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Study of oxidative stress and hsCRP in alcoholic and nonalcoholic fatty liver disorders

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Original Research Article

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Abstract: Alcoholic and nonalcoholic fatty liver disease is a very common global problem, in worldwide. During oxidative stress the pathological changes observed in AFLD and NAFLD. High CRP levels may put the patients, at increased risk for liver disease. The aim of this study was estimation of oxidative stress and hsCRP in pateint with alcoholic and nonalcoholic fatty liver disease. In the present study, total (n = 495) subjects were recruited for the study and divided in three groups. Group I; (n = 167) of alcoholic liver disease; (n = 158) of nonalcoholic fatty liver disease, and group III included (n = 170) healthy control subjects. HsCRP was measurement of immunoterbidy method. Plasma MDA and Blood activity of SOD, CAT were estimated by spectrophotometric method. The present study shown that levels of MDA and hsCRP was significantly increased ($p<0.001$) and blood SOD, CAT activity, and were significantly decreased ($p<0.001$) in AFLD patients as compared with healthy controls, and also significantly increased ($p <0.001$) and decreased in NFALD patients when they compared with healthy controls. Serum HsCRP and oxidative stress markers are the increased risk for liver disease.

Keywords: Superoxide dismutase (SOD), Catalase (CAT), High sensitive C-reactive protein (HsCRP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma Glutamyltransferase (GGT) Malondialdehyde (MDA), Reactive oxygen species (OS) Alcoholic fatty liver disease (ALD), Alcoholic fatty liver disease (AFLD) & Non Alcoholic fatty liver disease (NAFLD)

INTRODUCTION

Fatty liver (steatosis) is fat accumulated in the liver, and 5 to 10% of the liver weight is due to accumulation of fat which can be cause fatty liver disease.

Fatty liver is of two types; first is alcoholic fatty liver disease (AFLD), it is manifested by alcohol overconsumption [1]. Second is NAFLD where excessive fat accumulation occurs in liver [2], and associated with obesity and metabolic syndrome [3]. Excessive amount of alcohol results, liver damage, release of inflammatory cytokines, impaired oxidative stress, lipid peroxidation reaction, and also acetaldehyde toxicity. These can be causes inflammation of liver, apoptosis and finally fibrosis of liver cells [4]. Prevalence rate of the alcoholic liver disease is around 25-40% in the general Indian population [5]. Nonalcoholic fatty liver disease (NAFLD) is characterized by chronic liver disease that may be lead to end-stage liver disease. NAFLD enhances the progression of fibrosis, liver cirrhosis, and lastly liver failure [6]. The triglycerides (TG) accumulation in the hepatocytes is an indication of NAFLD [7]. The prevalence rate of NAFLD is around 9-32% in Indian general population, and with a higher incidence rate with patients of diabetic and obesity patients.

Oxidative stress is one of the pathogenic mechanisms contributing to the progression of steatosis (simple fatty liver) to nonalcoholic steatohepatitis (NASH-fatty) [8]. Oxidative stress is also the cause of development of liver disease [9]. Malondialdehyde (MDA) is produced by lipid peroxidation and also it is the key marker of oxidative stress [10]. Increased plasma MDA levels related to the excessive consumption of alcohol and also associated with pathogenesis and progression of liver disease. The HsCRP (high sensitive **C**-reactive protein) positively associated with excessive alcohol consumption diabetes and obesity, and it is an early biomarker of AFLD, NAFLD.

PATIENTS AND METHODS

The present study was carried out in the Department of Biochemistry, Sri Aurobindo Institute of Medical sciences (SAIMS) and P.G. Institute, Indore, M.P., India during July 2015 to Aug 2016. Patients were enrolled from Department of Medicine, Gastroenterology, SAIMS College and Hospital, Indore. Total (n = 495) subjects were enrolled for the study. Group I; included- 167 (97 males and 70 females) of alcoholic fatty liver disease pateints, group II; included- 158 (68males and 89 females) of nonalcoholic fatty liver disease pateints. The age between 30 to 75 years both gender, and group III include- 170 normal healthy controls (98 males and 72 females) without any complication. Essential hypertension, thyroid disease, pregnancy cardiac associated liver disease cancer, asthma, and other infectious diseases were excluded from the study. The study was approved by the Institutional Ethical Committee and patients were recruited for the study after taking their written informed consent. A detailed physical examination was done which included measuring of height, weight and blood pressure.

Sample collection and analysis of biochemical parameters

The overnight fasting blood was collected in anticoagulat tubes. Plasma, serum and hemolysate were prepared and stored at -4°C. Blood samples transferred in EDTA tubes were centrifuged at 3000 rpm for 15 min and their plasma fractions were stored at -20°C to measure MDA levels. Estimation of blood Catalase activity by Aebi (1984) method (11). Determination of Superoxide Dismutase activity (SOD) by Marklund and Marklund (1974) method (12). Plasma MDA was measured by spectrophotometric method at 531nm (13), and HsCRP was determination of immunoterbidy method.

STATISTICAL ANALYSIS

The statistical analysis was carried out by the SPSS statistics version 20.0. Values are presented as means ± standard deviation (Means ±SD). $P < 0.05$ was considered as significant level.

RESULTS

Age distribution in study groups

The age distribution in alcoholic and nonalcoholic fatty liver disease and healthy control subjects are listed in Table 1. Out of total (n = 495) subjects, 167 AFLD patients 158 were nonalcoholic fatty liver disease. According to age distribution, 61 to 75 years age group subjects were in higher numbers as compare to other age groups.

Physiological characteristic of AFLD compared to controls

The duration of disease, height, weight and basal metabolic rate (BMI), in AFLD and healthy control are shown in Table 2.

Physiological characteristic of NAFLD compared to controls

The duration of disease, height, weight and basal metabolic rate (BMI), in NAFLD and healthy control are shown in Table 3. The Duration of disease, weight and BMI were found significantly increased in NAFLD as compared to healthy controls.

Blood sugar in AFLD compared to controls

The blood sugar in AFLD patients and healthy control are shown in Table 4. The blood sugar found not significantly in AFLD.

Blood sugar in AFLD compared to controls

The blood sugar in NAFLD patients and healthy control are shown in Table 5. The blood sugar found to significantly increase in NAFLD.

Liver profile in AFLD compared to controls

The biochemical parameters- AST, ALT, ALP, GGT, total protein and total bilirubin, in AFLD patients and healthy control are shown in Table 6. The AST, ALT, ALP, GGT, and total bilirubin were found significantly increased and no significant difference of total protein in AFLD.

Liver profile in NAFLD compared to controls

The biochemical parameters- AST, ALT, ALP, GGT, total protein and total bilirubin, in NAFLD patients and healthy control are shown in Table 7. The AST, ALT, ALP, GGT, and total bilirubin were found significantly increased and no significant difference of total protein in NAFLD as compared to healthy controls

Lipid profile in AFLD compared to controls

Serum total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C in AFLD and healthy control are shown in Table 8. The serum total cholesterol, triglyceride, LDL-C and VLDL-C levels were found significantly increased and no significant difference of HDL-C AFLD as compared to healthy controls.

Lipid profile in NALFD compared to controls

Serum total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C in ALD and healthy control are shown in Table 9. The serum total cholesterol, triglyceride, LDL-C and VLDL-C levels were found significantly increased and no significant difference HDL-C in NAFLD as compared to healthy controls.

Blood activity of SOD, CAT and plasma MDA, and hsCRP levels in ALFD compared to controls

Blood activity of SOD, CAT and plasma MDA levels in AFLD and healthy control are shown in Table 10. The plasma MDA, and hsCRP levels significantly increased and blood activity of SOD and catalase significantly decreased in AFLD as compared to healthy controls.

Blood activity of SOD, CAT and plasma MDA, and hsCRP levels in NAFLD compared to controls

Blood activity of SOD, CAT and plasma MDA levels in NAFLD and healthy control are shown in Table 11.The plasma MDA, and HsCRP levels significantly increased and blood activity of SOD and catalase significantly decreased in NAFLD as compared to healthy controls.

Table-01: Age distribution of study groups and healthy controls

Age (years)	AFLD (n=167)	NAFLD (n=158)	Controls (n=170)
30-45	39	42	49
46-60	53	51	62
61-75	76	65	59
Total	167	158	170

Table-02: Physiological characteristic of AFLD

Parameters	AFLD (n=167)	Controls (n=170)	P value
Height (cm)	169.2±0.11	170.1±0.09	p<0.33
Weight (kg)	57.15±9.65	57.18±6.95	p<0.97
BMI (kg/m^2)	20.25±1.75	19.56±1.34	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

Table-03: Physiological characteristic of NAFLD

Parameters	NAFLD (n=158)	Controls (n=170)	P value
Height (cm)	169.6±0.09	170.1±0.09	p<0.06
Weight (kg)	126.39±15.23	57.18±6.95	p<0.001
BMI (kg/m^2)	44.80±7.9	19.56±1.34	p<0.001

Data are presented as mean ± SD, p <0.05 was considered as significant level

Table-04: Blood sugar in AFLD in compared to controls

Parameters	AFLD (n=167)	Controls (n=170)	P value
Blood Sugar (mg)	107.92±29.08	103.38±20.57	p<0.095

Data are presented as mean ± SD, p <0.05 was considered as significant level

Table-05: Blood sugar in NAFLD compared to controls.

Parameters	NAFLD (n=158)	Controls (n=170)	P value
Blood Sugar (mg)	159.22±62.28	103.38±57	p<0.001

Data are presented as mean ± SD, p <0.05 was considered as significant level

Table-06: Liver profile in AFLD as compared to controls

Parameters	AFLD (n=167)	Controls (n=170)	P value
ALT (IU/L)	115.66±34.28	29.18±8.46	p<0.001
AST (IU/L)	112.43±40.97	28.14±7.23	p<0.001
GGT (IU/L)	108.25±30.26	22.87±10.05	p<0.001
ALP (IU/L)	185.49±49.46	86.91±24.81	p<0.001
T.P (g/dl)	4.92±0.82	6.97±0.65	p<0.001
T.B.(mg/dl)	0.86±0.28	0.62±0.15	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

Table-07: Liver profile in NAFLD as compared to controls

Parameters	NAFLD (n=158)	Controls (n=170)	P value
ALT (IU/L)	82.87±30.94	29.18±8.46	p<0.001
AST (IU/L)	77.07±32.54	28.14±7.23	p<0.001
GGT (IU/L)	107.34±36.64	22.87±10.05	p<0.001
ALP (IU/L)	109.72±91.86	86.91±24.81	p<0.001
T.P (g/dl)	5.21±0.92	6.97±0.65	p<0.001
T.B.(mg/dl)	0.72±0.28	0.62±0.15	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

Table-08: Lipid profile in AFLD as compared to the controls

Parameters	AFLD (n=167)	Controls (n=170)	P value
T. Chol (mg/dl)	259.53±35.91	178.74±20.13	p<0.001
TG (mg/dl)	189.12±37.96	122.23±13.46	p<0.001
HDL-C (mg/dl)	42.08±7.70	41.08±7.37	p<0.99
LDL-C (mg/dl)	172.25±35.24	113.20±17.93	p<0.001
VLDL-C (mg/dl)	37.96±7.52	24.46±2.74	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

Table-09: Lipid profile in NAFLD compared to controls

Parameters	NAFLD (n=158)	Controls (n=170)	P value
T. Chol (mg/dl)	284.08±29.47	178.74±20.13	p<0.001
TG (mg/dl)	203.34±26.25	122.23±13.46	p<0.001
HDL-C (mg/dl)	43.96±8.79	41.08±7.37	p<0.001
LDL-C (mg/dl)	199.46±31.35	113.20±17.9	p<0.001
VLDL-C (mg/dl)	40.46±5.25	24.46±2.74	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

Table-10: Blood activity of SOD, catalase plasma MDA, and HsCRP levels in AFLD as compared to controls

Parameters	**AFLD (n=167)**	**Controls (n=170)**	**P value**
MDA µmol/L	8.01±2.56	2.81±0.53	p<0.001
SOD U/g of Hb	2.69±1.09	5.68±1.08	p<0.001
CAT U/g of Hb	3.17±1.20	6.77±1.07	p<0.001
HsCRP (mg/dl)	6.57±2.66	1.83±0.56	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

Table-11: Blood activity of SOD, catalase plasma MDA, and hsCRP levels in NAFLD as compared to controls

Parameters	NAFLD (n=158)	Controls (n=170)	P value
MDA µmol/L	7.85±3.07	2.81±0.53	p<0.001
SOD U/g of Hb	2.53±1.09	5.68±1.08	p<0.001
CAT U/g of Hb	6.77±1.07	3.69±1.48	p<0.001
HsCRP (mg/dl)	7.48±2.45	1.83±0.56	p<0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

DISCUSSION

In the our present study, we showed that physiological characteristic such as height, weight and BMI were increased in NAFLD pateints then AFLD, due to BMI was independently associated with NAFLD. BMI is an also independent predictor of fat infiltration of the liver and it does provide an indicator of body fatness for people and it is used to screen for weight that may lead to problem of health [14]. Our present study suggested high levels of BMI in obesity for development of NAFLD which leads to the liver cirrhosis. Pang Q *et al*., also reported that the increased BMI a strong association between obesity and NAFLD risk in the population compared with the western population [15]. The blood sugar was found to be no statistically significant (p<0.001) in AFLD and significantly increased in NAFLD as compared to controls. Similar results were reported by Babu Rao *et al.*, [16]. In the present study, uncontrolled hyperglycemia is a major cause of NAFLD and increase level of blood sugar is seen in fasting and postprandial state. However monitoring of blood sugar regularly, prevent ongoing of diabetes condition but lake of awareness also make a suitable condition of NAFLD.

In our study, we shows the total bilirubin, AST, ALT, ALP, and GGT were found significantly increased (p<0.001) and total protein was found to be non-significant in AFLD and NAFLD as compared to controls in both genders. Severity of liver damage is often converted to the amount of alcohol consumption in pateints with a history of heavy alcohol abuse [17]. However, liver disease doesn't only depend to the amount of alcohol consumption but also depend on the type 2 diabetes. Obesity is also play an important role in the development of liver disease.

In the present results, we observed that the lipid profile (TC, TG, and LDL, VLDL and HDL) were found to be statistically significant (p<0.001) and level of HDL was found to be not significant (p<0.005) in study groups in comparison to the control group in both genders. Similar results documented by Boemeke L *et al.*, [18] have found that markedly significantly increased (p<0.001) levels of total cholesterol (TC), triglyceride (TG), LDL-C and HDL-C in ALD. Sen A *et al.*, [19] reported the lipid profile significantly increase in nonalcoholic fatty liver disease, and NAFLD with diabetic patients may be attributed to increase in the

mobilization of free fatty acid from fat depots. The role of liver in lipid and lipoprotein metabolism, therefore hypertriglyceridemia has been correlated with hepatocyte fat accumulation [20]. Kelishadi R *et al.*, [21] have reported that NAFLD patients have elevated levels of total cholesterol, triglycerides, LDL-C and VLDL-C and reduced levels of HDL-C in NAFLD. Jin HB *et al.,* [22] found fatty liver positively correlated with levels of plasma triglyceride and negatively with level of plasma HDL-C, but not with levels of total cholesterol. Our results showed the obese T2DM patients were significantly higher levels of serum triglycerides, LDL-C and VLDL-C with significantly lower HDL-C levels in comparison to controls. Similar finding were seen in previous study Yadav NK *et al.*, [23] have demonstrated the mean levels of serum triglyceride higher in diabetics in comparison to obese control subjects.

In our study, we shows that the plasma MDA, which is the end product of lipid peroxidation, was found to be significantly increased ($p<0.001$) in AFLD group as compared to the control group. Similar results reported by Muller G *et al*. [24] found to be higher concentration of MDA in AFLD and NAFLD. The processes of liver damage and lipid peroxidation are closely connected. The oxidative stress has a causative role in liver fibro genesis and its mechanism first described by Chojkier *et al*, have demonstrated that *in vitro* evidence of a possible molecular linkage between enhanced lipid peroxidation and induction of collagen gene expression. We observed the levels plasma MDA was found to be significantly increased ($p<0.001$) in NAFLD groups as compared to the control group. Previous studies have shown that the plasma MDA levels significantly increased in NAFLS than that of healthy controls [25], and also reported that the MDA is an indicator of lipid peroxidation; explain the presence of an enough antioxidant pool in the early stage of the disease before the fibrosis development [26]. Therefore, MDA in hepatic and peripheral tissue would be an ideal approach in the pathogenesis of NAFLD.

In present study, the activity of erythrocyte SOD and catalase ($p<0.001$) were significantly decreased in AFLD in comparison to control group. Excessive consumption of alcohol is associated with changes in cell function and the oxidant-antioxidant system. Capacity of reduced antioxidant has been found in liver disease and may promote the free radical generation, lipid peroxide, and lipid peroxidation mediated by free radical is reasoned to damage of cell. According to some previous studies; Chen YL *et al*. Pujar S *et al*. [27, 28] have reported that the significantly decreased erythrocyte SOD ($p<0.05$), and catalase activity ($p<0.05$) in ALD patients. Chari S *et al*. Janani AV *et al*., [29, 30] demonstrated that the blood SOD activity were significantly lower ($p<0.001$) in alcoholic liver disease as compared to the controls. SOD is an antioxidant enzyme that changes superoxide anion radicals into hydrogen peroxide and molecular oxygen [31], with regard to lipid metabolism in liver. It is reported that increased lipid peroxidation and hepatic TG accumulation because of abnormal lipid metabolism in liver [32]. According to Bhandari S *et al.,* [33] reported that the activity of blood SOD was decrease significantly in NAFLD. Koek CM *et al*. Leghi GE *et al*. [34, 35] found that the reduced levels of catalase in NAFLD compared to the controls. Reduction of catalase activity may occur to its depletion or inactivation by reactive oxygen species. Since oxidative damage to cellular are associated with the decreased activity of catalase.

In the present study, the level of hsCRP was found to be significantly increased ($p>0.001$) in AFLD group in comparison to control group in the both genders. The C-reactive protein positively associated with excessive alcohol consumption, and it is an early biomarker of AFLD, and decrease with liver fibrosis [36]. Our findings are in line with previous studies such as Kogiso T *et al*. Riquelme A *et al.* [37, 38] have been found that the levels of hsCRP were significantly higher and independently associated with NAFLD as compared to controls. hsCRP may be a clinical feature not only distinguishes NASH from simple non progressive status, but also indicates the severity of fibrosis. In the cross sectional study reported by Park SH *et al*. [39] elevated hsCRP level was associated with NAFLS in apparently healthy non-obese Korean men.

CONCLUSION

The present study suggest that higher levels of malondialdehyde (MDA) and decreased antioxidant enzyme such as super dioxide dismutase (SOD) and catalase (CAT) activity may be considered as identifying markers and help to early detection of liver disease, and monitoring of the effective therapeutic treatment of patients. These are cost effective and can be easily assayed in the lab. High sensitive C-reactive protein (hsCRP) is most important biomarkers of liver disease, therefore these markers are also useful for early detection of liver diseases. Improving the liver function may have significant clinical implications for the prevention and treatment of AFLD and NAFLD. This fact is to be kept in mind when planning strategies for prevention of complications of diseases for better quality of life.

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