Turk J Gastroenterol 2005; 16 (3): 129-133

Importance of anticoagulant proteins in chronic liver diseases

Kronik karaciğer hastalıklarında antikoagülan faktörlerin önemi

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Background/aims: *This study was conducted to elucidate the importance of anticoagulant proteins in chronic liver disease and their possible role as markers in determining the severity of the liver disease.* ***Methods:*** *This study was conducted on 35 patients with cirrhosis, 15 patients with chronic active hepatitis and 10 healthy controls. Coagulation inhibitor proteins such as protein C, protein S and antithrombin, as well as D-dimer level and thrombin time, which reflect fibrin degradation products, were measured. Cirrhotic patients were categorized as Child A, B and C and chronic active hepatitis patients as mild or moderate activity according to the modified Knodell histopathologic classification. The parameters were compared between patient groups and healthy controls.* ***Results:*** *In comparison with controls, the cirrhotics had significantly decreased protein C, protein S, antithrombin levels and increased D-dimer levels. The Child B and Child C patients differed significantly with respect to protein C and antithrombin levels only. In the chronic active hepatitis patients, protein S, protein C and fibrinogen were within normal limits, whereas antithrombin was low.* ***Conclusions:*** *In chronic active hepatitis, the antithrombin level may be used as an early marker of hepatocellular damage. In cirrhotics, protein C and antithrombin may be useful for assessment of hepatocellular damage, whereas D-dimer may be important for the transition to decompensation.*

Key words: Anticoagulant proteins, chronic active hepatitis, liver cirrhosis

Amaç: *Kronik karaciğer hastalığında antikoagülan proteinlerin önemini aydınlatmak ve karaciğer hastalığının derecesini belirlemede bir gösterge olarak kullanılıp kullanılamayacaklarını tespit etmek amacıyla çalışmamızı planladık.* ***Yöntem:*** *35 karaciğer sirozlu, 15 kronik aktif hepatitli hasta ve 10 sağlıklı gönüllü çalışıldı. Hastalarda ve gönüllülerde protein C, protein S, antitrombin gibi doğal koagülasyon inhibitör proteinleri, fibrin yıkım ürünlerini yansıtan D-dimer düzeyi ve trombin zamanına bakıldı. Sirozlu hastalar Child-Pugh sınıflamasına göre Child A, B ve C olarak, kronik aktif hepatitli hastalar ise modifiye Knodell histopatolojik sınıflandırmaya göre hafif ve orta olarak gruplandırıldı. Bakılan parametreler gruplar arasında birbirleriyle ve kontrol grubunun sonuçları ile karşılaştırıldı.* ***Bulgular:*** *Kontrol grubu ile karşılaştırıldığında, sirozlu hastalarda protein C, protein S, antitrombin düzeyleri istatistiksel olarak anlamlı şekilde düşük, D-dimer düzeyleri yüksek bulundu. Child B ve C grupları arasında ise sadece protein C ve antitrombin düzeylerinde istatistiksel olarak anlamlı fark vardı. kronik aktif hepatitli hastalarda protein S, protein C ve fibrinojen düzeyleri normalken, antitrombin düşük değerlerde tespit edildi.* ***Sonuç:*** *Kronik aktif hepatitli hastalarda antitrombin düzeyleri hepatosellüler hasarın derecesinin erken dönemde tespit edilmesinde bir belirteç olarak kullanılabilir. Sirozlu hastalarda ise hepatosellüler hasarın değerlendirilmesinde protein C ve antitrombin yanısıra özellikle sirozlu hastalarda dekompanze döneme geçiş açısından D-dimer önemli bir gösterge olabilir.*

Anahtar kelimeler: Antikoagülan proteinler, kronik aktif hepatit, karaciğer sirozu

INTRODUCTION

Chronic active hepatitis and the consequent cirrhosis are severe hepatic parenchymal diseases with potentially life-threatening complications. The acute and chronic inflammation in the liver is associated with a bleeding diathesis due to deficiency in the synthesis of coagulation factors as well as a procoagulant state due to the defects in the synthesis of anticoagulant factors by the liver. End-stage cirrhosis is frequently associated with clinical findings of bleeding tendency. The degree and the clinical significance of the decrease in anticoagulant factors due to liver parenchymal damage are currently unclear.

In this study, the levels of coagulation factors as well as anticoagulant factors such as protein C

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Manuscript received: 09.03.2005 **Accepted:** 24.05.2005

(PC), protein S (PS) and antithrombin (AT) synthesized in the liver were investigated in patients with chronic liver disease. The objectives were to elucidate the significance of anticoagulant proteins in chronic liver disease and to determine whether they can be used as markers of hepatic parenchymal damage.

MATERIALS AND METHODS

After approval of the Ethics Committee of Erciyes University, School of Medicine, this study was conducted involving 50 patients with hepatic parenchymal damage and 10 healthy subjects who attended hospital for a check-up.

The medical histories of the patients and the healthy controls were taken and physical examinations were performed. After an overnight fasting, venous blood samples were taken for whole blood count (hemoglobin, leukocyte and platelet) and biochemical measurements. The presence of the serological markers for hepatitis viruses was investigated by ELISA. HBV-DNA was measured by the hybrid-capture method in HBsAg-positive patients and HCV-RNA was measured by polymerase chain reaction (PCR) in anti-HCV positive patients. In patients with increased transaminases but negative serology for hepatitis viruses, autoimmune markers (ANA, ASMA, LKM-1, AMA), and the other etiological factors were measured. The prothrombin time (PT), partial thromboplastin time (PTT), PC, PS, fibrinogen, thrombin time (TT), AT and D-dimer (D-d) were measured.

Ultrasonography was performed to assess liver size and echo structure, spleen size, portal vein diameter and the presence of ascites. All patients with abnormal liver function tests underwent upper gastrointestinal endoscopy to evaluate the possible presence of varices in the esophagus and the fundus and congestion in the stomach and the duodenum.

In patients who had suitable coagulation parameters and consented to the procedure, liver biopsy was performed with a Wimm-Silverman needle. All specimens were evaluated by the same pathologist. The modified Knodell score was used (1).

In patients with unsuitable coagulation parameters, the diagnosis of cirrhosis was based on the positivity of one of the virological markers, physical examination findings (splenomegaly, ascites, peripheral signs of cirrhosis), laboratory data (decreases in platelets and/or leukocytes), ultrasonographic findings (irregular liver surface, small liver size, coarseness of liver echogenicity, dilatation of the portal vein, splenomegaly and ascites) and endoscopic findings (esophageal and/or fundic varices, congestive gastroduodenopathy).

Patients with diabetes mellitus, renal disease, hyperlipidemia, hepatosteatosis and history of drug or chronic alcohol abuse were excluded from the study.

Cirrhotic patients were grouped according to the Child-Pugh classification. Patients with chronic viral hepatitis were grouped with respect to the necroinflammatory activity as expressed by the histological activity index (HAI): 1-6: mild, 7-12: moderate, 13-18: severe (1).

The results were expressed as mean± standard deviation.

The PC, PS, AT, fibrinogen, and fibrin degradation product levels of the cirrhotic patients, chronic active hepatitis patients and healthy controls were compared with the Kruskall-Wallis test and the Mann-Whitney U test as appropriate. Probability values less than 0.05 were considered significant.

RESULTS

Cirrhosis was diagnosed in 35 patients and chronic active hepatitis in 15; the diagnosis was made by liver biopsy in 35 patients and clinical and laboratory findings in 15. The characteristics of the patients and controls, etiological factors and the degrees of parenchymal damage are presented in (Table 1).

Table 1. The characteristics and results of the patients and the controls

	n	Age	ALT	Scoring			Etiology		
							HBV	HCV	Autoimmune
CAH	15	44.4±9.1 (19-55)	118.0±62.5 (37-274)	Mild* 6	Moderate* 9	Severe* -	5	10	-
Cirrhosis	35	52.9±11.0 (21-73)	63.5±50.6 (19-204)	Child A 15	Child B 8	Child C 12	10	20	5
Controls	10	42.1±11.7 (28-60)	19.9±10.2 (8-40)						

*Histological activity index (HAI)

Table 2. The anticoagulant levels of the patient groups and healthy controls

	PC	PS	AT	Fibrinogen	TT	D-d	PT	Albumin	Bilirubin
CAH	103.9	77.6	86.5	288.8	16.9	0.4	13.1	4.4	1.0
	±35.2	±23.8	±11.2	±34.5	±1.4	±0.3	±0.7	±0.3	±0.6
Cirrhosis	44.3	45.0	57.1	233.4	17.2	2.3	17.6	3.2	2.2
	±32.9	±20.7	±22.3	±76.1	±2.0	±1.9	±4.0	±0.8	±2.0
Controls	120.2	70.0	107.0	303.8	16.7	0.3	12.8	4.3	0.8
	±10.6	±16.7	±4.0	±75.6	±1.5	±0.4	±0.6	±0.2	±0.2

Normal reference levels: PC: 70-130%, PS: 65-140%, AT: 80-120%, fibrinogen: 200-400 mg/dl, TT: 15-22 sn, D-d: 0.0-0.5 µg/ml, PT: 12-14 sec, albumin: 3.5-5 mg/dl, total bilirubin: 0.2-1.3 mg/dl

The prothrombin time was prolonged in 94% (33/35) of the cirrhotic patients, the bilirubin level was elevated in 60% (21/35), and the albumin level was decreased in 60% (21/35). PC was below normal range in 83% (29/35), PS in 86% (30/35), fibrinogen in 37% (13/35), and AT in 86% (30/35). TT was within normal limits in all cirrhotic patients, whereas D-d was high in 28 patients (80%).

The PT, albumin, bilirubin, PC, fibrinogen and TT results of all chronic active hepatitis patients were within normal limits. PS was low in 26% (4/15) and AT in 40% (6/15).

The PC, PS, AT, fibrin degradation product, PT, albumin and bilirubin results of the patients and healthy controls are presented in (Table 2).

Statistical Comparison Yielded The Following Results

1. When the patients with chronic active hepatitis were compared with healthy controls,

- The AT level was significantly lower in the patients (p: 0.031).

- The two groups did not differ significantly with respect to the other parameters.

2. When the cirrhotic patients were compared with healthy controls,

- Significant differences were found in all parameters.

3. When cirrhotic patients were compared with chronic active hepatitis patients,

- Significant differences were found in all parameters.

The investigated parameters were also evaluated with respect to the severity of cirrhosis. PC, PS, AT, and fibrinogen showed a direct relationship with the degree of parenchymal damage. The TT was within normal limits; D-d levels showed an inverse relationship with the degree of parenchymal damage.

The anticoagulant levels of the patients with different degrees of parenchymal damage are presented in (Table 3).

Statistical Analysis Yielded The Following Results

1. When Child A cirrhotic patients were compared with Child B cirrhotic patients,

- The D-d levels were significantly higher in Child B patients (p: 033).

- The two groups did not differ significantly with respect to the other parameters.

2. When Child A cirrhotic patients were compared with Child C cirrhotic patients,

- Significant differences were found in all parameters.

3. When Child B cirrhotic patients were compared with Child C cirrhotic patients,

- PC was significantly lower in Child C patients (p: 0.045).

- AT was significantly lower in Child C patients (p: 0.010).

The same parameters were compared between chronic active hepatitis patients with different levels of necroinflammatory activity (Table 4). When

Table 3. The anticoagulant levels of the patients with varying degrees of parenchymal damage

	PC	PS	AT	Fibrinogen	TT	D-dimer
Child A	62.7±36.4	52.4±19.3	69.7±22.4	273.3±56.3	17.0±2.3	1.5±2.4
Child B	42.2±29.2	46.7±29.2	56.8±17.7	293.7±81.3	16.4±1.6	2.3±1.1
Child C	22.8±12.4	34.0±12.4	34.6±14.8	179.4±65.3	17.9±1.8	3.5±1.6

Normal reference levels: PC: 70-130%, PS: 65-140%, AT: 80-120%, fibrinogen: 200-400, mg/dl, TT: 15-22 sec, D-d: 0.0-0.5 µg/ml

Table 4. Mean coagulation inhibitor levels in patients with chronic hepatitis

	PC	PS	AT	Fibrinogen	TT	D-dimer
Mild CAH	93.1±34.6	85.5±20.8	88.6±16.1	300.6±35.0	16.6±1.7	0.3±0.4
Moderate CAH	73.5±24.8	73.5±24.8	85.1±7.3	280.3±33.1	17.1±1.3	0.4±0.2

Normal reference levels: PC: 70-130%, PS: 65-140%, AT: 80-120%, fibrinogen: 200-400 mg/dl, TT: 15-22 sec, D-d: 0.0-0.5 μg/ml

the groups with mild and moderate liver injury were compared, there was no statistically significant difference. Although PC and PS were within normal range, the levels were lower in the group with moderate liver injury. The fibrosis scores and AT levels in these groups were compared with correlation analysis tests, and there was no statistically significant correlation.

DISCUSSION

The liver is an important organ that synthesizes coagulation factors, except the von Willebrand factor; fibrinolytic system proteins, except the tissue plasminogen factor; and the urokinase-type plasminogen activator; as well as coagulation and fibrinolysis inhibitors (2-4). In patients with hepatic parenchymal disease, the loss of functional parenchyma results in decreased synthesis of both coagulation factors and natural anticoagulant proteins (2, 5-9).

The levels of coagulation factors and natural anticoagulant proteins synthesized in the liver are valuable in diagnosis and treatment, as they reflect the degree of hepatocyte damage.

In this study, the levels of protein inhibitors and fibrin degradation products were measured in patients with parenchymal damage to investigate whether they can be used as markers of parenchymal damage.

In studies on patients with viral hepatitis and cirrhosis, PC levels were less markedly decreased in comparison with AT; thus, PC may be less sensitive than AT as a marker of hepatocellular damage (10-12). In patients with viral hepatitis, the decreased PC level recovers during resolution of inflammation and returns to normal before PT, and thus may be used as an early marker for recovery of synthetic functions of the liver (12).

In one study on alcoholic liver disease, the PC and AT levels were found be significantly lower in Child C cirrhotic patients (13). In other studies, PC levels were within normal limits in patients with steatosis only, but correlated with the degree of parenchymal damage in cirrhotics; the authors concluded that PC was an important marker of hepatocellular damage (13-15). In the present study on cirrhosis due to viral causes, with increasing severity of parenchymal damage, antithrombin was the first protein to decrease, followed by PC and finally PS. One point that should be kept in mind in interpreting blood levels of PS is that PS is synthesized not only by hepatocytes, but also by endothelial cells, megakaryocytes and Leydig cells, although their respective contributions are uncertain.

The deficiency in coagulation factors, platelet dysfunction, thrombocytopenia, dysfibrinogenemia and increased fibrinolysis in patients with liver disease generally present with increased bleeding diathesis (5, 11, 16); conversely, decreases in antithrombin and other natural anticoagulants increase the risk of thrombosis. Some authors argue that since both coagulation factors as well as antithrombin and natural coagulation inhibitor factors are equally decreased, coagulation is not observed in patients with liver disease (5, 11). In a study from Turkey, no significant association was found between activities of PC, PS and AT and presence of portal thrombosis (17). Tripodi et al. (18) suggested that bleeding is mainly due to the presence of hemodynamic alterations and that conventional coagulation tests are unlikely to reflect the coagulation status of cirrhotic patients.

Hyperfibrinolysis is considered to be another important cause of bleeding tendency in cirrhotic patients (13, 19). Fibrinogen is low whereas plasminogen, alpha-2-antiplasmin and fibrin degradation products are high. In some studies on patients with acute and chronic viral hepatitis, cirrhosis and hepatocellular carcinoma, the fibrinogen levels were within normal limits and fibrin degradation products were increased (3, 16), whereas in another study, the fibrinogen level was low, and the fibrinogen molecule was found to contain supernormal sialic acid levels with resultant functional impairment (2). Recent studies have shown that although thrombin-activatable fibrinolysis inhibitor concentrations were low in cirrhotics, there was no sign of increased plasma fibrinolytics, and the deficiency of antifibrinolytics was compensated by the decrease in profibrinolytics

(20). In the present study, D-d, a marker of fibrin degradation products, was found to be increased with increasing severity of hepatocyte damage. Since D-d was the only parameter that differed significantly between Child A and Child B patients, D-d levels may be considered as an important sign of decompensation in cirrhotic patients.

In the present study, in patients with chronic active hepatitis, PS and AT levels were decreased in comparison with healthy controls. However, the decrease in AT was statistically significant in comparison with controls, whereas the decrease in PS was not. The fibrosis scores and AT levels were compared with correlation analysis tests in mild and moderate hepatitis groups, and there was no statistically significant correlation. It was suggested that this confusion might be connected with similarity of fibrosis scores between the two groups.

In a study by Dong et al. (21) in 1999 in patients with chronic active hepatitis, the antithrombin levels were similarly decreased. Papatheodoridis et al. (22) investigated prothrombotic risk factors in patients with chronic viral hepatitis and argued that increased GGT levels and decreased AT levels may be markers of fibrosis.

In the chronic active hepatitis patients in the present study, the PC, PS, AT, fibrinogen and fibrin degradation product levels of the mild and moderate groups did not differ significantly. In cirrhotic patients, the impairment of synthetic function correlates with the increased hepatocellular damage. The absence of differences between the two subgroups of chronic hepatitis may be ascribed to the fact that the histological activities of the two groups were similar.

The levels of PC, PS and coagulation factors were within normal limits in patients with chronic active hepatitis, but AT was significantly decreased; therefore, AT may be considered as an early marker of hepatocellular damage. In addition, D-d may be a marker of decompensation in addition to PC and AT.

REFERENCES

1. Ishak K, Babtista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22: 696-9.
2. Mammen EF. Coagulation defects in liver disease. Med Clin North America 1994; 78: 545-54.
3. Takahashi H, Tatewaki W, Wada K, et al. Thrombin and plasmin generation in patients with liver diseases. Am J Hematol 1989; 32: 30-5.
4. Ito K, Niwa M, Kawada T, Tanaka Y. Plasma antithrombin III, alpha 2- plasmin inhibitor and plasminogen activities in cases of various liver diseases (Abstract). Tokai J Exp Clin Med 1986; 11(5): 345-52.
5. Rak K. Thrombosis promoting changes in chronic liver diseases. Folia Haematol 1988; 114(3): 333-9.
6. Roberts HR, Cederbaum AI. The liver and blood coagulation: physiology and pathology. Gastroenterology 1972; 63: 279.
7. Francis RB, Thomas W. Behavior of protein C inhibitor in intravascular coagulation and liver diseases. Thromb Haemost 1984; 52: 71.
8. Cordova C, Violi F, Alessandri C, et al. Prekallikrein and factor VII as prognostic indexes of liver failure. Am J Clin Pathol 1986; 85(5): 579-82.
9. Romero Gomez M, Suarez Garcia E, Lopez Lacomba D, et al. Antiphospholipid antibodies are related to portal vein thrombosis in patients with liver cirrhosis. J Clin Gastroenterol 2000; 31(3): 237-40.
10. Zurbon KH, Kirch W, Bruhn HD. Immunological and functional determination of the protease inhibitors, protein C and antithrombin III, in liver cirrhosis and in neoplasia. Thromb Res 1988; 52: 325-36.
11. Vigano S, Mannuccio P, D'Angelo A, et al. The significance of protein C antigen in acute and chronic biliary disease. AJCP 1985; 84(4): 454-8.
12. De Caterina M, Tarantino G, Farina C, et al. Haemostasis unbalance in Pugh-scored liver cirrhosis: characteristic changes of plasma levels of protein C versus protein S. Haemostasis 1993; 23: 229-35.
13. Raya-Sanches JM, Gonzales-Reimers E, Rodriguez-Martin JM, et al. Coagulation inhibitors in alcoholic liver cirrhosis. Alcohol 1998; 15: 19-23.
14. Bell H, Odegaard OR, Andersson T, et al. Protein C in patients with alcoholic cirrhosis and other liver diseases. J Hepatol 1992; 14(2-3): 163-7.
15. Kloczko J, Mian M, Wojtukiewicz MZ, et al. Plasma protein C as a marker of hepatocellular damage in alcoholic liver diseases. Haemostasis 1992; 22: 340-4.
16. Takahashi H, Tatewaki W, Wada K, et al. Fibrinolysis and fibrinogenolysis in liver diseases. Am J Hematol 1990; 34: 241-5.
17. Sezgin O, Oguz P, Ayaz S. Alterations in serum levels of the natural anticoagulants in chronic liver diseases and their relations to portal thrombosis. Turk J Gastroenterol 1998; 9(4): 313-6.
18. Tripodi A, Salerno F, Chantarangkul V, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology 2005; 41(3): 553-8.
19. Francis RB, Feinstein DI. Clinical significance of accelerated fibrinolysis in liver disease. Haemostasis 1984; 14: 460.
20. Lisman T, Leebeek FW, Mosnier LO, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology 2001; 121(1): 131-9.
21. Dong L, Peng Y, Wang R. Expression and its clinical significance of tissue factor pathway inhibitor and antithrombin III in patients with hepatic inflammatory diseases (Abstract). Chung Hua Nei Ko Tsa Chih 1999; 7(4): 224-5.
22. Papatheodoridis GV, Papakonstantinou E, Andrioti E, et al. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. Gut 2003; 52(3): 404-9.