Serum Paraoxonase and Arylesterase Activities in Various Forms of Hepatitis B Virus Infection

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The aim of this study was to determine and evaluate the activity of paraoxonase and arylesterase enzymes in various clinical forms of hepatitis B infection and to investigate the correlation between these parameters and chronic disease course/fibrosis. Overall, 40 patients diagnosed as hepatitis B carriers (CIHBV), 40 chronic active hepatitis B (CAHBV) patients, and 40 healthy adults (control group) between 18 and 65 years of age were enrolled the study. Serum paraoxonase and arylesterase activities were measured spectrophotometrically. Their activities were significantly lower in patients with CAHBV compared with CIHBV patients or with control group patients ($P < 0.001$). There was a negative correlation between alanine aminotransferase levels and the

activity of paraoxonase and arylesterase ($r = -0.38$, $P = 0.001$ and $r = -0.28$, $P = 0.002$, respectively). A statistically significant negative correlation was found between arylesterase activity in the sera of CAHBV patients and HBV DNA levels ($\rho = -0.33$, $P = 0.03$). On the contrary, no correlation was found between paraoxonase levels and HBV DNA levels ($P > 0.05$). The histology activity index of CAHBV patients did not correlate with paraoxonase and arylesterase activities ($P > 0.05$). In light of these findings, it may be assumed that during the progression of an inactive hepatitis B carrier to being actively infected, reduced paraoxonase and arylesterase activities may be observed. J. Clin. Lab. Anal. 25:311–316, 2011. © 2011 Wiley-Liss, Inc.

Key words: paraoxonase 1; arylesterase; hepatitis B

INTRODUCTION

Human serum arylesterase, also known as paraoxonase 1 (PON1), is an ester hydrolase glycoprotein that is synthesized predominantly in the liver (1–3). PON1 is also a high-density lipoprotein (HDL)-associated enzyme that catalyzes the hydrolysis of organophosphates such as neurotoxins, pesticides and arylesters; also, it is recognized as an antioxidant enzyme (4). Studies have indicated that PON1 can prevent lipid peroxide accumulation on low-density lipoprotein (LDL) (5,6). PON1 activity is decreased in some diseases such as rheumatoid arthritis, *Helicobacter pylori* infection, and in renal failure (7–9).

Hepatitis B virus (HBV) infections have different clinical appearances, ranging from asymptomatic infection to fulminate liver disease. In approximately 15–40% of chronic hepatitis B patients, disease progresses to cirrhosis and end-stage liver disease (10). In cases of chronic viral hepatitis whose pathogenesis is not entirely clear, experimental studies have demonstrated oxidative stress-mediated cell destruction and DNA and RNA

damage (11). Various biochemical, serological, and nucleic acid amplification tests are utilized to diagnose HBV infection. Diagnosis is confirmed by the histopathological examination of a liver biopsy, which is an invasive procedure that carries a risk of complications (12).

The liver has a key role in PON1 synthesis, and many studies have investigated whether serum PON1 activity could be useful for understanding the functional status of the liver (13). Several studies have also revealed that there is a marked decrease in serum paraoxonase or arylesterase activity in patients with liver diseases (14,15).

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The aim of this study was to evaluate the activity of paraoxonase and arylesterase in the sera of patients with various clinical forms of hepatitis B infection and to investigate the correlation between these parameters and chronic disease course/fibrosis.

METHODS

Patient Selection

This study was conducted at the Harran University Faculty of Medicine Infectious Diseases and Clinical Microbiology Outpatient Clinic between January and June 2009 in Sanliurfa, in Turkey. In total, 40 patients diagnosed as hepatitis B carriers, 40 chronic active hepatitis B patients, and 40 healthy adults between 18 and 65 years of age were enrolled in the study; gender preference was not used in patient selection.

Description of Study Groups

Study groups were defined as the following: Group 1, patients diagnosed with chronic inactive HBV (CIHBV) infection; Group 2, patients diagnosed with chronic active HBV (CAHBV) infection, and Group 3, the control group. Patients were diagnosed according to the 2009 American Association for the Study of Liver Diseases Viral Hepatitis Guidelines criteria (16). The diagnosis of chronic, active hepatitis was made using histopathological evaluations based on the modified Knodell score (17). Patients who requested to be discharged and those under 18 or over 65 years of age were excluded from the study. Additionally, patients with accompanying chronic diseases such as dyslipidemia, coronary artery disease, diabetes mellitus, hypertension, chronic renal insufficiency, and chronic alcohol consumption were excluded from the study; anemia, malignancy, cirrhosis, pregnancy, and smoking were also excluding criteria.

Healthy adults who applied to various outpatient clinics of the Harran University Faculty of Medicine Hospital and were between 18 and 65 years of age were enrolled in the control group; individuals in the control group matched the study groups in terms of age and gender.

This study was approved by the Harran University Faculty of Medicine Ethics Committee.

Sample Preparation

After informing patients about the study, signed informed consent forms were obtained from each patient.

From each patient, an 8–10 cc blood sample was drawn into heparinized tubes. Blood samples were centrifuged for 10 min at 3,500 rpm to separate plasma.

Separated plasma samples were transferred to dry, sterile tubes and kept at -80°C until analysis.

Study Design

Measurement of paraoxonase and arylesterase activities

Paraoxonase and arylesterase activities were measured spectrophotometrically by using commercially available kits (Relassay[®], Gaziantep, Turkey) (18–20). Paraaxon (0,0-diethyl-0-*p*-nitrophenyl phosphate) was used as a substrate for the measurement of paraoxonase, and phenyl acetate was used as a substrate for arylesterase measurements. Using spectrophotometry, the absorbance values of *p*-nitrophenol, which was formed through the enzymatic hydrolysis of paraoxonase, were recorded each minute at 412 nm. The absorbance values of phenol, which was formed through the enzymatic hydrolysis of arylesterase, were measured each minute at 270 nm. Enzyme activity was calculated as U/l.

Statistical Analysis

To analyze the results, descriptive statistics, such as the percentage, mean, and standard deviation, were utilized. Quantitative values were designated as mean $\pm$ standard deviation ($\bar{X} \pm \text{SD}$). Gender distribution in control and study groups was determined using Chi-square tests. In intergroup evaluations, one-way ANOVA tests were used because the groups met parametric requirements. Differences were considered statistically significant when $P < 0.05$. The Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc., Chicago, IL) was used for all statistical analyses.

RESULTS

A total of 120 subjects, between 18 and 65 years of age were enrolled; of these, 43.3% ($n = 58$) were female and 56.7% ($n = 62$) were male.

Group comparisons according to age and gender are shown in Table 1. When compared for age and gender, no statistically significant differences were observed between the groups ($P > 0.05$).

Paraoxonase activity levels were evaluated in all three groups and found to be lower in patients with CIHBV infection compared with the control group and in patients with CAHBV infection compared with patients with CIHBV infection. The lowest values were observed in patients with CAHBV infection, compared with the other study groups. The differences between the three groups were statistically significant ($P < 0.05$; Table 2). Results are also depicted as box plots for visual clarification (Fig. 1). Paraoxonase and arylesterase levels for each of the groups are presented in Figure 1.

TABLE 1. Age and Gender Analysis of Patient and Control Groups

	Inactive Hepatitis B (n = 40)	Active Hepatitis B (n = 40)	Control group (n = 40)
Gender (M/F)	24/16	22/18	21/19
Age (year)	29.6 ± 10.6	35.5 ± 12.3	30.7 ± 10.7

TABLE 2. Results Achieved in Study Groups and P-Values

Values	Active Hepatitis B	Inactive Hepatitis B	Control group	F	P
Paroxonase (U/l)	104.7 ± 58.9	138.4 ± 67.6	184.6 ± 9.2 ^a	13.4	0.000
Arylesterase (kU/l)	140.6 ± 20.7 ^a	164.2 ± 19.2 ^a	161.6 ± 28.9 ^a	26.7	0.000
ALT (IU/ml)	92.8 ± 72.0 ^a	23.7 ± 11.8	23.2 ± 11.5	35.1	0.000
HDL (mg/dl)	39.0 ± 9.7	43.2 ± 10.0	48.0 ± 6.1 ^a	10.3	0.000
LDL (mg/dl)	100.6 ± 23.1	94.9 ± 18.0	96.1 ± 18.6	0.8	0.437

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aGroup which created a difference.

Fig. 1. Paraoxonase and arylesterase levels in groups.

Arylesterase levels were also compared among the groups and were also found to vary between the groups. Arylesterase levels were lower in patients with CIHBV infection compared with control group individuals; arylesterase levels were also reduced in patients with CAHBV infection compared with patients with inactive hepatitis B. These differences were statistically significant. Consistent with the paraoxonase results, the lowest arylesterase value was detected in patients with

chronic active hepatitis B. The differences between the groups were statistically significant ($P < 0.05$; Table 2; Fig. 1).

Alanine aminotransferase (ALT) value, which is an indicator and diagnostic criteria for chronic hepatitis infection, was evaluated and intergroup comparisons were performed. Higher ALT values were observed in patients with inactive hepatitis B infection compared with the control group; increased ALT values were also recorded in patients with chronic active hepatitis B infection compared with patients with inactive hepatitis B infection. These differences were statistically significant ($P < 0.05$; Table 2).

Correlations between serum levels of ALT, paraoxonase and arylesterase were investigated. A negative correlation was noted between ALT and paraoxonase and arylesterase enzyme activities ($r = -0.38$, $P = 0.001$ and $r = -0.28$, $P = 0.002$, respectively).

HDL and LDL values were also examined. Even though the levels of HDL and LDL were higher in patients with active hepatitis B compared with the other groups, the differences were not statistically significant ($P > 0.05$; Table 2; Fig. 2).

HBV DNA levels in chronic active hepatitis B patients were measured as $52,683,500 \pm 47,324,320$ (min 110,000, max 587,000,000) IU/ml. A statistically significant negative correlation was detected between patient arylesterase activity levels and HBV DNA levels ($\rho = -0.33$; $P = 0.03$). However, a statistically significant correlation was not found between paraoxonase activity levels and HBV DNA values ($P > 0.05$).

The mean Knodell histology activity index (HAI) of chronic active hepatitis B patients was 6.2 ± 0.61 (range, 4–9). The HAI index of the patients with CAHBV did

Fig. 2. HDL values in groups. HDL, high-density lipoprotein.

not correlate with paraoxonase and arylesterase activities ($P > 0.05$ for all).

DISCUSSION

HBV, which causes chronic viral hepatitis, is an important pathogen that leads to fibrosis, cirrhosis, and hepatocellular cancer through necroinflammation and replication in liver cells. Even though an effective vaccine is available, chronic hepatitis B infection affects more than 400 million people worldwide, and it represents an important public health issue (21,22).

In a healthy organism, total oxidant and antioxidant levels are balanced. In the case of an increase in endogenous and exogenous oxidative substances above a certain level, or an insufficiency in antioxidative substances, this balance is disrupted in favor of oxidants, and oxidative stress occurs. Free oxygen radicals cause damage to proteins, lipids, carbohydrates, nucleic acids, and beneficial enzymes, which can lead to permanent damage (23).

Paraoxonase and arylesterase, which are synthesized in the liver, are esterases with an antioxidative effect; they protect cells against the harmful effects of the free radicals (24). In cases of chronic viral hepatitis whose pathogenesis is not entirely clear, experimental studies have demonstrated the role of oxidative stress in cell destruction and DNA and RNA damage (11).

Ali et al. found that PON1 activity in HCV-induced cirrhotic patients was lower compared with patients with chronic HCV infection (25). Keskin et al. evaluated the

correlation between PON1 and arylesterase and disease severity in cirrhotic liver patients and found lower enzyme activities in chronic hepatitis patients compared with the control group (26). In another study, Aslan et al. reported significantly lower paraoxonase and arylesterase levels in chronic hepatitis B and C patients compared with controls (14,27). In these studies, hepatitis B and C were evaluated in the same group. However, in our study, they were treated as two different diseases. Thus, different clinical forms of hepatitis B were compared with healthy controls. In this study, we observed that the enzyme levels of the patient groups were significantly lower compared with the control group. In patients with chronic hepatitis B infection, paraoxonase and arylesterase enzymes were significantly lower compared with patients with inactive hepatitis B; the highest enzyme levels were recorded in control group individuals. This finding suggests that in chronic active hepatitis B infection, these antioxidant enzymes are reduced to very low levels; most likely, disease progression to chronicity has a role in this decrease. Hence, we hypothesize that these enzymes may be used as an indicator for disease progression to a chronic state. In other words, in light of our data, it may be assumed that the reduced enzyme levels could indicate disease progression, from being an inactive hepatitis B carrier to having active hepatitis B infection. However, more comprehensive work requires to clarify this conclusion.

ALT levels are valuable in the diagnosis of chronic hepatitis B. ALT levels that are double the normal values for a duration of >6 months are a well-known indicator for disease chronicity (16). During inactive hepatitis B infections, ALT levels are expected to be normal. In this study, ALT levels in chronic hepatitis B patients were approximately three times higher compared with patients with inactive infection and the control group. These results are consistent with other studies and guidelines that describe hepatitis B infections (16). In this study, a significant negative correlation between ALT values and paraoxonase/arylesterase values was also observed.

PON1's function is mainly dependent on HDL. Although protecting HDL from the deleterious effects of oxidative stress, it also inhibits LDL oxidation (28). As in other studies where paraoxonase and arylesterase enzymes were evaluated in cases of liver damage and hepatitis, we observed that a decrease in PON1 was dependent on HDL concentration. These results suggest that PON1 enzymes exert an antioxidative effect to prevent oxidative stress and that reduced levels are found because of this consumption.

In a model of CCl_4 -induced liver disease in mice, Camps et al. determined that PON1 levels both in the

serum and liver tissue were decreased, and that this decrease was most prominent in fibrotic septa and inflamed regions of liver (29). The fact that the levels of both of these liver-based enzymes are decreased in hepatic disorders may suggest that enzymes are not synthesized in sufficient amounts because of cell damage. On the other hand, in previous studies, an increase in oxidative parameters was observed indicating that an oxidative damage is present in addition to infectious and inflammatory damage (27). Although antioxidants may increase as a response to this damage, their consumption may also be high in parallel with the severity of damage; hence they may have considerably low blood levels. The findings in our study are consistent with these previous results and indicate that a similar explanation is most likely valid for chronic hepatitis B infection.

Various biochemical, serological, and nucleic acid amplification tests are used to diagnose HBV infection with chronic progression. Nevertheless, a final diagnosis is confirmed by the invasive procedure of a liver biopsy and histopathological examination. This procedure carries certain risks and complications, requires monitoring after the procedure, and the final results are attained much later (30). Therefore, this study is important because we found that paraoxonase and arylesterase enzyme activities display significant changes in regards to disease prognosis. We detected significantly altered paraoxonase and arylesterase enzyme activities, suggesting that these levels may possibly be used to monitor disease progression.

However, several limitations of this study should be considered. Liver biopsies from patients with CAHBV infection did not exhibit advanced degrees of fibrosis. Thus, we probably could not see a significant correlation between the increase in HAI values and the levels of paraoxonase and arylesterase activities. Because patients with cirrhosis were not included into this study, we could not compare them with the hepatitis B patients; only different forms of hepatitis B were compared with the control group.

Serum PON1 activity is strongly influenced by genetic polymorphisms, in particular its activity toward paraoxon. Serum PON1 has two genetically determined polymorphic sites, one at position 192 and other at position 55 (31). It would have been interesting to determine the influence of genetic variability on serum PON1 activity in these types of patients. However, this measurement was not possible and represents a limitation of our study.

In this study, paraoxonase and arylesterase enzyme activity levels were measured in various clinical forms of hepatitis B infection. The results revealed that as the disease progresses to the chronic state, these two

antioxidant enzymes are used in larger amounts to prevent oxidative damage from oxidative stress and hence, the activities of both enzymes are decreased.

In light of these findings, it appears possible that these biochemical parameters, paraoxonase and arylesterase enzymes, may be used in the diagnosis and monitoring of hepatitis B infection. Nevertheless, further large-scale experimental and clinical studies are needed to verify this conclusion.

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