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Does Prolidase Indicate Worsening of Hepatitis B Infection?

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Background: Hepatitis B infection is a health problem that affects more than 400 million people all over the world. We aimed to evaluate the usability of prolidase enzyme that plays an important role in collagen synthesis. Prolidase levels increase in hepatic damage, which can be used as diagnostic parameters in the progressions to chronic hepatitis B (CHB) infection by evaluating it in different clinical forms of hepatitis B infection. *Methods:* A total of 69 patients who presented to our clinic with chronic hepatitis B (CHB) infection, 72 patients with inactive hepatitis B infection (IHB), and 45 healthy volunteers were included into this study. Alanine transaminase (ALT), Aspartate aminotransferase (AST) and prolidase levels of patients were measured. Hepatic biopsy was performed in patients with CHB infection. Prolidase levels were evaluated in three different groups, and its correlations with fibrosis were investigated. *Results:* Prolidase was different between all groups ($P < 0.001$). Prolidase level was found to be higher in CHB and IHB compared to the control group. There was no correlation between this enzyme, fibrosis, and histological activity index. *Conclusion:* In this present study, it is shown that prolidase levels increase in hepatitis B infection. It may be used as a biochemical marker in the chronic hepatitis B. J. Clin. Lab. Anal. 27:398–401, 2013.

Key words: prolidase; chronic hepatitis B; inactive hepatitis B

INTRODUCTION

Hepatitis B virus (HBV), etiological agent of chronic viral hepatitis, is an important pathogen that may lead to cirrhosis, hepatic decompensation, and hepatocellular carcinoma as a result of necroinflammation and replication that it has caused in hepatic cells. Chronic hepatitis B (CHB) infection is a problem affecting more than 400 million people all over the world. Despite its effective vaccine and improvements in diagnostic and therapeutic methods, its severity is still prevalent. Five percent of the world population is HBV carrier, and CHB infection is ranked ninth among mortality causes by the World Health Organisation (WHO). HBV-related diseases result in the three most important mortality causes in Asia, Africa, and Pacific coasts (1–3).

Collagen is a protein that forms the building blocks of connective tissue. It is composed of hepatic basal membrane, collagen, glycoprotein, and proteoglycan (4). Prolidase enzyme (EC: 3.4.3.7, iminodipeptidase) destroys iminopeptides formed by breaking of collagen in the organism (5). Prolidase plays an important role in recycling of proline from imidodipeptides (mostly derived from degradation products of collagen) for resynthesis of collagen and other praline-containing proteins. Prolidase also plays an important role in collagen metabolism, matrix remodeling, and cell growth (6).

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Although the pathogenesis of CHB infection is not clearly known, prolidase is known to be increased in diseases affecting collagen metabolism, hepatic damage, and fibrosis (7).

Liver biopsy is the gold standard in the evaluation of hepatic fibrosis. Being an invasive intervention, it has disadvantages, for example not repeatable, complicated, and inconvenient in some situations such as mass occupying lesions and coagulation disorders (8). We aimed to evaluate the usability of prolidase enzyme that has an important role in collagen synthesis. Prolidase levels increase in hepatic damage, which can be used as diagnostic parameters in the progressions to CHB infection and cirrhosis by evaluating it in different clinical forms of hepatitis B infection.

MATERIALS AND METHODS

Patient Population

A total of 69 patients with CHB infection, 72 patients with inactive hepatitis B (IHB) infection, and 45 healthy volunteers in the age group of 17–66 were enrolled in this study. All patients underwent a baseline evaluation including a detailed medical history, typical physical examination, and blood tests.

Patients with Hepatitis B surface antigen (HBsAg) positivity longer than 6 months, negative Hepatitis B "e" antigen HBeAg, serumHBV DNA $<$ 2.000 IU/ml ($<10^4$ copies/ml), normal ALT/AST, and negative anti-HDV were included as inactive HBsAg carriers; whereas patients with HBsAg positivity longer than 6 months, HBV DNA $>$ 2.000 IU/ml, persistent or intermittent high transaminases, moderate or high necroinflammation, and/or fibrosis in the liver biopsies were included as having CHB infection (8).

Liver biopsy was performed in all CHB patients. It was not performed in other groups. Histopathologic evaluations were performed according to modified Knodell system that was proposed by Ishak et al. (9). Histologic activity index (HAI) and fibrosis levels were recorded (9).

Control group was composed of healthy adults aging between 15 and 65 years, who were chosen according to age and gender distributions in the study group.

Exclusion Criteria

Individuals aging $<$15 years and $>$65 years; with autoimmune hepatitis, α1-antitrypsin deficiency and Wilson disease, coronary artery disease, diabetes mellitus, uncontrolled hypertension, dyslipidemia, chronic renal failure; with alcohol and smoking habits; who were diagnosed with malignancy; with hepatitis D and hepatitis C infections; with morbid obesity and pregnancy were excluded from the study.

In total, 8–10 cc of blood sample was withdrawn from all the participants and serums of samples were separated by centrifugation. Serums were stored at -80°C until the day of measurement.

Prolidase Measurement

Prolidase activity was determined by a photometric method based on the measurement of the proline levels produced by prolidase (10).

Serum samples (100 μl) were mixed with 100 μl of serum physiological. A total of 25 μl of the mixture was preincubated with 75 ml of the preincubation solution (50 mmol/l Tris HCl buffer pH 7.0 containing 1 mmol/l glutathione, 50 mmol/l $MnCl_2$) at 37°C for 30 min. The reaction mixture, which contained 144 mmol/l gly-pro, pH 7.8 (100 ml), was incubated with 100 ml of preincubated sample at 37°C for 5 min. To stop the incubation reaction, 1 ml glacial acetic acid was added. After adding 300 ml Tris HCl buffer, pH 7.8, and 1 ml ninhydrin solution (3 g/dl ninhydrin was melted in 0.5 mol/l orthophosphoric acid), the mixture was incubated at 90°C for 20 min and cooled with ice. Absorbance was then measured at a 515 nm wavelength to determine proline value.

Intraassay and interassay coefficient of variations (CVs) were lower than 10%. We measured the serum prolidase activity by the method optimized by Gültepe (11), which is a modification of Myara and Chinard's methods (12,13) based on the spectrophotometric determination of proline levels liberated from glycyl-L-proline by prolidase enzyme.

The authors have confirmed in writing that they have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. The study was approved by the local ethics committee.

Statistical Analyses

Pearson's chi-square test was used to compare the categorical variables between groups. Categorical variables were presented as counts and percentages. The Kolmogorov–Smirnov test was used to evaluate whether the distribution of variables was normal. Mann–Whitney U test was used to compare continuous variables between the two groups. Continuous variables were presented as median interquartile range (IQR). A *P*-value of less than 0.05 was considered to be statistically significant. Pearson correlation test was used to explore the relationship between the oxidative stress and the liver histopathology (Knodell fibrosis score and HAI).

SPSS software 17.0 for Windows (Chicago, IL) was used for all statistical analysis.

TABLE 1. Demographic Characteristics, Prolidase Levels of the Study Groups

	Inactive	Chronic	Control	*P*
Age, median (IQR)	33.5 (27.2–44.75)	30 (21–40)	33 (26.5–40)	>0.05
Male (*n*, %)	35 (48.6%)	42 (60.9%)	25 (55.6%)	>0.05
ALT, median (IQR)	21 (16–28.5)	82 (56–118)	20 (12–28)	<0.01[a]
Prolidase (U/l), median (IQR)	660.40 (655.89–664.58)	652.01 (632.33–660.84)	612,61 (598.23–638.43)	<0.01[b]

IQR, interquartile range.
[a]Difference forming group CHB.
[b]There was a statistically significant difference among all groups.

RESULTS

Age, gender, ALT, and prolidase levels of patient and control groups are presented in Table 1.

The ALT levels were found to be higher in CHB compared to the other two groups ($P < 0.001$).

There was no correlation between histopathological evaluations of liver biopsies (HAI and Knodell fibrosis score) of CHB patients and these enzymes ($P > 0.05$).

DISCUSSION

Prolidase has been shown to increase in CHB in this first study investigating prolidase activity in CHB and the role of it in progression to CHB. Prolidase, required for collagen resynthesis, has an important role in the breakdown of collagen and intracellular protein, and its activity is increased. Correlation between diseases affecting collagen metabolism, such as chronic liver diseases, osteoporosis, osteoarthritis, and left ventricular hypertrophy, and prolidase enzyme activity has been confirmed by the conducted studies (7).

In their experimental study, Abraham et al. performed hepatic fibrosis in 12 rats by tetrachloride and they composed a control group from six rats that received phenobarbital. Treatment was stopped at the seventh day. Then, histopathological examination was performed and prolidase levels were measured. Fibrotic tissue was more prominent in the group that received carbon tetrachloride, and prolidase activity was also higher in this group. They proposed that there was a correlation between collagen accumulation in the tissues and prolidase levels at the early stage of hepatic fibrosis (14).

Myara et al. demonstrated that prolidase enzyme levels increased as the hepatic fibrosis developed due to liver damage via CCl4 infusion in rats (15).

In three different clinical studies, which have evaluated prolidase activities in steatohepatitis, alcoholic liver disease, chronic hepatitis C infection, and chronic liver disease, prolidase levels were shown to increase as the hepatic damage developed (16–19).

However, our study is the first trial to measure prolidase levels in CHB patients. In our study, increased prolidase activity was observed in chronic hepatitis infection, in which increased collagen turnover has already been known. Results have indicated that prolidase levels are higher in IHB infection than those in CHB.

Hepatic fibrosis or cirrhosis is caused by increased synthesis and storage or decreased breakdown of extracellular matrix elements, especially collagen. Liver biopsy is the most valuable method to show changes in the liver. Being an invasive method, it is not employed and it cannot be repeated to show increased fibrosis or the efficacy of antifibrotic treatment. In addition to this, measurements of serum connective tissue proteins and some enzymes can reflect the fibrosis in liver by showing progression of disease and efficacy of treatment on fibrosis (20).

In this present study, we evaluated whether prolidase can be employed as biologic parameters in CHB in order to indicate fibrosis. Results have indicated that there is no correlation between fibrosis and this enzyme. We believe that affecting factors can be moderate degree of chronic hepatitis, which constitutes the majority of participants; exclusion of patients with cirrhosis from the study; and similarities in histopathological evaluations of patients. In our opinion, a clear decision cannot be made unless studies evaluating cirrhotic patients will not be conducted.

We consider that prolidase has roles in the pathogenesis of hepatitis B; it can be employed as a parameter indicating chronicity of hepatitis B infection, and this fact should be supported by further large-sized population studies.

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