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Evaluation of Oxidant and Antioxidant Levels in Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

***Aim:** Oxidative stress (OS) plays an important role in the pathogenesis of Nonalcoholic fatty liver disease (NAFLD). The aim is to determine the levels of oxidants, antioxidants and oxidative stress index (OSI) in NAFLD and its relation with disease severity and steatohepatitis.*

***Materials/Methods:** Sixty patients with liver steatosis in ultrasonography (USG) and 20 healthy volunteers were included. NAFLD patients were classified as grade1, grade2 and grade3 based on the increase in echogenicity in USG. Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Superoxide Dismutase (SOD) and Lipid Hydro Peroxidase (LPO) levels and Oxidative Stress Index (OSI) were evaluated.*

***Results:** Body mass index (BMI), waist circumference (WC), systolic/diastolic blood pressures, TOS levels was significantly higher in the NAFLD group ($p<0.05$). While the LPO level was higher than the control group, the difference was not significant ($p=0.33$). TAS and SOD levels were lower with no significance ($p=0.91$, $p=0.49$, respectively). OSI was significantly higher in NAFLD ($p<0.05$). Although TOS/LPO levels increased and TAS/SOD decreased with the increasing severity of fatty liver, no significant differences were between the two groups ($p > 0.05$). Higher TOS and LPO levels and OSI levels were correlated with high levels of ALT*

***Conclusion:** OS increases in patients with NAFLD. The oxidation of proteins and lipids created under OS can have an important role in the pathogenesis of NAFLD, and treatment options that reduce OS, ie reduce TOS levels or increase TAS levels, may be considered as a therapeutic option.*

Keywords: *Nonalcoholic fatty liver disease (NAFLD); Total Oxidant Status (TOS); Total Antioxidant Status (TAS); Superoxide Dismutase (SOD); Lipid Hydro Peroxidase (LPO); Oxidative Stress Index (OSI).*

Introduction and Purpose

Nonalcoholic fatty liver disease (NAFLD) is a liver disease displaying the histologic characteristics of fatty liver disease associated with alcohol seen in individuals who do not consume alcohol (1). NAFLD presents in two forms, namely, the simple fatty liver (steatosis), which is the milder form, and the more severe form, "nonalcoholic steato-hepatitis" (NASH) including inflammation and hepatocyte damage. While progressive fibrosis and the risk of cirrhosis are present in 20% to 30% of patients with NASH, prognosis is good in simple steatosis (1).

Many diverse theories have been suggested for the pathogenesis of NAFLD. Also, causes of progression

of NAFLD to severe liver damage have not been understood fully yet. The theory of pathogenesis that has been supported the most is insulin resistance (2). Another model suggested is the double impact theory. Based on "the double impact theory," it has been suggested that the period of first impact that involves the appearance of initial lesions is associated with the excessive increase of free fatty acids produced with the destruction of triglycerides (TG), and subsequently, rendering the liver vulnerable against the second impact caused by oxidative stress and proinflammatory cytokines. Consequently, steatosis transforms into steeatohepatitis through inflammation in hepatocytes and creation of fibrosis (1).

Free radicals are high-energy and unstable compounds containing one or more unpaired electron in their outer atomic orbitals. This unpaired electron provides the free radical with great reactivity and results in damaging of various biologic materials including proteins, lipids, DNA and nucleotide coenzymes (3,4,5). Many defense systems cut in to prevent the oxidative damage caused by the increase of free radicals and other damages resulting from this. These enzymes with superoxide dismutase (SOD) and glutathione peroxidase in the first place, which are known briefly as antioxidants as a group, constitute an important part of the defense system (6).

Reactive oxygen and nitrogen species are known as the pathologic mediators of many diseases including atherosclerosis, cancer, inflammatory diseases and diabetes. Endogenous- and exogenous-origin free radicals reacted with the unsaturated fatty acids of lipids on the cellular membrane causes oxidative destruction of membranes (7, 8).

In this study, determining the oxidant and antioxidant levels was aimed at with the purpose of assessing the oxidant and antioxidant statuses of patients with NAFLD and the relation between the grades of NAFLD and oxidative stress and evaluating the role of oxidative stress in NAFLD with elevated levels of ALT.

Materials and Methods

Criteria for Inclusion and Exclusion of Patients

The study was designed as a single-center prospective study. After obtaining the ethical committee, 60 patients with steatosis in their livers determined with ultrasonography (USG) in the outpatient clinics of Bozok University, Medical School, Internal Diseases Department between 1st December 2016 and 30th June 2017 and 20 healthy volunteers were included in the study.

Exclusion criteria:

- Patients under 18 years of age,
- Females with alcohol consumption exceeding 15 g/day and males with alcohol consumption exceeding 20 g/day
- Patients with liver diseases related to viruses,
- Patients with previous or newly-diagnosed autoimmune hepatitis,
- Patients who have been taking any of the drugs used in the treatment of NAFLD (biguanides, thiazolidinediones, gemfibrozil, ursodeoxycholic acid, vitamin E, beta carotene, N-acetyl cysteine or selenium)
- Pregnancy,
- Patients who had lost 20% of their body weight within the last three months,
- Patients who had undergone any gastrointestinal surgical operation, gastropexy, jejuno-ileal bypass, small intestine resection, or biliopancreatic diversion operation,
- Patients with renal insufficiency or atherosclerotic vascular disease,
- Patients with previous or newly-diagnosed Diabetes Mellitus.

Anthropometric Measurements

Heights, body weights, and waist and hip circumferences were measured on all the cases. Body MASS Index (BMI) was calculated based on the formula finding the ratio of body weight to square of height in square meters (weight/$height^2$ $height^2$, kg/m^2m^2).

Blood pressure measurements of cases were carried out following the Guidelines of the International Hypertension Society. Blood pressure was measured three times with one-minute means of intervals and systolic and diastolic values at second and third measurements were recorded.

Biochemical Measurements

Serum AST, ALT, alkaline phosphatase, gamma-glutamile transferase, total bilirubin, albumin,

ferritin, LDL cholesterol, HDL cholesterol, triglyceride, hsCRP, fasting insulin level, and viral, metabolic and autoimmune markers were determined in all the patients. Total Oxidant Status (TOS) levels and Total Antioxidant Status (TAS) levels were measured using the Rel Assay Diagnostic kit in Abbott Architect ci 4100 auto-analyzer with spectrophotometric method, and Superoxide Dismutase (SOD) levels was measured using the Cayman Assay kit and Lipid Hydro-peroxide (LPO) levels was measured using the Cayman Assay kit in Biotek ELx 808 device with spectrophotometric method at 490 nanometer wavelength.

Oxidative Stress Index (OSI) was calculated as the ratio of TOS to TAS (OSI= TOS/TAS).

Insulin resistance was determined with Homeostasis Model Assessment (HOMA) method. The formula (fasting plasma glucose mg / dl x fasting insulin level μU/ml / 405) was used for the calculation.

Patients with ALT values exceeding the reference range of the biochemistry laboratory of our hospital (5 - 45 IU/L) (>45 IU/L) were classified as NAFLD patients with increased ALT.

Diagnosis of NAFLD

USG in the diagnosis of fatty liver is an imaging technique that is easily accessible together with high accuracy and reliability. It was carried out using a convex USG probe of 2.0-5.0 MHz by a radiologist blinded for the study. Diagnosis of NAFLD was evaluated based on the echogenicity increase consistent with fatty infiltration of the liver with or without increased ALT levels, and was grouped as mild (grade 1), medium (grade 2) and severe (grade 3) fatty liver.

Statistical Analysis

The SPSS 18 program was used for data analysis. Results were presented as mean ± standard deviation and median (minimum-maximum). Distributions were considered as regards normality. T-test was used for data, for which the number of groups was two for normal distribution, and Mann Whitney-u test for abnormal distribution. In cases where the number of groups exceeded two, ANOVA was used for normal distribution, and Kruskal Wallis test was used for abnormal distribution. When the p value was found as significant in the tests applied, multiple-comparison test was used to find the groups that were the source of difference. $p<0.05$ was accepted for statistical significance.

Results

Eighty individuals in total were included in the study, out of which 37 were males (46.3%) and 43 were females (53.8%), with ages raging between 22 and 70. The mean age of individuals was calculated as 47.55 ±10.21 years. The control groups consisted of 10 females and 10 males, and their mean age was 47.2 ± 8.4 years. The NAFLD consisted of 60 individuals, out of which 33 were females (55%) and 27 males (45%), and their mean age was 48.3 ± 11.3.

Grade 1 hepatosteatosis was present in 20 patients out of 60 with NAFLD (33.3%), grade 2 was present in 20 (33.3%),, and grade 3 was present in 20 (33.3%).

Data Related to the Comparison of NAFLD and the Control Groups

BMI, waist circumference, weight, and systolic and diastolic blood pressures were found significantly higher in the NAFLD group as compared to the control group ($p<0.05$). AST, ALT, ALP, GGT values and lipid parameters were found significantly higher in the individuals with liver steatosis as compared to individuals without steatosis ($p<0.05$). There were significant increases also in fasting blood sugar, insulin level, calculated insulin resistance and uric acid in the patient group as compared to the control group ($p<0.05$). Demographic, clinical and biochemical results of the Patient and Control Groups are given in Table I.

Table1. *Demographic, clinical and biochemical data of patient and control groups.*

	CONTROL group (N:20)	NAFLD group (N:60)	P value
Age (year)	47,2±8,4	48,3±11,3	0,72
Gender (F/M)	10/10	33/27	0,68
Height(cm)	166,6±6,1	166,7±10,2	0,80
Weight (kg)	71,2±7,9	88,9±12,6	**0,008**
WC (cm)	94,4±9,6	108,5±12,4	**0,004**
BMI (kg/m2)	25,7±3,2	32,2±5,4	**0,001**

SBP (mmHg)	118,2±9,8	125,8±12,7	**0,002**
DBP (mmHg)	63,1±8,1	77,5±10,5	**0,001**
Glucose (mg/dl)	94,0±7,6	109,4±29,0	**0,012**
AST(IU/L)	16,4±3,8	39,2±35,5	**0,007**
ALT(IU/L)	16,9±8,2	58,5±50,6	**0,003**
ALP(IU/L)	58,8±12,4	80,2±25,4	**0,01**
GGT(IU/L)	19,1±10,8	54,4±45,4	**0,005**
Total bilirubin (mg/dl)	0,6±0,3	0,8 ±1,1	0,58
Direct bilirubin (mg/dl)	0,2±0,1	0,2±0,1	0,47
Albumin (g/dl)	4,1±0,3	4,4±0,3	0,64
Creatinine (mg/dl)	0,7±0,1	0,8±0,1	0,78
TSH (uIU/ml)	1,6±0,9	1,8±1,7	0,612
Sedimentation (mm/st)	11,0±7,2	13,1±9,8	0,484
CRP (mg/L)	3,3±0,2	6,4±6,6	0,52
HDL (mg/dl)	46,8±10,8	42,9±7,6	0,48
LDL (mg/dl)	99,1±31,7	131,8± 29,6	**0,037**
TG (mg/dl)	137,8±80,0	186,6± 81,3	**0,042**
Total cholesterol (mg/dl)	172,4±38,6	210,0±32,4	**0,012**
Uric acid (mg/dl)	4,7±0,9	5,8±1,3	**0,039**
Insulin (mIU/L)	8,5±4,0	15,0±16,3	**0,022**
HOMA-IR	2,0±1,0	4,2±5,4	**0,032**

WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure , AST: aspartate aminotransferase , ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gama glutamyl transferase , TSH: , thyroid-stimulating hormone CRP: C-reactive protein, HDL: high-density lipoproteins, LDL: low-density lipoprotein, TG: triglyceride, HOMA-IR: homeostatic model assessment for insulin resistance.

TOS level in the NAFLD group was found significantly higher as compared to the control group ($p<0.05$). No statistically significant difference was found between the two groups as regards LPO levels ($p=0.33$). While the TAS and SOD levels were lower as compared to the control group, the difference was not statistically different ($p=0.91$ and $p=0.49$, respectively) (Figure 1).

OSI was found statistically higher in the fatty liver group than the control group ($p<0.05$) (Table II).

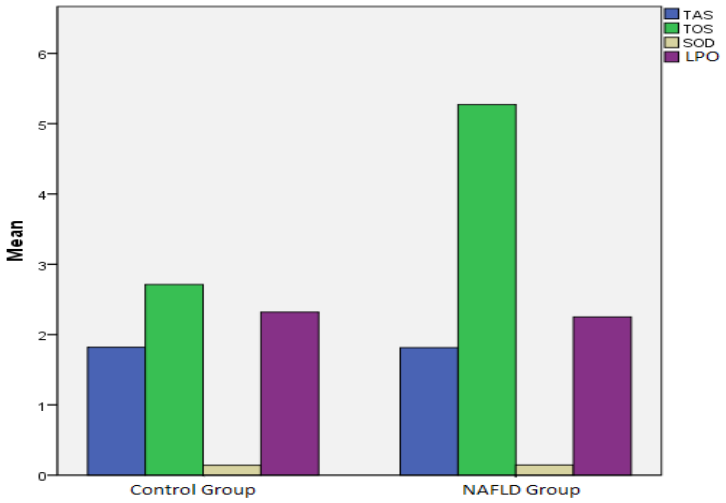

Figure1. *TAS, TOS, SOD and LPO levels of NAFLD and the control group.*

Table2. *TAS, TOS, SOD, LPO and OSI levels of NAFLD and the control group*

	CONTROL group (N:20)	NAFLD group (N:60)	P value
TOS (µmol H2O2 equiv/L)	2,71±0,75	5,27±2,80	**0,014**
LPO (nmol/L)	2,319±0,320	2,251±0,254	0,33
TAS (mmol Trolox equiv/L)	1,820±0,158	1,813±0,232	0,91
SOD (U/ml)	0,144±0,036	0,140±0,025	0,49
OSI	1,51±0,46	2,90±1,36	**0,014**

Data Related to Comparison of NAFLD Groups Based on the Severity of Steatosis in Liver

When the groups were considered separately based on the severity of liver steatosis, it was found that GGT levels decreased with the increasing steatosis severity, while the LDL, TG and total cholesterol levels increased with statistically significant differences ($p<0.05$). Demographic, clinical and biochemical data found upon grouping of patients with liver steatosis based on the severity of steatosis are given in Table III.

TOS sand LPO levels increased with the increasing severity of liver steatosis and TAS and SOD levels decreased with the same; however, no statistically significant differences were found (Figure 2).

OSI levels increased with the increasing severity of liver steatosis; however, no statistically significant difference was found ($p=0.15$) (Table IV).

Table3. *Demographic, clinical and biochemical data of NAFLD groups according to the fatty liver severity.*

	Grade 1 (N:20)	Grade 2 (N:20)	Grade 3 (N:20)	P value
Age (year)	50,53	47,52	48,73	0,289
Gender (F/M)	10/10	10/10	13/7	0,72
Height(cm)	168,95±10,75	166,40±7,98	163,30±10,07	0,58
Weight (kg)	82,53±11,42	84,65±14,42	81,90±11,17	0,49
WC (cm)	104,25±15,98	108,70±13,67	109,45±14,93	0,62
BMI (kg/m2)	31,05	32,87	31,03	0,05
SBP (mmHg)	124,25±11,84	124,75±15,85	128±11,52	0,16
DBP (mmHg)	77,25±12,19	77,75±13,42	76±7,71	0,19
Glucose (mg/dl)	108,6±7,20	106,1±6,58	118,05±15,12	0,58
AST(IU/L)	42,24±6,1	39,59±3,5	33,00±9,2	0,54
ALT(IU/L)	63,41±8,4	59,44±6,5	46,36±8,07	0,15
ALP(IU/L)	87,18±9,8	76,65±7,9	87,09±8	0,308
GGT(IU/L)	60,59±5,6	54,59±8,1	44,36±7,8	**0,019**
Total bilirubin (mg/dl)	0,79±0,12	0,70±0,15	0,66±0,11	0,335
Direct bilirubin (mg/dl)	0,32±0,1	0,35±0,09	0,24±0,12	0,448
Albumin (g/dl)	4,36±1,6	4,48±1,4	4,54±1,5	0,412
Creatinine (mg/dl)	0,83±1,1	0,79±0,6	0,82±0,7	0,103
TSH (uIU/ml)	1,58±1,2	2,31±0,8	1,42±2,1	0,102
Sedimentation (mm/st)	10,35±6	14,70±4,5	13,20±5	0,232
CRP (mg/L)	6,85±1,8	6,98±5	5,93±4,2	0,113
HDL (mg/dl)	45,25±8,2	44,35±6	43,35±4,6	0,97
LDL (mg/dl)	117,70±15	123,75±12	145,45±10,8	**0,003**
TG (mg/dl)	176,55±6,9	182,90±12	168,75±9,7	**0,007**
Total cholesterol (mg/dl)	197,3±12	201,1±15,4	223,4±18,2	**0,012**

Uric acid (mg/dl)	5,77±0,9	5,76±0,8	5,29±1,6	0,48
Insulin (mIU/L)	18,71±9	14,17±6,4	12,47±12	0,301
HOMA-IR	4,0±1,1	4,2±0,8	5,1±1,4	0,416

WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure , AST: aspartate aminotransferase , ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gama glutamyl transferase , TSH: , thyroid-stimulating hormone CRP: C-reactive protein, HDL: high-density lipoproteins, LDL: low-density lipoprotein, TG: triglyceride, HOMA-IR: homeostatic model assessment for insulin resistance.

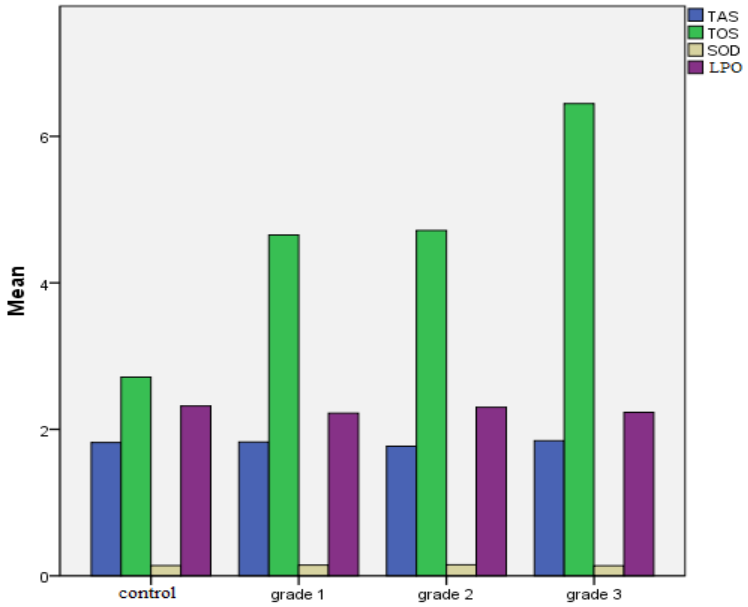

Figure2. *TOS, TAS, SOD and LPO levels according to the fatty liver severity.*

Table4. *TOS, TAS, SOD, LPO and OSI levels according to the fatty liver severity.*

	Grade 1 (N:20)	Grade 2 (N:20)	Grade 3 (N:20)	P value
TOS (µmol H2O2 equiv/L)	4,650±1,2	4,716±2,1	6,447±1,6	0,59
LPO (nmol/L)	2,221±1,04	2,300±1,42	2,332±0,78	0,141
TAS (mmol Trolox equiv/L)	1,827±1,16	1,768±0,89	1,845±0,78	0,457
SOD (U/ml)	0,146±0,12	0,140±0,10	0,131±0,18	0,57
OSI	2,54±1,2	2,72±2,1	3,45±2,4	0,15

Data Related to Comparison between NAFLD Groups with and without Elevated ALT Levels

When patients with NAFLD were grouped based on ALT levels, significant increases in systolic and diastolic blood pressure were found in the NAFLD with elevated ALT levels ($p<0.05$). AST, ALT, GGT and uric acid levels in the NAFLD group with elevated ALT were significantly higher as compared to the NAFLD group with normal ALT ($p<0.05$). Demographic, clinical and biochemical data of groups with and without elevated ALT levels in Table V.

No statistically significant differences were found in TAS, SOD, TOS and LPO levels between the NAFLD group with elevated ALT levels and the group with normal (Figure 3).

While OSI was higher in the NAFLD group with elevated ALT levels as compared to the NAFLD group with normal ALT levels, there was no statistically significant difference was found ($p=0.872$) (Table VI).

Table5. *Demographic, clinical and biochemical data of NAFLD with and without elevated ALT levels.*

	NAFLD Group with elevated ALT levels (n=27)	NAFLD Group with normal ALT levels (n=33)	P value
Age (year)	46,25±11,77	47,33±9.18	0,77
Gender (F/M)	15/12	18/15	0,343
Height(cm)	166±12,13	166,39±7,54	0,878
Weight (kg)	89,09±12,05	83,51±17,89	0,172
WC (cm)	110,74±11,66	104,78±16,66	0,123
BMI (kg/m2)	32,55±4,78	30,30±6,91	0,158
SBP (mmHg)	129,07±13,01	122,87±9,71	**0,048**
DBP (mmHg)	80,37±10,86	73,03±10,01	**0,011**
Glucose (mg/dl)	119±42,56	103±17,61	0,063
AST(IU/L)	47,71±26,8	20,06±8,2	**0,002**
ALT(IU/L)	82±42	22,63±10,8	**0,007**
ALP(IU/L)	80,59±22,64	76,33±21,67	0,45
GGT(IU/L)	64,29±48,06	35,23±24,05	**0,04**
Total bilirubin (mg/dl)	0,78±0,30	0,65±0,28	0,08
Direct bilirubin (mg/dl)	0,28±0,12	0,25±0,12	0,27
Albumin (g/dl)	4,51±0,39	4,41±0,37	0,30
Creatinine (mg/dl)	0,81±0,13	0,77±0,15	0,38
TSH (uIU/ml)	1,80±1,22	1,73±1,39	0,82
Sedimentation (mm/st)	11,29±9,47	13,11±10,23	0,30
CRP (mg/L)	6,05±5,9	7,01±7,3	0,58
HDL (mg/dl)	42,66±7,36	45,66±9	0,17
LDL (mg/dl)	133±27,9	125±33,6	0,36
TG (mg/dl)	170,74±72,1	180,42±85,7	0,64
Total cholesterol (mg/dl)	208,9±35,9	205,9±34,5	0,74
Uric acid (mg/dl)	6±1,4	5,2±1,2	**0,02**
Insulin (mIU/L)	11,79±4	17,8±2,1	0,16
HOMA-IR	4,4±4	4,1±3,4	0,21

WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure , AST: aspartate aminotransferase , ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gama glutamyl transferase , TSH: , thyroid-stimulating hormone CRP: C-reactive protein, HDL: high-density lipoproteins, LDL: low-density lipoprotein, TG: triglyceride, HOMA-IR: homeostatic model assessment for insulin resistance.

Table6. *TAS, TOS, SOD, LPO and OSI levels in patients with and without elevated ALT levels.*

	NAFLD group with elevated ALT levels (n=27)	NAFLD group with normal ALT levels (n=33)	P value
TOS (µmol H2O2 equiv/L)	5,37±1,6	5,18±1,8	0,793
LPO (nmol/L)	2,26±1,9	2,23±1,72	0,644
TAS (mmol Trolox equiv/L)	1,85±0,89	1,77±0,72	0,190
SOD (U/ml)	0,149±0,12	0,141±0,09	0,378
OSI	2,93±1,12	2,87±1,24	0,872

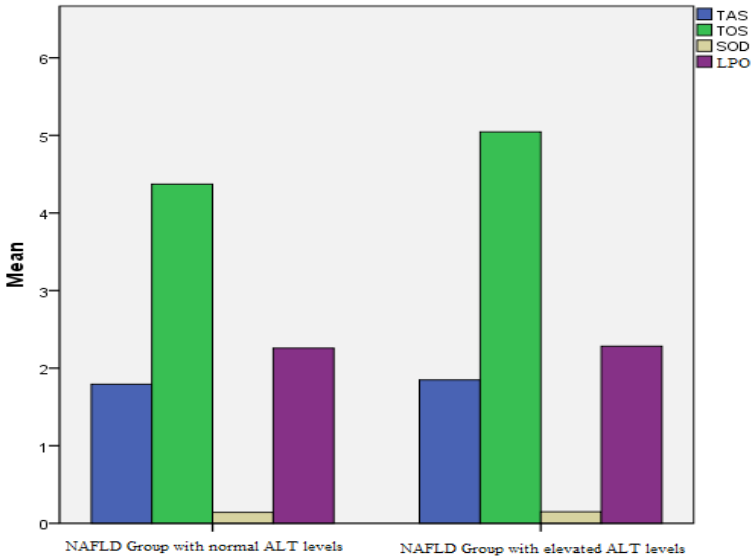

Figure3. *TAS, TOS, SOD and LPO levels in patients with and without elevated ALT levels.*

Discussion

NAFLD is a liver disease displaying the histologic characteristics of alcoholic fatty liver disease in seen individuals who do not consume alcohol. NAFLD covers a large spectrum of liver diseases including simple steatosis, steatohepatitis (NASH), hepatic fibrosis, hepatic cirrhosis and hepatocellular carcinoma (HCC) (1).

It is suggested in recent studies that oxidative stress plays a role in the pathologic process of NAFLD. Oxidative stress and insufficient antioxidant response has been suggested to play an important role in the activity of the disease (9).

The number of studies focusing on the increasing importance of oxidative stress in NAFLD is increasing (10). ROS and lipid peroxidation directly damage hepatocytes through their effects on membranes, proteins and DNA. Consequences of oxidative stress include the lipid peroxidation on cellular membranes, activation of satellite cells resulting in fibrosis of the liver, chronic inflammation and apoptosis (11). In addition to the membrane damage caused by lipid peroxidation, protein damage is a result of oxidative stress. The imbalance between the free radical production and the antioxidant defense causes cellular damage that will result in protein oxidation. Proteins are sensitive to oxidant-mediated damage that create cross links resistant against proteolysis and aggregation products (12).

It is believed I oxidative stress is an important factor in the development of hypertension in NAFLD. Lau et al. (13) found that baseline and follow-up, the risk of increased blood pressure was 3 folds in individuals with NAFLD if alanine transaminase concentrations were increased. Although the mechanism explaining the relationship between NAFLD and increased blood pressure has not been clarified yet, Cassidy et al.(14) have shown that the left ventricular wall is thickened in NAFLD in general, and concentric remodeling is followed. In our study, we found significant increases in systolic and diastolic blood pressures in the group with NAFLD as compared to the control group (systolic blood pressure in NAFLD was 125±12.7 and 118.2±9.8 in the control group, p=0.002; diastolic blood pressure in NAFLD was 77.5±10.5 and 63.1±8.1 in the control group, p=0.001).

In a literature review on studies investigating the relation between oxidative stress and NAFLD, it is seen that studies mostly focus on the relation between the oxidative stress and NASH. In their study carried out on 34 patients with NAFLD and 19 healthy volunteers measuring the levels of nitrates and advanced oxidation protein products (AOPP) as oxidant markers and nitrite levels as antioxidant markers, Çiftçi et al. (15) found that levels of nitrates and advances oxidation protein products (AOPP) were significantly higher in patients with NAFLD, while the nitrite levels were similar. Horoz et al. (16) have reported that OSI levels, which they calculated with a different method (total

peroxide level/ total antioxidant response), increased in patients with NASH and was correlated with fibrosis scores. They found that OSI levels were 0.64 ± 0.14 and 0.19 ± 0.11 in NASH and control groups, respectively ($p < 0.05$). In the same study, Horoz and colleagues found that TAS levels were significantly lower in patients with NASH as compared to the healthy control group (0.85 ± 0.11 and 1.88 ± 0.32, respectively; $p < 0.05$). In a study comparing the NASH group and the control group, Başkol et al. (17) found statistically significant increases in TOS in the NASH group (8.9 and 5.9, respectively; $p < 0.041$). Again in the same study, while no statistically significant TAS levels ($p<0.05$), OSI levels were significantly higher in patients with NASH (8.0 and 5.5 respectively; $p<0.039$). In our study, we found that TOS levels in the NAFLD group were significantly increased as compared to the control group (5.27±2.80 and 2.71±0.75, respectively; $p = 0.014$). Furthermore, we also found that OSI levels were significantly higher in patients with NAFLD (2.90±1.36 and 1.51±0.46, respectively; $p = 0.014$). Although we found that TAS levels were lower in patients with NAFLD, we did not find any significant difference (1.813±0.232 and 1.820±0.158, respectively; $p=0.91$). These data we have obtained in the study indicate the increased oxidative stress in NAFLD.

While we have found TOS and LPO levels higher in the NAFLD group with elevated ALT levels as compared to the NAFLD with normal ALT levels, no statistically significant differences were found (TOS value in the NAFLD group with elevated ALT levels was 5.37±1.6, and 5.18±1.8 in the NAFLD group with normal ALT levels, $p=0.793$; LPO value in the NAFLD group elevated ALT levels was 2.26±1.9, and was 2.23±1.72 in the NAFLD group with normal ALT levels, $p=0.644$). While OSI was found higher in the NAFLD group with elevated ALT levels, we did not find any statistically significant difference (2.93±1.12 in the NAFLD group with elevated ALT levels and 2.87±1.24 in the in the NAFLD group with normal ALT levels, $p=0.872$).

In our literature review, we find any studies evaluating the relationship between the oxidative stress and radiologic stages of NAFLD. In this study we have evaluated the relationship between the oxidative stress and radiologic stages of NAFLD, although we have found that OSI value increased with the increasing radiologic stage of NAFLD, we did not find statistically significant differences in TOS, LPO, TAS and SOD levels, including the OSI levels ($p<0.05$ for all).

Park et al. (18) reported increased TAS levels in patients with NASH. Başkol et al. (17) found no marked differences between patients with NASH and the control group in their study. Chitapanarux et al. (19), found increasing TAS levels in patients with NASH after oral supplementation with whey proteins. Again Horoz et al. (16) found TAS levels in patients with NASH lower as compared to the control group. Sin our study, although we found lower TAS values in the comparison of the NAFLD group with the control group, we did not find any statistically significant differences (1.820±0.158 in the control group, and 1.813±0.232 in the NAFLD group, $p=0.91$). Furthermore, we again did not find any statistically significant differences upon comparison of TAS levels in NAFLD groups with elevated and normal ALT levels and the groups based on NAFLD grades ($p < 0.05$ for all).

SOD catalyzes dismutation of superoxide anion to hydrogen peroxide. SOD, glutathione peroxidase and catalase are the principal enzymatic defense mechanisms against damages created by oxygen radicals. It is expected that the role of SOD on the pathogenesis of oxidative stress, included in the etiology of NAFLD will diminish with the increasing grade of steatosis in liver (20). In their study on 31 control individuals and 40 patients with fatty liver, Krautbaher et al. (9) found no differences of SOD levels in the control group and the group with NAFLD. In their study on 18 patients diagnosed with pathologic NASH diagnosis and a control group of 16 individuals, again Koruk et al. (21) found significantly lower SOD levels in the NASH group ($p < 0.05$). Videla et al. (22) found statistically lower SOD levels in the NAFLD group in their study they carried out on 31 patients with NAFLD and a control group of 25. Although we found lower SOD levels in our study in the NAFLD group as compared to the control group, there were no statistically significant differences (0.140±0.025 in the NAFLD group and 0.144±0.036 in the control group, $p=0.49$). Furthermore, we found that SOD levels decreased with increasing steatosis grades; however, there were no statistically significant differences (SOD levels were 0.146±0.12 in grade 1, 0.140±0.10 in grade 2 and 0.131±0.18 in grade 3; $p=0.57$).

LPO is generally in the form of polyunsaturated fatty acids (PUFA). The membrane damage related to lipid peroxidation is irreversible. Lipid peroxidation occurs with the attack of the hydroxyl radicals (OH-) to fatty

acid side chains of membrane phospholipids at areas close to membranes (23). Selek et al. (24) statistically significant increases in LPO levels in the NAFLD group in their study they carried out on 32 patients with NAFLD and a control group of 28 individuals ($p < 0.05$). Although we found LPO levels higher in the NAFLD group as compared to the control group in our study, we did not find any statistically significant differences (2,251±0.254 in the NAFLD group and 2,319±0.320 in the control group; $p=0.33$).

In conclusion, while the TOS and OSI values were found statistically higher in patients with NAFLD in our study, no statistically significant differences were found in LPO, TAS and SOD levels. Furthermore, no statistically significant difference were found in TOS, LPO, TAS, SOD and OSI levels between NAFLD groups based on the level of steatosis in liver. Again, we found no statistically significant differences in TOS, LPO, TAS, SOD and OSI levels in the group with NAFLD with and without elevated ALT levels. Our findings indicate that oxidative stress increases in patients with NAFLD. Therefore, we have the opinion that protein and lipid oxidation through oxidative stress can have an important role in in the pathogenesis of NAFLD, and treatment options that reduce oxidative stress, ie reduce TOS levels or increase TAS levels, may be considered a therapeutic option.

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