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ORIGINAL ARTICLE

Antithrombin-III as a Non-Invasive Marker of Chronic Liver Disease

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Background and Aims: **Recently, many studies have reported that the plasma concentrations of natural anticoagulants, such as Antithrombin-III (AT-III), are altered in patients with chronic liver disease. In addition, the changes in the synthesis of AT-III occur in liver tissue and are associated with the extent of chronic hepatitis and cirrhosis.**

Methods: **In this study, we analyzed the plasma level of AT-III and serum activity of aminotransferase in 60 participants: 20 patients with chronic hepatitis, 20 patients with cirrhosis, and 20 healthy individuals (controls).**

Results: **Low levels of AT-III and elevated levels of aminotransferase activity were associated with both chronic hepatitis and cirrhosis. We found that among patients with elevated gamma glutamyl transferase (GGT) activity and chronic liver disease, the level of AT-III in plasma was significantly lower in patients with chronic cirrhosis than in patients with chronic hepatitis ($P < 0.05$) and the level of AT-III in plasma was lower in patients with liver disease in comparison to healthy participants ($P < 0.001$).**

Conclusions: **The level of AT-III in patients with chronic liver disease may be used as a non-invasive factor for the laboratory diagnosis of cirrhosis.**

Keywords: **Chronic Liver Disease, Antithrombin-III, Hepatitis, Cirrhosis**

Introduction

Chronic hepatitis is the most common cause of cirrhosis [1]. Knowledge of the presence of cirrhosis is important for the management of patients with chronic hepatitis. Hepatitis C is a major cause of liver-related morbidity and mortality worldwide, represents a major public health problem [2], and is often associated with complex defects in humeral homeostasis. Virtually all patients with advanced liver disease have coagulopathies due to dysfunction of hepatic synthesis because many components of clotting factors are synthesizing in the liver tissue [3]. Antithrombin-III (AT-III) is a natural anticoagulant that is synthesized exclusively in the parenchymal cells of the liver [4, 5].

AT-III neutralizes thrombin and several other activated serine proteases of the coagulation system [6]. Deficiencies of AT-III can be hereditary or acquired. The hereditary pattern of AT-III deficiency is autosomal dominant and patients are generally heterozygous [7]. Acquired deficiency of AT-III can be caused by decreased synthesis due to damage to hepatic cells [8] and reduced transcapillary flux ratios [9]. Plasma concentrations of this physiological inhibitor of the coagulation system are low in severe chronic liver disease [10]. In addition, thromboembolism and disseminated intravascular coagulation (DIC) may occur in patients with AT-III deficiency [11, 12]. Therefore, determining the level of AT-III may be clinically useful in these patients for monitoring coagulopathies and making a differential diagnosis between chronic liver diseases. Often an elevation of one or more of the enzymes included in

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a screening panel is the first indication of asymptomatic liver disease. Even though the composition of liver function panels may differ between institutions, these panels typically include the following enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and possibly gamma glutamyl transferase (GGT). We studied the statistical difference of the levels of AT-III and transaminase activity in patients with chronic hepatitis, chronic cirrhosis, and no known liver disease to determine the utility of these markers in making differential diagnoses.

Materials and Methods

The present study included 60 patients who were 30 years of age or older. Patients were categorized into three groups: 20 patients with chronic hepatitis (Group I), 20 patients with cirrhosis (Group II), and 20 healthy participants (Group III). None of the patients received anticoagulant therapy. The local ethics committee approved the study. All samples from the Department of Gastroenterology, Shalimov Institute, Kiev, Ukraine were tested at the biochemical laboratory for AT-III, ALT, AST, and GGT activity. For all patients, hepatic infection was tested for with enzyme-linked immunosorbent assay (ELISA) assays and liver biopsies (Ishak Fibrosis Score). Plasma and serum were obtained from fasting blood samples drawn by venipuncture with Vacutainer® tubes; specifically, plasma was obtained from whole blood, which was collected by drawing into heparin zed tubes, and serum was obtained from blood clotting and incubated for 2 hours at 37°C prior to centrifugation and subsequent determination of aminotransferase activity.

AT-III and aminotransferase activity were then measured using commercially available assays. The AT-III level was determined by using a chromogenic substrate method with a reactive test standard (Technology Standard, Barnaul, Russia). In this assay, thrombin is first added to a plasma dilution containing excess heparin; after incubation, a thrombin-specific chromogenic substrate is used to determine AT-III concentration by a photometric method at 405 nm by a photometer analyzer (Screen Master Plus, Germany) [13]. The activity of transeaminases was determined with a reactive test standard (Cormay, Lublin, Poland). The GGT activity was determined by a kinetic method [14] with L-glutamyl-3-carboxy-4-nitroanalid and glycylglycine at 37°C; the rate that the absorbance changed at 405 nm was measured by a biochemical analyzer (Perestige 24 I, Tokyo, Japan) and is directly proportional to GGT activity. ALT and AST activity were determined by an optimized, modified method according to the International Federation of Clinical Chemistry (IFCC); the method did not include pyridoxal phosphate, the rate that the absorbance changed was measured at 340 nm.

The values of each parameter in each group were expressed as parameter averages, accounting for the standard deviation (mean ± SD). We ran a two-sample test and assumed a normal distribution. The statistical analysis was performed using the Statistica Instat plus v3.36 for windows XP professional (Tulsa-OK 74104, USA). $P < 0.05$ was considered significant.

Results

The mean age of the patients in Group I (8 males and 12 females) was 44.8 ± 9.2 (range: 35-60), in Group II (11 males and 9 females) was 43 ± 5 (range: 38-63), and in Group III (10 males and 10 females) was 45.8 ± 5.4 (range: 38-52). The levels of AT-III and transaminase activity for the three study groups are presented in Table 1. Based on the reference values by the laboratory, the normal ranges were 75 to140% for AT-III, 7 to 50 IU/I for GGT, 32 to 42 IU/U for ALT, and 31 to 37 IU/U for AST.

The levels of AT-III and GGT activity were significantly different in the study groups compared to the healthy participants. AST activity was only significantly different from the control group for Group II. AT-III levels were significantly different between Group I and Group II ($P < 0.05$). Table 2 shows the statistical differences between the study groups.

Discussion

A clinical model based on standard laboratory tests that could accurately detect the presence of cirrhosis would be useful and could reduce the requirement for liver biopsy in clinical practice. Current models to predict cirrhosis have relied upon a combination of clinical features, serum biochemical tests, an array of fibrosis markers, radiological studies, and other measures of hepatic function [15, 16]. Liver biopsy is the standard method used for the assessment of cirrhosis. However, biopsy is invasive and costly and is associated with patient discomfort and risk of major complications, including death [17, 18]. Thus, the need exists for a noninvasive, inexpensive, and accurate method for diagnosing cirrhosis [16]. We evaluated the association between AT-III levels and

Table 1. Laboratory test measurements for each group of patients.

Characteristics	Chronic hepatitis (n = 20)	Cirrhosis (n = 20)	Control (n = 20)
Ishak Fibrosis Score	0	5-6	---
ALT (IU/I)	66.8 ± 11.6	64.0 ± 10.7	61.4 ± 3.4
AST (IU/I)	73.0 ± 28.6	71.6 ± 22.4	61.4 ± 3.4
GGT (IU/I)	97.0 ± 16.3	182.8 ± 14.2	10.5 ± 2.6
AT-III (%)	70.4 ± 2.4	61.4 ± 3.4	97.2 ± 1.5

Data are presented as the mean ± the standard deviation (SD).

Table 2. Statistical tests of means against the reference group.

Characteristics	P value for Group I	P value for Group II
ALT	0.457	0.974
AST	0.026*	0.035*
GGT	0.000*	0.000*
AT-III	0.007*	0.008*

*P values of 0.05 or less are considered statistically significant.

serum aminotransferase activity in patients with chronic liver disease to determine whether these biochemical markers could be used in the diagnosis of fibrosis.

Many patients with chronic liver disease have coagulopathy [13] because the majority of the liver-coagulation factors are adversely affected [19]. The extent of coagulation abnormalities due to natural anticoagulants like AT-III depends upon the degree of altered liver function. Natural anticoagulants are intimately related to liver function [4] and AT-III is only synthesized by liver tissue [4, 5]. Acute or chronic liver diseases may decrease the concentration of AT-III [20]. Several studies have investigated the reduction of AT-III in chronic liver disease [21-24]. A plasma concentration of AT-III is too low in cirrhosis [25]. Kont *et al.* concluded that altered plasma concentration of AT-III in cirrhosis was due to reduced transcapillary flux ratios in his sample [26]. It is clear that damage to hepatic tissue, particularly damage to the endothelium, triggers the included inflammation [10] and upsetting of the physiological anticoagulant mechanism [27]. Our data show that patients with chronic hepatitis tend to have cirrhosis leading to decreased levels of AT-III (Table 2). We have suggested AT-III as a marker for fibrosis, but this hypothesis should be tested in future studies. The mechanisms behind the diminution of AT-III in liver disease are complex, but insufficient hepatic synthesis, an altered transcapillary flux ratio, and a low diffuseness DIC [12] may take part in the process.

When tissue damage occurs, cellular enzymes may be released into the serum, and the elevation of certain enzymes is often associated with damage to specific tissue or organs. Although the enzymes previously mentioned are present in tissues throughout the body, their elevation is most often associated with liver injury or disease. Elevation of aminotransferases like AST and ALT often reflect hepatocellular damage. GGT is present in decreasing concentrations in the kidney, liver, pancreas, and intestines, and elevations have been reported in several clinical conditions including pancreatic disease, myocardial infarction, renal failure, chronic obstructive pulmonary disease, rheumatoid arthritis, hyperthyroidism, congestive heart failure, diabetes, and alcoholism. In this study, the GGT activity in serum was significantly lower in patients with chronic cirrhosis than in patients with chronic hepatitis (Table 2), although the National Academy of Clinical Biochemistry (NACB) guidelines do not recommend routine use of GGT because of its low

predictive value of 32% for liver disease. GGT is very sensitive to ingestion of alcohol and many prescription and non-prescription drugs, including non-steroidal anti-inflammatory drugs, lipid-lowering drugs, antibiotics, antiepileptic drugs, antifungal agents, and antidepressants. Even small amounts of alcohol ingested 24 hours prior to the test may cause a temporarily elevated GGT.

ALT activity was not significantly different in patients with cirrhosis and chronic hepatitis compared to the control group; however, according to the NACB, the lack of a significant finding for ALT could be due to low specificity of the test and the lack of availability of a more specific alternative biomarker of necrosis. The activity of AST in study groups was significantly different in patients with cirrhosis and hepatitis compared to the healthy participants. AST can be elevated in patients with skeletal muscle disease, pulmonary emboli, hepatic disease, and also patients who have had intramuscular injection [28]. Therefore, the AT-III level in patients who have chronic liver disease and a high level of GGT activity due to tissue necrosis damage may be used as a non-invasive, inexpensive, and accurate method for the diagnosis of cirrhosis and prevention monitoring of these patients, provided they are not taking anticoagulants.

Conclusions

Decreased levels of plasma AT-III and increased levels of serum GGT activity are present in patients with chronic liver disease. The concentration of AT-III in patients with high GGT and AST activity was significantly lower for patients with cirrhosis than for patients with chronic hepatitis. Therefore, determining the levels of AT-III and aminotransferase activity in patients with liver disease may be used for differential diagnoses and the monitoring of disease progression.

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