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A Study on Changes of Coagulation Inhibitors and Fibrinolysis Inhibitors in Patients with Liver Cirrhosis and Hepatoma

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The authors conducted an investigation focusing mainly on the activities of the inhibitory factors of the coagulation and fibrinolysis processes in 35 normal adults and 72 liver cirrhosis and/or hepatoma patients.

The activities of antithrombin III, protein C, and α2−plasmin inhibitor were reduced to less than 50% in patients with decreased hepatic synthetic function while lupus anticoagulant was detected in more than 50% of patients with decreased hepatic synthetic function.

Hemostatic abnormalities in advanced lived diseases may be caused partly by a decrease of coagulation and fibrinolysis inhibitors and the presence of lupus anticoagulant.

Key Words : *Coagulation and fibrinolysis inhibitors, Liver cirrhosis*

INTRODUCTION

Hemostatic problems are detected in approximately 58−75% of patients with liver disease (Kelly and Tuddenham 1986). Hemostatic abnormalities in liver disease are both complex and multifactorial and known to be related to the balance between hepatic synthesis and clearance of activated coagulation factors and their inhibitors ; a synthesis of abnormal coagulation factors ; accentuated fibrinolysis and/or alteration in the fibrinolysis mechanism ; and disseminated intravascular coagulation. However, most studies have been done focusing mainly on the changes of the coagulation factors and on fibrinogen or fibrin degradation products (Lee, 1982 ; Rock, 1984 ; Choi et al., 1985 ; Colman et al., 1987). Considering the role of coagulation or fibrinlysis inhibitors in hemostasis, the inhibitors (Brandt, 1984) might also play important roles in the pathogenesis of hemostatic abnormalities in liver disease. The aim of this study is to define the changes of the inhibitors in patients with liver cirrhosis and hepatoma and to gather valuable informations for understanding the pathogenesis of hemostatic abnormalities in these diseases.

We measured the levels of the coagulation inhibitors and/or fibrinolysis inhibitors : antithrombin III (AT III), protein C(PC), Cl-inhibitor (Cl-INH) α2−plasmin inhibitor(α2−PI), α2−macroglobulin(α2−M) and lupus anticoagulant in liver cirrhosis and hepatoma patients. We also analyzed the relationships among the inhibitors, hepatic synthetic capability, and the extent of coagulation factor activities.

MATERIALS AND METHODS

Materials

Patient samples were drawn from 72 patients with pathological diagnosis of liver cirrhosis and/or

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hepatoma admitted to the Department of Internal Medicine of Seoul National University Hospital or Hallym University Hospital. Normal control samples were taken from 35 healthy persons among laboratory employees and persons confirmed to be normal with routine screening tests at the out-patient clinic.

The 4.5ml of whole blood was mixed with 0.5ml of 3.8% sodium citrate. Platelet poor plasma prepared by centrifugation (1500g, 10 minutes) was divided into aliquots in five microtubes and stored in a freezer (—80℃, 4—30 days). The frozen plasma was tested within two hours after thawing (37℃, 10 minutes).

Methods

1. Measurements of inhibitors

AT III, PC, C1-INH, and α2—PI were measured by synthetic chromogenic substrate method (Musgrace, 1984 ; Berichrome® Behring, Germany). α2—M was measured by radial immunodiffusion (N-partigen, Behring, Germany). The levels of the above inhibitors were expressed as the percentage of the mean of the normal control being 100%.

2. Detection of lupus anticoagulant

The presence of a circulating anticoagulant was detected by a mixing test, an APTT determination (Actin® Dade, USA) with a 1 : 1 mixture of patient plasma, and pooled normal plasma.

A diluted tissue thromboplastin inhibition test (DTTI test) was performed by a PT reagent (Thromborel-S® Behring, Germany), which was diluted 1 : 50 and 1 : 500 with saline. The 0.1ml of the 1 : 50 or 1 : 500 dilution was added to 0.1ml of patient plasma, and after incubation for five minutes at 37℃, the clotting time was measured with $CaCl_2$.The presence of lupus anticoagulant was suggested by a five-second prolonged APTT (which was not corrected by a mixing test) and then confirmed by the DTTI test in which the ratio of the clotting time of the patient mixture to that of the normal control was 1.3 or greater.

3. Other assays

The activity of thrombin generation in the extrinsic and intrinsic pathways was screened with PT (Thromborel-S® Behring, Germany) and APTT(Actin® Dade, USA). The results of PT were expressed as INR (International Normalized Ratio) and those of APTT as the ratio of the clotting time of the patient to that of the normal control.

Cholinesterase activity was determined by the spectrophotometric method using acetylthiocholine as a substrate (Test-combination Cholin-esterase® Behringer Mannheim, Germany).

4. Groups of patients

The patients were divided into four groups according to the levels of cholinesterase : 0—600 u/l (25%) ; 601—1200 u/l(50%) ; 1201—1800 u/l (75%) ; and more than 1801 u/l, which represented hepatic synthetic activity

To represent the activities of the intrinsic, extrinsic, and common pathways, the patients were also divided into four groups according to the levels of PT(INR) and APTT ratio : Group I : less than 1.19(75%) ; Group II : from 1.19 to 1.60(50%) ; Group III : form 1.60 to 2.88(25%) ; and Group IV : more than 2.88.

Statistical analysis was carried out using Student's t test and r correlation coefficient.

RESULTS

1. Reference ranges of coagultion and fibrinolysis inhibitors(Table 1)

The reference ranges of coagulation and fibrinolysis inhibitors obtained from 35 normal Korean adults with the synthetic chromogenic substrate method were AT III : 74.4—125.6% ; PC : 64.4—135.6% ; C1-INH : 70.8—129.2% ; and α2—PI : 69.8—130.2%. The α2—M analyzed with the radial immunodiffusion method was 0.92—3.12 g/l in males and 1.67—3.19 g/1 in females.

2. Coagulation and fibrinolysis inhibitors in liver cirrhosis and hepatoma (Table 1)

AT III, PC and α 2—PI levels significantly decreased in liver cirrhosis and hepatoma patients. C1-INH slightly decreased in liver cirrhosis and hepatoma with lobectomy. The α 2—M slightly decreased only in hepatoma with lobectomy.

Lupus anticoagulants were detected in 26 patients(51.0%) among the 51 patients of liver cirrhosis and in three patients(27.3%) among the 11 patients of hepatoma associated with cirrhosis.

3. Relationship between the levels of the inhibitors and the hepatic synthetic function. (Table 2)

The activities of AT III, PC, and α 2—PI were well-correlated with the levels of cholinesterase (hepatic synthetic function). The levels of C1-INH and α 2—M showed no significant correlation with the levels of cholinesterase. (Fig.1).

Table 1. Plasma Levels of Coagulation and Fibrinolysis Inhibitors in Liver Cirrhosis and Hepatoma

		Normal Control (n−35) Mean± SD	Liver Cirrhosis (n−51) Mean± SD	Hepatoma c̄ Cirrhosis (n−11) Mean± SD	Hepatoma s̄ Lobectomy (n−6) Mean± SD	Hepatoma c̄ Lobectomy (n−4) Mean± SD
Cholinesterase	(%)	100± 12.6	28.3± 12.1	28.8± 11.9	65.5± 28.5	19.5± 16.7
PT(INR)		1.0± 0.05	1.86± 0.93	1.95± 1.05	1.16± 0.15	1.80± 0.12
APTT(ratio)		1.0± 0.12	1.99± 0.74	1.15± 0.30	1.15± 0.30	2.15± 0.46
Antithrombin III	(%)	100± 12.8	30.4± 17.0	32.8± 21.2	73.3± 23.8	19.6± 10.8
Protein C	(%)	100± 17.8	28.8± 14.8	26.6± 13.7	79.0± 29.5	17.0± 5.3
C1-inhibitor	(%)	100± 14.6	84.8± 24.7	97.7± 23.9	109.0± 17.5	72.3± 11.0
α 2-plasmin inhibitor	(%)	100± 15.1	46.5± 18.1	54.1± 21.4	91.2± 12.7	31.7± 8.0
α 2-macroglobulin	(%)	100± 20.4	109.0± 42.7	98.3± 32.1	90.0± 31.5	65.8± 15.7
Lupus anticoagulant, No. (%)		−	26 (51.0)	3 (27.3)	−	−

Table 2. Levels of Inhibitors in Subgroups Classified According to Cholinesterase Levels.

Test		Normal Control (n−35) Mean± SD	Cholinesterase Levels 0-600(25%) (n−35) Mean± SD	601-1200(50%) (n−29) Mean± SD	1201-1800(76%) (n−6) Mean± SD	1801- (n−2) Mean± SD
Albumin	(g/dl)	−	2.96± 3.33	2.63± 0.46	2.88± 0.79	3.3± 0.57
PT	(INR)	1.0± 0.05	1.95± 1.06	1.69± 0.70	1.86± 0.80	1.18± 0.25
APTT	(ratio)	1.0± 0.12	2.14± 0.76	1.73± 0.84	1.97± 0.88	1.10± 0.33
Antithrombin III	(%)	100± 12.8	24.0± 11.5	36.8± 19.3	57.0± 26.4	90.4± 36.6
Protein C	(%)	100± 17.8	22.2± 10.1	36.3± 20.9	48.1± 23.7	91.3± 35.4
C1-inhibitor (%)		100± 14.6	85.8± 23.3	86.3± 23.3	109.6± 36.6	88.7± 1.6
α 2-plasmin inhibitor	(%)	100± 15.1	41.4± 16.6	54.6± 20.3	69.8± 26.3	93.8± 21.2
α 2-macroglobulin	(%)	100± 20.4	98.0± 37.8	109.4± 46.6	106.5± 21.3	129.0± 1.0
Lupus anticoagulant, No. (%)		−	19(54.3)	8(27.6)	2(33.3)	−

Lupus anticoagulants were detected in 19 patients(54.3%) out of 35 with cholinesterase levels less than 25%, in eight patients(27.6%) out of 29 with cholinesterase levels from 25% to 50%, and in two patients(33.3%) out of six with cholinesterase levels more than 50%.

4. Relationship between the levels of the inhibitors and plasma coagulation activities. (Table 3)

The levels of AT III, PC, C1-INH, and α2−PI were-correlated with PT(INR) and APTT(ratio) representing the intrinsic, extrinsic, and common pathway activities. However, α 2−M had little correlation. (Fig.2).

Lupus anticoagulants were detected more frequently in the group of decreased coagultion activities.

5. Correlations between inhibitors (Table 4)

There were significant correlations between AT III and PC, AT III and α 2−PI, and PC and α 2−PI.

6. Lupus anticoagulant (Table 5)

Lupus anticoagulant was detected in 29 (46.8 %) out of 62 patients with liver cirrhosis, but not in the 10 patients with hepatoma only. The frequency of lupus anticoagulant positivitiy was higher in those patients with more decreased hepatic synthetic function. Patients with lupus anticoagulant showed lower activities of AT III, PC, CI-INH and α2−PI than the patients without it.

Table 3. Levels of Inhibitors in Subgroups Classified According to INR and APTT Ratio.

		Normal Control	Grade I (n-8)	Grade II (n-17)	Grade III (n-40)	Grade IV (n-7)
		(n−35) Mean± SD	-1.19(75%) Mean± SD	1.19-1.60(50%) Mean± SD	1.60-2.88(25%) Mean± SD	2.88- Mean± SD
Cholinesterase	(%)	100± 12.6	53.8± 25.8	34.8± 17.0	26.0± 11.9	24.7± 14.3
PT	(INR)	1.0± 0.05	1.18± 0.23	1.36± 0.11	1.79± 0.33	3.75± 1.80
APPT	(ratio)	1.0± 0.12	1.19± 0.56	1.41± 0.12	2.03± 0.39	3.48± 1.46
Antithrombin III	(%)	100± 12.8	77.2± 18.6	44.0± 12.1	24.2± 10.9	13.4± 4.8
Protein C	(%)	100± 17.8	72.4± 27.0	40.6± 9.4	23.7± 11.8	12.0± 8.2
C1-inhibitor (%)		100± 14.6	111.8± 14.1	93.1± 24.1	85.6± 24.0	62.9± 10.7
α 2-plasmin inhibitor	(%)	100± 15.1	81.4± 13.6	61.3± 17.2	44.0± 17.2	26.7± 15.1
α 2-macroglobulin	(%)	100± 20.4	97.3± 17.8	131.6± 52.3	96.5± 32.9	76.3± 19.8
Lupus anticoagulant, No. (%)		−	−	2(11.8)	21(52.5)	6(85.7)

Table 4. Correlation between Cholinesterase, PT, APTT, Inhibitors and Platelet Number (n=72)

	Cholinesterase	PT	APTT	AT II	PC	CI-INH	α2-PI	α2-M
PT	-0.22 #3							
APPT	-0.36 #1	-0.84 #1						
AT III	0.72 #1	-0.48 #1	-0.60 #1					
Pro-C	0.72 #1	-0.49 #1	-0.58 #1	0.89 #1				
C1-inhibitor	0.19 NS	-0.35 #1	-0.38 #1	0.46 #1	0.42 #1			
α 2-plasmin inhibitor	0.57 #1	-0.55 #1	-0.63 #1	0.82 #1	0.79 #1	0.63 #1		
α 2-macroglobulin	0.16 NS	-0.26 #2	-0.30 #2	0.22 #2	0.20 #2	0.19 NS	0.29 2#	
Platelet No.	0.36 #1	-0.27 #2	-0.25 #2	0.50 #1	0.51 #1	0.22 #2	0.42 #1	-0.07 NS

1 : P<0.005 ; 2 : P<0.005 ; 3 : P<0.01 ; NS : not significant

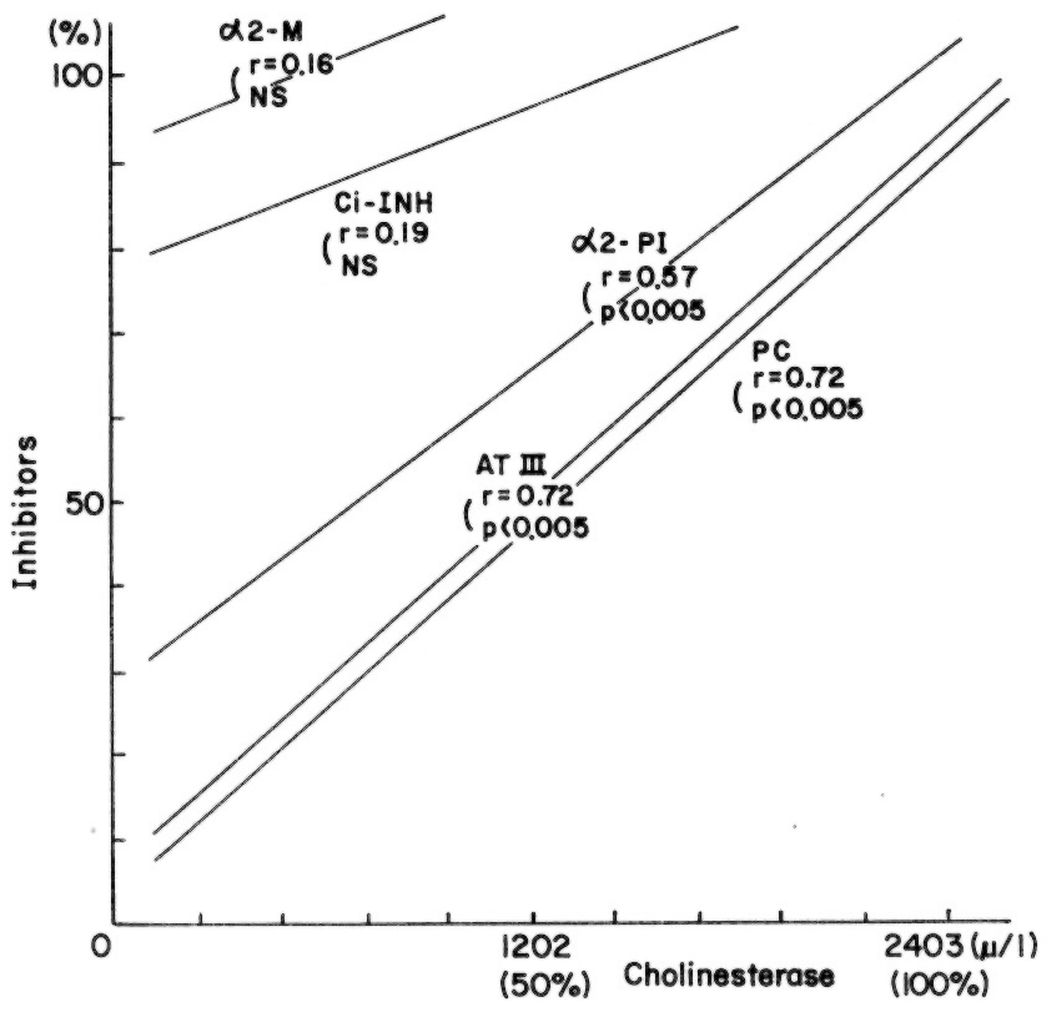

Fig .1. Relationship between Coagulation Inhibitors and Cholinesterase Activity in Liver Diseases.(NS : statistically not significant)

DISCUSSION

Hemostatic abnormalities in liver disease are mostly caused by the reduced and/or defected hepatic synthesis of protein (Canoso et al., 1979) and partly influenced by increased consumption (Stein and Harker, 1982).

The changes of coagulation and fibrinolysis inhibitors in liver cirrhosis and hepatoma have been individually reported. The mean level of AT III was about 50% in liver cirrhosis and hepatoma (Lee, 1982) and 62% in liver cirrhosis (Lurie and Creter, 1981). The PC was also depressed in liver disease (Griffin et al., 1982). The mean level of α 2−PI was 68% in liver cirrhosis with no ascites, 41% in liver cirrhosis with ascites, and 45% in hepatoma associated with cirrhosis (Aoki and Yamanaka, 1978).

The mean level of α 2−M, the protein with the widest spectrum of protease inhibiting activity, was 75%(Lurie and Creter, 1981) and 129% (Aoki

Table 5. Inhibitors and Other Parameters in Patients with or without Lupus Anticoagulant.

		Normal Control	Liver Disease with or without Lupus Anticoagulant		
		(n−35) Mean± SD	Neg. (n−43) Mean± SD	Pos. (n−29) Mean± SD	Statistical Significance
Cholinesterase	(%)	100± 12.6	35.1± 19.4	25.1± 12.2	P<0.05
PT	(INR)	1.0± 0.05	1.48± 0.38	2.31± 1.18	P<0.005
APPT	(ratio)	1.0± 0.12	1.56± 0.41	2.48± 0.95	P<0.005
Antithrombin III	(%)	100± 12.8	42.7± 22.0	20.4± 12.0	P<0.005
Protein C	(%)	100± 17.8	40.1± 22.0	19.9± 13.0	P<0.005
C1-inhibitor	(%)	100± 14.6	97.2± 24.1	74.5± 19.0	P<0.005
α 2-plasmin inhibitor	(%)	100± 15.1	61.5± 19.6	34.3± 13.9	P<0.005
α 2-macroglobulin	(%)	100± 20.4	105.7± 45.2	100.3± 33.0	Not significant
Platelet No.	($\times 10^3/mm^3$)	−	15.3± 10.8	10.5± 6.9	P<0.1

Neg. : without lupus anticoagulant, Pos. : with lupus anticoagulant

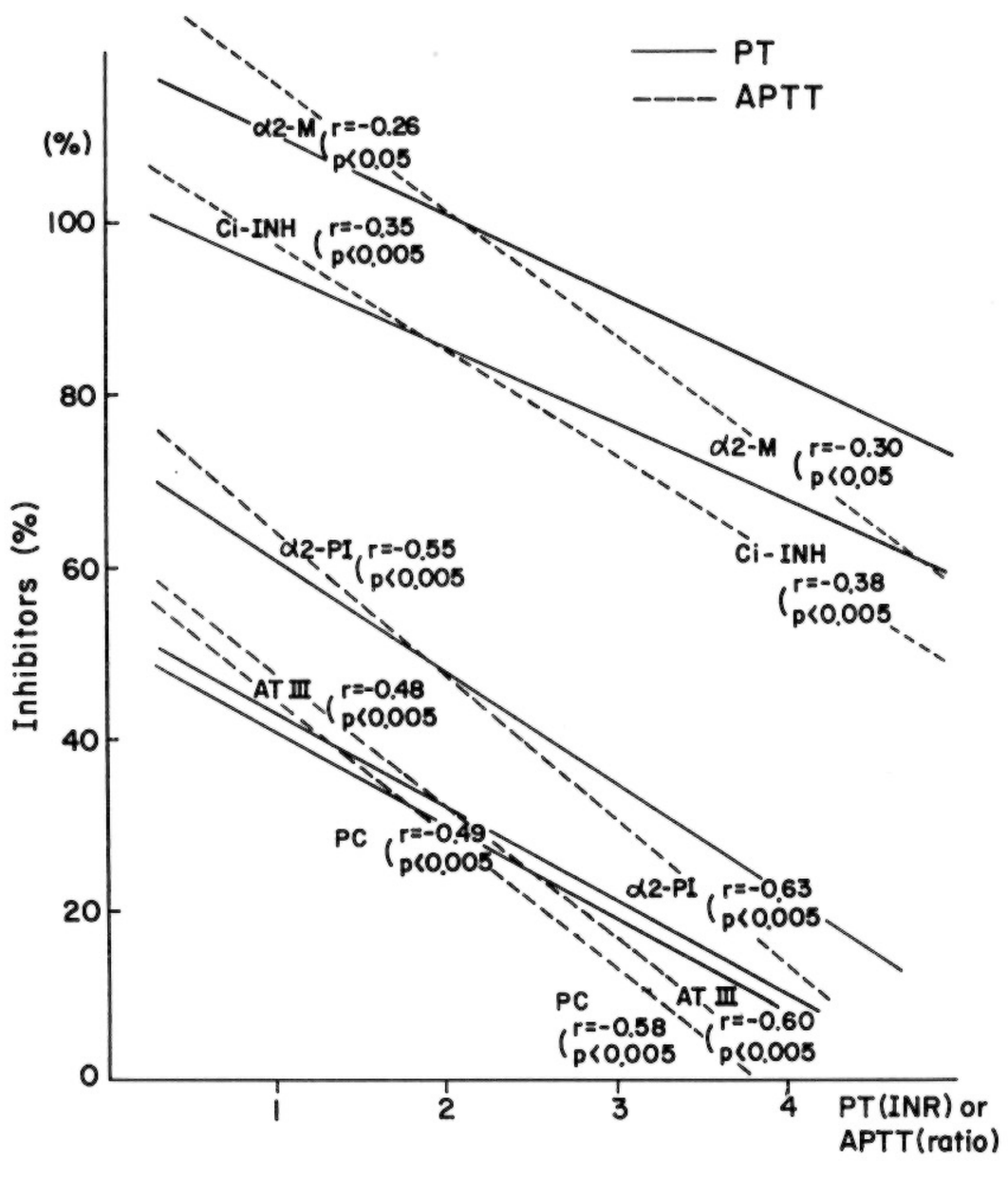

Fig .2. Relationship between Coagulation Inhibitors and PT or APTT

and Yamanaka, 1978) in liver cirrhosis. Recently, Sinclair et al. (1988) measured the major plasma protease inhibitors in alcoholic liver cirrhosis. The level of AT III and α 2−PI were significantly reduced, the mean levels of them being 65% and 70% each. The levels of α 2−M and CI-INH were elevated, the mean levels of them being 148% and 191% each.

In this study, the mean level of AT III in liver cirrhosis was 30%, a slightly lower value than those reported by others ; α 2−PI 47%, similar to those reported by others ; α 2−M 109%, near normal value : and C1-INH 85%, a lower value than that reported by Sinclair et al. (1988). A possible explanation for the difference of the levels of C1-INH could be the difference of measuring methods.

The pathogenesis of changes of coagulation and fibrinolysis inhibitor in liver disease has been explained as the depression of AT III mainly caused by reduced hepatic synthesis (Knot et al., 1984) and in part by DIC(Laursen et al., 1981). Aoki and Yamanaka(1978) reported that the levels of α 2−PI in patients with hepatocellular damage were significantly correlated with the levels of albumin and cholinesterase which are good indicators of hepatic synthetic function. But there was a report that the α 2−PI was depressed due to DIC in liver disease(Marongiu et al., 1985). Sinclair et al. (1988) reported that the levels of both AT III and α 2−PI significantly correlated with the serum albumin concentration. This study revealed AT III and PC were well-correlated with hepatic synthetic function, and acute DIC was considered in only one patient. The levels of C1-INH and α 2−M, being inhibitors, were slightly elevated despite the failure of hepatic synthesis in liver disease. These findings suggest their increased production from other sites(CI-INH from platelets and α 2−M from lymphocytes).

THe effects of changes of inhibitors on hemostatic abnormalities in liver disease have also been suggested (Lee, 1982, Lurie and Creter, 1981 ; Sinclair et al., 1988). Bocks et al. (1985) reported that among patients with liver disease, those with capillary bleeding were explained as having a defi-

ciency of coagulation factors, along with enhanced fibrinolysis caused by an increased concentration of tissue plasminogen activator. Hiller et al. (1983) reported that the mean level of AT III measured in 15 patients with acute esophageal varix bleeding was 51% of the normal control, and this could be a risk factor of the varix bleeding associated with hypercoagulability (Bertalgia et al., 1983). Therefore, the depression of AT III and PC due to hepatic synthetic failure might alleviate the bleeding tendency induced by the deficiency of defects of coagulation factors and aggravate the hypercoagulability induced by the deficiency of plasminogen and DIC.

Oh and Cho(1987) reported 11 cases with lupus anticoagulant of which two cases (18.2%) were liver cirrhosis and hepatoma. Kim et al. (1988). reported that among 17 cases with lupus anticoagulant four cases(23.5%) had liver disease. But Rosove et al.(1986) did not detect lupus anticoagulant in eight cases of hepatic failure. In this study, lupus anticoagulants were detected in 29 (46.8%) out of 62 patients with liver cirrhosis. Considering that autoimmunity is a component of the pathogenesis of liver cirrhosis, the lupus coagulant detected in liver cirrhosis is understood as a autoantibody. Lupus anticoagulant was more frequently detected in cases with reduced hepatic synthetic function, prolonged PT and APTT and reduced levels of AT III, PC, C1-INH and α2−PI. There might be two possible explantions for this finding : the lupus anticoagulant is more frequently detected in more advanced liver cirrhosis and there are false positive cases due to markedly reduced levels of coagulation factors. The latter should be confirmed by anticardiolipin antibody test.

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