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Leptin Insensitivity in NAFLD Is Produced by Oxidative Stress and Cytokine Overexpression

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) pathogenesis is caused by an imbalance in the biochemistry and immunology of the liver. The aim of the present study was to evaluate the role of oxidative stress and inflammatory factors in the serum concerning leptin concentration.

Methods: This study was carried out on 67 females, including 20 healthy and 47 patients diagnosed by liver scan. Anthropometric analysis and routine biochemical tests, including alanine and aspartate transaminase, triglycerides, cholesterol, fasting blood sugar, low-density lipoprotein, high-density lipoprotein, and their ratio, were carried out. Also, the concentration of malondialdehyde and total antioxidant capacity concentration as well as the activity of catalase, superoxide dismutase, and glutathione peroxidase, were estimated. In addition, the concentration of leptin, interleukin-6, and tumor necrosis factor-α was analyzed. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with $P<0.05$ considered statistically significant.

Results: Patients with NAFLD had significantly higher BMI (33.63±6.37 kg/m² vs. 25.33±2.53 kg/m²), waist circumference (90.6±9.85 cm vs. 81.38±5.44 cm), and hip circumference (93.43±10.06 cm vs. 86.14±5.57 cm) compared with controls ($P<0.05$). Their lipid profile showed elevated triglycerides (209.12±73.41 mg/dl vs. 121.19±38.51 mg/dl) and cholesterol (229.73±43.42 mg/dl vs. 171.19±22.91 mg/dl), as well as a higher prevalence of diabetes mellitus (30%). Although total antioxidant capacity did not differ ($P>0.05$), MDA levels were significantly increased from controls (1.01±0.22 nmol/L) to Grade 1 (1.24±0.41 nmol/L), Grade 2 (1.33±0.32 nmol/L), and Grade 3 NAFLD (1.43±0.44 nmol/L), while SOD, CAT, and GPx activities were reduced (all $P<0.05$). IL 6, TNF α, and leptin concentrations were markedly elevated from grade 1 to 3, with the highest at Grade 3 (17.63±10.19 pg/mL, 104.63 ± 14.43 ng/mL, and 2.23±0.92 µg/mL, respectively, for each one; $P<0.05$).

Conclusion: Although leptin controls fat metabolism via satiety, results revealed that the overproduction of leptin might be a consequence of oxidative stress and inflammatory response to fat accumulation in the liver. Therefore, measuring leptin along with IL-6 and TNF-α might help to diagnose liver injury.

Keywords: NAFLD, Oxidative stress, IL-6, TNF-α, Leptin, Transaminases

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Key Messages:

↑What is "already known" in this topic:

NAFLD is a common metabolic liver disorder marked by elevated oxidative stress and reduced antioxidant defenses, leading to hepatocellular injury and activation of inflammatory pathway. Oxidative stress contributes to immune dysregulation, promoting hepatic inflammation and fibrosis. Leptin, a key adipokine, further modudlates metabolic imbalance and immune mediated inflammation.

→What this article adds:

This study identifies a significant correlation between oxidative stress markers and immunological mediators, particularly IL 6 and leptin, in NAFLD. The integrated biochemical-immunological highlights how oxidative injury aligns with cytokine activation and adipokine disturbance, revealing combined oxidative immune mechanisms that may support early detection or therapeutic targeting.

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Introduction

In today's world, Non-alcoholic fatty liver disease (NAFLD) as a common liver disease can be seen in 25% and 80% of the general and obese population (1). NAFLD is a metabolic syndrome,

where its strong links to obesity, insulin resistance, increased systemic inflammation, and advanced atherosclerosis (2) have been well documented. The pathogenesis of NAFLD is usually explained by lipid accumulation, which causes non-alcoholic steatohepatitis (NASH) and cirrhosis due to the production of inflammatory cytokines and dysregulation of carbohydrate and lipid metabolism (1), but its exact mechanism has not been fully elucidated.

It is generally accepted that pro-inflammatory cytokines, including IL-6 and TNF-α, are central contributors to NAFLD development (3, 4). But other factors such as uncontrolled oxidative stress and leptin as lipid specific hormone, also could play their part in the pathogenesis of NAFLD. The liver is the main organ responsible for metabolizing many compounds that generate reactive oxygen radicals (ROS), which can cause liver tissue damage. The level of ROS may be increased by fat accumulation in liver tissue, which causes liver malfunctioning to detoxify the toxicant. Due to their high reactivity, ROS attack cellular components, leading to nucleic acid damage, lipid oxidation, and protein modification. Although hepatic lipid accumulation in NAFLD is mostly related to the accumulation of triglyceride (TG) droplets (5), some investigators considered this protective rather than deleterious (6, 7) and blamed free fatty acids (FFAs) to be the actual culprit of hepatopathy (8, 9). Hepatic FFAs originated from lipolysis in adipose tissue, de **novo** synthesis from carbohydrates in the liver, and dietary lipids (10). Whether their presence in TGs is taken seriously or not, the oxidation of unsaturated FFAs is the most important process to generate different forms of aldehydes in the liver (8, 11). In addition to reactive ROSs, one of the lipid peroxidation products is 4-hydroxynonenal (4-HNE), which interferes with DNA, RNA, and protein synthesis and triggers inflammatory responses in the affected and surrounding tissues (12).

Leptin, as a peptide hormone, is released from adipose tissue and plays a regulatory role in appetite control, neuroendocrine activity, and overall energy balance. It also seems to influence several other physiological processes, including metabolism, endocrine regulation, and immunological functions. Altered leptin signaling is linked to several metabolic disorders, most notably obesity. The most significant factors affecting circulating plasma leptin concentrations include total body fat mass index (BMI), metabolic hormones, and gender (13). Leptin is considered to be a pro-inflammatory adipokine that stimulates the activity of monocytes/macrophages as well as the production of pro-inflammatory cytokines. It has also been reported that leptin increases ROS production and induces oxidative stress in cardiovascular and diabetic patients (14).

Therefore, the aim of this study was to examine oxidative stress, leptin concentration, and the levels of IL-6 and TNF-α in the serum of patients with non-alcoholic fatty liver disease (NAFLD). Furthermore, we classified NAFLD into different grades according to Brunt's criteria (15) using ultrasonic analysis and performed subgroup analyses to elucidate the essential association between the investigated parameters and NAFLD.

Methods

Study design and sampling

The present study was conducted in Al-Husseini Hospital, Karbala, Iraq, where 67 females participated in this study, including 20 controls (who did not have any sign of NAFLD) and 47 patients (who were divided into four groups, namely grade one, grade two, and grade three based on the ultrasound examination). All the participants were informed of the nature of the research, and their consent was obtained by signing the form previously confirmed by the moral committee of Arak University IR.ARAKU.REC.1401.129. From the cephalic arm vein of all the eligible subjects, 10 mL of blood was taken and kept at 37 °C till it was coagulated. The serum was separated using centrifugation at 300 g for 5 minutes, then preserved at -20 °C till further analysis. It is important to note that all patients who had taken medication or dietary supplements were excluded from the study to minimize potential confounding factors. However, individuals who engaged in daily exercise were included as a study variable to evaluate its potential impact, irrespective of the specific type of exercise performed.

Routine biochemical test

Estimation of aspartate transaminase (AST) (Lot #: 7293001), alanine transaminase (ALT) (Lot #: 71671801), Bilirubin total and direct, triglycerides (TG) (Lot #: 72472501), cholesterol (Col) (Lot #: 73304401), low density lipoprotein (LDL), high density lipoprotein (HDL) (Lot #: 63382401) and LDL/HDL ratio, fasting blood sugar (FBS) (Lot #: 66105401), protein (Lot #: 72256101) and hemoglobin-A1c was carried out in the serum of the participant using automated analyzer (cobas c-311 analyzer, Roche company, Switzerland). The ALP (Lot #: 1065B), HbA1c (Lot #: 1063B), LDL (Lot #: 2538), and bilirubin (Lot #: 2604) kits were from Bekman company, Germany, and the rest of the commercial kits used in this study were purchased from Roch company, Germany. To conduct the estimation, the recommended procedure by the company was followed.

Analysis of oxidative stress

Catalase activity assay

Catalase (CAT) activity was estimated using a commercial kit (KiaZist company (KCAT 96), Iran). According to the company instructions, the enzymatic reaction was carried out using 20 µL of sample. The absorbance was taken at 540 nm using a spectrophotometer, and a standard curve was plotted. The concentration of the formaldehyde (µM) was calculated using the linear formula

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Y=0.0003X+0.0585 with R2=0.9721, and the activity of CAT was reported as mU/mg of protein.

SOD activity assay

Using this commercial kit (KiaZist company (KSOD 96), Iran), the activity of superoxide dismutase (SOD) was estimated. The reaction was conducted according to the company procedures using 20 µL of sample, and the absorbance was taken at 570 nm. The activity based on % inhibition rate was expressed as U/mg of protein.

Glutathione peroxidase Activity assay

Glutathione peroxidase (GPX) activity was estimated by a commercial kit (KiaZist company (KGPX 96), Iran). The estimation was conducted using 20 µL of samples at 340 nm, and the activity of GPX was reported as mU/mg of protein, taking 0.00622 μM^{-1} cm^{-1} as the extinction coefficient of NADPH.

Total antioxidant capacity assay

Total antioxidant capacity (TAC) of the serum was estimated by a commercial kit (KiaZist company (KTAC 96), Iran). According to the company protocol, 30 µL of the sample was used to conduct the measurement, and the absorbance was taken at 450 nm using a spectrophotometer. Using the provided Trolox as standard, a standard curve was plotted, and the concentration of the samples was calculated from the formula Y=0.0013X+0.0857 with R2=0.9991. The results were presented as nmol of Trolox equivalent/mg of protein.

Malondialdehyde concentration assay

Malondialdehyde (MDA) concentration was estimated by a commercial kit (KiaZist company (KMDA 96), Iran). According to the company protocol, 200 µL of sample, blank, and standard were added to 600 µL of TBA Solution and overlaid with 200 µl mineral oil. The mixture was incubated for 60 minutes at 95°C, then kept on ice for 10 minutes. Measurement was carried out at 546 nm using 200 µL of the reaction mixture and using the linear formula y=0.0187x+0.0102 and R2=0.9991; the concentration of MDA in the samples was expressed as µM/ml.

Tumor necrosis factor-α, interleukin-6, and leptin estimation

We measured the concentration of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and leptin using a commercial kit (ARS-BIOCHEM, PRS-01568hu, China), (IL E-3200, LND company, Germany), and (ARS-BIOCHEM, PRS-00916hu, China), respectively, based on the sandwich model of ELISA, where the monoclonal antibodies were coated to the plate. Sensitivity and specificity of the kits were 87 %, 89 %, and 87 %, as well as 92 %, 97 %, and 92 %, respectively. The secondary antibody conjugated to horseradish peroxidase also directly reacted with the captured protein, and then the HRP enzymes produced color by catalyzing the chromogen. According to the company protocol, 50 µl of each calibrator, Control, and Sample was taken, and using an ELISA reader, the developed color was measured at 450 nm. The concentration of TNF-α, IL-6, and leptin was determined using the linear formula Y=0.0085X+0.1286 with R^2=0.9298, Y=0.0006X+0.0172 with R^2=0.9998, and Y=0.4044X+0.2522 with R^2=0.9374, respectively. The concentration of TNF-α, IL-6, and leptin was expressed as ng/L, pg/ml, and µg/L, respectively.

Statistical analysis

Data were analyzed with SPSS software (version 19) using one-way ANOVA, Tukey test as a post-hoc test for data with more than two groups, and unpaired Student t-test for data with only two groups with $p<0.05$ as the minimum level of significance. Also, the graphs were plotted using GraphPad Prism software (version 8.4.3).

Results

Anthropometric analysis and daily habit

Data analysis showed that the patients suffering from NAFLD had significantly ($P<0.0001$) higher body weight, waist circumference, hip circumference, and body mass index when compared to healthy individuals (Figure 1). In addition, we found that the incidence of NAFLD was significantly correlated with exercise, since only 26.8 % of the patients were exercising when compared to the healthy individuals (52.4 %). But no correlation was found between smoking habit, restaurant food consumption, fast food consumption, and incidence of NAFLD (Table 1).

Biochemical analysis

Activity of liver enzymes and bilirubin concentration

Data analysis showed that the activity of alanine transaminase (ALT) and aspartate transaminase (AST) in the serum of the NAFLD patients was significantly ($P<0.05$) higher than the activity of the same enzymes in the serum of healthy subjects (Figure 2A), but their ratio was significantly lower (Figure 2B). Also, we found the activity of alkaline phosphatase (ALP) was significantly ($P<0.05$) lower in patients when compared with the control group of the

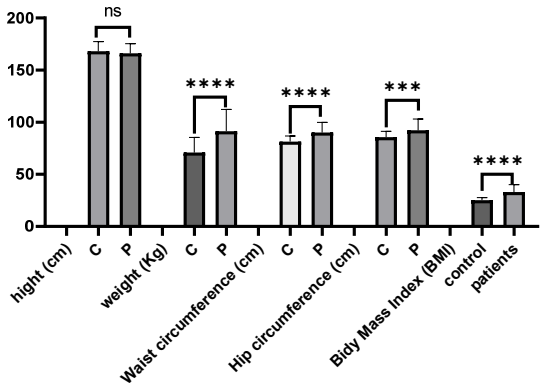

Figure 1. Anthropometric analysis: comparison between the height (cm), Weight (Kg), Waist circumference (cm), Hip circumference (cm), Body Mass Index, and Age (years) of the healthy individuals and patients suffering from NAFLD. Data were analyzed using Unpaired t-test and were shown as mean ± SD. Statistical significance is represented as follows: (ns) not significant, (***) $P< 0.001$ and (****) $P< 0.0001$.

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Table 1. Percentage comparison of the daily habits in healthy individuals and patients suffering from NAFLD.

Variable	Healthy individuals (%)	Patients suffering from NAFLD (%)
Age (years)	21-47	21-51
Exercise (%)	52.8	26.8
Smoking habit (%)	28.6	10.7
Restaurant food consumption (%)	66.7	42.8
Fast food consumption (%)	61.9	28.6

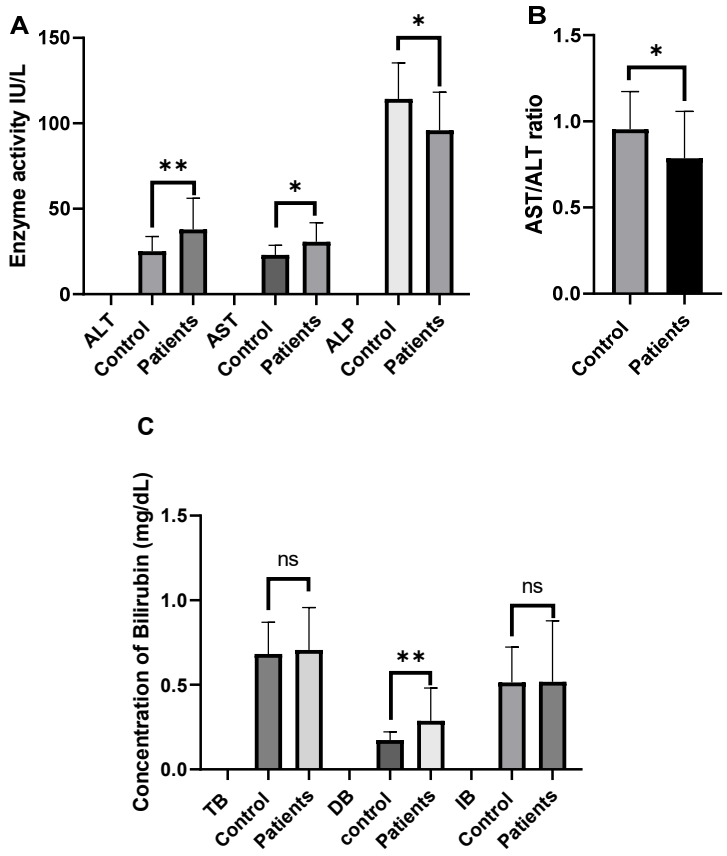

Figure 2. Comparison between the A) activity of ALT, AST and ALP, B) AST/ALT ratio and concentration of bilirubin in healthy individuals and patients suffering from NAFLD of different grades. Data were shown as mean ± SD and statistical significance is represented as follows: (ns) p>0.05, (*) *P*< 0.05 and (**) *P*< 0.01.

individuals participating in the study (Figure 2A). It was also revealed that the levels of total (TB) and indirect bilirubin (IB) showed no significant difference (*P*>0.05) between the patients suffering from NAFLD and healthy individuals, while the level of direct bilirubin (DB) was significantly (*P*<0.05) higher in the patients when compared with healthy individuals (Figure 2C).

Lipid Profile and blood sugar concentration

Analysis of data showed that the patients suffering from NAFLD had significantly (*P*<0.01) higher total cholesterol (TC) and triglycerides (TG) as compared to healthy individuals, but no variation in the concentration of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) was observed (Figure 3A). In addition, we found that there was no significant variation (*P*>0.05) in the ratio of LDL/HDL, while a significant (*P*<0.01) increase in the ratio of TC/HDL was found in the serum of patients compared to healthy individuals (Figure 3B). We also found that the level of fasting blood sugar (FBS) and hemoglobin A1c (HbA1c) was significantly higher in the serum of patients suffering from NAFLD as compared to healthy individuals (Figure 3C and D).

Oxidative stress analysis

Results of the present study showed that the level of total antioxidant capacity (TAC) showed no significant (*P*>0.05) change in the serum of the NAFLD patients when compared with the healthy subjects. Whereas a significant increase in the malondialdehyde (MDA) concentration was found in the patients with NAFLD compared to the control group. Also, we found that the maximum concentration of MDA was statistically more in grade III compared to grades II and I (Figure 4A and B).

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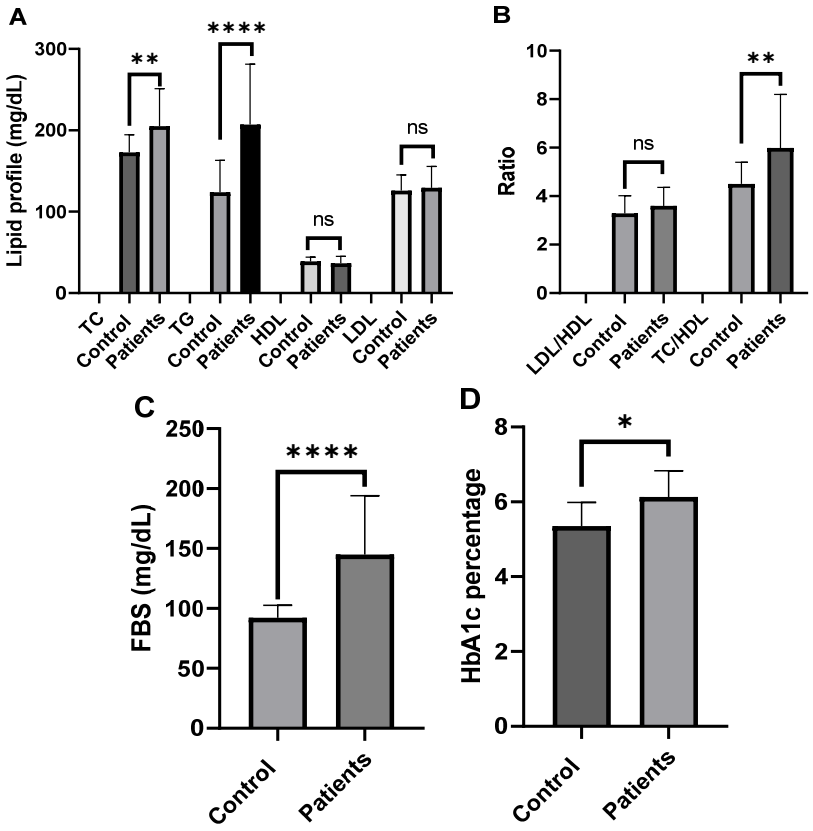

Figure 3. Comparison between A) the concentration of TC, TG, HDL and LDL, B) the ratio of LDL/HDL and TC/HDL, C) fasting blood sugar (FBS) concentration and D) percentage of HbA1c in healthy individuals and patients suffering from NAFLD of different grades. Data were shown as mean ± SD and statistical significance is represented as follows: (ns) $p>0.05$, (*) $p<0.05$, (**) $P< 0.01$ and (***) $P< 0.001$.

On the other hand, the results of the present study showed that the activity of glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) enzymes in the serum of the NAFLD patients was significantly reduced ($P<0.05$) when compared with the activity of the same enzymes in the serum of the healthy subjects. The maximum reduction was found in the activity of the CAT, which was found to be highly significant ($P<0.001$) (Figure 4 C, D, and E).

Inflammatory and hormonal variation

When the data were analyzed, we found that the level of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and leptin in the serum of the NAFLD patients increased highly significantly ($P<0.0001$) when compared with the level of the same cytokines and hormone in the serum of the healthy subjects. No significant ($P>0.05$) variation in the level of the same factors was found with respect to the progression of the disease grade (Figure 5 A, B, and C).

Discussion

NAFLD is a pathological condition of fat accumulation in the liver of overweight or obese (16), although normal persons also develop NAFLD (17). In the present study, the patients with NAFLD were overweight which their physical activity also was lesser than healthy individuals. In addition, the patients' waist and hip circumference as well as BMI were also significantly higher when compared with the control group. Other investigations also confirmed the relation between the mentioned parameters and NAFLD (4, 5). Higher weight and higher hip and waist circumferences might correspond to lower physical activity, as the practice and duration of exercise of patients compared to control individuals were much lower.

Lin et al. (2017) carried out a secondary data analysis from December 2012 to September 2013 in southwestern Taiwan, in which they found that the activity of AST and ALT in patients with NAFLD was significantly higher than that of healthy individuals (6). In addition to a previous study, Eslami et al. (2021) showed that the activity of the SGOT and SGPT in NAFLD patients was significantly higher than in the control group, but the activity of ALP was lower (7). Many investigations have shown that the ratio of AST and ALT is an important laboratory indicator to diagnose the different types of liver disease (22, 23, 24). In general, if the ratio is more than 1, in 92 % of the patients and more than 2 in 70 % of the patients, it shows alcoholic

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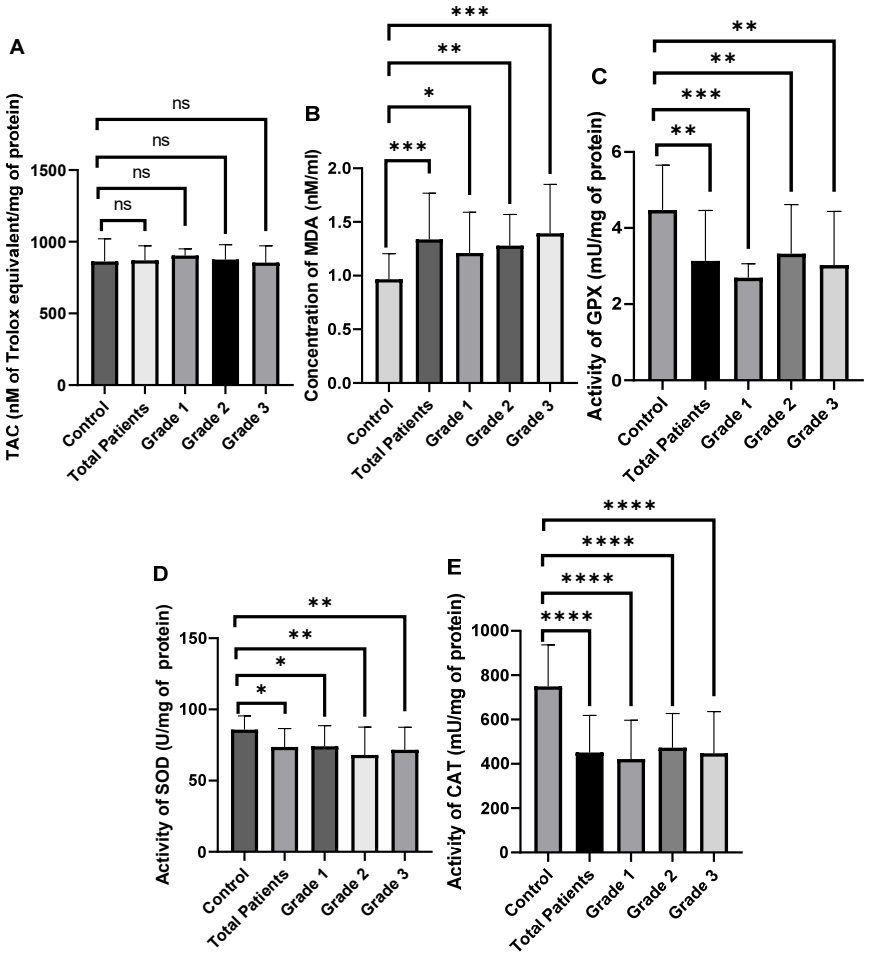

Figure 4. Comparison between the A) level of the TAC, B) level of MDA, C) activity of GPX, D) activity of SOD and E) activity of CAT in healthy individuals and patients suffering from NAFLD of different grades. Data were shown as mean ± SD and statistical significance is represented as follows: (ns) p>0.05, (*) p<0.05, (**) p< 0.01, (***) p< 0.001 and (****) p<0.0001.

fatty liver disease, but when the ratio is less than 1, the patient is more likely to suffer from NAFLD (25). Our results also confirm that the patients participating in the present study were suffering from NAFLD since the activity of ALT and AST showed elevation, but the ALP activity was diminished, and the ratio of AST/ALT was less than 1. This means that the liver tissue of the patients was damaged since these enzymes have been released into the serum. In addition, we found that the concentration of total bilirubin and indirect bilirubin in the patient's serum showed no variation when compared to a control group of individuals, while the direct bilirubin was significantly higher in the patient's serum. Direct bilirubin is a conjugated form of bilirubin (26) classified as the glucuronated and delta forms of bilirubin, which the second one is bounded to albumin in the serum (26). While total bilirubin is normal, an increase in direct bilirubin has only been reported in patients with plasma cell dyscrasias (27). Since no other investigation has mentioned such variation, this could be due to interference with the bilirubin assays; therefore, further studies are needed to see if this phenomenon may have a clinical implication in diagnosing NAFLD. In terms of lipid profile analysis, we found that the concentration of triglycerides, cholesterol, and the ratio of cholesterol/HDL was significantly higher in patients with NAFLD. Our data were confirmed by some investigations with respect to total cholesterol and triglyceride levels as well as the ratio of cholesterol/HDL (28), but no study was found to confirm our results regarding the level of LDL and HDL as well as the LDL/HDL ratio, as we observed no change in the said parameters. This variation might be due to geographical differences or cultural variation, as the present study was carried out in Iraq, whereas the other two studies have been conducted in Iran and the USA. The present investigation showed that the level of FBS and HbA1c was significantly higher in patients suffering from NAFLD when compared to the control group, these data also supported by other investigations (18, 20, 28). Overall, the individuals suffering from NAFLD in Iraq and Karbala were showing the same anthropometric and clinical characteristics as the patients from other parts of the world.

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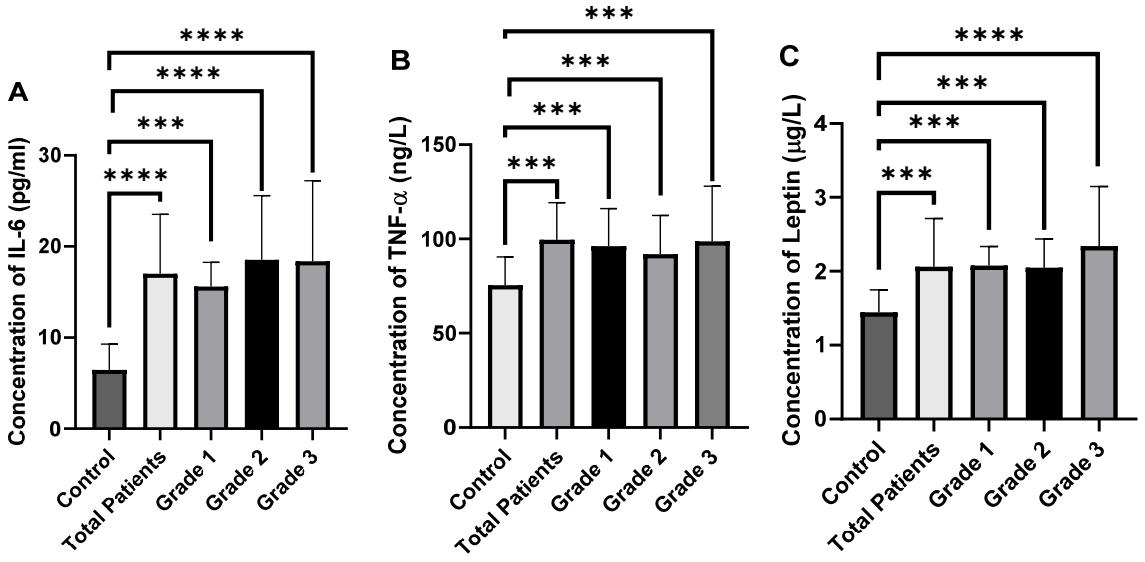

Figure 5. Comparison between A) the level of IL-6, B) the level of TNF-α, and C) the level of leptin in healthy individuals and patients suffering from NAFLD of different grades. Data were shown as mean ± SD, and statistical significance is represented as follows: (***) $P< 0.001$ and (****) $P< 0.0001$.

Although reactive oxygen species (ROSs) are formed naturally, the antioxidant defense system, including enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and antioxidant molecules such as vitamin C, glutathione, coenzyme Q10, and vitamin A are constantly working to reduce these species and prevent their oxidative effect (29). Oxidation of biomolecules like unsaturated fatty acids, nucleic acids, and proteins (30) by ROSs results in metabolic and structural malfunctioning (31). The cell membrane contains a large amount of unsaturated fatty acids, which, upon oxidation by ROSs, produce malondialdehyde (MDA) that can be measured as an oxidative stress marker (32).

In the present study, we found no variation in the TAC level of the serum of the patients with NAFLD as compared to healthy ones, but the main objective was the significant increase in MDA concentration and reduction of the antioxidant enzymes. SOD catalyzes the dismutation of superoxide to produce hydrogen peroxide as a by-product (33), and in coordination, CAT acts to convert hydrogen peroxide to oxygen and water, which nullifies the effect of hydrogen peroxide (34). Besides these enzymes, glutathione peroxidase and glutathione reductase perform oxidation and reduction of glutathione to remove ROSs. These enzymes altogether, are the key players to scavenge ROSs (35) and prevent the production of MDA, which is the product of lipid peroxidation (36).

The role of oxidative stress in NAFLD is controversial, as some of the investigation, such as those by Świderska et al. (2019), report elevation of SOD and GPX activity (37), while others believe the activity of these enzymes is low in patients with NAFLD (38, 39). Since the activity of AST and ALT in the serum of patients with NAFLD is significantly higher than in normal individuals, the liver cells must have been damaged. The destruction of the cell membrane is the main factor in the release of these metabolic enzymes into the serum. Oxidation of unsaturated fatty acids in the membrane lipid bilayer is the main reason for destabilizing the membrane; thus, the reduction in antioxidant enzyme activity is more likely to happen in NAFLD. If we look at the results of the anthropometric analysis, it is obvious that the patients who participated in this study were under medical care and most of them were not using restaurant meals or fast food; therefore, their routine behavior has changed, which might be the main reason for TAC level not changing. But, in the case of antioxidant enzyme activity variation, their gene expression might have been unbalanced, which would take more time to normalize their activity.

Acute-phase protein concentrations in plasma either increase or decrease in response to inflammation, which brings about an acute-phase response to accelerate the production of peripheral leukocytes, circulating neutrophils, and their precursors (40). In response to tissue injury, cells such as neutrophils and macrophages secrete a number of cytokines like IL-1, IL-6, and TNF-α. The liver tissue also responds to injury by producing many acute-phase proteins, which may also contribute to the promotion of tissue damage (40). IL-6 and TNF-α are pro-inflammatory release by adipocytes, and their concentration correlates with the percentage and distribution of fat tissue in the body (41). In the present investigation, the level of both IL-6 and TNF-α increased significantly, which promotes the production of acute-phase proteins. IL-6 is thought to stimulate hepatic mINDY expression by binding to its receptor and activating

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STAT3, which then interacts with the mINDY promoter. This signaling pathway enhances citrate uptake and promotes hepatic lipogenesis (42). IL-6 strongly stimulates the acute-phase reaction and also acts as a mitogenic factor for hepatocytes; therefore, activation of the IL-6 signaling pathway might cause the liver to ultimately develop liver cancer (43). Therefore, from two angles, the elevation of IL-6 with respect to NAFLD is important: 1) lipogenesis and 2) tumorigenesis, which both have been documented in acute fatty liver pathogenesis (44).

TNF-α is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis (45). TNF-α is both an adipokine and a cytokine; as an adipokine, TNF-α promotes insulin resistance and is associated with obesity-induced type 2 diabetes. As a cytokine, TNF-α is a macrophage-derived inflammatory mediator that activates multiple intracellular signaling pathways during acute inflammation (46). In the present study, the elevation of FBS and TG as well as TC showed that TNF-α might be considered an adipokine, but an increase in serum metabolic enzymes such as AST and ALT would also raise the speculation to interpret the role of TNF-α as a cytokine too. Overall, an increase in the level of IL-6 and TNF-α in NAFLD would lead us to conclude that the inflammation induced by oxidative stress due to the reduction of antioxidant enzyme activity is detrimental to liver tissue.

Leptin, which is predominantly produced by adipocytes, is a protein hormone that influences appetite and satiety and helps in the maintenance of energy reserves (47). The amount of circulating leptin correlates with the amount of energy reserves, mainly triglycerides stored in adipose tissue. High leptin levels are interpreted by the brain as indicating that energy reserves are high, whereas low leptin levels indicate that energy reserves are low (48). In obesity, high BMI is highly correlated with elevated leptin levels (49), especially in females (50), and a decreased sensitivity to leptin occurs (similar to insulin resistance in type 2 diabetes), resulting in an inability to detect satiety despite high energy stores and high levels of leptin (51). Overproduction of leptin in patients with NAFLD might be due to leptin insensitivity. In obesity, leptin resistance may arise from reduced transport of leptin into the brain and disruptions in downstream neuronal signaling (52, 53). In addition, although leptin primary amino acid sequences show differences in vertebrates, its secondary and tertiary structures are similar (54), and we should take into consideration that it very much resembles the long-chain helical structure of interleukin (IL) 6 (55), which makes leptin a responsible molecule to be indirectly involved in the inflammatory response in NAFLD (56).

The findings indicate that leptin enhances insulin's ability to suppress hepatic glucose production in rodents. This effect reflects a functional overlap between leptin and insulin signaling, suggesting that both hormones may act through partially shared metabolic pathways (53). However, impairment of leptin signaling in pancreatic β cells might cause insulin resistance and decrease insulin suppression of hepatic gluconeogenesis, which elevates the production of glucose in the liver (57). What we have observed in the present study might be of interest, since irrespective of BMI elevation, an increase in FBS, TG, and TC on one side and overproduction of inflammatory factors as well as inhibition of antioxidant enzyme activity from the other side might be a key factor in increasing the leptin level.

Conclusion

In a developing society, reduction in physical activity and diversion from the traditional food regime lead to accumulation of fat in the liver, which causes oxidative stress and induction of liver damage leading to overproduction of inflammatory cytokines such as IL-6 and TNF-α, which work side by side to imbalance the metabolic activity. Uncontrolled food habits and a sedentary lifestyle along with inflammatory response may cause severe liver conditions. The results of the present investigation might help the physician to diagnose fatty liver prior to acute injury. Although there are strong correlations between high BMI and overproduction of leptin, we showed that there might still be a strong correlation between reduction of antioxidant enzymes, elevation of inflammatory cytokines, and increase in leptin, which can be considered as a laboratory consideration to diagnose NAFLD. While further studies with a larger patient cohort are necessary to confirm these findings, the present study provides critical insights for medical experts into the intricate relationship between NAFLD, oxidative stress, and immunological responses. These findings contribute to the growing body of evidence supporting the role of oxidative stress in disease progression and may help inform future research and clinical approaches.

Acknowledgment

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Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contributions

Abnosi MH. Conceived the study and was in charge of overall direction and planning, designed the experiments, analyzed the data, and wrote the manuscript. Al-Janabi AHA. Diagnosed the disease and confirmed NAFLD in patients using ultrasound. Al-Saeed MMS carried out the laboratory experiments.

Ethical Considerations

The study was approved by Arak Medical University ethics committee, Iran, and the approval number is IR.ARAKU.REC.1401.129.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

AI Use Statement

No AI tool was used to generate, alter, or fabricate data.

References

1. Duan Y, Pan X, Luo J, Xiao X, Li J, Bestman PL and Luo M. Association of Inflammatory Cytokines with Non-Alcoholic Fatty Liver Disease. Front. Immunol. 2022; 13:880298. doi: 10.3389/fimmu.2022.880298.
2. Stols-Gonçalves D, Tristão LS, Henneman P, Nieuwdorp M. Epigenetic Markers and Microbiota/Metabolite-Induced Epigenetic Modifications in the Pathogenesis of Obesity, Metabolic Syndrome, Type 2 Diabetes, and Non-alcoholic Fatty Liver Disease. Curr Diabetes Rep. 2019; 19(6): 31. doi: 10.1007/s11892-019-1151-4
3. Rafaqat S, Gluscevic S, Mercantepe F, Rafaqat S, Klisic A. Interleukins: Pathogenesis in Non-Alcoholic Fatty Liver Disease. Metabolites. 2024;14(3):153. doi: 10.3390/metabo14030153.
4. Al-hussaniy HA, Al-Shammari AH, Sameer HN, Oraibi AI. The relationship between statin therapy and adipocytokine/inflammatory mediators in dyslipidemic nondiabetic patients: A comparative study. Pharmacia. 2023;70(3):581-5. DOI 10.3897/pharmacia.70.e109800
5. Scorletti E, Carr RM. A new perspective on NAFLD: Focusing on lipid droplets. Journal of Hepatology. 2022; 76(4): 934-945.doi.org/10.1016/j.jhep.2021.11.009.
6. Walther TC, Farese Jr RV. Lipid droplets and cellular lipid metabolism. Annu. Rev. Biochem., 2012; 81: 687-714.
7. Greenberg AS, Coleman RA, Kraemer FB, McManaman JL, Obin MS, Puri V, Yan QW, Miyoshi H, Mashek DG. The role of lipid droplets in metabolic disease in rodents and humans. J. Clin. Invest., 2011; 121: 2102-2110.
8. Smirne C, Croce E, Di Benedetto D, Cantaluppi V, Comi C, Sainaghi PP, Minisini R, Grossini E, Pirisi M. Oxidative Stress in Non-Alcoholic Fatty Liver Disease. Livers. 2022; 2(1):30-76. https://doi.org/10.3390/livers2010003
9. Li M, Xu C, Shi J, Ding J, Wan X, Chen D, Gao J, Li C, Zhang J, Lin Y, Tu Z, Kong X, Li Y, Yu C. Fatty acids promote fatty liver disease via the dysregulation of 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. Gut. 2018 Dec;67(12):2169-2180. doi: 10.1136/gutjnl-2017-313778.
10. Saponaro C, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. Nutrients. 2015; 7: 9453–74. doi: 10.3390/nu7115475.
11. Galer EL, Grace PM. Reactive aldehydes: a new player in inflammatory pain. Ann Transl Med. 2015; 3(Suppl 1): S23. doi: 10.3978/j.issn.2305-5839.2015.03.27.
12. Li Y, Zhao T, Li J, Xia M, Li Y, Wang X, Liu C, Zheng T, Chen R, Kan D, Xie Y, Song J, Feng Y, Yu T, Sun P. Oxidative Stress and 4-hydroxy-2-nonenal (4-HNE): Implications in the Pathogenesis and Treatment of Aging-related Diseases. J Immunol Res. 2022 Mar 23;2022:2233906. doi: 10.1155/2022/2233906.
13. Montes-de-Oca-García A, Perez-Bey A, Corral-Pérez J, Marín-Galindo A, Calderon-Dominguez M, Velázquez-Díaz D, Casals C, Ponce-Gonzalez JG. Influence of Gender on Plasma Leptin Levels, Fat Oxidation, and Insulin Sensitivity in Young Adults: The Mediating Role of Fitness and Fatness. Nutrients. 2023;15(11):2628. https://doi.org/10.3390/nu15112628
14. Al-Hussaniy HA, Alburghaif AH, AL-Zobaidy MA, Alkuraishy HM, Mostafa-Hedeab G, Azam F, Al-Samydai AM, Al-tameemi ZS, Naji MA. Chemotherapy-induced cardiotoxicity: a new perspective on the role of Digoxin, ATG7 activators, Resveratrol, and herbal drugs. Journal of med life. 2023;16(4):491.
15. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999;94:2467-2474. Doi: 10.1111/j.1572-0241.1999.01377.x.
16. Jung I, Koo DJ, Lee MY, Moon SJ, Kwon H, Park SE, Rhee EJ, Lee WY. Increased Risk of Nonalcoholic Fatty Liver Disease in Individuals with High Weight Variability. Endocrinol Metab. 2021; 36(4):845-854.
17. DiStefano JK, Gerhard GS. NAFLD in normal weight individuals. Diabetol Metab Syndr . 2022; 14: 45. doi.org/10.1186/s13098-022-00814-z.
18. Abdallah HR, Youness ER, Bedeir MM, Abouelnaga MW, Ezzat WM, Elhosary Y, El-Hariri HM, Hussein MAEA, Ahmed HR, Eladawy R. Clinical and diagnostic characteristics of non-alcoholic fatty liver disease among Egyptian children and adolescents with type1 diabetes. Diabetol Metab Syndr. 2023 Mar 21;15(1):52. doi: 10.1186/s13098-023-01029-6..
19. Sheng, G., Xie, Q., Wang, R. et al. Waist-to-height ratio and non-alcoholic fatty liver disease in adults. BMC Gastroenterol. 2021; 21: 239. https://doi.org/10.1186/s12876-021-01824-3.
20. Lin MS, Lin TH, Guo SE, Tsai MH, Chiang MS, Huang TJ, Chen MY. Waist-to-height ratio is a useful index for nonalcoholic fatty liver disease in children and adolescents: a secondary data analysis. BMC Public Health. 2017; 17: 851. https://doi.org/10.1186/s12889-017-4868-5.
21. Eslami B, Aletaha N, Maleki-Hajiagha A, Sepidarkish M, Moini A. Evaluation of the predictive value of body mass index (BMI), waist circumference, and visceral fat to differentiate non-alcoholic fatty liver (NAFLD) in women with polycystic ovary syndrome. J Res Med Sci. 2022; 27: 37. doi: 10.4103/jrms.JRMS_292_20.
22. Treeprasertsuk S, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, et al. The predictors of the presence of varices in patients with primary sclerosing cholangitis. Hepatology. 2010;51(4):1302–10.
23. Xuan Y, Wu D, Zhang Q, Yu Z, Yu J, Zhou D. Elevated ALT/AST ratio as a marker for NAFLD risk and severity: insights from a cross-sectional analysis in the United States. Front. Endocrinol. 2024; 15:1457598. doi: 10.3389/fendo.2024.1457598
24. Hall P, Cash J. What is the real function of the liver 'function' tests? Ulster Med J. 2012 ;81(1):30-6.
25. Malnick SDH, Alin P, Somin M, Neuman MG. Fatty Liver Disease-Alcoholic and Non-Alcoholic: Similar but Different. Int J Mol Sci. 2022 Dec 19;23(24):16226. doi: 10.3390/ijms232416226.
26. Zhao D, Wu GY. Prolonged Direct Hyperbilirubinemia Following Acute Hepatitis: When Not to Worry?J Clin Transl Hepatol. 2024;12(12):985-987. doi: 10.14218/JCTH.2024.00265.
27. Ball M, Miller I, Cotten SW, Direct Bilirubin Higher Than Total Bilirubin?, Clin Chem.2015; 61(6): 889, https://doi.org/10.1373/clinchem.2014.237040.
28. Altalebi RR, Al-Hussaniy HA, Al-Tameemi ZS, Al-Zobaidy MA, Albu-Rghaif AH, Alkuraishy HM, Hedeab GM, Azam F, Al-Samydai AM, Naji MA. Non-alcoholic fatty liver disease: relation to juvenile obesity, lipid profile, and hepatic enzymes. J Med Life. 2023;16(1):42. doi: 10.25122/jml-2022-0091
29. Long He, Ting He, Shabnam Farrar, Linbao Ji, Tianyi Liu, Xi Ma; Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 2017; 44 (2): 532–553. https://doi.org/10.1159/000485089.
30. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018; 13:757–772.
31. Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. Circ Res. 2018;122(6): 877-902. doi: 10.1161/CIRCRESAHA.117.311401.
32. Singh Z, Karthigesu IP, Singh P, Kaur R. Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review. Iran J Public Health. 2014; 43(3): 7-16.
33. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol. 2018 Jun 4;217(6):1915-1928. doi: 10.1083/jcb.201708007.
34. Vitolo M. Decomposition of hydrogen peroxide by catalase. World J Pharm Pharm Sci. 2021; 10(8): 47-56.
35. Dey S, Sidor A, O'Rourke B. Compartment-specific Control of Reactive Oxygen Species Scavenging by Antioxidant Pathway Enzymes. J Biol Chem. 2016 May 20;291(21):11185-97. doi: 10.1074/jbc.M116.726968.
36. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J Biol Chem. 2003 Aug 15;278(33):31426-33. doi: 10.1074/jbc.M212549200.
37. Świderska M, Maciejczyk M, Zalewska A, Pogorzelska J, Flisiak R.,

[DOI: 10.47176/mjiri.40.69]

Chabowski A. Oxidative stress biomarkers in the serum and plasma of patients with non-alcoholic fatty liver disease (NAFLD). Can plasma AGE be a marker of NAFLD? Oxidative stress biomarkers in NAFLD patients. Free Radic. Res. 2019, 53, 841–850.
38. Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med 2012;52(1):59–69.
39. Hardwick RN, Fisher CD, Canet MJ, Lake AD, Cherrington NJ. Diversity in antioxidant response enzymes in progressive stages of human nonalcoholic fatty liver disease. Drug Metab Dispos 2010;38(12):2293–301.
40. Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. J Pharm Bioallied Sci. 2011; 3 (1): 118–27. doi:10.4103/0975-7406.76489.
41. Popko K, Gorska E, Stelmaszczyk-Emmel A, Plywaczewski R, Stoklosa A, Gorecka D, Pyrzak B, Demkow U. Proinflammatory cytokines Il-6 and TNF-α and the development of inflammation in obese subjects. Eur J Med Res. 2010; 15(Suppl 2): 120-2. doi: 10.1186/2047-783x-15-s2-120.
42. von Loeffelholz C, Lieske S, Neuschäfer-Rube F, Willmes DM, Raschzok N, Sauer IM, et al. The human longevity gene homolog INDY and interleukin-6 interact in hepatic lipid metabolism". Hepatology. 2017; 66 (2): 616–630. doi:10.1002/hep.29089.
43. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. J Hepatol. 2016; 64(6):1403-15. doi: 10.1016/j.jhep.2016.02.004.
44. Dhamija E, Paul SB, Kedia S. Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: An increasing concern. Indian J Med Res. 2019; 149(1):9-17. doi: 10.4103/ijmr.IJMR_1456_17.
45. Kokolakis G, Sabat R, Krüger-Krasagakis S, Eberle J. Ambivalent Effects of Tumor Necrosis Factor Alpha on Apoptosis of Malignant and Normal Human Keratinocytes. Skin Pharmacol Physiol. 2021; 34 (2): 94–102.
46. Sethi JK, Hotamisligil GS. Metabolic Messengers: tumour necrosis factor. Nat Metab. 2021; 3 (10): 1302–1312. doi:10.1038/s42255-021-00470-z.
47. Al-Hussaniy HA, Alburghaif AH, Naji MA. Leptin hormone and its effectiveness in reproduction, metabolism, immunity, diabetes, hopes and ambitions. J Med Life. 2021; 14 (5): 600–605. doi:10.25122/jml-2021-0153.
48. Hebebrand J, Hildebrandt T, Schlögl H, Seitz J, Denecke S, Vieira D, et al. The role of hypoleptinemia in the psychological and behavioral adaptation to starvation: Implications for anorexia nervosa. Neurosci Biobehav Rev. 2022; 141: 104807. doi:10.1016/j.neubiorev.2022.104807.
49. Cheng J, Luo Y, Li Y, Zhang F, Zhang X, Zhou X, Ji L. Sex- and body mass index-specific reference intervals for serum leptin: a population based study in China. Nutr Metab (Lond). 2022; 19: 54. https://doi.org/10.1186/s12986-022-00689-x
50. Abd Hasan1 S, Abdul Hammed BS, Banjah QM. Association Between BMI and Leptin Levels in Women with Primary Infertility: A Cross-Sectional Study. JOGCR. 2024; 9(5): 492-497. doi.org/10.30699/jogcr.9.5.492
51. Pan H, Guo J, Su Z. Advances in understanding the interrelations between leptin resistance and obesity. Physiol Behav. 2014; 130: 157–169. doi:10.1016/j.physbeh.2014.04.003.
52. Engin A. The Mechanism of Leptin Resistance in Obesity and Therapeutic Perspective. Adv Exp Med Biol. 2024;1460:463-487. doi: 10.1007/978-3-031-63657-8_16.
53. Amitani M., Asakawa A., Amitani H., Inui A. The role of leptin in the control of insulin-glucose axis. Front. Neurosci. 2013; 7:51. doi: 10.3389/fnins.2013.00051.
54. Blanco AM, Soengas, JL. Leptin signalling in teleost fish with emphasis in food intake regulation. Mol Cell Endocrinol. 2021; 526: 111209. doi.org/10.1016/j.mce.2021.111209.
55. Vadacca M, Margiotta DP, Navarini L, Afeltra A. Leptin in immuno-rheumatological diseases. Cell Mol Immunol. 2011 May;8(3):203-12. doi: 10.1038/cmi.2010.75.
56. Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of Leptin in Inflammation and Vice Versa. Int J Mol Sci. 2020; 21(16):5887. doi: 10.3390/ijms21165887.
57.s Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. Ann N Y Acad Sci. 2018 Jan;1411(1):21-35. doi: 10.1111/nyas.13435.

[DOI: 10.47176/mjiri.40.69]