Association of paraoxonase activity and atherosclerosis in patients with chronic hepatitis B

*Karsen H[1], Binici I[2], Sunnetcioglu M[2], Baran AI[2], Ceylan MR[2], Selek S[3], Celik H[3]

1. Harran University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Sanliurfa, Turkey
2. Yuzuncu Yil University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Van, Turkey
3. Harran University School of Medicine, Department of Biochemistry, Sanliurfa, Turkey

Abstract

Background: The hepatitis B virus is a significant pathogen that causes cirrhosis, and hepatocellular cancer as a result of the damage it causes to liver cells. Its infection affects more than 400 million people globally. Although there is an effective vaccine and treatment methods, almost 1, 000, 000 people die every year.

Objective: To investigate paraoxonase and arylesterase activities along with oxidative status parameters and serum lipid levels, and to find out if there is any increased susceptibility to atherogenesis.

Methods: Thirty-four subjects with chronic hepatitis B and 39 healthy subjects as control were enrolled in the study. Age, body mass index and gender, Serum Triglycerides (TG), High-density Lipoprotein (HDL) and Low-Density lipoprotein (LDL) levels, serum paraoxonase-1 and arylesterase activities were determined. Oxidative and antioxidative statuses were evaluated by measuring serum-free sulfhydryl groups, lipid hydroperoxide levels, total antioxidant capacity, total oxidant status, and oxidative stress index.

Results: Serum TG and LDL levels were higher while serum HDL levels were lower in patients with chronic hepatitis B than in controls but the differences did not reach statistical significance. Serum paraoxonase-1 and arylesterase activities, plasma free sulfhydryl groups, and total antioxidant capacity were significantly lower in patients than in controls ($p=0.018$, $p=0.005$, $p<0.001$, $p=0.037$ respectively), while lipid hydroperoxide, total oxidant status, and oxidative stress index were significantly higher (for all $p<0.001$).

Conclusion: The diminution in the paraoxonase-1 and arylesterase activities could contribute to the accelerated development of atherosclerosis in patients with chronic hepatitis B.

Key words: Chronic hepatitis B, paraoxonase activity, oxidative status, atherosclerosis

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Introduction

There is an increasing knowledge about the oxidative status in subjects with Chronic Hepatitis B (CHB) virus infection. In several studies, increase in oxidative components or decrease in antioxidants or both, have been reported in subjects with either acute or CHB virus infection[1,2]. Oxidative stress, owing to increased lipid and protein oxidation products and decreased antioxidants is associated with cardiovascular diseases and down-regulates Paraoxonase-1 (PON1) and arylesterase activities[2].

The PON1 is an high-density lipoprotein (HDL) -associated protein synthesized in the liver, and reduces oxidative stress in lipoproteins, in macrophages, and in the atherosclerotic lesion. It protects against atherosclerosis development, and this phenomenon could be related to its antioxidative properties[3]. The PON1 serum activity is related to systemic lipid peroxidation stress and atherogenesis[4].

The aim of this study was to investigate paraoxonase and arylesterase activities along with oxidative status parameters and serum lipid levels, and to find out if there is any increased susceptibility to atherogenesis, which might be reflected with increased oxidative stress and decreased serum PON1 activity in patients with chronic hepatitis B.

***Corresponding author:**
Hasan Karsen
Harran University School of Medicine
Department of Infectious Diseases and Clinical Microbiology
Sanliurfa, Turkey
Tel: 00904143183000
E-mail: hasankarsen@hotmail.com

Methods

Study design

A group of patients with CHB and a control group of otherwise healthy volunteers without acute or chronic hepatitis B were matched for age, BMI, and gender. The diagnosis of CHB was based on the guidelines for chronic hepatitis B diagnosis of the American Association for the Study of Liver Diseases[5]. Patients who had an infection with other hepatitis viruses, a history of autoimmune disease, a

history of hepatotoxic drug use or a history of nucleoside anti-HBV drug or interferon use were excluded. The study protocol was approved by the Regional Committee for Medical Research Ethics.

Outcome parameters

Serum Triglycerides (TG), HDL and low-density Lipoprotein (LDL) levels, serum PON1 and arylesterase activities were determined. Oxidative and antioxidative status were evaluated by measuring serum free Sulfhydryl (-SH) groups, lipid Hydroperoxide (LOOH) levels, Total aAtioxidant capacity (TAC), total oxidant status (TOS) and Oxidative Stress Index (OSI).

Analytical methods

Lipid parameters were measured using commercially available assay kits (Abbott®, Illinois, USA) with an autoanalyzer (Aeroset®, Abbott®, Illinois, USA).

Paraoxonase activity

The PON1 activity was measured in the absence (basal activity) and presence of NaCl (salt-stimulated activity) [6]. Briefly, the rate of paraoxon hydrolysis was measured by the increase of absorbance at 412 nm at 25 °C. Paraoxonase activity was expressed as U/L serum.

Arylesterase activity

Phenylacetate was used as a substrate to measure the arylesterase activity[7]. The reactionwas started by the addition of the serum and the increase in absorbance was read at 270 nm. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. Arylesterase activity was defined as kU/L serum.

Free sulfhydryl serum levels

Free sulfhydryl serum levels were measured by the method of Ellman[8]. Briefly, 1 mL of buffer containing 0.1M Tris, 10 mM EDTA, pH 8.2, and 50 uL serum was added to cuvettes followed by 50 uL 10 mM DTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as millimolars.

Serum LOOH levels

Serum LOOH levels were measured with the ferrous oxidation with xylenol orange assay[9]. The principle of the assay depends on the oxidation of ferrous ion to ferric ion via various oxidants and the produced ferric ion is measured with xylenol orange. The LOOH's are reduced by Triphenyl Phosphine (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels.

Total antioxidant capacity

Total Antioxidant Capacity (TAC) levels were measured by Erel's TAC method, which is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) radical cation by antioxidants[9,10]. In the assay, ferrous ion solution, which is present in the Reagent 1 is mixed by hydrogen peroxide, which is present in the Reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The results were expressed in mmol Trolox Eq/L.

Total oxidant status

Total Oxidant Status (TOS) serum concentrations were measured using Erel's TOS method, which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange[9,12]. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The results were expressed in ìmol H_2O_2 Eq/L.

Oxidative Stress Index (OSI)

Percentage ratio of serum TOS level to serum TAC level was accepted as OSI. To perform the calculation, the resulting unit of TAC (mmol Trolox Eq/L) was changed to ìmol Trolox Eq/L, and the OSI value was calculated according to the following formula[12]. OSI = [(TOS, ìmol H_2O_2 Eq/L) / (TAC, ìmol Trolox Eq/L)]/100.

Statistical analysis

All analyses were conducted using SPSS 15 (SPSS for Windows 15, Chicago, IL). The Student's t-test for paired samples was used to compare the blood samples, assuming a 95% confidence interval. All statistical tests were two-sided. Continuous variables were expressed as mean ± standard deviation. P-values <0.05 were considered to be statistically significant.

Results

Thirty-four subjects with chronic hepatitis B and 39 healthy subjects as control were enrolled in the study. There were no significant differences (p>0.05) in female/male ratio, age or BMI between patients and controls (table 1). The mean age of patients in the study sample was 36.24±14.20 years, compared with a mean age of 35.21±14.13 years in patients in the control group. Serum TG and LDL levels were higher while serum HDL levels were lower in CHB subjects than in controls but the differences did not reach statistical significance.

Serum PON1 and arylesterase activities, free sulfhydryl serum levels, serum LOOH, TAC, TOS levels and oxidative stress index are given in table 1. Serum PON1 and arylesterase activities of CHB subjects were significantly lower than the control group (p=0.018 and p=0.005; respectively). Serum PON1 activity of patients and controls are shown in figure 1. Plasma free sulfhydryl groups and TAC levels were significantly lower in CHB subjects than in controls (p<0.001 and p=0.037 respectively). Serum LOOH and serum TOS levels of the patient group were significantly higher than the control group (p<0.001 for both). The OSI was significantly higher in patients than controls and is shown in figure 2. (p<0.001).

Table 1: Demographic characteristics, serum lipid parameters, PON1 activity, oxidant and antioxidant parameters in patients and controls

Parameters	Patients (n=34) Mean±SD	Controls (n=39) Mean±SD	p value
Female/Male	18/16	22/17	Ns
Age	36.24±14.20	35.21±14.13	Ns
BMI (kg/m^2)	20.10±3.12	21.28±2.16	Ns
TG	137.09±56.74	125.46±65.98	Ns
HDL	39.55±17.50	43.18±12.60	Ns
LDL	113.50±31.11	102.95±27	Ns
Paraoxonase-1 (U/L)	140.18±68.06	169.49±72.22	0.018
Arylesterase (kU/L)	136.42 ±60.15	162.57± 40.06	0.005
-SH (mmol/L)	0.43±0.11	0.54±0.06	<0.001
LOOH (μmol H_2O_2 Eq/L)	43.17±54.19	6.93±3.62	<0.001
TAC (mmol Trolox Eq/L)	1.17±0.21	1.38±0.16	0.037
TOS (μmol H_2O_2 Eq/L)	58.06±29.76	15.05±10.42	<0.001
OSI (arbitrary unit)	4.75±2.24	1.10±0.79	<0.001

BMI = body mass index; TG = triglyceride; HDL = High density lipoprotein; LDL = Low density lipoprotein; -SH = Free sulfhydryl groups; LOOH = Lipid hydroperoxide; TAC = Total antioxidant capacity; TOS =Total oxidant status; OSI = Oxidative stress index; Ns = Not statistically significant.

Figure 1: The OSI level of patients and control groups

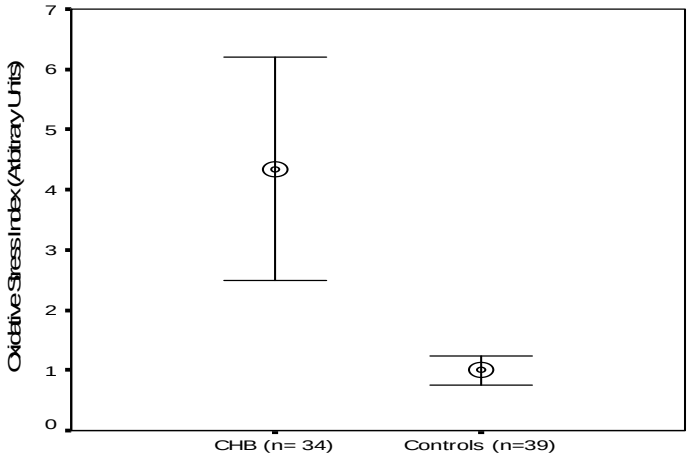
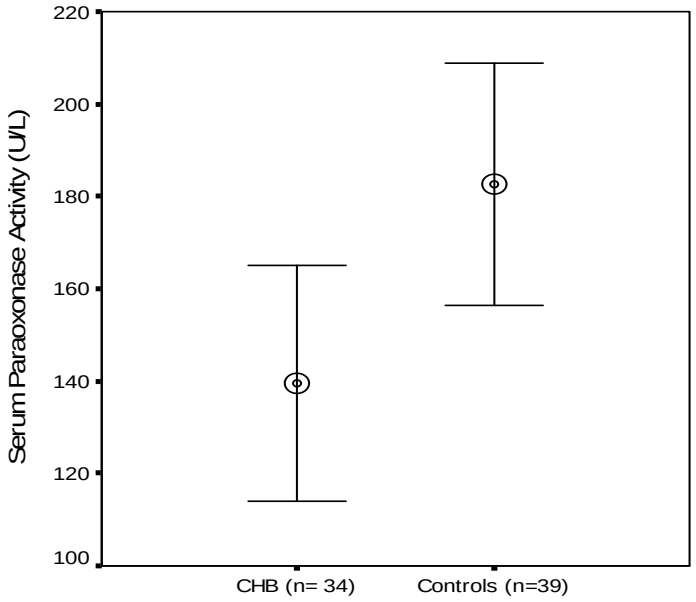


Figure 2: The Paraoxonase activity of patients and control groups



Discussion

In the present study, serum paraoxonase, arylesterase activities, TAC and total free sulfhydryl group levels were significantly lower in patients with CHB than controls, while LOOH levels, TOS and OSI were significantly higher. The results show that patients with CHB are exposed to potent oxidative stress and they have decreased PON1 activity. These predisposal factors may, in part, play a role in the pathogenesis of atherosclerosis in patients with CHB.

The PON1 both prevents the formation of oxidized LDL and inactivates LDL-derived oxidized phospholipids once they are formed. It also protects phospholipids in HDL from oxidation[13]. These actions suggest a role of PON1 in cardiovascular diseases and atherosclerosis. The PON1 has also been shown to metabolize a number of drugs and pro-drugs via its lactonase activity[14]. Serum PON1 activity is shown to be associated with modulation of endothelial functions and regulation of coronary vasomotor tone[11,15]. Increased incidence of subclinical atherosclerosis in carotid arteries has been reported in patients with CHB[16]. On the other hand, others have failed to demonstrate any difference between CHB and controls and stated that HBsAg seropositivity was not associated with increased mortality risks of atherosclerosis-related/ cardiovascular diseases[17]. Decrease in PON1 activity under oxidative stress is an independent risk factor for coronary arter disease and mostly attributed to changes in the redox status of the free sulfhydryl groups of proteins since sulfhydryl compounds prevent the inhibition of PON1 activity[18]. Free sulfhydryl groups of proteins constitute the main antioxidant component of serum and have been shown to be associated with the coronary heart disease[19]. In our study, serum PON1 and arylesterase activities were lower in patients with CHB subjects than in controls.

Free radicals are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms. Organisms are protected against oxidative stress via enzymatic and nonenzymatic antioxidative mechanisms. Under normal conditions, a delicate balance exists between rates of free radical formation and their removal by antioxidant enzymes and molecules[18]. Although determination of either oxidants or antioxidant components alone may give information about the oxidative stress, determination of oxidants along with antioxidants is more useful in this context[18,19]. We studied the total antioxidant and oxidant parameters instead of individual antioxidant compounds such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, which act in combination with each other affecting TAC producing synergistic or antagonistic effects. As in the case of the TOS, the knowledge of TAC, which is the cumulative capacity of antioxidant components to scavenge free radicals, was claimed to be useful for epidemiologic purposes by many researchers. The ratio of the serum TOS level to TAC, regarded as OSI and an indicator of oxidative

stress, reflects the redox balance between oxidation and anti-oxidation. Recently, it has been reported that OSI may reflect the oxidative status more accurately than TAC or TOS alone[20]. In our study, plasma free sulfhydryl groups, and TAC levels were significantly lower in CHB subjects than in controls, while LOOH, TOS levels and OSI were significantly higher. These results represent the severe oxidative stress in patients with chronic hepatitis B infection.

On one hand, chronic hepatitis B is the most common cause of hepatocellular carcinoma but on the other hand it could contribute to the development of atherosclerosis.

Conlusion

In the light of the findings of this study, we concluded that oxidative stress is increased, while serum PON1 and arylesterase activity is decreased, in CHB patients. The diminution in the paraoxonase and arylesterase activities could contribute to the accelerated development of atherosclerosis in patients with CHB. Further studies are needed to clarify the possible mechanisms underlying the decreased enzyme activities.

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