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Serum Prolidase Enzyme Activity and its Relation to Histopathological Findings in Patients with Non-Alcoholic Steatohepatitis

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The aim of this study was to investigate serum prolidase enzyme activity and to find out its association with liver biopsy specimens' histopathological findings in patients with nonalcoholic steatohepatitis (NASH), which may progress to liver fibrosis and cirrhosis. Thirty-six patients with biopsy-proven NASH and 29 healthy controls were enrolled. Serum prolidase enzyme activity was measured spectrophotometrically. Serum prolidase enzyme activity was significantly higher in patients with NASH than controls ($P = 0.016$). A significant correlation was observed between serum prolidase enzyme activity and fibrosis score in patients with NASH ($r = 0.661$, $P < 0.001$). Serum prolidase activity seems to be correlated with the level of fibrosis. Monitoring serum prolidase activity may be a useful adjunctive tool in predicting liver fibrosis, especially in the absence of advanced fibrosis and other conditions, which may affect the interpretation of prolidase activity. J. Clin. Lab. Anal. 24:207–211, 2010.

Key words: fatty liver; liver biopsy; fibrosis; dipeptidases

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) histologically resembles alcohol-induced liver damage and observed in patients without a history of significant alcohol consumption (1). Its prevalence is 2.1–6.3% in the general population, increasing to 9–40% in obese individuals with a body mass index (BMI) of 30 kg/m^2 or more (2). The pathogenesis of NASH seems to be multifactorial, including derangement in metabolic parameters, endotoxin-induced cytokine release, and oxidative stress (3,4,5). NASH can be progressive, cause fibrosis and cirrhosis, and can ultimately lead to liver failure and hepatocellular carcinoma in a minority of patients (2,6).

Prolidase is a manganese-requiring homodimeric iminodipeptidase, which releases carboxy-terminal proline or hydroxyproline from oligopeptides and was first identified in 1937 (7). Prolidase activity has been documented in erythrocytes, leukocytes, plasma, dermal fibroblasts, kidney, brain, heart, thymus, and uterus (8). It has been shown that prolidase enzyme activity was correlated with collagen turnover rate (9). In addition, prolidase enzyme activity has been also suggested to be increased in chronic viral hepatitis and alcoholic liver disease (10,11). However, little is known about serum prolidase enzyme activity and its relationship with the degree of fibrosis in patients with NASH (12).

Therefore, in this study, we aimed to investigate prolidase enzyme activity in patients with biopsy-proven NASH and to find out whether prolidase enzyme activity is correlated with histopathological findings in those NASH patients.

NASH, non-alcoholic steatohepatitis; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation.

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MATERIALS AND METHODS

Enrollment of Study Population

A total of 36 consecutive patients with biopsy-proven NASH and 29 healthy controls were enrolled in this study. The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989 and approved by the local research committee for ethics. All the subjects were informed about the study protocol and the written consent was obtained from each one.

Initial Evaluation

The major indications for liver biopsy in those 36 subjects were ultrasonographically diagnosed fatty liver and elevation in alanine aminotransferase (ALT). Diagnosis of NASH was made according to the following criteria:

- Existence of hepatic steatosis ($\geq 10\%$ of hepatocytes affected) with acinar zone 3 hepatocellular injury (ballooning degeneration), and/or lobular inflammation, with or without Mallory's hyaline and pericellular, and/or sinusoidal fibrosis on liver byopsy. Necroimflamatory grading and fibrosis scoring were based on a modification of the scoring system proposed by Brunt et al. (2).
- Negative serological markers for viral infection such as HBsAg or anti-HCV, and immunological disorders such as antinuclear anti-bodies, anti-smooth muscle antibodies, and anti-liver/kidney microsomes type 1 antibody.
- No evidence that favors metabolic liver disease such as Wilson's disease and hemochromatosis and α-1 antitrypsin deficiency.

Exclusion Criteria

Exclusion criteria included recent gastrointestinal bypass surgery, pregnancy, serum total bilirubin level higher than 2 mg/dl, usage of estrogen, tamoxifen, glucocorticoids, and methotrexate in the past 6 months, existence of coronary artery disease, rheumatoid arthritis, renal diseases, cancer, systemic or local infection, and history of excess alcohol ingestion averaging more than 30 g/day (three drinks per day) in the past ten years, or history of alcohol intake averaging greater than 10 g/day (one drink per day: seven drinks per week) in the past one year.

Blood Sample Collection

Blood samples were obtained following an overnight fasting state. Samples were withdrawn from an antecubital vein into blood tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min and they were analyzed.

Determination of Prolidase Activity

Serum was diluted 40 fold with 2.5 mmol/l Mn^{2+}, 40 mmol/l trizma HCl buffer (pH: 8.0) and preincubated at 37°C for 2 hr. The reaction mixture containing 30 mmol/l gly-pro, 40 mmol/l trizma HCl buffer (pH: 8.0), and 100 µl of preincubation serum in 1 ml was incubated at 37°C for 30 min. A total of 0.5 ml of 20% trichloroacetic acid solution was then added to stop the incubation reaction. The supernatant was used for measurement of proline by the method proposed by Myara et al. (11,13), which is a modification of Chinard's method (14). Intra-assay coefficient of variation of the assay was 3.8%.

Other Parameters

Serum aspartate aminotransferase (AST) and ALT level and lipid parameters were determined via an autoanalyzer (Aeroset®, Wiesbaden, Germany) using commercially available assay kits (Abbott®, Abbott Park, IL).

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Qualitative variables were assessed by χ^2 test. Continuous variables were compared using Student *t*-test. Pearson correlation analysis was used to find out the correlations of prolidase enzyme activity with ALT level. Spearman correlation analysis was used to find out the correlations of prolidase enzyme activity with liver biopsy specimens' histological findings. P value of less than 0.05 was regarded as significant.

RESULTS

The demographic and clinical data of study population are shown in Table 1.

There were no statistically significant differences between two groups in regard to age, gender, and BMI (all $P > 0.05$). Serum triglyceride ($P < 0.001$), serum cholesterol ($P = 0.025$), AST ($P < 0.001$), and ALT ($P < 0.001$) levels, and serum prolidase enzyme activity ($P = 0.016$) were significantly higher in patients with NASH than controls.

Necroimflamatory grades and fibrosis scores of the 36 subjects with NASH were as follows:

- Grade 1 (mild) necroimflamatory changes, in 14 subjects; grade 2 necroimflamatory changes (moderate), in 16 subjects; grade 3 (severe) necroimflamatory changes, in 6 subjects.

TABLE 1. The Clinical and Demographic Data of the Study Groups

	Patients with NASH	Control subjects	*P* values
Age (years)	42.1±7	39.1±7	NS
Gender (M/F)	23/13	19/10	NS
BMI (kg/m^2)	28.4±3	28.1±2.3	NS
Triglyceride	225±83.5*	149.8±42.4	<0.001
Cholesterol	198±44.5*	171±25.1	0.025
ALT (U/l)	77.8±38.4*	23.7±5.9	<0.001
AST (U/l)	52.1±29.2	21.3±3.9	<0.001
AST/ALT	0.78±0.29	0.92±0.25**	0.042
Prolidase (U/l)	99.8±19*	80.7±16	0.016

Data were presented as mean±SD. NASH, non-alcoholic steatohepatitis; ALT, alanine aminotransferase; BMI, body mass index; NS, non-significant. *$P<0.05$ vs. Controls; **$P<0.05$ vs. Patients with NASH.

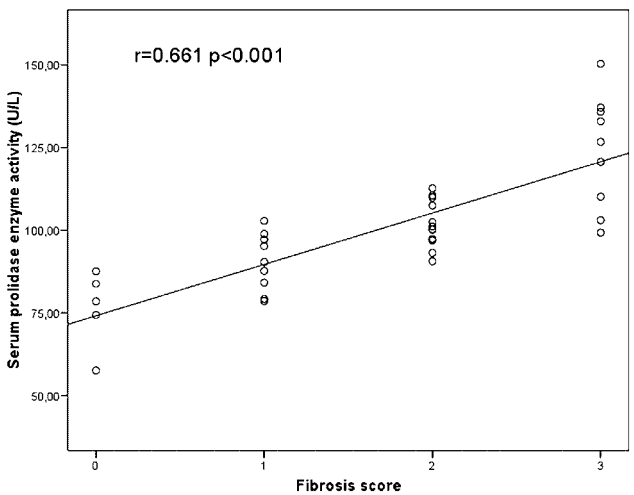

Fig. 1. The correlation of serum prolidase enzyme activity with liver fibrosis score in patients with non-alcoholic steatohepatitis.

- Stage 0 Zone 3 perisinusoidal/pericellular fibrosis, in 5 subjects; stage 1, in nine subjects; stage 2, in 13 subjects; stage 3, in 9 subjects.
- None of the subjects with NASH had stage 4 fibrosis.

Spearman correlation analysis revealed a significant correlation between serum prolidase enzyme activity and fibrosis score in patients with NASH ($r = 0.661$, $P<0.001$) (Fig. 1). However, serum prolidase enzyme activity was not correlated with neither AST and ALT levels nor AST/ALT ratio in patients with NASH ($P>0.05$). Furthermore, neither fibrosis score nor necroinflammatory grades was correlated with serum AST and ALT levels, or AST/ALT ratio in patients with NASH (all $P>0.05$).

DISCUSSION

In this study, we observed that serum prolidase enzyme activity is significantly higher in patients with NASH than healthy controls, and significantly associated with liver biopsy specimens' histopathological findings in those patients with NASH.

The value of laboratory tests to diagnose liver fibrosis is limited. Thus, liver biopsy is still the gold standard method for evaluating the severity of liver fibrosis and cirrhosis (15). However, liver biopsy is an invasive procedure and may cause potential complications including bleeding, pneumothorax, and perforation of colon or gallbladder (16). Thus, it is difficult to perform liver biopsy for all patients who need to be assessed repeatedly. In addition, biopsy samples are usually too small to diagnose the disease accurately and diagnostic opinions often differ among pathologists (17). As a result of these limitations, there is a need for simple, inexpensive, and reliable noninvasive monitoring methods for evaluating the severity of hepatic fibrosis.

In recent years, many noninvasive tests, e.g., AST/ALT ratio, AST/platelet ratio index, hyaluronic acid, YKL-40, N-terminal propeptide of type III collagen, FibroTest, SteatoTest, and NashTest (18–25), have been evaluated in clinical practice to replace liver biopsy for the assessment of the degree of fibrosis in patients with chronic liver disease due either to chronic viral hepatitis or NASH. Besides these markers, transient elastography, 13C-caffeine breath test, and DNA sequenced-based serum protein glycomics have also been proposed for the evaluation of liver fibrosis in chronic liver diseases (26–28). However, many of these tests are not widely available, and nonspecifically elevated in the presence of various circumstances. Thus, the recent trend is the use a combination of these tests in order to improve the accuracy of fibrosis prediction (29).

Prolidase plays an important role in the recycling of proline for collagen synthesis and cell growth (9). Prolidase enzyme activity increases in important quantities during collagen biosynthesis (30). In only few studies, serum prolidase enzyme activity has been investigated in patients with chronic liver diseases. Myara et al. (11) investigated serum prolidase enzyme

activity in patients with chronic hepatitis and cirrhosis. All the patients with high prolidase activity had chronic liver diseases in the study of Myara et al. (11). However, of 27 patients with biopsy-proven cirrhosis, they found increased prolidase activity only in 5 patients. Thus, they speculated that plasma prolidase activity might be high in the early stage of fibrosis and might subsequently drop in advanced fibrosis. Furthermore, Brosset et al. (10) investigated prolidase enzyme activity in patients with alcoholic liver disease and they observed that cirrhotic patients with alcoholic hepatitis had significantly higher enzyme activity in comparison with those without alcoholic hepatitis. Besides chronic liver diseases, recently, we showed that serum prolidase enzyme activity may be affected in patients with *Helicobacter pylori* infection and in patients with bronchial asthma (31,32).

In this study, in an attempt to search for a suitable marker for the prediction of liver fibrosis severity in patients with NASH, we measured the serum prolidase activity in these patients. We observed a significant increase in serum prolidase enzyme activity in patients with NASH compared with healthy controls and a significant correlation of serum prolidase enzyme activity with fibrosis score determined on liver biopsy specimens. However, as none of the patients in this study had cirrhosis, we were unable to evaluate prolidase activity in patients with cirrhosis due to NASH.

Data related to serum prolidase enzyme activity and its usefulness in evaluating patients with NASH are limited to a recently published study of Kayadibi et al. (12). In accordance with our findings, they also reported a significant association for serum prolidase enzyme activity with stage of fibrosis. In contrast to our findings, they also reported a nearly significant association between AST/ALT ratio and stage of fibrosis, and a significant association between AST or ALT levels and lobular inflammation. It is well known that serum AST and ALT are elevated in almost 90% of patients (33), as also observed in our patients. However, normal serum aminotransferases do not exclude the presence of advanced histologic features, as also illustrated in a study of Mofrad et al. (34). They included 51 patients with a fatty liver and a normal ALT who had undergone a liver biopsy, and they found a total of 12 subjects had bridging fibrosis, while 6 had cirrhosis (34). Combining with the results of this study, it can be suggested that clinical utility of serum aminotransferases levels and AST/ALT ratio seems to be limited in the prediction of liver histological changes in patients with NASH.

In conclusion, although serum prolidase activity is not specific for liver fibrosis and may be affected from other conditions, it seems to be correlated with the level of fibrosis. Monitoring serum prolidase activity may be a useful adjunctive tool in predicting liver fibrosis, especially in the absence of advanced fibrosis and other conditions, which may affect the interpretation of prolidase activity.

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