Antithrombin III as a criteria marker in chronic liver disease.

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Citation

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Abstract

Recently, many studies have reported that the plasma concentrations of natural anticoagulants, such as antithrombin III (AT III), are altered in chronic liver diseases. In addition, the changes in synthesis of AT III occur in liver tissue and are associated with the extent of chronic hepatitis and cirrhosis. In this study, we analyzed the plasma level of III and serum activity of aminotransferase in 60 participants: 20 patients with chronic hepatitis, 20 patients with cirrhosis, and 20 healthy individuals (control). Low levels of AT III and elevated levels of aminotransferase activity were associated with both chronic hepatitis and cirrhosis (P< 0.05). We found that among patients with elevated GGT activity and chronic liver disease, the level of III in plasma is significantly lower in patients with chronic cirrhosis than in patients with chronic hepatitis (P< 0.05) and the level of III in plasma was lower in patients with liver disease in comparison to those in healthy participants (P< 0.05).. Therefore, ATIII level in patients with chronic liver disease may be used as a exist factor for cirrhosis laboratory diagnosis.

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List of Abbreviations

AT III - Anti Thrombin III
ALT - Alanin Aminotransferase
AST - Aspartate Aminotransferase
GGT - ?-Glutamiltransfrease
DIC - Disseminated Intravascular Coagulation

INTRODUCTION

Chronic hepatitis is the most common cause of cirrhosis (Liang TJ et al, 2000), knowledge of the presence of cirrhosis is important for the management of patients with chronic hepatitis. Chronic liver disease is a major cause of morbidity and mortality in many countries and is often associated with complex defects in humeral homeostasis. Virtually all patients with liver advance disease have coagulopathies due to dysfunction of hepatic synthesis because many components of clotting factors are synthesizing in liver tissue (Mammen.EF, 1992). Antithrombin III (AT III) is a natural anticoagulant that is synthesized exclusively in parenchymal cells of the liver (Leon M et al ,1983, Ewa Marciniak et al ,1974). AT III neutralizes thrombin and several other activated serine proteases of the coagulation system (Saxena V et al, 2004). Deficiencies of III can be hereditary or acquired. The hereditary pattern of AT III deficiency is autosomal dominant and patients are generally heterozygous (Jesty J, 1979). Acquired deficiency of III can be caused by decreased synthesis due to damage to hepatic cells (Kaul V V et al, 2000) and reduced transcapillary flux ratios (Müller G, 1992). Plasma concentrations of this physiological inhibitor of the coagulation system are low in severe chronic liver disease (Wada H et al, 1993). In addition, thromboembolism and disseminated intravascular intravascular coagulation (DIC) may occur in patients with AT III deficiency (Maly J et al , 1997, Barkagan Z.C ,2002, Sheikh Sajjadieh. M.R, 2008). 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 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), Alanin aminotransferase (ALT), and possibly gamma glutamyl transferase (GGT).

We studied the correlation between of the levels of ATIII and transaminases 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 subjects did have to be older than 30 years. the study group consisted three groups ,of 20 patients with chronic hepatitis C (group I), 20 patients with cirrhosis due to viral infection (group II) , and 20 healthy participants (group III) . None of the patients were receiving anticoagulant therapy. The local ethics committee approved the study. All samples from department of Gastroenterology of Shalimov Institute (Kiev, Ukraine) were tested at biochemical laboratory for AT III and ALT, AST and GGT activity. Hepatic infection in group I, approved by Elisa assay and cirrhosis in group II detected by liver biopsy. Plasma and serum were obtained from fasting blood samples drawn by venipuncture with Vacutainer® tubes , plasma was obtained from whole blood which collected by drawing into heparin zed tube and serum was obtained from blood clotting and incubated for 2 hr at 37°C prior to centrifugation and subsequent determination of aminotransferases activity.

AT III and aminotransferases activity were then measured using commercially available assays.

The III level was determined by using a chromogenic substrate method with a reactive test-standard (company 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) (Burtis C.A et al,1999). The activity of transeaminases was determined with reactive test standard (company Cormay, Lublin, Poland). The activity GGT was determined by a kinetic method (Flute PT, 1977) with L-glutamyl-3-carboxy-4-nitroanalid and glycylglycine at 37°C; the rate that the absorbance changes at 405 nm is measured by a biochemical analyzer (Perestige 24 I, Tokyo, Japan) and is directly proportional to GGT activity. The activity of ALT and AST were determined by optimized, modified method according to International Federation of Clinical Chemistry (IFCC), without pyridoxal phosphate, the rate that the absorbance changes at 340 nm.

The values of each parameter in each group were expressed as parameter averages and standard deviation (mean ± sd). It was applied t-student test single sample, for correlation between various measurements, linear regression analysis was used. Analysis was done used Statistica Demo version 8 for windows XP professional (Tulsa-OK 74104, USA). $P<0.05$ was consider significant.

RESULTS

The mean age of the patients in group I (8 male,12 female) was 44.8 (range 35-60), in group II (11 male,9 female) was 43 (range 38-63),and in group III (10 male,10 female) was 45.8 (range 38-52).The levels of AT III and transaminases activity in study groups are presented in table 1 . The normal ranges based on the reference value by the laboratory of AT III is 75 to140%; and of GGT is 7 to50 IU/I, ALT is 32 to 42 IU/U, AST is 31 to 37 IU/U.

In patients with liver disease level of AT III and GGT and ALT activity in this research are different significant with compared to the healthy participants. In patients with chronic hepatitis and cirrhosis level of AST activity not different significant with compared to the healthy participants. The plasma level of AT III was 71.8 ± 5.4% in patients with chronic hepatitis, 60.6 ± 5.9% in patients with cirrhosis, and 97.52 ± 2 % in the healthy participants ($P < 0.05$). Table 2 shows statistical tests of means against reference constants, regression and correlations for the coagulation activity in each study group.

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 (Sheth SG et al, 1998, Anna S. F et al, 2005). 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 (Cadrane JF et al, 2000, Janes CH et al, 1993). Thus, the need exists for a noninvasive, inexpensive, and accurate method for diagnosing cirrhosis (Anna S. F et al, 2005).

We evaluated the association between AT III levels, and serum Aminotransferase activity, in chronic liver disease, to determine whether these biochemical markers could be used in diagnosis. Many patients with chronic liver disease have coagulopathy (Burtis C.A, 1977) because the majority of the liver coagulation factors are adversely affected (R C van

Nieuwenhuizen et al, 1999), 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 (Leon M, 1983) and AT III is only synthesized by liver tissue (Leon M, 1983, Ewa Marciniak, 1974). Acute or chronic liver diseases may decrease the concentration of AT III (Castellino DJ et al, 1997). Several studies have investigated the reduction of AT III in chronic liver disease (Bick RL, 1982, Abildgaard U, 1981, Cong YL, 2005, Kerr R et al, 2003). A plasma concentration of AT III is too low in cirrhosis (Gomez , 2000). E. Kont concluded altered plasma concentration of AT III in cirrhosis due to reduced transcapillary flux ratios (E Knot et al, 1984). It is clear tissue liver damage, particularly damage to the endothelium, triggers the included inflammation (Wada H et al, 1993) and embarrassment to physiological anticoagulant mechanism (Michal J et al, 1998). Our data show that concentration of AT III was significantly lower in patients with cirrhosis than in patients with chronic hepatitis ($P< 0.05$). So, the sensitivity and specificity of the AT III as a necrosis marker for the detection liver tissue damage is known in our study and suggested these hypothesis more will approve by any educational commentary as a biochemical marker for necrosis determination damage of tissue liver. However mechanism of diminution of AT III in liver disease is complex, insufficient hepatic synthesis, altered transcapillary flux ratio and low diffuse Disseminated Intravascular Coagulation (Sheikh Sajjadieh. M.R, 2008) may take part it .

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 the aminotransferases AST and ALT often reflect hepatocellular damage.

Gamma glutamyltransferase (GGT) is present in decreasing concentration in kidney, liver, pancreas, and intestine 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, our data has shown that patients with chronic liver disease, the activity of GGT in serum is significantly lower in patients with chronic cirrhosis than in patients with chronic hepatitis, although 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 no steroidal anti-inflammatory drugs, lipid-lowering drugs, antibiotics, antiepileptic, antifungal agents, and antidepressants. Even small amounts of alcohol ingested 24 hours prior to the test may cause a temporary elevated GGT.

The activity of ALT was significant different in patient with cirrhosis ($P=0.026$) and chronic hepatitis ($P=0.035$) compare with control group , however according to the NACB, ALT do to low specificity and availability of more specific alternative biomarker of necrosis. The activity of AST in study groups was not different significant with compared to the healthy participants, AST can be elevated in patients with skeletal muscle disease, pulmonary emboli, hepatic disease and also by intramascular injection (Contir R, 1999). Therefore, AT III level in patients with chronic liver disease under condition of high aminotransferase activity as a tissue necrosis damage may be used as a exist factor that non invasive, inexpensive and accurate method for diagnosis cirrhosis in both diagnosis and prevention monitoring of these patients, provided that they are not taking anticoagulants.

CONCLUSIONS

Decreased levels of plasma III and increased levels of serum aminotransferase activity in patients with liver disease are present in patients with chronic liver disease. The concentration of AT III under condition of high GGT and ALT activity was significantly lower in patients with cirrhosis than in 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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