body mass index (BMI), but inversely with high-density lipoprotein (HDL-C).

• **high-density lipoprotein (HDL-C):** it was of high statistical significance with an acceptable result, demonstrating its effect

Table 4. Analysis of variables by gender in each group.

Variables	Con=29		D1=29		D2=32		p-value
	Males = 13	Females=16	Males=17	Females=12	Males=14	Females=18	$p < 0.05$
GPX1 ($pmol.ml^{-1}$)	9.211 ± 2.162 a	8.546 ± 1.606 ab	7.569 ± 1.867 c	7.872 ± 2.035 c	8.221 ± 1.953 bc	7.583 ± 1.914 c	0.036 ∗
95 % CI	7.95 , 10.0	7.29 , 9.19	7.20 , 9.23	6..68 , 8.48	6.64 , 8.49	6.77 , 8.97	
CAT ($ng.ml^{-1}$)	0.5161 ± 0.193 a	0.5138 ± 0.1076 a	0.6570 ± 0.5800 a	0.5357 ± 0.1339 a	0.5494 ± 0.0894 a	0.4650 ± 0.1043 a	0.473
95 % CI	0.362,0.669	0.375,0.652	0.401,0.697	0.334,0.595	0.523,0.791	0.376,0.695	
TC (mg/dL)	171.3 ± 35.23 c	184.8 ± 35.76 bc	176.5 ± 34.76 c	192.9 ± 33.64 b	196.7 ± 38.60 b	238.3 ± 43.30 a	0.0003 ∗∗∗
95 % CI	150, 191	166, 203	158, 194	171, 214	176, 216	220, 255	
TG ($mg.dL^{-1}$)	189.5 ± 33.80 c	190.5 ± 37.40 c	220.0 ± 40.40 b	207.4 ± 44.20 bc	374.4 ± 32.40 a	230.9 ± 35.60 b	0.039 ∗
95 % CI	102 , 276	112 , 269	143 , 296	116 , 298	263 , 431	157 , 304	
HDL-C ($mg.dL^{-1}$)	34.75 ± 7.410 b	38.11 ± 7.160 a	32.06 ± 6.110 b	38.62 ± 7.240 a	34.83 ± 7.610 b	40.87 ± 7.590 a	0.009 ∗∗
95 % CI	30.7, 38.7	34.5, 41.6	28.6, 35.5	34.4, 42.7	31.0, 38.6	37.5, 44.2	
GLUC ($mg.dL^{-1}$)	95.40 ± 11.20 c	124.5 ± 24.80 b	129.9 ± 24.90	162.2 ± 37.70 a	152.1 ± 22.30 a	136.7 ± 26.60 a	0.026 ∗
95 % CI	56.5, 134	89.5, 159	95.9, 163	121, 202	114, 189	103, 169	
ALT ($U.L^{-1}$)	12.06 ± 2.702 c	10.87 ± 2.678 c	17.03 ± 4.470 b	12.19 ± 2.550 c	24.62 ± 5.010 a	16.78 ± 3.740 b	0.0003 ∗∗∗
95 % CI	7.36, 16.7	6.63, 15.1	12.9, 21.1	7.30, 17.0	20.0, 29.1	12.7 , 20.7	
AST ($U.L^{-1}$)	16.66 ± 2.406 c	16.93 ± 3.309 c	20.92 ± 4.700 b	16.09 ± 2.897 c	24.00 ± 5.840 a	19.85 ± 4.840 b	0.009∗∗
95 % CI	13.0, 20.3	13.6, 20.2	17.7, 24.1	12.2, 19.8	20.7, 27.8	16.7, 22.9	
BMI ($kg.m^{-2}$)	27.83 ± 3.500 b	28.01 ± 5.840 b	29.59 ± 4.460 b	32.13 ± 3.920 a	32.37 ± 5.880 a	32.71 ± 7.060 a	0.032∗
95 % CI	24.8, 30.7	25.3, 30.6	26.9, 32.1	29.0, 35.2	29.5, 35.2	30.1, 35.3	

The letters a, c, b, and a indicate the presence/absence of differences between groups, with the letter a taking the highest value. The compound letters ab and indicate no difference between groups. The above data is represented by the mean ± standard deviation at the statistical significance level of $p < 0.05$ and the mark $(*)$ indicates statistical power. (ALT) Alanine aminotransferase, (AST) aspartate aminotransferase, (GPX1) glutathione peroxidase, (CAT) catalase, (Cho) total cholesterol, (HDL-C) high-density lipoprotein, (GLUC) fasting glucose, (TG) triglycerides.

on NAFLD disease and the highest arithmetic mean was in women in the D2 group women.

Concentration Trends:

Figure 1 presents the higher concentration of HDL-C in patients compared to healthy individuals, with this trend being more noticeable in females than in males . As for the Figure 2 it shows that when comparing males it increased in D1 group by 25% and decreased in D2 group by 7.3%, while in females it decreased in D1 group by 27% and increased in D2 group by 17%.

Finally, from Table 6 it is clear that it has a strong and inverse association with TG.

• **Fasting sugar (GLUC):** was statistically significant and with an acceptable result, demonstrating its effect on NAFLD disease and the highest arithmetic mean of the D1 group women.

Concentration Trends:

Figure 2 presents the higher concentration of GLUC in patients compared to healthy individuals, and was between males and females by an equal 50%. As for Figure 2 it shows that when comparing males it increased in D1 group by 43% and in D2 group by 41%, while in females it decreased in D1 group by 2% and increased in D2 group by 19%.

Finally, from Table 6 it is clear that it has a strong and positive association with ALT, TC and a negative association with AST.

• Alanine Aminotransferase (ALT): The statistical significance was high and the result is very acceptable, which indicates its significant effect on NAFLD disease and the highest arithmetic mean in males in the D2 group.

Concentration Trends:

Figure 1 presents the higher concentration of ALT in patients compared to healthy individuals, with this trend being more noticeable in males than in females. As for the Figure 2 it shows that when comparing males it increased in D1 group by 45% and in D2 group by 54%, while in females it decreased in D1 group by 15 and increased in D2 group by 42%.

Finally, from Table 6 shows a very strong and positive association with the enzyme AST and also with fasting sugar.

• Aspartate Aminotransferase (AST): The statistical significance was high with a very acceptable result, which indicates its significant effect on NAFLD disease and the highest arithmetic mean in the D2 group males.

Concentration Trends:

Figure 1 presents the higher concentration of AST in patients compared to healthy individuals, with this trend being more noticeable in males than in females. As for the Figure 2 it shows that when comparing males it increased in D1 group by 39% and decreased in D2 group by 36%, while in females it decreased in D1 group by 28% and increased in D2 group by 24%.

Finally, from Table 6 it is clear that it has a positive and strong association with the enzyme ALT, but inverse in order with fasting sugar and TC.

• Body mass index (BMI): The BMI was statistically significant and the result is acceptable, indicating its association with NAFLD disease and the highest arithmetic mean of females in the D2 group.

Levels Trends:

Figure 1 presents the higher level of BMI in patients compared to healthy individuals, especially in females, and males and females were roughly equal to some extent with a difference of 1%. As for the Figure 2 it shows that when comparing males it increased in D1 group by 28% and in D2 group by 20%, while in females it decreased in D1 group by 15% and increased in D2 group by 22% . Interestingly, when calculating the body mass index (BMI) of the participants, 11 of them were within the normal range and the majority were within the abnormal range and from Table 6 it is clear that it is related strong positive with TG.

BMI and Age Distribution:

Table 5 presents the mean age of people in individual group is this study, which contributes to providing age-related data to understand the effect of age on the variables measured in this study and interpret them more accurately. In addition to the number of people who show an increase in body mass index (BMI), which was associated with NAFLD, the highest increase in the D2 group was especially in (17) females aged 70-30 years and (14) males aged 50-30 years.

Table 6 presents bilateral correlations between of the biochemical indicators through the use of Spearman's correlation (r), and the strength of statistical significance p-value, through this, it is possible to identify the extent of their impact on NAFLD disease.

Table 7 presents the normal range of routine indicators, which are an essential means of assessing public health when comparing the measured practical results with the normal range and distinguishing them from the pathological condition or the onset of disease conditions.

4. Discussion of Results:

4.1 Interpretation of Analytical Results:

In this experimental study, the results of the detection of very low concentrations of GPX1 (4 pmol.mL^{-1}) and CAT (0.3 ng.mL^{-1}) showed a high degree of accuracy and sensitivity with the least practical error. A very low detection limit value shows that the analytical method is able to detect very low concentrations of enzymatic antioxidants, even if they are in very low quantities by observing the quantitative estimate limit value.

The results were with a high degree of accuracy and repetitivity where they were (RSD % $<$ 1 %) any good repeatability of readings within one experiment with the least possible dispersion of the measured values, which reflects the stability of analytical performance and ensure the reliability of measurements.

Unknown concentrations can be found in the sample serum based on a relationship obtained to draw the standard curve when preparing a series of known standard solutions of different concentrations, the linear ratio was high (R^2 = 99%) and indicates the quality and accuracy of the experimental results of enzymatic antioxidants and their great ability to understand and explain the causes of the disease in patients NAFLD.

4.2 Interpretation of Vital Results:

An unhealthy diet leads to a disturbance in the metabolic process thus increasing the incidence of high blood pressure, insulin-resistant diabetes, accumulation of visceral fat in the abdomen [14] and liver, and also infection with NAFLD. A recent study also showed that it is related to metabolic disturbance , and it seems that the oxidative process plays a pivotal

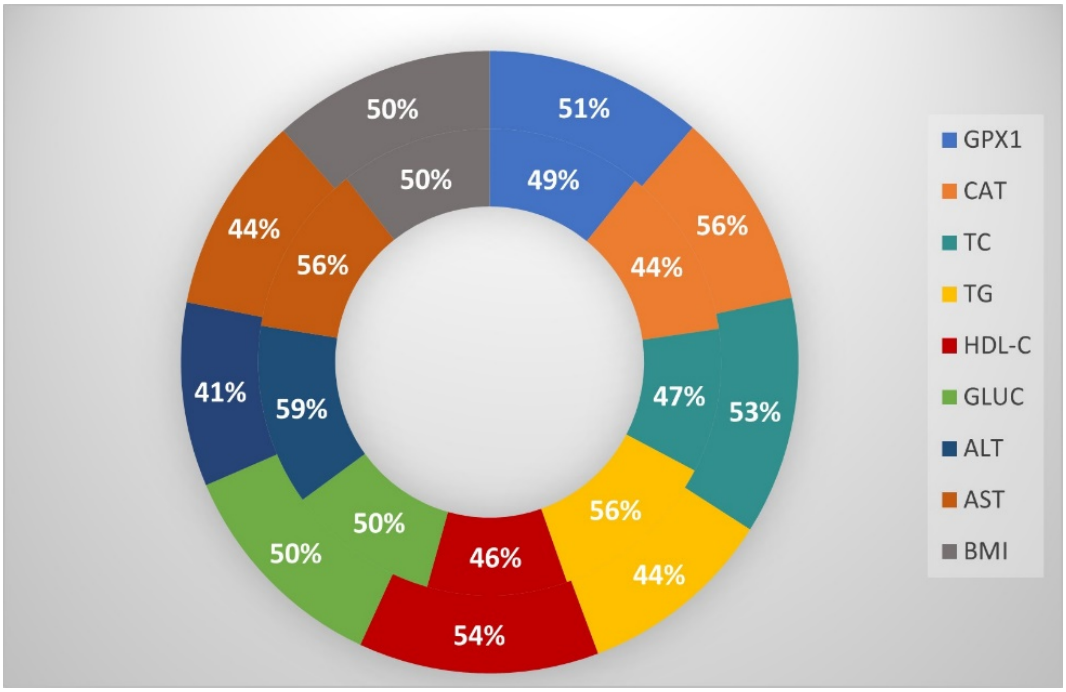

Figure 1. Represents the percentages in males (external circle) and females (inner circle) for the indicators of enzymatic antioxidants, lipids, fasting sugar and body mas.

role in the development of the pathological condition. Under these conditions, enzymatic antioxidants play an important role as a defense against oxidative damage [7].

4.2.1 The Relationship between Lipids Accumulation and Glutathione Peroxidase (GPX1) and Catalase (CAT):

The current study showed the imbalance in the antioxidant system versus the production of free radicals by observing changes in GPX1 and CAT activity/concentration, worsening the pathological condition. In the context of the results of this study, NAFLD disease was associated with GPX1 level/concentration but not CAT level/cocentration. The CAT enzyme did not show a significant difference between patients and healthy individuals, although a difference in its levels was observed. The concentrations were very low in all individuals, and close to zero, not sufficient to show a significant difference, this indicates that in some cases it is not related to the disease.

The results of this study were consistent with the results of a previous study conducted in 2020 year on obese individuals, where both CAT and GPX1 did not show statistical significance [15]. However, GPX1 was shown in the current study to increase the incidence of NAFLD disease. The correlations in this study showed that the relationship between GPX1 and CAT is very strong, because together they contribute to the removal of hydrogen peroxide resulting from lipid oxidation, and other reports have reported their association with the membrane that coats hemoglobin, making it protected from oxidative attacks [16].

In this study, different levels of GPX1 and CAT in patients compared to healthy people were associated with age and sex differences and the amount of lipids.

A. Group - simple lipids in the liver : Antioxidant molecules are depleted differently in both sexes. The level of GPX1 and CAT increased in males (mean age 45 years) compared to healthy people. In females (mean age 50 years), these enzymes showed an inverse pattern as their levels decreased.

Two types of estrogen receptor genes in females plays a defensive role in fighting oxidative agents, which makes no need for enzymatic antioxidants to exert a great effort in the defense system against oxidative stress [17], this is proof that females have a stronger defense system than males in fighting oxidative agents.

As studies have shown that elevated levels of antioxidant enzymes are associated with oxidative stress markers, which rise with increased production of reactive oxygen and its types in the early stages of oxidative damage. In this case, antioxidant enzyme activity is stimulated to combat free radical chain reactions. Therefore, this led to elevated levels of both GPX1 and CAT In males with simple fatty liver [18].

B. Group - moderate to severe lipids in the liver: The production of oxidative agents in the mitochondria increases,

Figure 2. Shows total concentration of measured indicators for males and females in (Con) healthy group, (D1) group with mild fatty deposits, (D2) group with moderate to severe fatty deposits , and the indicators represent (TC) total cholesterol ($mg.dL^{-1}$), (HDL-C) high-density lipoprotein ($mg.dL^{-1}$), (TG) triglycerides ($mg.dL^{-1}$), (ALT) alanine aminotransferase ($U.L^{-1}$), (AST) aspartate aminotransferase ($U.L^{-1}$), (BMI) body mass index ($kg.cm-2$), (GPX1) glutathione peroxidase ($pmol.mL^{-1}$), (CAT) catalase($ng.mL^{-1}$), (GLUC) sugar ($mg.dL^{-1}$).

Table 5. Life expectancy and percentage of body mass index (BMI) in the healthy and patient groups.

Age	Con = 29		D1 = 29		D2 = 32	
	Males =13	Females=16	Males =17	Females=12	Males =14	Females=18
(30-40)	3	10	8	2	7	5
(41-50)	3	2	2	4	6	2
(51-60)	2	4	7	4	0	7
(61-70)	5	0	0	2	1	4
	Average lifespans (range)					
	51(31-66)	43(30-60)	45(30-60)	50(33-66)	42(30-67)	51(30-70)
BMI ($kg.m^{-2}$)	Percentage % increase in body mass index (BMI)					
BMI ≥ 25	(11) 84	(12) 70	(14) 82	(11) 91	(14) 100	(17) 94

Con:- Healthy groups, D1:- Patients with mild fatty accumulation, D2:- Patients with moderate to severe fatty accumulation.

a difference in GPX1 and CAT levels was observed in males (median age 42 years), where GPX1 level/concentrati -on decreased compared to healthy people by a difference of 1.2%, while CAT compared to healthy subjects increased by a difference of 12%. It seems that CAT at this stage is still fighting oxidative factors due to the increase in accumulated fats that lead to an increase in oxidative factors, and that causes oxidative stress without the appearance of hepatitis or to an increase in the rate of oxidation of unsaturated lipids that increase its activity [19], as age and metabolic disorder and insulin resistance also play an important role [20].

Previous studies have reported that in males the formation of reactive oxygen of the super-oxygen type occurs in the mitochondria, this causes more oxidative damage than in females , with lower levels of enzymatic antioxidants . It has also been shown that aging contributes to the production of free radicals in the mitochondria which causes damage to its constituent DNA a defect in its normal functions, and damage to proteins and fats in the body of the organism [21]. Under these conditions, when mitochondria are under reactive oxygen stress, it leads to targeting the genes that produce antioxidant enzymes and their depletion thus reducing their production [22].

In this study, it was observed that in males in conjunction with lipid accumulation CAT showed a greater attack than GPX1 in fighting oxidative agents, resulting in CAT constituent genes being targeted more than GPX1 genes. So quantitatively the CAT concentration was very low compared to GPX1, and this was shown in males. On the other hand, most of them suffer from chronic diseases and take medications to lower blood pressure and diabetes, which led to lower lipid levels a littel percentage, consequently, a slight decrease in GPX1 concentration was observed. However, the activity of these antibiotics can decrease when the lipid level is low due to the lack of amounts of oxidative agents (reactive oxygen) [23]. While in females (mean age 51 years), the level of GPX1 and CAT is higher compared to healthy people, especially GPX1. It is known that estrogen activity decreases with age in ovaries, blood vessels, adipose tissue. when the level of inflammatory cytokines elevated in adipose tissue it inhibit enzymes (estrogen producers) that enter into interactions with estrogen receptors , this increases free fatty acids and insulin resistance and promote oxidative agents (e.g. in case of hypertension), thus stimulating the activity of antibiotics in maintaining liver healt [24]. It appears that females in this group have increased the concentration of selenium in the GPX1 installation, and led to increased lipids accumulation, because selenium is one of the essential components in the GPX1 architecture, which plays a paradoxical role, either it is an antioxidant and removes damaged fats or contributes to the production of reactive oxygen and its types that cause an increase in the percentage of fat in muscle cells, insulin resistance, and the last role occurs in the case of high concentrations of selenium (sourced from food) [25].

In summary, the concentration/level/amount of GPX1 was higher than CAT in the serum of both sexes, and showed greater resistance to oxidizing agents thus maintained it's level serum in blood, but CAT was targeted by oxidative factors leading to a decrease in its production in the body. On this basis, the higher the CAT level in males, the higher the amount of fat in the liver, while the higher the level of GPX1, the lower the amount of fat in the liver. In females, a reverse pattern appears, where the higher the level of GPX1 and then CAT, the greater the amount of fat in the liver significantly.

Table 6. Spearman's correlation between the measured indicators.

	GPX1	ALT	AST	TC	HDL-C	GLUC	BMI
CAT	p=0.000 r=0.97						
ALT						p=0.003 r=0.50	
AST		p=0.000 r=0.72		p=0.017 r=-0.44		P=0.009 r=-0.48	
TG		p=0.035 r=0.38	p=0.020 r=0.41	p=0.002 r=0.54	p=0.002 r=-0.54		p=0.036 r=0.40
GLUC				p=0.039 r=0.39			

p:- Represents statistical significance, r :- Spearman's correlation.

4.2.2 The Relationship between Lipids Accumulation and Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST):

In this study, the results showed that the association is very strong between ALT and AST, and that there is a small increase in liver enzymes ALT and AST associated with NAFLD, and both are positively correlated with glucose and TG. the results also showed that different levels of ALT and AST in patients compared to healthy people are associated with age and sex differences and the amount of lipids.

A. Group - simple lipids in the liver: In males (the mean age 45 years), it was observed that the level of ALT rises more than AST by a difference of 6% compared to healthy people, while in females (the mean age of 50 year) , it was observed that the level/concentration of ALT decreases below the level/concentration of AST by a difference of 13% compared to healthy people.

Elevation of these enzymes indicates liver cell damage resulting from high lipid biomarkers in the case of obesity, NAFLD, hypertension and insulin-resistant diabetes [26], because in the case of cell resistance to insulin, which is mainly seen in NAFLD disease, leads to increased sedimentation of unsaturated fatty acids in fat cells, which later accumulate in the liver, and thus lead to stress and weakening of the process of beta oxidation and mitochondria, and at the same time leads to weakening the inner lining of blood vessels and it causes atherosclerosis [27].

In this case, the hepatocytes become inflamed and may be damaged and release ALT at a greater rate and AST at a lower rate to the bloodstream, because ALT is mainly created in the liver and its elevation indicates an increase in the level of fat in the liver, while high AST may indicate several injuries, including the liver and the accumulation of bile fluid and muscles. Moreover, the difference in the level of enzymes is due to the difference in sex hormones, the male hormone (androgen) that stimulates the activity of these enzymes, while the female hormone (estrogen) reduces the activity of these enzymes before menopause [28], [29].

B. Group - moderate to severe lipids in the liver:

This stage witnesses an increase in the production of oxidative agents in the liver. Moreover, it was observed in males (mean age 42 years) and females (mean age 51 years) that the level of ALT rises more than AST by a difference of 18% compared to healthy people, this rise was more noticeable in males compared to females. Which indicates the presence of oxidative damage related to liver cell damage.

Although all of this group are obese, obesity is directly associated with the elevation of these enzymes. Males suffered from high blood pressure and insulin-resistant diabetes more than females, and usually these patients suffer from a significant incidence of cell damage and the release of ALT and AST enzymes at a greater rate, and this has been proven in a previous study that high levels increase the risk of these chronic diseases. Moreover, the percentage of lipids accumulated (abdominal tissue) in males is higher compared to females (subcutaneous tissue), and leads to a greater impairment of liver enzyme [30]. Also, older females show gradual stages of decline in estrogen hormone and elevated insulin

Table 7. The normal range and results of routine indicators using the Copas 6000 device.

Routine indicators	The range of practical results	Natural range
ALT ($U.L^{-1}$)	(3.8 – 64.9)	[Females $\leq$ 33] [Males $\leq$ 41]
AST ($U.L^{-1}$)	(10.8 – 55.5)	[Females $\leq$ 32] [Males $\leq$ 40]
TC ($mg.dL^{-1}$)	(116 – 318)	$<$ 200
TG ($mg.dL^{-1}$)	(75 – 1224)	$<$ 150
HDL-C ($mg.dL^{-1}$)	(20.4 – 61.4)	3.09 - 150
GLUC ($mg.dL^{-1}$)	(81 – 429.4)	74 - 106
BMI ($kg.m^{-2}$)	(20.3 – 50.6)	18.5 – 24.9

(ALT) Alanine aminotransferase, (TC) total cholesterol, (BMI) body mass index, (HDL-C) high-density lipoprotein, (TG) triglycerides, (AST) aspartate aminotransferase, (GLUC) sugar.

resistance, which are factors that promote the accumulation of fat in the liver as mentioned earlier. There are many studies that resort to the treatment of NAFLD disease in these females with estrogen-based pharmacological intervention [31]. The results of ALT and AST in this study are consistent with the results of a previous study [32].

In summary, elevated concentration/ level of ALT and AST gradually leads to an increase in the amount of liver fat in males, and significantly in females.

4.2.3 The Relationship between Lipids Accumulation and Body Mass Index (BMI):

The results of this study showed that fat accumulated in the liver is associated with a higher BMI in NAFLD patients compared to healthy people, observed especially in females, with a very small difference from males, and is positively associated with the level of TG. In addition to the above, the vast majority of participants in this study were outside the normal range of BMI by 87%; however, (6) of the healthy people were within the normal weight range, but the disease has appeared in (5) people of normal weight (3 males and one female, suffering from mild fatty liver disease, and one female suffering from severe fatty liver disease). Previous studies have reported that people with and without obesity had a great similarity in the factors causing inflammation, but it is certain that insulin resistance is the factor that greatly affects the emergence of NAFLD [33].

As the results showed that the difference in the level of BMI in patients compared to healthy people was associated age and sex difference and the amount of lipids.

A. Group - simple lipids in the liver:

In males (mean age was 45 years), it was observed that the level of BMI increases compared to healthy people, in females (mean age is 50 years), the BMI level was observed to decrease slightly compared to healthy people. The role of androgen and estrogen hormones in showing this difference is highlighted, Where estrogen is active before menopause, which contributes to preventing the accumulation of lipid in females, as for the increased activity of androgen hormone in males, it has a strong relationship with the accumulation of lipid in the liver and thus, obesity associated with chronic diseases [34], such as insulin-resistant diabetes and high blood pressure associated with NAFLD [35]. There were (8) of males and (4) of females had chronic diseases, so chronic diseases, unhealthy dietary pattern and androgen hormone were all factors that led to higher BMI in males compared to females.

B. Group - moderate to severe lipids in the liver: In males (mean age is 45 years), in females)mean age of males is 51 years(, it was observed that the level of BMI is increases compared to healthy people, but in females more than in males. Body mass is associated with chronic diseases, it ‘s found that (13) of males and (9) of females had the chronic diseases mentioned above, and were committed to taking antihypertensive drugs, diabetes and a healthy diet, as stated in a recent study conducted in Scotland that antihypertensive drugs in diabetics led to weight loss with modification of the dietary pattern [36]. For those who do not have chronic diseases, there seem to be other factors that affect them, including muscular dystrophy, aging, lack of physical activity, it seems that there are other factors more influential in these females, including muscle atrophy, aging, lack of physical activity. It appeared in another study that muscle atrophy is inversely related to body weight, where with age and lack of body movement activity, the accumulation of liver fat associated with many chronic diseases increases, and in this case the function of adipose tissue in the muscles is disrupted and reduces the secretion of the protein ”adiponectin”, which promotes two processes: - Cell absorption of glucose by insulin and oxidation of unsaturated fats, leading to glucose formation and insulin resistance to a decrease in the protein ”myosin” that makes up the mus-

cles, increase the amount of free fatty acids and thus weaken muscle strength [37]. In summary, as the BMI level rises the amount of fat gradually increases in males, and significantly in females.

4.2.4 The Relationship between Lipids Accumulation and Triglycerides (TG):

In this study, the concentration/level of TG in patients, especially in males, was elevated and was influential in NAFLD, which is positively associated with body mass, TC, AST and ALT, but negatively with HDL-C lipoprotein.

As the results showed that the difference in concentration/level of TG in patients compared to healthy people was associated with age and sex differences and the amount of lipids.

A. Group - simple lipids in the liver: In males (mean age 45 years), the concentration/level of TG increased compared to healthy people, while in females (mean 50 years), the concentration/level of TG decreased compared to healthy people.

The concentration /level of TG is mostly associated with the rate of movement of the body movement and dietary patterns, in return males were less interested in exercise and more interested in an unhealthy dietary pattern than females, resulting in TG accumulation. It was found that lack of physical activity or after a fatty meal increases the level of TG in the blood, and positively affects body mass and also came in a study that it negatively affects lipoprotein HDL-C [38], this is because HDL-C particles are consumed after the breakdown (TG) by lipoprotein lipase present in HDL-C particles, leading to a decrease in HDL-C levels and an increase in TG levels [39]. In addition, in females the level of TG was lower, it seems that estrogen to negatively affect their TG level [40]. It was found that there is a link between people with NAFLD and insulin-resistant diabetes [41]. This is because insulin mainly affects the fat in the liver, and consequently, sex-specific changes occur in liver enzymes ALT and AST and the level of lipids associated with each other positively. In contrast, the appearance of symptoms of high blood pressure and insulin-resistant diabetes [42].

There were (8) males and (4) females with chronic diseases, which led to a disorder of the level of TG in males more than females.

B. Group - moderate to severe lipids in the liver: In males (mean age 42 years) and in females (mean age 51 years), the concentration/level of TG increased compared to healthy people, but in males more than in females. We mentioned earlier that (13) of males and (9) of females suffer from diabetes and high blood pressure, so it is normal for the level of TG to rise in males more than females.

From the observation of Table 5, we find that (11) females are over 51 years old, and as females age and physical activity decreases, symptoms of insulin resistance appear more and estrogen activity decreases, and this negatively affects the level of TG. In summary, elevated concentration/level of TG leads to a gradual increase in the amount of liver fat in males, and significantly in females.

4.2.5 The Relationship between Lipids Accumulation and High-density lipoprotein (HDL-C):

The results in this study showed that the concentration of HDL-C is moderately high and has a strong effect in NAFLD patients, especially in females, and as we mentioned earlier, the reasons for its negative association with TG. As the results showed that the difference in the level of HDL-C in patients compared to healthy people was associated age and sex differences and the amount of lipids.

A. Group - simple lipids in the liver: In males (mean age 45 years) the concentration/level of HDL-C increased compared to healthy people, and in females (mean age 50 years) decreased compared to healthy people. The number of Males with chronic diseases were more than females in the same conditions, and the results of a previous study reported that the level of HDL-C rises in the case of patients with high blood pressure, they have ranged from ”40-80” mg.d^{L-} [43], and this was somewhat close to the results of the current study, which amounted to ”20.4 – 61.4”, but it was more close to the results of another study (63-44) mg.d^{L-}, and the mean age of males was (44) years old with diabetes [44]. In females, a few had high blood pressure; however, the level of HDL-C was low, as usual, HDL-C levels are low in patients with NAFLD [45].

B. Group - moderate to severe lipids in the liver: In males (median age 42 years) and females (median age 51 years), the concentration/level of HDL-C compared to healthy people, but it was in females increased more than in males.

Males with chronic diseases are more than females in the same conditions, they had high concentrations/levels of TG, there will certainly be a decrease in HDL-C concentration/level, as we mentioned earlier the negative relationship between them.

Appear in females show greater sensitivity to the absorption of TG in the muscles than in males, as reported by previous reports close relationship between estrogen and the level of TG, as it works as an antioxidant of fats and inflammation in addition to regulating the level of lipids in serum. It is known that with aging, low estrogen levels and menopause cause inhibition of TG levels, and thus rise the level of HDL-C, [46], [47] as stated in the current study that the relationship is inverse between TG and HDL-C.

As the genetics factor also plays an important role in the high levels of HDL-C in the blood and can change the function of HDL-C, whether positively provides protection or nega-

tively increases the risk of disease, or genetic mutations may occur for the protein that makes up it, and work to increase its production, which leads to high levels [39].

In summary, elevated concentration/level of HDL-C leads to a gradual increase in the amount of liver fat in males, and significantly in females.

4.2.6 The Relationship between Lipids Accumulation and Total cholesterol (TC):

the results of the current study showed high TC levels that were very influential in NAFLD patients, especially in women, but were positively associated with TG, AST and glucose. As the results showed that the difference in the level of TC in patients compared to healthy people is associated with different age and sex and the amount of lipids.

A. Group - simple lipids in the liver: In males (mean age 45 years) an increase in TC concentration/level was observed compared to healthy people. While in females (mean age 50 years), a lower concentration/level of TC was observed compared to healthy people. As mentioned earlier, the diseases suffered by males and poor dietary pattern are higher compared to females, so it certainly leads to a higher concentration/level of TC in males than in females.

Also, fatty foods that contain fats (unsaturated, trans) and sugars of all kinds increase cholesterol, as it has a negative effect even if it is present in small quantities. Increased cholesterol increases (TG levels, insulin resistance, body mass) and thus liver enlargement. In an experimental study, these factors were less affected by the absence of cholesterol in dietary foods [48].

Patients with high blood pressure, and diabetes usually suffer from a rise in the level of TC, due to the weakness of the inner lining of blood vessels and the amount of free fatty acids (cholesterol), caused by oxidative factors causing the secretion of inflammatory cytokines [49].

B. Group - moderate to severe lipids in the liver: In males (mean age 42 years) and females (mean age 51 years), it was observed that the concentration/level of TC increased compared to healthy people, but it was higher in females than in males.

Premenopausal females are also known to be less likely to have dyslipidemia than males, as were the females in group D1, but after menopause, blood lipids rise due to higher insulin resistance by a greater percentage compared to males and thus increase the incidence of NAFLD, high blood pressure and insulin-resistant diabetes In short, insulin resistance varies according to age and gender [50].

In addition, the lack of inhibition of high cholesterol is associated with insulin resistance that causes inflammation, as the fattier accumulations increase, this prevents the transfer of cholesterol out of the cell because in this case there are receptors that work opposite instead of receiving natural low-density lipoprotein (LDLR) that receive the oxidizing form of this protein (oxLDL), which leads to the accumulation of cholesterol in the liver [51].

It appeared in a study conducted in India observed elevated changes in liver enzymes and positively related with lipid disorder dysfunction where AST was positively associated with both TC and TG, has been observed that cells, enzymes and the process of lipid metabolism in the liver is affected by an excessive amount of cholesterol, which increases the incidence of hypertension and insulin-resistant diabetes [52].

In summary, elevated concentration/level of TC leads to a gradual increase in the amount of liver fat in males, and significantly in females.

4.2.7 The Relationship between Lipid Accumulation and Glucose (GLUC):

The results of the current study showed high fasting GLUC levels that were influential in NAFLD patients, especially in females, and was positively associated with ALT, AST and TC. As the results showed that the difference in the level of GLUC in patients compared to healthy people is associated with different age and sex and the amount of lipids.

It is also known that genetics factor and a high-calorie dietary pattern have two crucial roles in impaired mitochondrial function, which in turn leads to sequential and interrelated disorders. Disorders are represented by increased stimulation of beta cells in the pancreas to increase insulin secretion, and cell intolerance this stress leads to the appearance of a sign of insulin resistance, which in turn leads to an increase in the level of GLUC in the blood instead of being absorbed by the cell.

Next, a change metabolism in lipids occurs (conversion of saturated and unsaturated fatty acids to TG and free fatty acids to cholesterol) it increases its levels in the blood and disturbed liver enzymes. These factors are inflammatory causes in tissues and fat cells in muscles and liver, leading to increased obesity, hyperlipidemia, NAFLD and insulin-resistant diabetes [53].

Group - simple lipids in the liver: In males (median age 45 years), and females (median age 50 years), higher concentration/level of GLUC was observed in males compared to

healthy people, but decreased in females by a very small percentage compared to healthy people. Poor dietary pattern, lack of physical activity and chronic diseases in males were higher compared to females, these factors lead to insulin resistance and GLUC intolerance, and thus increase its concentration in the blood.

Foods containing high calories, including cholesterol, this leads to stimulating the liver to increase its synthesis, and when it begins to accumulate, it negatively affects the process of insulin secretion and disrupts pancreatic beta cells, and thus leads to disruption of GLUC metabolism and accumulation in the blood, and increases the incidence of chronic diseases that we mentioned earlier [54].

B. Group - moderate to severe lipids in the liver: In males (median age 42 years) and females (median age 51 years), a 41% increase in GLUC concentration/level was observed in males compared to healthy people, and 19% in females compared to healthy people, but the increase was noticeable in females compared to males.

As mentioned earlier, (13) of males have the aforementioned chronic diseases and suffer from a disorder in the level of TG and TC, and therefore there is an increase in the concentration/level of GLUC compared to healthy people. In contrast, (9) of females have chronic diseases, and most of them are elderly after 51 years, and we mentioned that insulin resistance appears after menopause at a greater rate compared to males. Therefore, genetic factors, health status, dietary pattern, lack of physical activity and insulin resistance are all major reasons for the high concentration/level of GLUC in the blood.

In summary, elevated concentration/level of fasting GLUC leads to a gradual increase in the amount of liver fat in males, and significantly in females.

5. Conclusion:

In the current study, the analytical method used showed accuracy, sensitivity and high reproducibility. After examining all chemical biomarkers, it was found that - by sex and age - in conjunction with the accumulation of fat in NAFLD disease show different effects in this study. Elevated the concentration/level GPX1, TC, HDL-C, BMI and fasting GLUC, increase the development of NAFLD disease in females, while elevated TG, ALT and AST, increase the development of NAFLD disease in males, and despite the elevated concentration/level of CAT in males, it statistically does not show any association with NAFLD disease.

6. Future Studies:

The results of this study may differ from previous studies due to the design of the study, ethnicity, geographical location of the population and health status of the participants.

Future studies require first, a study compared the accuracy and sensitivity of this method with other analytical methods to ensure the efficiency of analytical performance, especially when applied in different operating environments. Second, there are few studies dealing with the effect of enzymatic antioxidants in NAFLD patients, and a consistent change in CAT concentration with lipid intake was observed, and reflects a biological process, and this suggests the importance of further studies to confirm this observation and understand its mechanism more deeply.

In this study, exact data were not available about, physical activity and consumption of fatty and sugary foods that increase the accumulation of lipid in the liver. Third, it is recommended to include a detailed information on medications, physical activity and dietary pattern in future studies.

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Ethical approval: Approval was also obtained from the University of Kirkuk and the Children's Hospital not to take samples from patients suffering from low blood levels, case number 305, dated 5/3 /2024.

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دراسة تحليلية لمضادات الاكسدة الانزيمية (*GPX1, CAT*) لدى مرضى الكبد الدهني الغير الكحولي، العلاقة مع العمر والجنس

هفين عادل قادر [*1] ، عمر صالح حسن [1]

[1] قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة تكريت، تكريت، العراق.

[*] الباحث المسؤول: *ha230020pep@st.tu.edu.iq*

الخلاصة

تقلل مضادات الاكسدة الانزيمية كلوتاثايون بيروكسيديز والكاتالاز لدى مرضى الكبد الدهني الغير الكحولي من تأثيرات عوامل الاكسدة عن طريق تحويلها الى جزيئات الماء. تهدف هذه الدراسة التجريبية الى تقييم دقة وحساسية الطريقة التحليلية في الكشف عن تراكيز منخفضة جداً من مضادات الاكسدة الانزيمية والعلاقة بمرض الكبد الدهني الغير الكحولي. شملت الدراسة ذكوراً واناثاً بنسبة 46 : 44 وبعد فحصهم بالموجات الفوق الصوتية تم تقسيمهم الى ثلاثة مجاميع منهم مجموعة الاصحاء ومجموعة المرضى يعانون من دهون متراكمة خفيفة ومجموعة المرضى يعانون من دهون متراكمة متوسطة الى شديدة. تم تقييم مضادات الاكسدة الانزيمية بطريقة التحليل المناعي المرتبط بالانزيم ، اما الكوليستيرول الكلي والدهون الثلاثية والسكر الصائم وبروتين دهني عالي الكثافة و الانين امينوترانسفيراز و اسبارتات امينوترانسفيراز بتقنية الكوباس 6000 . تم حساب مؤشر كتلة الجسم بنسبة الوزن بالكيلوغرام الى مربع الطول بالمتر. أظهرت النتائج الكمية لاقل تركيز لمضادات الاكسدة الانزيمية انها بدرجة عالية من الدقة والحساسية والتكرارية وبنسبة خطية 99% للمنحني القياسي . كانت الدلالة الإحصائية عالية ومقبولة للتراكيز المرتفعة في المرضى مقارنة بالافراد الاصحاء لكل من السكر الصائم والكوليستيرول الكلي والبروتين الدهني العالي الكثافة ومؤشر كتلة الجسم بالاخص لدى الاناث ، اما الكلوتاثايون بيروكسيدز و الكاتالاز و الدهون الثلاثية و الانين امينوترانسفيراز و اسبارتات امينوترانسفيراز بالأخص لدى الذكور، ولكن الكاتالاز لم يكن مهماً احصائياً ($p > 0.05$) اكدت النتائج ان الطريقة التحليلية عالية الدقة ، كما كانت المؤشرات الحيوية ذو علاقة وثيقة ومؤثرة على مرض الكبد الدهني الغير الكحولي، وكان تأثير كلوتاثايون بيروكسيديز اكثر من الكاتالاز في ذلك.

الكلمات الدالة : الكبد الدهني الغير الكحولي ؛ انزيمات الكبد ؛ اضطراب الدهون ؛مضادات الاكسدة الانزيمية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: أُجري هذا البحث وفقًا للمبادئ الأخلاقية لإعلان هلسنكي. نُفذت الإجراءات بعد الحصول على موافقة المشاركين الشفهية والكتابية المستنيرة قبل جمع العينات. كما تمت الموافقة على بروتوكول البحث واختيار المرضى من قِبل لجنة الأخلاقيات الطبية في دائرة الصحة، ومن مستشفى آزادي التعليمي بمحافظة كركوك.

مساهمات المؤلفين: تولت المؤلفة هفين عادل قادر دور محوري في هذه الدراسة، تولت جمع العينات، إجراء التحاليل اللازمة، تفسير النتائج المستخلصة. بالإضافة الى كتابة المخطوطة وتدققيها لغوياً وقواعدياً. بينما تولى المؤلف عمر صالح حسن تصميم الفكرة الأساسية للبحث، والإشراف على جميع خطوات البحث.