Original Article

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Oxidative stress biomarkers among Saudi patients with non-alcoholic steatohepatitis versus chronic hepatitis C

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ABSTRACT

Objective: As oxidative status may be an influential factor for increasing the progress of the disease and decreasing the effectiveness of the treatment, the aim of this study was to measure the oxidative stress and anti-oxidative markers among Saudi patients with chronic hepatitis C (CHC) versus nonalcoholic steatohepatitis (NASH).

Methods: This study was carried out on a sample of 150 consecutive patients (75 with CHC and 75 with NASH), selected from gastroenterology outpatient clinic, King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Also, this study included 75 healthy volunteers, mostly, relatives of the patients who came with them to the clinic. Measurements of oxidative stress and anti-oxidative stress markers were measured for all participants.

Results: The mean values of oxidative stress markers included conjugated dienes (CD), malondialdehyde (MDA), nitric oxide (NO) and myeloperoxidase (MPO) were significantly elevated in patients with CHC and patients with NASH when compared with Healthy controls group. Also, these parameters were significantly elevated in patients with NASH than patients with CHC. However, the mean values of anti-oxidative stress markers include glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione (GSH), arylesterase (AE) and paraoxonase (PON) were significantly reduced in patients with CHC and patients with NASH when compared with Healthy controls group. Also, these parameters were significantly reduced in patients with NASH than patients with CHC.

Conclusion: In this study, patients with NASH have higher levels of oxidative and lower anti-oxidant markers than patients with CHCs.

Keywords: chronic hepatitis C, oxidative stress, nonalcoholic steatohepatitis

INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of liver disease and has a current global prevalence rate of approximately 2.35%, which accounts for approximately 160 million infected individuals (1). Approximately 70– 80% of infected individuals develop chronic HCV infection. The hallmarks of chronic HCV infection are inflammation and liver fibrosis and 20–30% of patients develop cirrhosis with a risk of hepatocellular carcinoma that are responsible for high morbidity and mortality rate (2-5). A combination of pegylated interferon-a and ribavirin is standard treatment for chronic HCV infection which proven efficacy (6-9). However, lower likelihood of sustained virological response after antiviral therapy, reported in CHC with high oxidative stress (10).

Chronic hepatitis C virus infection may progress toward chronic hepatitis, liver cirrhosis, and hepatoma (11). The probability of hepatoma in CHC patients is ten folds higher than healthy people. The mechanisms of CHC progression to liver cirrhosis and hepatoma are complex. Many studies indicate that there are higher oxidative stress and lower anti-oxidative materials such as retinol, α-tocopherol, γ-tocopherol, ß-cryptoxanthin, lycopene, alpha-carotene, beta-carotene, glutathione, zinc and selenium in CHC patients than in healthy individual (12, 13). The increased oxidative stress will cause hepatic stellate cells to proliferate, and produce collagen (14) and it also induces liver cell injury and genemutation, which progresses toward liver cirrhosis and hepatoma (15).

Non-alcoholic steatohepatitis (NASH) is a chronic progressive liver disease characterized by accumulation of fat in the liver accompanied by necroinflammation and hepatocellular injury (16, 17). NASH prevalence is estimated between 2–3% respectively of the general population in Western societies (18). In all probability NASH prevalence figures will rise in the future as NASH is considered the hepatic manifestation of the metabolic syndrome and the number of overweighed individuals is growing. However, the exact mechanism of the progression from a benign steatotic liver to an inflamed organ remains a point of interest as NASH is projected to be the leading cause of liver transplantation in the United States by 2020 (19, 20).

Nonalcoholic steatohepatitis (NASH) is a liver disease characterized by diffuse fatty acid infiltration and inflammation. The exact cause of NASH is unclear, but it is increasingly becoming more evident that the disease is much more common than was previously thought (21). NASH is seen in patients of all ages, including children, and is associated with over-nutrition and underactivity, insulin resistance, and genetic factors. Lipotoxicity, oxidative stress, cytokines, and other pro-inflammatory mediators may each play a role in transition of steatosis to NASH (22, 23).

Oxidative stress is often defined as an imbalance between pro-oxidants and anti-oxidants that further lead to oxidative damage. Hepatitis C virus has the capacity to generate substantial oxidative stress within hepatocytes (24, 25). Subsequently, oxidative stress has been identified as a significant mechanistic pathway culminating in the development of hepatic cirrhosis, liver failure and liver cancer (26).

Oxidative stress has been shown to play an important role in the pathogenesis of NAFLD/NASH in animal and human studies (27-30). In the absence of alcohol intake, patients who either have metabolic syndrome or any of its components with insulin resistance, develop hepatic steatosis due to increased lipolysis and increased delivery of fatty acids from adipose tissue to liver (31).

The aim of this study was to measure the oxidative stress and anti-oxidative markers among Saudi patients with chronic hepatitis C virus infection versus nonalcoholic steatohepatitis.

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MATERIALS AND METHODS

Statistical Assessment

This study was carried out on a sample of 150 consecutive patients (75 with CHC and 75 with NASH), were selected according to the available patients from gastroenterology outpatient clinic, King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Patients with CHC (mean age 40.29 ± 6.13 year and body mass index 25.18 ± 3.25 kg/m2) were characterized by the presence of anti-HCV and HCV RNA as assessed by polymerase chain reaction analysis, they had been diagnosed with chronic hepatitis C on the basis of abnormal serum alanine aminotransferase (ALT) and liver histology of chronic hepatitis in the last year or more. However, patients with NASH(mean age 39.84 ± 7.15 year and body mass index 26.05 ± 3.12 kg/m2) diagnosed according to findings of ultrasound examination. Also, this study included 75 healthy volunteers, mostly, relatives of the patients who came with them to the clinic. They shared the same socio-economic status of the patients group. This group included age, sex, BMI-matched normotensive healthy subjects without a family history of diabetes with normal abdominal ultrasound, normal AST and ALT levels and fasting plasma glucose. The healthy subjects didn't have any clinical or laboratory renal insufficiency, liver damage, neoplasia or neurological disorders.

Exclusion criteria were: (1) advanced liver cirrhosis (Child-Turcotte-Pugh B and C); (2) hepatocellular carcinoma; (3) other causes of liver disease or mixed etiologies (excessive alcohol consumption, hepatitis B, autoimmune liver disease, Wilson's disease, hemochromatosis, or alpha1-antitrypsin deficiency); (4) human immunodeficiency virus infection; (5) previous treatment with antiviral therapy, immunosuppressive drugs, and/or regular use of drugs influencing lipid metabolism and/or oxidative stress; (6) active intravenous drug addiction. This study was approved by the Ethics Committee of King Abdulaziz University Hospital, Jeddah, Saudi Arabia and all patients gave informed consent for participation in this study. All subjects underwent a routine clinical examination, including physical examination, biochemical tests, and liver ultrasonography.

Clinical and Laboratory Assessment

In all subjects clinical and anthropometric data were collected at the time of enrollment. Body mass index (BMI) was calculated on the basis of weight (kilograms) and height (metres), and subjects were classified as normal weight (BMI 18.5–24.9 kg/m2), overweight (BMI 25–29.9 kg/m2), and obese (BMI ≥30 kg/m2). Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. Also, between 07:30 and 09:00, after an overnight fast of 12 h fasting blood sample was drawn. The plasma lipid profile (total cholesterol, total triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL), plasma glucose concentration, insulin and markers of hepatic function including (ALT), aspartate amino- transferase (AST), and g-glutamyl transpeptidase (GGT) were determined (Roche Diagnostics GmbH, Mannheim, Germany) using commercially available assay kits. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). HOMA-IR = (fasting blood glucose (mmol/l) _ fasting insulin (mIU/ml))/22.5 (32). However, all HCV patients were tested at the time of biopsy for HCV RNA by qualitative polymerase chain reaction (Roche Diagnostics, Indianapolis, NJ, USA).

Ultrasound Evaluation

In the present study, liver ultrasonography was examined in all participants by the same ultrasound operator. Each patient underwent abdominal US (Siemens Antares equipment with CH4-1 MHz transducer; Siemens Medical, Erlangen, Germany) in a fasting state. The presence of hepatic steatosis was assessed independently by radiologists according to findings such as hepatorenal contrast, blurring of the vascular wall, and profound attenuation of the diaphragm. However, patients with NASH diagnosed according to findings of ultrasound examination.

Measurement of Oxidative Stress Markers and Anti-oxidant Status

During the same hospital admission in CHC & NASH patients and on outdoor basis in both healthy volunteers, serum (from 10 mL blood in plain vial) and plasma (from 5 mL blood in EDTA vial) were separated from the sample within 30 min of collection and was stored in pyrogen free polypropylene cryotubes at - 80ºC until analysis. Oxidative stress was studied by markers of lipid peroxidation which included determining plasma levels of malondialdehyde (MDA), conjugated dienes (CD), nitric oxide (NO) and Myeloperoxidase (MPO). MDA and CD were assayed in plasma by the method of Buege and Aust. (33) and were expressed as mmol/L. Also, Nitric oxide was determined as described previously by Vodovotz (34) and Myeloperoxidase was quantified spectrophotometrically by the method described by Buchmann et al. (35). However, Anti-oxidant status was studied by glutathione (GSH), glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD), Arylesterase (AE) and Paraoxonase (PON). GSH was assayed by the method of Beutler et al. (36), SOD was assayed by the method of Nishikimi et al. (38), AE was measured using p-nitrophenyl acetate as substrate as described by Kao et al. (39) and PON was quantified spectrophotometrically by the method described by Gil et al. (40).

Statistical Analysis

Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. Means of different groups were compared using one-way ANOVA. All data were expressed as the mean ± SD. $P<0.05$ indicated statistical significance.

RESULTS

Study group included 87 males (43.5%) and 113 females (56.5The demographic and clinical characteristics of the all participants are shown in Table 1. The mean age of the healthy controls group was 38.61 ± 7.28 year, and the mean age of patients with CHC was 40.29 ± 6.13 year, where the mean age of patients with NASH was 39.84 ± 7.15 year. There was no significant age, gender, hemoglobin, body mass index (BMI), waist circumference and waist hip ratio between the three groups. However, systolic blood pressure, diastolic blood pressure, aspartate aminotransferase, alanine aminotransferase, cholesterol, low density lipoprotein, high density lipoprotein, triglycerides, total bilirubin, glucose, insulin, hemostasis of insulin resistance index (HOMA-IR) were significantly different between the healthy controls, patients with CHC and patients with NASH.

The mean values of oxidative stress markers included conjugated dienes (CD), malondialdehyde (MDA), nitric oxide (NO) and myeloperoxidase (MPO) were significantly elevated in patients with CHC and patients with NASH when compared with Healthy controls group. Also, these parameters were significantly elevated in patients with NASH than patients with CHC (Table 2). However, the mean values of anti-oxidative stress markers include glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione (GSH), aryl esterase (AE) and paraoxonase (PON)were significantly reduced in patients with CHC and patients with NASH when compared with Healthy controls group. Also, these parameters were

Table 1: *Comparison of clinical data between healthy controls, patients with CHC and NASH*

	Healthy controls	CHC patients	NASH patients
Age (year)	38.61 ± 7.28	40.29 ± 6.13	39.84 ± 7.15
Gender (M/F)	53:22	51:24	49:26
Hb (gm/dl)	13.85 ± 1.64	10.17± 1.51	10.94 ± 1.48
BMI (kg/m2)	24.72 ± 3.14	25.18 ± 3.25	26.05 ± 3.12
Waist circumference (cm)	82.12 ± 6.83	81.45 ± 7.12	83.13 ± 7.64
WHR	0.80 ± 0.06	0.83 ± 0.08	0.85 ± 0.09
SBP (mmHg)	113.17 ± 6.43	124.56 ± 8.32*	139.22 ± 12.65*$
DBP (mmHg)	75.84 ± 5.13	84. 41 ± 6.11*	92.81 ± 10.15*$
AST(IU/l)	25.32 ± 4.19	78.43 ± 6.59*	75.81 ± 6.12*$
ALT (IU/L)	29.12 ± 3.87	98.43 ± 7.86*	92.51 ± 6.71*$
Cholesterol, mg/dl	137.23 ± 14.65	161.47± 11.82*	214.56 ± 17.53*$
LDL (mg/dL)	74.16 ± 5.84	108.22± 8.95*	134.97± 11.76*$
HDL (mg/dL)	51.28 ± 5.92	40.83± 4.88*	35.21± 3.54*$
Triglycerides (mg/dL)	92.94 ± 7.44	78.64 ± 6.32*	109.55±8.81*$
Total Bilirubin (mg/dl)	0.64 ± 0.19	1.73 ± 0.86*	1.13 ± 0.32*$
Glucose (mmol/L)	4.91 ± 0.53	5.34 ± 0.65 *	5.96 ± 0.72*$
Insulin (mU/L)	12.16 ± 2.98	21.13 ± 3.96*	27.82± 4.33*$
HOMA-IR index	2.29 ± 0.62	5.34 ± 0.97*	7.68 ± 1.15*$

Hb: Hemoglobin; **BMI:** Body mass index; **WHR:** Waist hip ratio; **SBP:** Systolic blood pressure; **DBP:** Diastolic blood pressure; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **LDL:** Low density lipoprotein; **HDL:** High density lipoprotein; **HOMA-IR:** Hemostasis of insulin resistance index; (*): indicates a significant difference relative to healthy controls; ($): indicates a significant difference relative to HCV, P < 0.05

Table 2: *Mean value and significance of oxidative stress and anti-oxidant status markers among healthy controls, patients with CHC and NASH*

	Healthy controls	CHC patients	NASH patients
CD (mmol/L)	15.89 ± 3.15	21.92 ± 4.26*	25.17 ± 5.18*$
MDA (mmol/L)	15.75 ± 3.42	22.31± 4.11*	25.83 ± 5.36*$
NO (µmol/l)	28.43 ± 4.97	35.92 ± 6.43*	45.16 ± 8.95*$
MPO (nmol/ml/min)	10.31 ± 2.14	15.25 ± 3.21*	20.73 ± 4.81*$
GPx (units/gHb)	17.86 ± 3.16	23.51± 4.82*	26.86 ± 4.53*$
SOD (units/mL)	62.45 ± 7.34	46.71± 6.54*	55.42± 6.13*$
GSH (mmol/gHb)	3654.23 ± 228.45	2914.39 ± 187.38*	2028.36 ± 165.32*$
AE (nmol/ml/min)	1045.21 ± 112.41	562.49 ± 76.53*	434.27± 48.36*$
PON (nmol/ml/min)	683.52 ± 65.94	458.32 ± 64.85*	178.44 ± 28.42*$

CD: conjugated dienes; **MDA:** Malondialdehyde; **NO:** Nitric oxide; **MPO:** Myeloperoxidase; **AE:** Arylesterase; **PON:** Paraoxonase; **GPx:** Glutathione peroxidase; **SOD:** Superoxide dismutase; **GSH:** Glutathione; (*): indicates a significant difference relative to healthy controls; ($): indicates a significant difference relative to HCV, P < 0.05.

significantly reduced in patients with NASH than patients with CHC (Table 2).

RESULTS

Increased levels of oxidative stress play a role in the pathogenesis of liver diseases, including chronic hepatitis C virus infection (CHC) and nonalcoholic steaohepatitis (NASH) (41). However, Patients with HCV and HCV-NAFLD groups had altered blood lipid profiles, higher IR, elevated liver enzyme activities, imbalances in anti-oxidant defense (42). To our knowledge, this is the first study to compare the level of oxidative stress and anti-oxidative markers among patients with chronic hepatitis C virus infection versus nonalcoholic steatohepatitis. The main finding of our study proved excess level of oxidative and lower anti-oxidant markers in patients with NASH than patients with CHC and healthy volunteers, these results are in line with many previous studies (42, 43).

Previous studies had shown that CHC patients had higher levels of serum malondialdehyde (MDA) than healthy individuals. The imbalance of antioxidant and oxidant status may be influential in determining the efficacy of the treatment for CHC patients (44). Also, El-Kannishy et al. proved that patients with CHC had significantly higher oxidative stress, which can lead to chronic inflammation (45). Moreover, Dikici et al. found that patients with CHC had increased oxidative stress and decreased levels of some important antioxidants such as GSH and ß- carotene are in accordance with the findings of other investigators (46-50). Indeed, it has been shown that the amount of reactive oxygen species (ROS) found in healthy human livers was significantly lower than values found in the liver affected hepatitis C (51). MDA was also elevated in the liver and the blood of patients with hepatitis C as reported by De Maria et al. (52). Also, Boya et al. reported that, the peripheral blood mononuclear cells separated from chronic hepatitis C patient had increased MDA concentrations (53). Moreover, Ali et al. found that cirrhotic patients with HCV had higher serum MDA, NO levels and MPO activity while lower AE and PON1 activities than the patients with CHC (54). Some studies are in agreement with the present investigation such as Gangadharan et al. who found a decreasing in serum AE and PON1 activities in chronic and cirrhotic HCV patients compared with healthy controls (55).

Many previous studies support our findings regarding excess level of oxidative and lower anti-oxidant markers among patients with NASH than healthy control as Leach found that NASH was an independent predictor of decreased GSH levels and patients with NASH had significantly lower levels of antioxidants: reduced and total glutathione, GSH/GSSG ratio, GPx and an increased level of MDA (a marker of lipid peroxidation) vs. the control group, which reflects the presence of oxidative stress in patients with NASH (41). Similar results have been shown earlier with both CD and MDA and other markers of lipid peroxidation in patients with NAFLD (56-58). As GSH plays an integral role in the coordination of cellular anti-oxidant defense processes and its levels are shown to vary inversely with susceptibility to oxidative stress (59). Decreased GSH levels have been shown earlier in patients with NAFLD as well as in experimental models of NAFLD (60-62). Moreover, Sanyal et al. stated that excessive fatty oxidation by the mitochondrial peroxisomes under the over expression of CYP2E1 enzymes, in the steatotic livers lay the foundation of oxidative stress (63).

Our results thus suggest higher oxidative stress in patients with NASH in comparison to patients with chronic viral hepatitis and may indirectly also suggest that even though the oxidative stress is initiated at a same pace in both NASH and CHC patients, over a period it may get lower in patients with CHC, these results agreed with Kumar et al. compared the presence of oxidative stress and cytokines in 25 patients with NAFLD with 25 age, sex and BMI-matched patients with chronic viral hepatitis (CVH) and 25 healthy volunteers (HV). Patients with NAFLD had significantly higher levels of malondialdehyde (MDA) and conjugated dienes (CD) in comparison to HVs. Patients with NAFLD also had significantly higher MDA levels in comparison to CVH patients. Patients with NAFLD had significantly lower GSH levels in comparison to HVs. Patients with NAFLD had higher GPx activity in comparison to HVs. Catalase activity was significantly decreased in both NAFLD and CVH patients in comparison to HVs. Patients with NAFLD had significantly higher SOD activity in comparison to CVH patients (64).

CONCLUSION

Increased levels of oxidative stress play a role in the pathogenesis of liver diseases, including chronic hepatitis C virus infection.

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