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RESEARCH ARTICLE

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Determination of some Antioxidant Activities (Superoxide Dismutase, Catalase, Reduced Glutathione) and Oxidative Stress Level (Malondialdehyde Acid) in Cirrhotic Liver Patients

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Abstract

Objective: The aim of this study was to determine the levels of malondialdehyde (MDA) and antioxidants such as reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) in the blood serum of liver cirrhosis patients.

Methods: İn This investigation, we took blood from 31 healthy individuals, and 30 patients with Cirrhosis in both males and females. In this study, serum MDA levels, SOD, GSH, and CAT activities were measured spectrophotometrically. In paired group comparisons in terms of continuous variables; the T-test was utilized where normal deviation was achieved, and Mann-Whitney U statistics was utilized where it was not. In addition, ROC curve analysis was performed to evaluate their performance in differentiating the patient group from the control group.

Results: SOD, CAT, and GSH activities were significantly decreased in the patient groups compared to the healthy control group ($p<0.05$). MDA levels were significantly higher in the patient group compared to the healthy control group ($p <0.05$).

Conclusion: In conclusion, in this study, oxidative stress may play an important role in the development of liver cirrhosis. This study is the first one to show the relationships of MDA, SOD, CAT, and GSH in liver cirrhosis. Further studies are essential to investigate antioxidant enzymes and oxidative stress status in liver cirrhosis.

Keywords: Liver Cirrhosis, SOD, GSH, CAT, MDA

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INTRODUCTION

A free radical is basically an element with an atom or molecule that has an unpaired electron in its outer orbit. Due to the unpaired electron, it contains, the atom, ion, or molecule of the free radical is very reactive. This free electron is harmful to various biological systems as it is capable of "stealing" an electron off a stable molecule and causing it to become a free radical as well. The tissue may lose some of its functionality (1).

Reactive oxygen species (ROS) occur as a result of normal cellular metabolism. Oxidative stress (OS) is known to play a role in the development of various diseases such as Alzheimer's disease, Parkinson's disease (2), diabetes-induced pathologies, rheumatoid arthritis and motor neuron diseases, and neurodegeneration (3).

Oxidative damage in DNA can cause cancer. Various antioxidant enzymes such as SOD, CAT, GSPx, GR, GST, etc. protect DNA from OS (4).

Cirrhosis is an advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature. It leads to the shunting of the portal and arterial blood supply directly into the hepatic outflow (central veins), compromising the exchange between hepatic sinusoids and the adjacent liver parenchyma, i.e., hepatocytes (5).

Cirrhosis is characterized by vascularized fibrotic septa that link portal tracts with each other and with central veins, leading to hepatocyte islands that are surrounded by fibrotic septa and are devoid of a central vein. The major clinical consequences of cirrhosis are impaired hepatocyte (liver) function, increased intrahepatic resistance (portal hypertension), and the development of hepatocellular carcinoma (HCC). Cirrhosis and its associated vascular distortion are traditionally considered to be irreversible but recent data suggest that cirrhosis regression or even reversal is possible (6,7).

The aim of this study is to measure antioxidants GSH, CAT, and SOD and level OS such as MDA in patients with cirrhosis.

METHODS

Materials

In our investigation, we took blood from 31 healthy individuals, and 30 patients with Cirrhosis in both males and females. From each healthy patient individuals, we took 4 ml of blood from an antecubital venous vein and added 2 ml to the biochemistry tube and the other 2 ml to the serum tube.

Analysis Methods

Sample analysis

The study starts, with brachial vein blood samples (4cc), which were taken from the cases in the patient group (liver cirrhosis) and the control groups. The tubes serum was separated from plasma by centrifugation in "Nuve NF 800 centrifuge" at (5000 rpm) for 5 minutes and obtained serums were conserved (at 20-°C)

until they are processing. Local ethics committee approval was obtained before starting the study. Informed consent forms were obtained from patients and healthy individuals. When adequate numbers of samples were obtained, serum MDA levels and SOD, GSH, and CAT activities were measured spectrophotometrically in the Biochemistry laboratories of the Department of Chemistry, Faculty of Science, Van Yuzuncu Yil University

Determination of SOD activity

SOD activity was determined by using the proposed method of Popov et al. (8). According to this method, xanthine oxidase was used as substrate. Blank and sample tubes were read against bidistilled water at 560 nm. Results were expressed as U/L.

Determination of CAT activity

CAT enzyme activity was determined according to Aebi's method (9). The principle of the test is based on the determination of the H2O2 decay rate at 240 nm. Results were expressed as U/L.

Determination of GSH level

Glutathione level was determined according to the method Beutler et al., 1963 (10). 800 µl of phosphate buffer was added to 200 µl of serum. The first absorbance (OD1) at 412 nm was recorded. 100 µl of Ellman's reagent was added to the same tube, and the 2nd absorbance (OD2) was recorded.

Determination of MDA level

MDA level was determined according to the method reported by Gutteridge (11). The absorbances were read in a UV/Vis spectrophotometer at 532 nm.

Statistical Analysis

Mean and standard deviation was used in descriptive statistics of the data. In paired group comparisons in terms of continuous variables; the T-test was utilized where normal deviation was achieved, and Mann-Whitney U statistics was utilized where it was not. In addition, ROC curve analysis was performed to evaluate their performance in differentiating the patient group from the control group. The statistical significance level was taken as $p<0.05$ in the calculations and SPSS (ver:13) package program was used for the calculations.

RESULTS

The results obtained in the present study were from a total number of 61 subjects out of which 31 were healthy controls and 30 were liver cirrhosis cases. Contains descriptive statistics and comparative results for SOD, MDA, GSH, and CAT in Table 1. When samples were examined, the difference between the patient and control group mean SOD, MDA, GSH, and CAT were statistically significant ($p <0.05$). When Table 1 was examined for SOD, GSH, and CAT levels, the mean of the patient group was lower than the average of the healthy control group (Figure 1, 3, 4). When Table 1

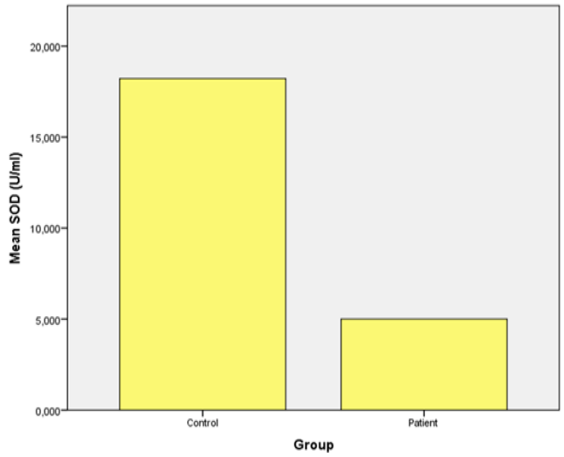

Figure 1. The level of SOD enzyme for control and cirrhosis patient

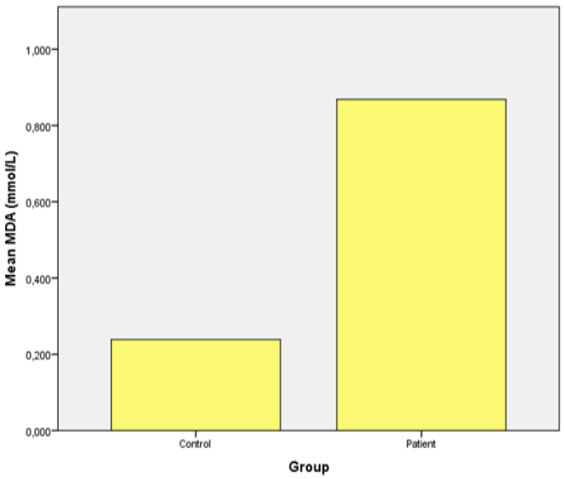

Figure 2. The level of MDA for control and cirrhosis patient

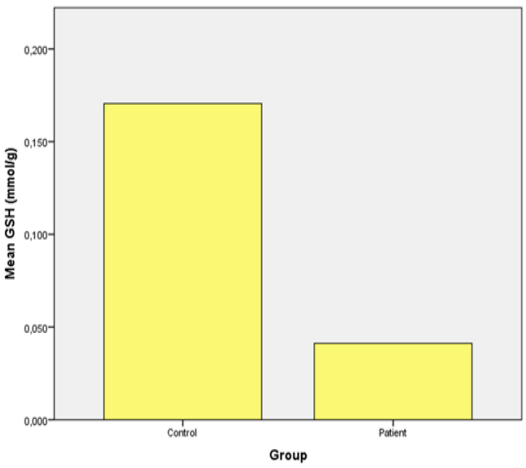

Figure 3. The level of GSH for control and cirrhosis patient

was examined for MDA levels, the mean of the patient group was higher than the average of the healthy control group (Figure 2) ($p<0.05$).

According to the test results made with the ROC curve for MDA in the study; the area under the curve is 1.000±0.001. The cut-off value for MDA was found to be 0.454 (sensitivity 100%, specificity 100%) (Figure 5).

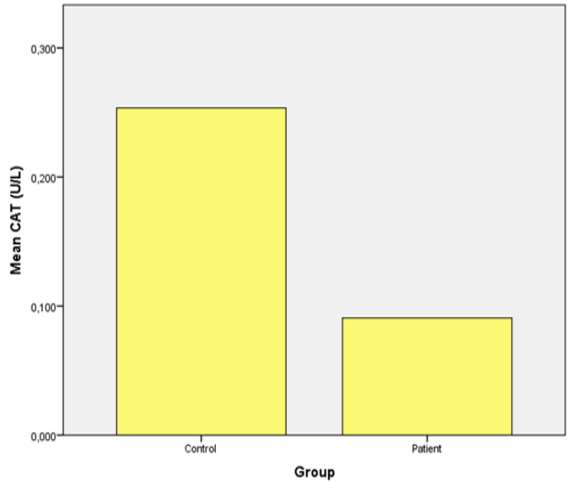

Figure 4. The level of CAT enzyme for control and cirrhosis patient

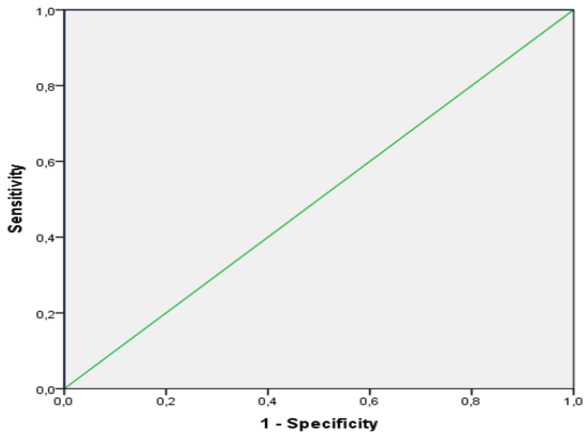

Figure 5. ROC Curve

Table 1. Comparison according to the control group and patients with liver cirrhosis

Group		n	Mean ± Std. deviation	P
SOD **(U/L)**	Control	31	18.213 ± 1.382	0.001
	Patient	30	5.008 ± 1.303	
MDA **(µmo/L)**	Control	31	0.239 ± 0.045	0.001
	Patient	30	0.869 ± 0.256	
GSH **(µmo/L)**	Control	31	0.171 ± 0.017	0.001
	Patient	30	0.041 ± 0.022	
CAT **(U/L)**	Control	31	0.254 ± 0.018	0.001
	Patient	30	0.091 ± 0.009	

Table 2. ROC curve analysis

	Group	cut-off value	area under the curve	St. Error	Sensitivity	Specificity	p
MDA (µmol/L)	Patient-Control	0.45400	1.000	0.001	1.000	1.000	0.001

DISCUSSION

ROS are associated with many diseases; they can also cause potential diseases that can lead to death. These comprise long inflammation and autoimmune illness like rheumatoid arthritis, diabetes mellitus, and cardiovascular diseases such as atherosclerosis, hypertension, and ischemia. As well OS is found to have a significant effect on several kinds of cancer, including renal, lung, liver, and breast cancers (12). Oxygen and nitrogen are the basis of reactive species that cause the oxidation of cells and tissues. The cytochrome p450 enzymes act on the formation of reactive oxygen species in hepatocytes (mitochondria and endoplasmic reticulum), which attack both proteins and lipids hepatocytic in addition to DNA, due to a shortage of antioxidants and imbalance with OS factors (13).

Under the right conditions, maintains a balance between oxidant and antioxidant molecules and domination the level of ROS, it requires preparation of the cells with special molecular strategies. ROS is troubling an imbalance between oxidant agents and antioxidants (13). Oxygen-free radicals have useful functions in the body like phagocytosis, detoxification reactions, the killing of precancerous cells, and apoptosis. Besides, the normal composition of the ROS can control some metabolic cellular functions such as proliferation, migration, immunities, wound curative, and gene expression (14). Lipids, proteins, carbohydrates, and other cell components are exposed to oxidation when increased OS, which causes significant damage to cell structures. The cumulation of damage is called OS (15). Free radicals work to oxidize unsaturated fatty acids on the membranes

catalyzed, this process is called lipid peroxidation. MDA is a marker for OS, and one of the end products of lipid peroxidation (16).

Structural abnormalities, functional disorders, and other disorders (such as proliferative, metabolic, and inflammatory) occur in the liver, as a result of the Redox state which is infecting liver cells. Therefore, in light of the diseases that arise as a result of OS, should be checked for OS in liver disease, it may have a major role in fibrosis, as well as can determine the stages of cirrhosis, monitoring cells damage, and follow the actual results of drug treatments (13).

Antioxidants help to reach advanced stages of treatment, in addition, they have a great ability to protect liver cells from damage caused by free radicals, which causes OS, that in the case of high levels of ROS leads to damage to the cells through necrotic mechanisms. When an imbalance between oxidizing agents and antioxidants occurs, OS will increase, and this plays a role negatively in liver disease and degenerative and chronic disorders (17). Chronic alcoholism is a major cause of cirrhosis. Alcohol causes an increase in free radicals. Alcohol is also known to increase OS in experimental rat models. Enzymatic antioxidants such as CAT and SOD and systems such as non-enzymatic glutathione, vitamins A, C, and E prevent free radicals (18,19,20).

We found through the results obtained an increased level of MDA in serum due to increased severity of fibrosis and in contrast, found a significant reduction in the concentrations of vitamins E and C, which are important indicators of antioxidants. In several studies, compared with healthy controls, were observed a significant decrease in GSH levels in patients with alcoholic liver diseases. However, it is possible that the amount and time of alcohol consumption do not interfere in making a significant difference to the activity of SOD and CAT according to some reports that showed increases or the absence of changes or decreases in it, and this has caused controversy in the scientific community (21,22).

OS is one of the known pathological mechanisms and as mentioned earlier it has negative roles on the liver and is involved from the beginning of the disease until the development of the disease. Also, other factors affecting the liver such as alcohol, drugs, radiation, and pollutants all increase OS, which gradually destroys the liver and causes many diseases such as chronic viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis, which can develop to cirrhosis (23,24).

As a conclusion of this study, antioxidants levels like SOD, CAT, and GSH are decreased, and OS is increased (as evidenced by elevated levels of lipid peroxidation like MDA in patients with liver cirrhosis than healthy

controls (P<0.05). In liver cirrhosis patients, oxidants in high concentrations, which cause oxidative, are released by stress-activated macrophages and neutrophils. This can lead to damage to the DNA, proteins, lipids, and carbohydrates. Lipid peroxidation and MDA react with unsaturated fatty acids (released from cell membranes) that cause damage to cells and tissues (25).

The mean level of SOD activity showed a statistically significant decrease in liver cirrhosis cases when compared to the control group (P<0.05). Some studies suggested that lowered SOD activity may be caused by the inhibitory effects of hydrogen peroxide. This would demonstrate that the increased production of hydrogen peroxide during the dismutation reaction influences the process (26).

In this study, the resulting level of CAT activity was statistically and significantly decreased when it is compared to the control group in liver cirrhosis (P<0.05). Significant findings have been found in CAT activity in patients with cirrhosis (27). This decreased CAT activity in the liver cirrhosis group may have occurred due to catalase being inactivated by H2O2. Both of these also show reduced CAT activity in liver cirrhosis patients' serum. The change of H_2O_2 into H2O and O2 may be a cause of reduced catalase. Consequently, it preserves the cells from the harmful effects of accumulated hydrogen peroxide.

In this study, our result level of GSH was statistically and significantly decreased when it is compared to the control group in liver cirrhosis (P<0.05). Glutathione reductase also takes part as a peroxyl scavenging mechanism. GSH is a non-protein sulfhydryl molecule and is considered a very essential antioxidant defense system for body metabolism. The molecule acts as an intra-cellular reluctance in redox reactions by keeping the cellular element protected against potentially damaging ROS.

In conclusion, in this study, antioxidant enzymes such as SOD, CAT, and GSH were decreased and increased lipid peroxidation level such as MDA was increased in patients with cirrhosis. The results show that OS has related to liver cirrhosis and may increase the danger of cirrhosis liver. This study shows that OS affects tissue cellular damage very well in cirrhosis liver patients. While HBV, HCV, and alcohol are the main factors associated with it. ROS are produced in normal metabolism and living cells, also it is considered signaling molecules that mediate the response. DNA, proteins, and lipids are exposed to oxidative damage when the ROS level in the cell increases. Other problems include DNA damage, loss of enzyme activity, and inhibition of protein synthesis that leads to cell death. ROS is a key product in cells and contributes to the regulation of oxidation and reduction and signal transmission pathways. Liver cirrhosis patients may receive support from antioxidant

therapy along with therapeutic drugs and the cessation of drinking alcohol. Combined with catalase and SOD antioxidants helpful effects might be elevated. In this study, serum MDA level was found to be significantly higher in liver cirrhosis than in the healthy control group, and its specificity and sensitivity were found to be 100%. With these findings, it can be said that serum MDA level can be used as a biomarker in liver cirrhosis. This study is the first one to show the relationships of MDA, SOD, CAT and GSH in liver cirrhosis. Further studies are essential to investigate antioxidant enzymes and OS status in liver cirrhosis.

Ethics Committee Approval: Ethics committee approval was received for this study from Van Yuzuncu Yil University Clinical Research Ethics Committee (2018/103)
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Author Contributions: Concept: HH, HD, MA, CD; Design: HH, HD, MA, CD; Literature search: HH, HD, MA, CD; Data Collection and Processing: HH, HD, MA, CD; Analysis or Interpretation: HH, HD, MA, CD; Writing: HH, HD, MA, CD.
Conflict of Interest: No conflict of interest was declared by the authors.
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