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Evulation of prolidase enzyme, and galectin levels as a marker for fibrosis in patients with chronic hepatitis B

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Abstract. – OBJECTIVE: The fibrosis can be detected using non-invasive methods including prolidase activity, proline levels and galectin-3 (GAL-3) detection in the serum. The aim of this study was to investigate the liver fibrosis through non-invasive methods in chronic hepatitis B patients.

PATIENTS AND METHODS: This prospective case control study includes 56 patients with Chronic Active Hepatitis B (CAHB), 57 patients with Inactive Hepatitis B (IHB), and 60 healthy matched control subjects. The first group included the CAHB [hepatitis B surface antigen (HBsAg): positive; HBV DNA >2,000 IU/mL; normal or high alanine aminotransferase (ALT) value] undergo a liver biopsy, while the second group included the IHB (HBsAg: positive; HBV DNA: negative; normal ALT value). The third group comprised the healthy controls. Serum prolidase enzyme activities (SPEA), proline and galectin-3 levels were measured for each group.

RESULTS: Patients with CAHB had significantly higher SPEA levels (1,004.3±186.8 IU/L) than did the controls (196.5±306 IU/L) (p<0.001). Significantly higher serum GAL-3 levels were found in the CHB group compared with HBV carrier and the control groups (27.4±32.2 ng/mL, 6.5±13.4 ng/mL, 3.1±5.7 ng/mL, respectively, p<0.001). The relationship between serum prolidase activity, hidroxiprolyne and fibrosis (p<0.05). There were no significant differences in ALT levels between inactive HBV carriers and the control groups (p>0.05).

CONCLUSIONS: We suppose that hidroxiprolyne levels and prolidase enzyme activity might be an indicator as a marker for fibrosis in CAHB and the evaluation of response to treatment.

Key Words:
Prolidase enzyme, Galectin, proline, Chronic hepatitis B.

Introduction

The most important complications of the hepatitis B virus infection are chronic hepatitis and cirrhosis. The severity of the disease is closely related to the degree of fibrosis in chronic hepatitis B infection[1]. Definitive diagnosis and degree of fibrosis can only be determined by histological examination. Biochemical tests used in the follow-up of chronic hepatitis are insufficient to reveal the level of fibrosis[2]. Histological examination methods can lead to difficulties in the follow-up of patients with chronic hepatitis B, as they are both invasive and the method has its own difficulties. Therefore, various tests that are non-invasive and can give faster results in the follow-up of fibrosis development have been the subject of research[3,4]. For this purpose, in many studies[1], the structures forming the extracellular matrix and the mediators involved in the development of fibrosis have been investigated and their relations with fibrosis have been revealed. Prolidase enzyme is an enzyme that occur after collagen degradation and is responsible for the degradation of immuno peptides[5-10]. The increase in collagen production and destruction processes in the fibrosis stage causes an increase in galectin-3 and prolidase enzyme activity[2,3]. Studies have shown that there is a relationship between

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serum prolidase enzyme and galectin-3 levels and fibrotic activity[6-9]. The aim of this study is to determine the correlation of serum prolidase activity, hydroxyprolyne, GAL-3 and total oxidative stress-total antioxidant capacity measurements with liver biopsy in the follow-up of fibrotic activity as a non-invasive method in chronic hepatitis B patients. Thus, our goal was collecting information about the prognosis of the disease by determining the degree of fibrosis of the disease in patients who cannot have a liver biopsy.

Patients and Methods

A total of 173 patient (57 Chronic Active Hepatitis B (CAHB), 56 inactive hepatitis B (IHB), and 60 healthy controls) who were followed up with at the Infectious Diseases Clinics of Dicle University Hospital were included in this study. The diagnosis of CAHB infection was based on the following: persistent or intermittently elevated liver enzymes, serum alanine aminotransferase (ALT) greater than the normal value (normal ALT value is less than 40 IU/mL), a positive hepatitis B surface antigen (HBsAg) test result for more than 6 months, and serum HBV DNA>2,000 IU/mL. Liver biopsy was performed in all CAHB patients. It was not performed in other groups. Histopathologic evaluations were performed according to modified Knodell system that was proposed by Ishak et al[11]. Histologic activity index (HAI) and fibrosis levels were recorded. Patients with HBsAg positive, HBV DNA negative or <2,000 IU/mL, normal ALT and aspartate aminotransferase (AST) values were included in the study as inactive hepatitis B carriers. Patients in the control group had normal medical histories, physical examinations and blood biochemistry. None of these patients had positive hepatitis B serum markers.

In addition, patients presented as acute liver injury other than viral etiology such as alcoholic hepatitis, toxic hepatitis, autoimmune hepatitis, genetic disorders including Wilson's disease, biliary diseases, cirrhosis, and pregnant women were excluded.

HBV DNA was tested by the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v. 2.0 by Roche molecular systems (Pleasanton, CA, USA).

Measurement of Serum Prolidase Activity

Serum prolidase enzyme activity was determined using a commercially available quantitative enzyme-linked immune sorbent assay (ELISA) technique (Hangzhou Eastbiopharm Company, Hangzhou, China) according to the manufacturer's instructions. Serum prolidase enzyme activity levels were shown as 10 U/L. The lowest mean detection limit for prolidase level was 10 U/L.

Measurement of Serum Hydroxyproline

Serum hydroxyproline levels were measured by using a commercial quantitative enzyme-linked immune sorbent assay (ELISA) (Eastbiopharm Company, Hangzhou, China) according to the manufacturer's instructions. Serum hydroxyproline levels were shown as nmol/L. The lowest mean detection limit for hydroxyproline level was 10 nmol/L.

Measurement of Serum GAL-3

Serum GAL-3 levels were measured by using a commercial quantitative enzyme-linked immune sorbent assay (ELISA) (Eastbiopharm Company, Hangzhou, China) according to the manufacturer's instructions. Serum GAL-3 levels were shown as ng/mL. The lowest mean detection limit for GAL-3 level was 0.015 ng/mL.

Measurement of Total Antioxidant Capacity (TAC)

TAC of the supernatant fractions was determined using a novel automated measurement method developed by Erel[12]. In this method, the antioxidative effect of the sample against potent-free radical reactions (initiated by a hydroxyl radical) is measured. The results are expressed as μmol Trolox Eq./L.

Measurement of Total Oxidant Activity (TOA)

TOA of supernatant fractions was determined using a novel automated measurement method developed by Erel[13]. The assay is calibrated with hydrogen peroxide and the results are expressed as μmol H_2O_2 Eq./L.

The approval of the Dicle University Medical Faculty, Non-Invasive Clinical Studies Ethics Committee was obtained for the study.

Statistical Analysis

The calculations were performed using the Statistical Package for Social Sciences software version 22.0 (SPSS, IBM Corp., Armonk, NY, USA) for Windows. The Kolmogorov-Smirnov test was used to confirm that data were within the ranges of normal distribution in both groups.

Table I. Prolidase activity and Galectin-3 level in the study groups (mean ± standard deviation).

	Chronic active hepatitis B (n = 56)	Inactive hepatitis B (n = 57)	Controls (n = 60)	*p
SPEA (U/L)	1,004.3 ± 186.8[a,e]	221.3 ± 164.3	196.5 ± 306	< 0.001
Hidroxiprolyne	1,251.8 ± 513.6[a,h]	324.6 ± 333.5	199.5 ± 267.7	< 0.001
Galectin-3 (µm/L)	27.4 ± 32.2[a,h]	6.5 ± 13.4	3.1 ± 5.7	< 0.001
ALT (U/L)	48.7 ± 34[a]	32.2 ± 18.5	25.6 ± 7.6	0.001
AST (U/L)	37 ± 19.2[a]	26.9 ± 13.8	24.2 ± 7.7	< 0.001
TAC (µmol Trolox Eq./L)	1.48 ± 0.22[b]	1.46 ± 0.19	1.40 ± 0.15	0.015
TOS (µmol H_2O_2 Eq./L)	106.9 ± 128[c,f]	59.1 ± 71.5	110.8 ± 93.5	0.001

CAHB: Chronic Active Hepatitis B, IHB: Inactive Hepatitis B, SPEA: Serum prolidase enzyme activity, TAC: Total antioxidant capacity, TOS: Total oxidant status. *Difference between three groups with Kruskal-Wallis' test. Differences between pairwise groups with Mann-Whitney U-test: [a]Compared with group control ($p < 0.001$). [b]Compared with group control ($p = 0.004$). [c]Compared with group control ($p = 0.005$). [d]Compared with group control ($p = 0.002$). [e]Compared with group IHB ($p < 0.001$). [f]Compared with group IHB ($p = 0.001$). [g]Compared with group IHB ($p < 0.001$). [h]Compared with group IHB ($p < 0.001$).

A nonparametric test was employed for the variables outside the normal distribution. The comparison of the data between reciprocal groups was carried out through the Mann-Whitney U and Chi-square test. Pearson's correlation analysis was used to find out the correlations of prolidase enzyme activity with fibrosis. Spearman's correlation analysis was used to find out the correlations of prolidase enzyme activity with liver biopsy specimens' histological findings. Statistical significance was based on a value of $p<0.05$ with a 95% confidence interval.

Results

The study group included 57 CAHB patients (30 male/27 female), 56 IHB patients (33 male/23 female) and 60 healthy adult (32 male/28 female). There was no statistically significant difference between the groups in terms of the mean age and gender (p=0.410 and p=0.155, respectively).

The laboratory data of study population are shown in Table I. Patients with CAHB had significantly higher SPEA levels (1,004.3±186.8 IU/L) than did the controls (196.5±306 IU/L) (p<0.001). The relationship between serum prolidase activity, hidroxiprolyne levels and fibrosis score (p<0.05) (Figure 1). There was a statistically significant correlation of the serum prolidase activity, hidroxiprolyne level and the fibrosis score (r=0.391, r=0.369, p<0.05), and the fibrosis score was observed to increase together with the serum prolidase activity (Figure 2). Significantly higher serum GAL-3 levels were found in the CAHB group compared with IHB and the control groups (27.4±32.2 ng/mL, 6.5±13.4 ng/mL,

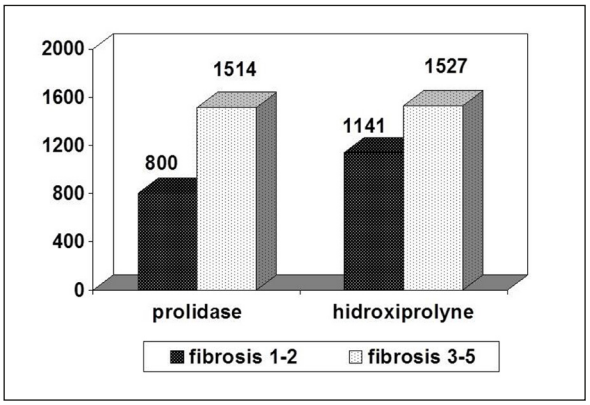

Figure 1. The relationship between serum prolidase activity, hidroxiprolyne levels and fibrosis score.

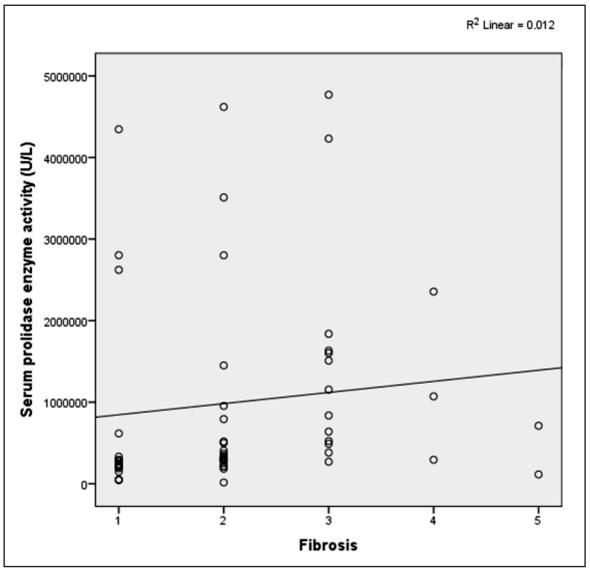

Figure 2. The relationship between serum prolidase activity and fibrosis (r=0.391, $p < 0.05$).

3.1±5.7 ng/mL, respectively, $p<0.001$). There was no relationship between the GAL-3 value and the fibrosis score. The GAL-3 value increase was not correlated with the fibrosis score increase. There were no significant differences in ALT levels between IHB and the control groups ($p>0.05$).

In cases of CAHB, no correlation was detected between the patient's fibrosis levels and the prolidase values and the total antioxidant status (TAS) ($p=0.015$). TAC and total oxidant status (TOS) was found higher in CAHB than IHB and control groups.

Discussion

There is no effective method rather than biopsy to understand the severity and fibrosis of the disease in chronic hepatitis B. In recent years, it has become increasingly important to determine the degree of fibrosis in chronic liver diseases, especially in patients who cannot or do not want to have a liver biopsy, by looking at various markers in the serum sample. For this purpose, studies[14-16] have been carried out on various molecules and enzymes, to have knowledge about the molecular involved in liver fibrosis in chronic hepatitis B. This study aimed at finding information about the prognosis of the disease, especially in patients for whom liver biopsy could not be performed, and to determine the degree of fibrosis by looking at the markers indicated in the serum sample in these patients. Thus, without an invasive procedure, fibrosis will be detected with faster methods and economic costs will be reduced.

Proline is a significant molecule involved in collagen production and it is not yet fully understood how it takes part in fibrosis in chronic liver diseases. It is thought that there is a relationship between proline level and fibrosis in chronic hepatitis B[17]. Ilhan et al[18] found a positive correlation between fibrosis level and prolidasis levels at the beginning of treatment. It was concluded that prolidase levels decreased significantly in the 12th month of treatment and that there were useful tests for monitoring fibrotic activity, on the other hand other parameters may be useful tests for monitoring treatment. Our results have shown positive correlation between prolidase enzyme activity and hidroxiprolyne levels and high levels of fibrosis. Furthermore, Horoz et al[19] investigated prolidase enzyme activity in patients with non-alcoholic steatohepatitis and they observed that non-alcoholic steatohepatitis patients had significantly higher enzyme activity in comparison with healthy control groups. Recently, Nazligül et al[20] investigated serum prolidase enzyme activity in patients with chronic viral hepatitis, and they founded serum prolidase enzyme activity was significantly higher in patients with chronic viral hepatitis than healthy control groups. We found increased prolidase enzyme activity and hidroxiprolyne levels in CAHB. Thus, we considered that serum prolidase anzyme activity could be a useful noninvasive diagnostic marker in routine clinical practice for determining the patients with CAHB.

GAL-3 may contribute to some cellular processing which may result in fibrosis development, cancer progression, and tissue remodeling[21]. This situation has a key role especially in diseases such as chronic hepatitis B. Studies[21-22] have suggested that galectin may be associated with the development of fibrosis and the severity of the disease. In the study by Henderson et al[22] the serum GAL-3 levels and the fibrosis of the liver were compared in rat model of reversible carbon tetrachloride (CCL_4) induced liver fibrosis. According to the results of this study, the serum GAL-3 levels were correlated with the fibrosis score of the liver and the GAL-3 levels increased together with the fibrosis level. Uluca et al[23] investigated serum GAL-3 levels in children with chronic viral hepatitis, and they founded GAL-3 levels were significantly higher in patients with chronic viral hepatitis than healthy control groups. We founded GAL-3 was significantly higher in patients with CAHB than IHB and healthy control groups. In our study, no correlation was observed between the serum GAL-3 levels and the fibrosis score. This result was attributed to the limited number of patients in our study.

TOS and TAC are important biochemical markers for determining oxidative status[12,13]. Yamamoto et al[24] found that high oxidative stress was combined with necroinflammation in CAH, cirrhosis and hepatocellular cancer cases. According to our results, increased TAC value and decreased TOS level in CAH cases confirm that oxidative stress is high in the cell and cell destruction with necro inflation.

Conclusions

In line with the results to be obtained by comparing the various methods used for fibrosis, we aimed to determine the most appropriate fibrosis

detection method that provides fast, accurate and reliable results that we can use in the clinic, which is practical and easy to apply and has cost-effective properties, and to find an alternative method in patients who cannot be biopsied. Proline and prolidase enzyme activity might be an indicator of fibrosis, follow-up, and the evaluation of response to treatment. In summary, the level of prolidase is closely correlated to the fibrosis of hepatitis patients, and this can be used to evaluate and predict the fibrosis of patients.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval
The study has been approved by the Medical Ethics Committee of the Dicle University Medical Faculty.

Authors' Contribution
Tekin R, Mermutluoglu C, Tekin S and Deveci O designed the meta-analysis; Canpolat Erkan RE, Tekin R and Ceylan Tekin R searched the literature; Aydogdu G and Celen MK analyzed the literature; Tekin R and Dayan S wrote and edited the manuscript.

Availability of Data and Materials
The datasets are available from the corresponding author on reasonable request.

Informed Consent
Patient consent forms were provided.

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