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Study of Correlation between Stress Marker Enzymes and Other Biochemical Parameters in Non-Alcoholic Fatty Liver Disease

Dr. Purnima Dey Sarkar[1], Surya Tiwari[2*]
[1]Professor, Department of Biochemistry, MGM Medical College Indore, Madhya Pradesh, India
[2]Department of Biochemistry, MGM Medical College Indore, Madhya Pradesh, India

Original Research Article

***Corresponding author**
Surya Tiwari

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Abstract: Oxidative stress and lipid accumulation are major contributors in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). The purpose of this study was to find the relation between oxidative stress parameters and histopathological findings in NAFLD patients. The study was designed to determine effects of stress marker enzymes and other biochemical parameters in non-alcoholic fatty liver disease patients with reference to the normal healthy individuals. 175 non-alcoholic fatty liver disease patients were enrolled for the study & were compared with 150 normal healthy individuals of the same age. Those fulfilling inclusion & exclusion criteria were enrolled for the study & the blood samples were analysed for lipid profile, LFT, plasma MDA and SOD**.** Significant higher concentrations of MDA (P<0.001), GGT (P<0.001) and lower concentration of SOD (P<0.001) & Protein (p<0.001) was demonstrated in patients with non-alcoholic fatt liver disease when compared with normal healthy individuals controls. This study shows that NAFLD correlates with enhanced oxidative stress. Histopathological disease severity significantly correlated with oxidative stress parameters.

Keywords: MDA, SOD, GGT, TG, HDL, NAFLD.

INTRODUCTION

According to the latest WHO data issued in May 2014 Liver disease related deaths in India reached 2.44% of overall deaths. Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasing global health concern, with an estimated prevalence of 20%-30% in Western countries and 15% in Asian countries. The increased prevalence of obesity, hypertriglyceridemia in the general population is considered the most common cause for NAFLD [1]. NAFLD is defined as an excessive accumulation of fat in the liver leads to chronic liver injury in which at least 5% of hepatocytes display lipid droplets that exceed 5–10 % of liver weight in patients who do not consume significant amounts of alcohol [2, 1**]**. It is a heterogeneous disease that may range from a relatively mild and subtle disease (hepatic steatosis) to a much more active and progressive disease, in which significant necroinflammatory fatty infiltration of hepatocytes are involved, designated non-alcoholic Steatohepatitis (NASH) [3**,** 4]. By poorly defined mechanisms, including free fatty acid and cholesterol accumulation accompanied by oxidative stress and inflammation, NAFLD may progress to the irreversible steatohepatitis (NASH) and further to cirrhosis or hepatocellular carcinoma.

The first step of the pathophysiology of NASH is the lipid accumulation in the liver causing steatosis. This increases the sensitivity of the liver to injury and inflammation. The second step involves oxidative stress and lipid peroxidation, in time leading to steatohepatitis [5, 6] which eventually might develop cirrhosis and hepatocellular carcinoma [7]. An inflammatory process in the liver with concomitant tissue damage and fibrosis mediates NASH progression. A number of factors point to the multi factorial nature of this disease, including derangement in the metabolic parameters, oxidative stress, etc. [8].

Disturbance in the balance between supply, formation, consumption and hepatic oxidation or disposal of triglycerides triggers fat accumulation in the Liver. Plasma non-esterified fatty acid pool from

adipose tissue, newly synthesized fatty acids in the liver via de novo lipogenesis and dietary fatty acids are considered the potential sources of lipids contributing to fatty liver. Approximately 60-80% of liver triacylglycerol procured from circulating free fatty acids and 25% of liver triacylglycerol obtained from increased de novo lipogenesis [9]. Transcriptional regulation of sterol regulatory element binding protein-1c (SREBP-1c) mediates de novo lipogenesis, up regulated by insulin and activated by hyperinsulinemia. Stimulation of these pathways has been involved in the pathogenesis of hepatic steatosis [10]. Dietary fat consumption is responsible for 15% of free fatty acids supply to the liver. Usually lipids are transported from the liver via very-low density lipoproteins (VLDL), which are formed by microsomal triglyceride transfer protein (MTP) incorporating triglyceride into apolipoprotein B (apo B). Reduced activity of MTP and apo B synthesis and secretion impairs hepatic lipid export and favour hepatic triglyceride accumulation [11].

The main pathway of hepatocellular injury is considered the oxidative stress induced lipid peroxidation. There is growing evidence implicating FFA in the production of oxidative stress within hepatocytes. Increased fatty acid β-oxidation as well as peroxisomal fatty acid oxidation can both lead to increase in reactive oxygen species generation and subsequent lipid peroxidation. Under normal conditions, hepatic aerobic metabolism involves a steady-state production of pro-oxidants such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are balanced by a similar rate of their consumption by antioxidants. Imbalance in the pro-oxidants/antioxidant equilibrium in favour of pro-oxidants constitutes the oxidative stress phenomenon, a condition that may induce a number of pathophysiological events in the liver. Hepatotoxicity by oxidative stress may be achieved through a direct attack of ROS and RNS on essential biomolecules with loss of their biological functions and cell viability [2]. The end products of lipid per oxidation malondialdehyde (MDA),is also involved in the pathogenesis of liver damage due to direct toxicity ,and can intervene in the formation of Mallory body and increase collagen synthesis due to stellate cells[12-14].

Laboratory tests that are routinely included in the evaluation of patients with suspected NAFLD include a serum panel of liver tests {alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma-glutamyl-transpeptidase (GGT), protein and albumin}. Elevated serum ALT and AST levels are the primary abnormality seen in patients with NAFLD and tend to be higher in patients with NASH as compared with NAFL. However, liver aminotransferase levels are seldom higher than 5 times the upper limit of normal, and typically fluctuate with normal levels seen in more than two-thirds of NASH patients at any given time [15]. Elevated gammaglutamyl transpeptidase (GGT) (double or triple) in 50% of patients is also frequent. Bilirubin and albumin usually remain normal until advanced stages of liver disease [16].

METHODOLGY

The present study was undertaken in the Department of Medical Biochemistry, MGM Medical College Indore (M.P.). The study group comprise of 175 histologically & ultrasound scan proven Non-Alcoholic Fatty Liver Disease patients and 150 Healthy Individuals matched for age and dietary habits were treated as controls. The study subjects were randomly selected irrespective of age or occupational status. The age of subjects ranged between 20-70 years and the educational status ranged from illiteracy to post-graduation. Non-Alcoholic Fatty liver disease patients selected for the study were, further confirmed by questionnaire, laboratory investigations and clinical findings. The patients suffering from renal disorders, diabetes mellitus, obesity, hypertension, thyroid disease, cardiovascular disease, viral hepatitis, hepatitis A & B, Asthma patients, Patients of lung cancer, any other malignancy, Gout, TB, HIV, Malnutrition, Malabsorption and or suffering from any other infectious diseases were excluded from the study, history of daily alcohol intake >20g(men) and >10g(women).

Prior to taking out blood samples a detailed and accurate history, including Anthropometric measurements of the subjects was done. Anthropometric evaluation included measurement of BMI. Blood sampling was, performed in the morning, following a not less than 12 hr. fasting period. Biochemical parameters analysed were Plasma MDA, serum SOD, AST, ALT, GGT, Protein, Albumin, Bilirubin, Cholesterol, Triglyceride, HDL, LDL and VLDL. The Institutional Ethics Committee granted ethical approval. All the data was computed and analysed by using statistical packages for social science (SPSS) software version 20. Results were expressed as Mean ± SD. $p<0.05$ is considered as Significant and $p<0.001$ is considered as highly significant.

RESULTS

The basic demographic details of the study subjects are shown in Table 1. Table 2 shows the lipid profile level between cases & controls. Table 3 shows lipid profile between stages of NAFLD. Table 4 shows Liver Function Test between cases and controls. Table 5 shows Liver Function Test between stages of NAFLD. Table 6 shows oxidative stress & antioxidant level between cases and controls. Table 7 shows oxidative stress & antioxidant level between stages of NAFLD.

Table-1: Baseline characteristics of controls and cases

S.No.	Variables	Controls	Cases
1	Age (Years)	41.2±7.9	42.58±8.95
2	BMI (kg/m^2)	21.11±1.54	43.29±6.8

Table-2: Lipid Profile level between controls and cases

S.No.	Variables	Controls	Cases	p-Value
1	Total Cholesterol (mg/dL)	160.93±24.74	204.57±39.11	<0.001
2	Triglyceride (mg/dL)	98.14±26.06	168.62±61.68	<0.001
3	HDL (mg/dL)	52.5±10	31.67±7.33	<0.001
4	LDL (mg/dL)	99.92±20.51	129.74±32.17	<0.001
5	VLDL (mg/dL)	19.63±5.21	33.62±12.25	<0.001

Table-3: Liver Function Test between stages of NAFLD

S.No.	Variables	Simple Steatosis	Moderate Steatosis	NASH	ANNOVA Significance
1	Total Cholesterol (mg/dL)	191.54±35.15	201.92±35.59	232.22±37.97	<0.001
2	Triglyceride (mg/dL)	139.06±51.4	170.1±55.58	217.2±55.91	<0.001
3	HDL (mg/dL)	37.03±7.09	31±3.63	23.21±2.1	<0.001
4	LDL (mg/dL)	116.26±32.68	129.51±27.05	154.67±24.41	<0.001
5	VLDL (mg/dL)	27.81±10.27	34.05±11.02	43.49±11.18	<0.001

Table-4: Liver Function Test between controls and cases

S.No.	Variables	Controls	Cases	p-Value
1	AST (IU/L)	25.57±6.27	54.19±22.71	<0.001
2	ALT (IU/L)	25.28±5.52	73.88±34.31	<0.001
3	GGT (IU/L)	26.54±8.23	53.59±31.45	<0.001
4	Bilirubin (mg/dL)	0.62±0.27	1.12±0.53	<0.001
5	Protein (mg/dL)	7.46±0.45	6.34±0.82	<0.001

Table-5: Liver Function Test between stages of NAFLD

S.No.	Variables	Simple Steatosis	Moderate Steatosis	NASH	ANNOVA Significance
1	AST (IU/L)	37.52±13.29	54.39±12.98	83.02±18.57	<0.001
2	ALT (IU/L)	48.37±13.32	73.77±21.64	118.94±29.92	<0.001
3	GGT (IU/L)	40±10.62	53.47±34.82	77.9±35.65	<0.001
4	Bilirubin (mg/dL)	0.85±0.39	1±0.36	1.83±0.37	<0.001
5	Protein (mg/dL)	7.01±0.43	6.39±0.19	5.08±0.48	<0.001

Table-6: Oxidative Stress & Antioxidant level between controls and cases

S.no.	Variables	Controls	Cases	p-Value
1	MDA (nmol/ml)	1.8±0.75	5.94±2.08	<0.001
2	SOD (U/g of Hb)	6.83±1.26	2.09±0.85	<0.001

Table-7: Oxidative Stress & Antioxidant level between stages of NAFLD

S.No.	Variables	Simple Steatosis	Moderate Steatosis	NASH	ANNOVA Significance
1	MDA (nmol/ml)	4.66±0.84	5.62±0.91	8.78±2.34	<0.001
2	SOD (U/g of Hb)	2.53±0.21	2.16±1.09	1.19±0.29	<0.001

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction, as determined by liver function testing. NAFLD is rapidly becoming an important problem. Undiagnosed, this condition may progress silently and result in cirrhosis, portal hypertension, and liver-related death in early adulthood.

In the present study it was observed that there was significant increase in the levels of serum total cholesterol ($p < 0.001$), LDL ($p < 0.001$), VLDL ($p <$

0.001) and triglycerides ($p < 0.001$) level when compared with the control group. Also there is significantly decreased level of HDL ($p < 0.001$) in NAFLD patients when compared with the control group. The present finding is accordance with study done by Mahamoud *et al*.[17] Kumar [18], Targher[19], Marchesini G [2], Bajaj [20], Nobili [21], Uchil [22] and Mouralidarane *et al.* [23], Simonen *et al*. [24]

In the present study we found significantly higher levels of AST, ALT, GGT and Bilirubin level in NAFLD patients when compared with the control group. Level of serum Protein decrease in NAFLD patients when compared with the control group. The primary laboratory abnormality in NAFLD is the elevated serum AST and ALT levels. However, liver aminotransferase levels are seldom higher than 3 or 4 times the upper limit of normal. The ALT levels are higher than the AST levels in most instances, but the AST level may occasionally be higher than the ALT level, especially in the presence of cirrhosis. In addition, the present finding is in accordance with the study done by Suzuki [25] and colleagues, Ahlgren, A *et al*. [26] J. D. Browning *et al*.[27], B. W. Smith *et al*.[28], D. M. Torres *et al.* [29], P. Angulo *et al*.[30].

In the present study, we found significantly increased level of MDA in NAFLD patients when compared with the control group. The level of antioxidant enzyme SOD decreases significantly in NAFLD patients when compared with the control group. Increased oxidative stress is considered a key trigger in the pathogenesis of NAFLD and one of the enzymes counteracting oxidative stress, copper/zinc (Cu/Zn) Superoxide Dismutase (SOD) depends on adequate copper availability, suggesting a potential link between copper availability and impaired antioxidant defence in NAFLD[31, 32].

Pessayre *et al.* [33] have shown that excess fat deposition in the liver is associated with lipid peroxidation and the degree of this peroxidation is directly related to the severity of steatosis. The end products of lipid peroxidation, malondialdehyde (MDA) covalently bind to hepatic proteins, and acts as potent agents for neutrophil chemotaxis and stimulating pro-inflammatory cytokines. Malondialdehyde also activates hepatic stellate cells to produce collagen, leading to fibrosis. The present finding is in accordance with the studies done by E. Albano *et al*. [34], S Seki *et al*. [35], K. Begriche *et al*.[36] demonstrating that oxidative stress is a major player triggering the progression of steatosis to steatohepatitis.

CONCLUSION

Non-alcoholic fatty liver disease is a very common problem that physicians encounter in their daily practice. All patients with NAFLD/NASH should be counselled regarding the benefits of lifestyle modification including dietary manipulation and regular exercise that would not only improve liver function but also result in surrogate benefits vis-a-vis metabolic syndrome and related cardiac and other problems. Finally, there is urgent need for research on the natural history of NAFLD in Indians and NAFLD epidemiology and disease burden including morbidity and mortality in Indians, besides developing effective treatment strategies for prevention of NAFLD in India.

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