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

*Karsen H1, Binici I2, Sunnetcioglu M2, Baran AP, Ceylan MR2, Selek S3, Celik H1

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

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 infection1,2. 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 activities3.

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 properties3. The PON1 serum activity is related to systemic lipid peroxidation stress and atherogenesis4.

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.

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 Diseases3. Patients who had an infection with other hepatitis viruses, a history of autoimmune disease, a

*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
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 Sulphhydryl (-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 activity7. The reaction was 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 Ellman8. 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 assay9. 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 antioxidants9,10. 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 orange9,12. 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 H2O2 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 formula12. OSI = [(TOS, ìmol H2O2 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 sulphydryl 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 sulphydryl 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) | Controls (n=39) | p value |
|--------------------------|----------------|----------------|---------|
| Female/Male              | 18/16          | 22/17          | Ns      |
| Age                      | 36.24±14.20    | 35.21±14.13    | Ns      |
| BMI (kg/m²)              | 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 H₂O₂ 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 H₂O₂ 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 sulphydryl 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
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 oxidation13. 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 prodrugs via its lactonase activity14. Serum PON1 activity is shown to be associated with modulation of endothelial functions and regulation of coronary vasomotor tone15.15. Increased incidence of subclinical atherosclerosis in carotid arteries has been reported in patients with CHB17. 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 diseases17. Decrease in PON1 activity under oxidative stress is an independent risk factor for coronary artery 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 activity18. Free sulfhydryl groups of proteins constitute the main antioxidant component of serum and have been shown to be associated with the coronary heart disease19. 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 molecules18. 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 context18,19. 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...
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. 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.

References
1. Irshad M, Chaudhuri PS, Joshi YK. Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. Hepatol Res 2002; 23:178-84.
2. Demirdag K, Yilmaz S, Ozdarendeli A, Ozden M, Kalkan A, Kilic SS. Levels of plasma malondialdehyde and erythrocyte antioxidant enzyme activities in patients with chronic hepatitis B. Hepatogastroenterology 2003; 50:766-70.
3. Rosenblat M, Karry R, Aviram M. Paraoxonase 1 (PON1) is a more potent antioxidant and stimulant of macrophage cholesterol efflux, when present in HDL than in lipoprotein-deficient serum: relevance to diabetes. Atherosclerosis 2006; 187:74.
4. Canales A, Sanchez-Muniz FJ. Paraoxonase, something more than an enzyme? Med Clin (Barc) 2003; 121:537-48.
5. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-2.
6. Seres I, Paragh G, Deschene E, Fulop T Jr, Khalil A. Study of factors influencing the decreased HDL associated PON1 activity with aging. Exp gerontol 2004; 39:59-66.
7. Eckerson HW, Wyte MC, La Du BN. The human serum paroxonase/arylesterase polymorphism. Am J Hum Genet 1983; 35:1126-38.
8. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70-7.
9. Ezel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004; 37:112-9.
10. Ezel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37:277-85.
11. Pasqualini L, Cortese C, Marchesi S, Siepi D, Pirro M, Vaudo G, et al. Paraoxonase-1 activity modulates endothelial function in patients with peripheral arterial disease. Atherosclerosis 2005; 183:349-54.
12. Ezel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38:1103-11.
13. Costa LG, Cole TB, Jarvik GP, Furlong CE. Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. Ann Rev Med 2003; 54:371-92.
14. Draganov DI, La Du BN. Pharmacogenetics of paraoxonase: a brief review. Nannya's Schmiedeberg's Arch Pharmacol 2004; 369:79-88.
15. Malin R, Knuuti J, Janatuinen T, Laaksonen R, VesaLainen R, Nuutila P, et al. Paraoxonase gene polymorphisms and coronary reactivity in young healthy men. J Mol Med 2001; 79:449-58.
16. Alkhouri N, Tamimi TA, Verian L, Lopez R, Zein NN, Feldstein AE. The inflamed liver and atherosclerosis: a link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk. Dig Dis Sci. 2010; 55(9):2644-50.
17. Wang CH, Chen Cj, Lee MH, Yang HL, Hsiao CK. Chronic hepatitis B infection and risk of atherosclerosis-related mortality: A 17-year follow-up study based on 22,472 residents in Taiwan. Atherosclerosis. 2010; 211(2):624-9.
18. Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN, et al. Paraoxonase status in coronary heart disease. Are activity and concentration more important than genotype? Arterioscler Thromb Vasc Biol 2001; 21:1451-7.
19. Rozenberg O, Aviram M. S-Glutathionylation regulates HDL-associated paraoxonase 1 (PON1) activity. Biochem Biophys Res Commun 2006; 351:492-8.
20. Rontu R, Karhunen PJ, Ilveskoski E, Mikkelsson J, Kajander O, Perola M, et al. Smoking-dependent association between paraoxonase 1 M/L55 genotype and coronary atherosclerosis in males: an autopsy study. Atherosclerosis 2003; 171:31-7.