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"A STUDY OF OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN NON-ALCOHOLIC FATTY LIVER DISEASE"

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a prevalent global health issue. Oxidative stress contributes to the pathological changes observed in this condition. Elevated CRP levels may increase the risk of liver disease in affected patients. This study aimed to assess oxidative stress and hsCRP levels in patients with non-alcoholic fatty liver disease.

Methods: Patients diagnosed with non-alcoholic fatty liver disease (NAFLD) based on clinical, biochemical, or imaging studies were included along with age and sex-matched healthy controls. The overnight fasting blood samples were obtained using the standard venipuncture techniques most of the morning after fasting through the night. All the variables were estimated as per the standardized techniques.

Results: In this study, a total of 100 subjects were recruited and divided into two groups: Group I (n = 50) with non-alcoholic fatty liver disease (NAFLD), and Group II (n = 50) comprising healthy controls. hs-CRP was measured using the immunoturbidimetric method, while plasma MDA and blood activities of SOD and CAT were estimated using spectrophotometry. The results showed a significant increase in MDA and hsCRP levels, and a substantial decrease in SOD and CAT activity in NAFLD patients compared to healthy controls. Elevated hs-CRP and oxidative stress markers indicate a higher risk of liver disease.

Conclusion: The results of the present study suggest that elevated malondialdehyde (MDA) levels and reduced antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), could serve as important markers for the early detection of liver disease and monitoring the effectiveness of treatments.

Keywords: Non-Alcoholic Fatty Liver Disease, malondialdehyde, superoxide dismutase (SOD), hs-CRP (high sensitivity C Reactive Protein)

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, with a prevalence of 23–25% in the general adult population. The highest prevalence is in the Middle East (32%) and South America (30%), while the lowest is in Africa (13%) [1]. North America, Europe, and Asia have reported prevalence rates of 24.1%, 23.7%, and 27.4%, respectively [2], increasing to 55.5% in those with type 2 diabetes [3]. The prevalence in the general population of India is estimated to range between 9% and 32%, with urban populations exhibiting higher rates due to lifestyle factors such as increased sedentary behavior, poor dietary habits, and rising obesity rates. NAFLD, characterized by hepatocyte steatosis and fat accumulation without excessive alcohol consumption, can range from benign simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) [4]. NASH, which can lead to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), is now the second leading cause of liver transplantation in Europe and the U.S. and is predicted to surpass hepatitis C [5-7]. Oxidative stress (OS) plays a crucial role in the progression of non-alcoholic fatty liver disease (NAFLD) from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) [8-10]. Increased reactive oxygen species (ROS) levels lead to lipid peroxidation, inflammation, and fibrogenesis by activating hepatic stellate cells (HSCs) and inhibiting VLDL secretion, which contributes to fat accumulation in the liver [11]. ROS also promotes hepatic insulin resistance, necroinflammation, and hepatocyte apoptosis. OS has been linked to atherothrombosis, potentially connecting NAFLD to cardiovascular disease [12]. Studies have shown that NAFLD is associated with altered redox status and increased metabolic risk [13-15]. Moreover, chronic overnutrition and high intake of saturated fats and carbohydrates can exacerbate OS through mechanisms such as NADPH oxidase (NOX) activation and mitochondrial oxidative phosphorylation, further linking NAFLD to systemic OS [16-18]. Malondialdehyde (MDA), a key marker of oxidative stress, is produced by lipid peroxidation. Elevated plasma MDA levels have been associated with alcohol abuse and the development and progression of liver diseases. High-sensitive C-reactive protein (hs-CRP) is another important biomarker that is positively associated with alcohol abuse, diabetes, and obesity and is an early indicator of non-alcoholic fatty liver disease (NAFLD). In the current study, we evaluated the markers of oxidative stress (hs-CRP) and MDA in non-alcoholic fatty liver disease.

MATERIAL AND METHODS

This cross-sectional study was conducted in the Department of General Medicine, Institute of Medical Sciences. Institutional Ethical approval was obtained for the study after following the protocol for human research. Written consent was obtained for the study after explaining the nature of the study in vernacular language. Those willing to participate in the study willingly were included in the study.

Inclusion Criteria:

1. Patients diagnosed with non-alcoholic fatty liver disease (NAFLD) based on clinical, biochemical, or imaging studies.
2. Aged 18 – 60 years
3. Males and Females
4. Patients who provided informed consent to participate

Exclusion Criteria:

1. Individuals with a history of alcohol intake
2. With liver diseases such as (HBV and HCV)
3. Autoimmune hepatitis or drug-induced liver injury
4. History of MI, stroke
5. Pregnant or breastfeeding females
6. Recent use of anti-inflammatory medications
7. Patients with CKD or malignancies

The overnight fasting blood samples were obtained using the standard venipuncture techniques most of the time in the morning after fasting through the night. Serum was also obtained by allowing the blood samples to clot and then spinning them in a centrifuge to sediment the clot. Serum was also tested for high-sensitivity C-reactive protein (hsCRP) for which an immunoturbidimetric method is common. For MDA estimation, blood was collected from the participants through venipuncture and centrifuged at 3000 rpm for 10 minutes after which plasma was isolated. MDA concentration was determined with the Thiobarbituric Acid Reactive Substances (TBARS) method. Antioxidants were determined by a method that involved the reaction of plasma with thiobarbituric acid (TBA) and heating the mixture at an acidic pH. MDA can react with TBA forming a pink colored MDA-TBA adduct. The intensity of the color formed was determined using a spectrophotometer at 532 nm while blood was prepared for the preparation of red blood cell lysate. SOD was extracted from red blood cells after the process of lysis with a hypotonic solution. SOD activity was determined using the NBT method which estimates the ability of SOD to impair the superoxide radical–dependent reduction of NBT. These superoxide

radicals were produced in vitro as described previously by employing the xanthine-xanthine oxidase system. The reaction mixture normally involved NBT, a superoxide radical generator, and the sample. SOD works opposite to NBT for superoxide radicals and therefore forms less of the blue formazan product of which formation is proportional to the intensity of light absorbance measured spectrophotometrically. NBT reduction was determined by using a spectrophotometer at 560 nm to monitor the decrease in NBT. Liver function tests were also performed as per the standard protocol for each patent. Similarly, the same number of age and sex-matched samples were obtained from the normal patients to act as controls.

Statistical analysis: All the available data was uploaded to an MS Excel spreadsheet and analyzed by SPSS version 22 in Windows format. The continuous variables were represented as mean, standard deviation, and percentage, and the categorical variables were calculated using the chi-square test for differences between the two variables. The values of p (<0.05) were considered as significant.

RESULTS

A total of 50 subjects of non-alcoholic fatty liver disease which included 35(70%) males and 15(30%) females in the study group. Similarly, the same number of age and sex-matched controls were taken for the study. The age-wise description is given in Table 1. The majority of participants in both groups are in the 41-60 age range, suggesting that older adults are more likely to be affected by NAFLD. A smaller proportion of participants in both groups are in the younger age ranges of 20 - 30 and 31- 40 years.

Table1: Age distribution of study groups and healthy controls

Age (years)	*NAFLD (n=50)*	*Controls (n=50)*
20 – 30	6 (12%)	5 (10%)
31 – 40	10 (20%)	10 (20%)
41 – 50	11(22%)	14 (28%)
51 – 60	23 (46%)	21 (42%)
Total	50 (100%)	50 (100%)

Table 2 shows the anthropometric profiles (height, weight, and BMI) of the two groups. There was no significant difference in height between the NAFLD group and the control group. The NAFLD group had a significantly higher average weight (95.88 kg) compared to the control group (61.25 kg). The NAFLD group also had a significantly higher average BMI (36.54 kg/m²) compared to the control group (23.20 kg/m²). The significant differences in weight and BMI between the two groups strongly suggest that obesity or overweight is a major risk factor for NAFLD.

Table 2: Anthropometric profile of cases and controls included in the study

Parameters	*NAFLD (n=50)*	*Controls (n=50)*	*P value*
Height (cm)	164.5 ± 0.19	166.51 ± 1.15	0.812
Weight (kg)	95.88 ± 12.34	61.25 ± 5.19	0.012*
BMI (kg/m^2)	36.54 ± 2.25	23.20 ± 0.59	0.001*

The Random blood sugar estimation in both groups of individuals revealed individuals with NAFLD had significantly higher blood sugar levels (160.12 mg/dL) compared to the control group (101.18 mg/dL). The difference in blood sugar levels between the two groups was statistically significant, with a p-value less than 0.001. This suggests that a strong association between NAFLD and elevated blood sugar levels exists. This finding aligns with existing research that highlights the link between NAFLD and insulin resistance, which can lead to hyperglycemia.

Table 3 presents a comparison of various liver function tests (LFTs) between individuals with Non-Alcoholic Fatty Liver Disease (NAFLD) and a control group. A critical analysis of the table shows individuals with NAFLD had significantly higher levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP) compared to the control group. The NAFLD group had a significantly lower level of total protein compared to the control group. There was a trend towards a higher level of total bilirubin in the NAFLD group, but the difference was not statistically significant.

Table 3: Liver profile in NAFLD as compared to controls

Parameters	*NAFLD (n=50)*	*Controls (n=50)*	*P value*
Alanine transaminase ALT (IU/L)	81.16 ± 20.21	30.32± 6.60	0.012*
Aspartate transferase AST (IU/L)	78.19 ± 21.71	29.41 ± 5.33	0.011*
Gamma-glutamyl transpeptidase GGT (IU/L)	105.61 ± 30.08	20.56± 9.35	0.0001*

Alkaline Phosphatase ALP (IU/L)	103.62 ± 49.57	82.15 ± 19.77	0.031*
Total Protein (g/dl)	5.21 ± 1.91	6.98 ± 0.94	0.049*
Total Bilirubin (mg/dl)	0.78 ± 0.29	0.64 ± 0.19	0.064

Table 4: Blood activity of SOD, catalase plasma MDA, and hs-CRP levels in NAFLD as compared to controls

Parameters	*NAFLD (n=50)*	*Controls (n=50)*	*P value*
MDA µmol/L	7.95 ± 2.12	2.89 ± 0.61	0.001*
SOD U/g of Hb	2.19 ± 1.06	5.78 ±1.32	0.021*
CAT U/g of Hb	6.80 ± 1.82	3.82 ±1.37	0.034*
hs-CRP (mg/dl)	7.34 ± 1.97	1.75 ± 0.67	0.001*

Table 4 shows the differences in biomarker levels between the NAFLD group and control group, including superoxide dismutase (SOD), catalase, malondialdehyde (MDA), and high-sensitivity C-reactive protein (hs-CRP). When the table was critically analyzed, it was found that NAFLD patients had elevated MDA levels which were associated with oxidative stress. Among enzymes, there was a statistically significant decrease in the levels of superoxide dismutase and catalase, which are antioxidant enzymes. It is also evident that NAFLD patients had significantly elevated hsCRP status, which is an inflammatory factor. Higher MDA in the NAFLD group supports the proposed oxidative stress as a characteristic feature of NAFLD. However, oxidative stress may also induce liver injury and inflammation. The lowered levels of SOD and catalase indicate that erythrocytes from NAFLD patients have diminished antioxidant reserves and this decrease in their efficiency leads to increased oxidative stress in NAFLD. The significantly enhanced hsCRP indicates increased inflammation in patients with NAFLD, and this inflammation can cause damage to the liver as well as a host of related issues.

DISCUSSION

In the current study, we observed that the mean levels of height, weight, and BMI were significantly higher among patients with NAFLD than among controls. This can be attributed to the fact that BMI is positively correlated with NAFLD even after controlling for other confounding factors. BMI, which is also an indicator of body fat, is used for screening obesity-related diseases and is often found to be associated with hepatic steatosis [19]. The results of this study are in concordance with the observations of Panag et al. [20] who also reported that increased BMI was strongly associated with NAFLD risk in the Asian population compared to the Western population. In this study, we found an increased mean blood sugar level in the NAFLD group compared with that in the control group however, the values were not statistically significant. Rao et al. [21] in a similar study also reported that blood sugar levels did not increase significantly in the NAFLD group. Increased fasting and postprandial blood sugar levels were observed in the NAFLD group. Regular monitoring of blood sugar levels may help prevent the occurrence of diabetes in the prediabetic population and in controlling sugar levels in the already diabetic population. The present study found that all liver function parameters, AST, ALT, ALP, and GGT, were significantly increased, although the total protein levels were not significantly altered in the NAFLD group. Liver functions are affected by the existence of type 2 diabetes, obesity, and the amount of fat deposition in liver cells, which impairs hepatocyte function. Boemeke et al. [22] in a similar study found dyslipidemia existent in patients with NAFLD. Similarly, Zhu et al. [23] found that lipid parameters were significantly increased in patients with NAFLD, and the increase was greater in cases of NAFLD with type 2 diabetes mellitus. In diabetes, increased mobilization of fats and free fatty acids could play a role in altering lipid parameters. Because liver hepatocytes play an important role in lipid metabolism, hepatocyte fat accumulation could affect the effective functioning of hepatocytes [24]. This study found that the plasma MDA levels were significantly increased in the NAFLD group. Muller et al. [25] have observed that the concentration of MDA was increased in both AFLD and NAFLD cases. Liver damage and lipid peroxidation are closely related. Oxidative stress plays a role in liver fibrogenesis, and Li S et al. [26] found in vitro evidence of a possible molecular connection between enhanced lipid peroxidation and the induction of collagen gene expression.

In our study, we found that the activity of erythrocyte SOD and catalase was significantly decreased in NAFLD compared to the control group. Reduced antioxidant capacity has been observed in liver diseases. This could lead to the formation of more free radicals and lipid peroxidation, which in turn could cause damage to the cells. Few studies in this field have reported similar decreases in SOD and catalase activity in NAFLD cases. [27, 28] SOD is the antioxidant enzyme that changes the superoxide anion radicals into hydrogen peroxide and molecular oxygen [29]. In the current study, we found that hsCRP levels significantly increased in the NAFLD group. C-reactive protein is an early biomarker of inflammation [30]. Previous studies have also found that hsCRP levels are significantly higher and independently associated with NAFLD compared to controls. Increased hsCRP is a feature that can distinguish NASH from a simple non-progressive status and is an indicator of the severity of fibrosis.

CONCLUSION

The results of the present study suggest that elevated malondialdehyde (MDA) levels and reduced antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), could serve as important markers for the early detection of liver disease and monitoring the effectiveness of treatments. These markers are cost-effective and can be easily assayed in laboratories. High-sensitivity C-reactive protein (hs-CRP) is also a key biomarker for liver disease, making it valuable for early detection. Improving liver function could have significant clinical implications in the prevention and treatment of NAFLD.

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