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"Assessment Of Serum Paraoxonase Activity And Malondialdehyde Levels In Nonalcoholic Fatty Liver Disease Patients"

KEYWORDS: Nonalcoholic fatty liver disease, Paraoxonase, Malondialdehyde.

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ABSTRACT *Background: Increased oxidative stress and inflammation play a fundamental role in the onset and development of liver diseases. Nonalcoholic fatty liver disease (NAFLD) is an emerging lesion in modern societies, and will become more prevalent in the future. Chemically, oxidative stress occurs as a result of increased level of lipid peroxides and free radical intermediates, as well as decrease in the total antioxidant capacity.*

Aim: In the present study, our aim was to investigate the lipid peroxidation and Paraoxonase antioxidant enzyme in NAFLD patients in Al- Quwayiyah region of Saudi Arabia.

Materials and methods: This study was conducted in Al- Quwayiyah Government General Hospital, Saudi Arabia. We took total 60 patients with NAFLD along with 76 age and sex matched healthy controls. Various biochemical parameters, PON and malondialdehyde were measured and compared.

Results: The significantly increased levels of malondialdehyde (MDA) and decreased antioxidant enzyme activity of Paraoxonase (P<0.001) in NAFLD patients when compared to control groups.

Conclusion: The study illustrated that in NAFLD patients there is an increased concentration of lipid peroxides may be contribute to decreased levels of antioxidant PON activity. Hence, monitoring of MDA and PON as a routine analysis in clinical biochemistry laboratories.

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH) are hepatic lesions which appear frequently in obese and diabetic individuals despite the fact that they may not have a history of alcohol abuse. Fatty liver, altered lipid metabolism induced deregulated cytokine production and oxidative stress, which could cause liver injury and finally also NASH. All these events could end to hepatic inflammation and fibrosis [1]. Several studies have shown that alternation in the β oxidation of fatty acid promotes the increased free radical production and lipid peroxidation (LPO) [2].

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to excess production of peroxides and free radicals. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidants and antioxidant enzymes. LPO is a free radical related process, which is potentially harmful because it's uncontrolled; self- enhancing process causes disruption of membranes, lipids and other cell components [3]. Thus LPO in the blood provides useful information for the prognosis of NAFLD patients.

Paraoxonase (PON) is an enzyme having both paraoxonase and aryl esterase activity. It hydrolyzes aromatic carboxylic acid esters and certain organophosphorus pesticides, especially paraoxon and nerve gas. The PON gene family contains three members, PON1, PON2 and PON3, which are located on chromosome 7q21.3—22.1. Serum PON1 is mainly synthesized in liver and it is tightly bound to HDL and it protects LDL and HDL from lipid peroxidation [4]. PON1 levels have been reported in a variety of diseases such as cardiovascular disease, diabetes mellitus, Alzheimer's disease, chronic renal failure, HIV, metabolic syndrome and liver diseases.

PON1 plays a major role in alleviating tissue damage due to formation of free radicals [5]. Therefore the aim of this study was to investigate the lipid peroxidation marker i.e. MDA and antioxidant enzyme PON1 activity in NAFLD patients and compared with normal healthy subjects.

2. MATERIAL AND METHODS

2.1 Study design and study area:

An open randomized study was case control in design. We have selected the patients as they are presented. Patients included in the present study were all admitted to gastroenterology unit and some were admitted to medicine unit or attending the Outpatient department (OPD) of medicine of the Al- Quwayiyah Government General Hospital, Shaqra University, Kingdom of Saudi Arabia. The study was taken during the period of November 2013 to December 2014.

2.2 Selection of patients:

The study group consisted of 60 patients with NAFLD of both the sexes (38 males and 22 females) they were between 45—55 years. The criteria for the diagnosis for NAFLD was made on the basis of clinical symptoms, sonographic, hepatic ultrasonography scanning, computed tomography and laboratory findings, whereas we didn't do the liver biopsy. Those patients whose body mass index (BMI) was >30 were considered as obese. 76 healthy volunteers both age and gender matched considered as controls. Subjects suffering from other known case of liver disease, viral hepatitis B and C, rheumatoid arthritis, autoimmune liver diseases, hemochromatosis, wilson disease, strokes, cerebrovascular accidents, any chronic or acute inflammatory illness, pregnancy and lactating mothers, alcoholics, and chronic drug consumption were excluded from

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the study. All NAFLD patients selected for these studies were on irregular treatment. All participants gave written informed consent and this study was approved by the institutional ethical and human research committee.

2.3 Blood sample and Biochemical analysis:
Blood samples were obtained after an overnight fast. 6 ml of plain blood was collected from each subject, the serum was carefully separated by centrifugation at 3000 x g RPM for 15 minutes and transferred to micro tubes and stored at + 4^0 C before analysis. The biochemical parameters such as fasting blood glucose, lipid profiles, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) levels were done by using fully automated biochemistry analyzer Cobas Integra 400 from ROCHE diagnostics, Germany. Serum levels of MDA were estimated by thiobarbituric acid (TBA) method. Autoxidation of unsaturated fatty acids involve in the formation of semi stable peroxides which then undergo a series of reactions to form short chain aldehydes like malondialdehyde. One molecule of MDA reacts with two molecules of TBA with the elimination of water to yield pink crystalline pigment with a maximum absorption of 535 nm [6]. Serum PON activity was estimated by using 5.5 Mm p- nitrophenyl acetate as a substrate. The change in the absorbance at 412 nm due to the formation of p- nitro phenol was measured by using ELICO spectrophotometer [7].

3. STASTISTICAL ANALYSIS
The statistical analysis was under taken using SPSS version 17.0 software. All values are expressed as mean ±SD. Differences between the mean were calculated by analysis of variance (ANOVA) test (multiple comparisons). Student t- test was used to estimate the significant difference between the groups. The level of significance was considered when p value <0.05.

4. RESULTS
The clinical characteristic of the NAFLD patients and normal subjects are presented in Table 1. In the present study the number of obesity and hypertensive were significantly high in the NAFLD patients compared to controls.

The biochemical parameters like serum fasting blood glucose, total cholesterol, triglycerides, and LDL-c were significantly increased, whereas decreased levels of HDL-c in NAFLD individuals when compared to controls ($p<0.001$). AST, ALT and GGT activities were significantly higher in NAFLD than normal subjects (Table 2). Significantly increased levels of MDA and however decreased PON activities was observed in NAFLD patients compared to healthy controls ($P<0.001$) respectively as shown in Table 3.

5. DISCUSSION
Non-alcoholic fatty liver disease (NAFLD) is a very common disorder and refers to a group of conditions where there is accumulation of excess fat in the liver. Although this is not normal, it is not serious but if it doesn't lead to inflammation or damage. NAFLD and NASH are increasingly causes of liver disease and liver related morality. It has been reported that NAFLD is one of the most common liver disease in world wide. Chronic exposure to increased levels of oxidative stress may result in an excess of ROS within the hepatocytes which can contribute to the deterioration from NAFLD to NASH [8]. Some experts estimate that about two thirds of obese adults and half of obese children may have fatty liver. We observed obesity and HTN were significantly high in NAFLD patients [9].

Increased oxidative stress and inflammation play a fundamental role in the onset and development of liver diseases. LPO level is an in index of oxidative stress. MDA is a natural product of LPO and reflects the oxidant status of the biological systems [10]. Several authors reported increased levels of MDA in NAFLD patients [11, 8]. In our study, we also observed the significant increased levels of MDA in NAFLD patients compared to controls, because greater degree of oxidative stress and poor enzymatic and non-enzymatic antioxidant defense system. The estimation of LPO in NAFLD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient.

PON1 is classified in a member of a super family of proteins that also consists of PON2 and PON3. Serum PON1 is synthesized mainly in the liver. The gene expression has been observed only in the liver. Arylesterase and PON1 activities have been shown to be functions of a single enzyme. There are several reports about the important of PON in liver disease, but only few of them has focused on the importance of its polymorphism in the liver disease mechanism. As mentioned earlier the liver plays a key role in the synthesis of PON1, and chronic liver diseases are associated with increased oxidative stress and inflammation. PON1 protects liver against inflammation, liver disease and fibrosis [12, 13]. Hussein et al [14] and Nguyen et al [15] revealed that decrease of PON1 activities in NAFLD patients. Similarly in the present study, we find significantly decreased levels of PON activity in NAFLD patients when compared to controls. But Mohammed Hashemi et al [4] reported that no significance difference between in NAFLD patients and controls in PON1 activity. Increased LPO and ROS in NAFLD consume antioxidants and can inactivate PON activity. Decreased antioxidant defense may increase hepatocytes susceptibility to liver injury leading to aggravate NAFLD to NASH [16].

There were some limitations in the present study, our sample size was small and we could not distinguish between NAFLD and NASH. There is needed to be proven in clinical trial studies.

6. CONCLUSION
This will be the first report to show the knowledge of non-alcoholic fatty liver disease among the Saudi population in Al-Quwayiyah region of Saudi Arabia. We hypothesized that reduced antioxidant PON activity and increased MDA levels may contribute to the increased susceptibility for the development of NAFLD. The present study illustrated that PON1 plays a very important protective role against oxidative stress and inflammation. Therefore, assessing of PON1 and MDA in NAFLD patients as routine analyses in clinical chemistry laboratories.

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Table 1. Clinical details of the study subjects

Particulars	Controls (n= 76) Mean ±SD	NAFLD (n= 60) Mean ±SD

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Age (yrs)	44.1 ± 6.2	45.3 ± 5.9 *
Sex (male / female)	43 / 33	38 / 22 *
BMI (kg/m^2)	24.8 ± 3.1	30.1 ± 4.1*
HTN %	3 %	44 % *

The values are mean ± Standard deviation (SD), * P<0.001, highly significantly compared to controls. BMI= Body mass Index, HTN= Hypertension,

Table 2. Various Biochemical parameters of the NAFLD patients and controls

Particulars	Controls (n= 76) Mean ±SD	NAFLD (n= 60) Mean ±SD
Fasting Blood Glucose (mg/dl)	83.3 ± 6.1	109.0 ± 7.3*
Total Cholesterol (mg/dl)	152.0 ± 21.3	220.0 ± 19.3*
Triglycerides (mg/dl)	109.2 ± 24.1	205.3 ± 21.2*
HDL-C (mg/ dl)	49.9 ± 6.1	41.3 ± 7.2*
LDL-C (mg/ dl)	98.2 ± 14.2	133.0 ± 15.8*
AST (U/ L)	19.2 ± 8.5	50.1 ± 11.2*
ALT (U/ L)	26.1 ± 7.7	62.8 ± 13.3*
GGT (U/ L)	23.5 ± 6.4	56.9 ± 15.2*

* P<0.001, highly significantly compared to controls. HDL- c=High density lipoprotein,

LDL-c = Low density lipoprotein, AST=Aspartate aminotransferase, ALT= Alanine

aminotransferase, GGT= Gamma glutamyl transferase.

Table 3. PON1 and MDA activities in the NAFLD patients and healthy controls

Particulars	Controls (n= 76) Mean ±SD	NAFLD (n= 60) Mean ±SD
PON 1 (U / L)	129.9 ± 18.5	98.3 ± 15.2*
MDA (nmoles / ml)	3.89 ± 0.8	6.21 ± 2.0*

* P<0.001, highly significantly compared to controls. PON1= Paraoxonase,

MDA= Malondialdehyde.

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