Original Article

Study of Circulating Oxidative Stress Markers and Level of Antioxidant in Non-Alcoholic Fatty Liver Disease (NAFLD)

Oxidative and Antioxidant level In Non Alcholic Fatty Liver Disease

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ABSTRACT

Objective: To document the markers of oxidative stress and state of endogenous and exogenous antioxidants levels in non-alcoholic fatty liver disease.

Study Design: Case control study.

Place and Duration of Study: This study was conducted at the Department of Medicine, Services Hospital Lahore from December 2018 to May 2019.

Materials and Methods: Fifty non-alcoholic fatty liver disease patients with elevated aminotransferases and presence of fatty liver on ultrasound were included in the study. Fifty healthy individuals of same age and sex matched healthy were selected as control. Patients with positive viral serology, alcohol use and known diabetics were excluded.

Results: Oxidative stress determinants including malondialdehyde (5.44 nmol/ml) and nitric oxide (15.5µmol/l) levels are found to be statistically significantly raised and endogenous antioxidants including glutathione (4.91 mg/dl), catalase (1.43 mmol/mol of protein), superoxide dismutase (0.24 nmol/ml) as well as exogenous antioxidants including vitamin C (0.33 mg/dl) and vitamin E (0.22 mg/dl) were reduced significantly in non-alcoholic fatty liver disease patients.

Conclusion: Non-alcoholic fatty liver disease is associated with derangement of multiple circulatory oxidative stress parameters and antioxident thus depicting a significant role of oxidative stress in disease mechanism.

Key Words: Non-alcoholic fatty liver disease, Oxidative stress, Antioxidants, Oxidative stress

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INTRODUCTION

It is an established fact that Non-alcoholic fatty liver disease (NAFLD) is the commonest disorder of liver in developed world.[1] NAFLD embraces a spectrum of disorders, from just steatosis in liver to steatohepatitis, Steatohepatitis can also lead to cirrhosis, liver failure and even hepatocellular carcinoma.[2] Fatty liver is characterized by macrovesicular fat accumulation in Hepatocytes. Whereas, non alcoholic steatohepatitis (NASH) is characterized by hepatocellular injury, inflammatory changes and varying degree of hepatic fibrosis.[3,4]

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In present times, NAFLD is known to affect 20% to 40% of general population in western industrialized countries.[5] However, NASH effects 10% to 30% of effected individuals only.[6] NAFLD is now also well recognized as a common problem in Asia Pacific region.[7] In general population of countries in Asia Pacific, the prevalence of NAFLD ranges from 2% to 37%. Larger surveys conducted in Japan, China and Korea showed a prevalence rate of 10% to 29%, which are comparable to the figures described in Western Surveys.[8,9]

The most common cause of elevated liver enzymes remains NAFLD.[10] Important associated factors associated with NAFLD are dyslipidaemias and toxins exposure.[11] Both dislipidaemia and toxin exposure cause oxidative stress and increased production of malondialdehyde (MDA) leading to mitochondrial damage.[12] In addition antioxidants like catalase and glutathione (GSH) are found to be reduced in these patients.[13] Reduced activity of antioxidant enzyme super oxide dismutase (SOD) is associated with increased reactive oxygen substances (ROS) production leading to increased susceptibility to NASH and hepatic fibrosis.[14] Research studies have shown that levels of antioxidant vitamins C and E decrease in NAFLD patients exhibiting a compromised antioxidant protection status.[15].

MATERIALS AND METHODS

Fifty NAFLD patients from outpatient department at Services Hospital Lahore were selected from 1st December 2018 to 31st May 2019. Detailed history of alcohol intake, diabetes mellitus, known hepatitis C or B clinical complications if any was collected by help of a questionnaire from all individuals of the study. Clinical diagnoses of the patient were also taken into consideration. Fifty age and sex-matched clinically apparently healthy individuals were included as controls. Patients with elevated LFTs especially ALT and AST having fatty liver on ultrasound examination were included in present study. Alcoholics, smokers and known diabetics and individuals with positive hepatitis B or C were not included in the study. 5ml blood was collected from control and study group in EDTA and red top vacutainers and processed. All chemical reagents used for analysis were sourced from Sigma Chemical Co. (St. Louis, Mo, USA). Results have been expressed as mean±SD. Independent sample t test was used for statistical analysis. The difference were considered significant at $p<0.05$.

RESULTS

Table No. 1: Oxidative stress markers profile of control vs NAFLD

Parameter	Groups	Mean±SD	No.	p value
MDA	Control	1.36±0.38	50	.000
	NAFLD	5.44±1.14	50	
NO	Control	11.28±1.34	50	.000
	NAFLD	15.50±1.64	50	

Table No. 2: Antioxidant profiles of control vs NAFLD

Parameter	Groups	Mean±SD	No.	p value
SOD	Control	0.73±0.25	50	.007
	NAFLD	0.24±0.11	50	
GSH	Control	9.77±1.17	50	.000
	NAFLD	4.91±1.11	50	
Catalase	Control	4.27±0.73	50	.000
	NAFLD	1.43±0.35	50	
Vitamin E	Control	0.29±0.067	50	.000
	NAFLD	0.22±0.073	50	
Vitamin C	Control	0.57±0.08	50	.000
	NAFLD	0.33±0.07	50	

The level of plasma Malondialdehyde (MDA) was determined to be 5.44±1.13 nmol/ml in NAFLD patients and 1.36±0.38 nmol/ml in control group. This difference in values between NAFLD and control group is found to be statistically significant ($p<0.05$). Level of nitric oxide (15.50±1.64 µmol/l) at ($p<0.001$), indirectly measured as nitrites and nitrates has also found to be statistically significant raised in NAFLD patient group (Table 1).

Data regarding stress antioxidants including superoxide dismutase (SOD), glutathion (GSH), catalase (CAT), vitamin C and vitamin E has been depicted in Table 2. Levels of endogenous antioxidant components of serum including SOD (0.243±0.1 nmol/ml) at ($p<0.05$), CAT (1.43±0.36 mmol/mol) at ($p<0.001$) and GSH (4.91±1.1 mg/dl) at ($p<0.001$) were found to be decreased in the NAFLD patient group than in the control group. This difference was statistically significant. Moreover in group having NAFLD effected patient, exogenous antioxidant level including vitamin C (0.34±0.07 mg/dl) at ($p<0.001$) and Vitamin E (0.22±0.07 mg/l) at ($p<0.001$) are statistically significantly decreased as compared to controls.

DISCUSSION

Oxidative stress is usually delineated as a condition which results because of either uncontrolled increase in reactive oxygen species or insufficient antioxidant defence system due to any pathological cause. Reactive oxygen species and reactive nitrogen species, mainly hydroxyl radical and nitric oxide, exert significant hazardous and toxic effects. Superoxide anion causes peroxidation of lipids in membranes thus causing formation of end products like MDA. These substances can directly injure hepatocytes and in turn leads to production of proinflammatory cytokines, spindle cell activation into fibroblast and myofibroblasts and fibrogenesis responsible for NASH.[16,17]

Parameters representing lipid peroxidation like MDA and NO show significant upward trend from control to NAFLD group with statistically significant increase in values in NAFLD patients as compared to control in present study. A previous study has document similar results and has associated high levels of these markers as a prognostic marker in patients with steatosis and NASH.[18] Deranged NO levels enhance the production of reactive oxygen substances and impose oxidative stress on liver cells in NAFLD patients.[19]

Glutathione, CAT and SOD are the best recognized constituents of the body's own antioxidant defence system. Glutathione prevent the oxidative damage of free radicals via direct non-catalytic reaction by helping SH group containing proteins to remain in reduced form. All these markers show statistically highly significant differences between the NAFLD and control group in present study. Both endogenous antioxidants like SOD, GSH and CAT as well as exogenous antioxidants like Vitamin E and Vitamin C have seen to be significantly decreased in NAFLD group in comparison to control group. Decreased levels of endogenous defence antioxidants like low vitamin C and decreased vitamin E play a significant role in pathogenesis of NAFLD.[20] Decreased hepatic GSH levels have been reported in most of NASH pathogenesis models.[21,22] Super oxide dismutase and Catalase has also found to be increased in plasma in another study conducted by Yesilova et al.[23]

Findings of the present study are consistent with the literature and show increased lipid peroxidation and consequential suppression of antioxidant capacity as

result of its consumption.[17] This imbalance between oxidative stress and antioxidant capacity of the body has a pivotal role in development of NAFLD and NASH.[24].

CONCLUSION

Non-alcoholic fatty liver disease accompanies a variety of changes in both oxidant and antioxidant system of the body. Increased lipid peroxidation, as depicted by elevated levels of malondialdehyde and nitric oxide and depletion of endogenous and exogenous antioxidants, importantly glutathione, catalase and vitamin C, occurs as a result of free radical formation secondary to excessive fat accumulation in liver.

Author's Contribution:

Concept & Design of Study:	Zunaira Kanwal
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