Looking for Our Ten Years Results for Coronary Heart Disease and Ischemic Stroke Group for the Standpoint of Haemostasis

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ABSTRACT

To evaluate the role the coagulation and fibrinolysis abnormalities in the pathogenesis of ischemic stroke of undetermined etiology, we assayed plasma concentration of fibrinopeptide-A and thrombin-antithrombin III complex, both sensitive markers for thrombin activation and fibrin formation, and D-dimer, a marker of plasmin activity and fibrinolysis. Hemostatic markers were measured in 32 patients with acute stroke and 20 patients with chronic stroke, and compared with 21 normal subjects. Fibrinopeptid-A and thrombin-antithrombin III complex levels were not elevated significantly, whereas the D-dimer level was markedly raised in acute ($p< 0.001$) and chronic ($p< 0.05$) phases of ischemic stroke in comparison with the control group. Prolonged elevation of D-dimer concentration suggests that hemostatic abnormalities have a primary role in the pathogenesis of ischemic stroke. The measurement of D-dimer concentration may help to better decide the indications for therapy of the patients with ischemic stroke of undetermined etiology.

Key Words: Hemostatic activation, Coronary artery disease, Molecular markers.

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INTRODUCTION

Endothelial cells, circulating platelets and proteins of the coagulation and fibrinolytic systems are known to contribute to the hemostatic processes. Among the various functional and biochemical alterations in the platelets and hemostatic systems of coronary artery disease and atherosclerotic diseases occupy on important place. The pathologic and physiologic activation of the hemostatic process results in generation of some markers of cellular and plasmatic origin. Physiological states (such as exercise, mechanical factors and pregnancy) may generate mediators during the hemostatic process.

Platelet factor (PF IV) and beta-thromboglobulin (ß-TG) are platelet granular storage products released upon activation of platelets. Elevated levels of these products have been found in association with myocarding infarction, venous thrombosis, diabetes and inflammating disease. Changes in the fibrinolytic system play on important role in coronary heart disease, atherosclerosis and diabetes. Thrombin antithrombin complex (TAT) formed during the thrombotic process circulates in the blood of patients. Fibrinopeptid A (FPA) is formed upon the activation of thrombin on fibrinogen. Tissue plasminogen activator (tPA) and plasminogen activator ihibitor-1 (PAI-1) are released from the endothelial cells tPA facilitates the digestion of onsite fibrin clots. Conversely, PAI-1 is one of the principal inhibitors of the fibrinolytic enzyme system. High levels of PAI-1 are associated with an increased risk of thromboembolic complications also D-dimer is a sensitive indicator of the formation of fibrin. A number of studies indicade that Lp(a) is an independent risk factor for the development of premature CAD. Lp(a) like plasminogen can bind to fibrin and that it competes with plasminogen and tPA for fibrin binding. Fibronectin is an early markers of connective tissue formation and increased levels of fibronectin, fibrinogen and fibrin together with Lp(a) are present in early atherosclerotic lesions. Transferrin is a major serum glycoprotein that transports iron between sites of absorption, storage and utilization. The main component of normal serum transferrin contains two biantennary glycans, each consisting of 2 mol of sialic acid. Recently several investigators indicated that LDL of most patients with coronary atherosclerosis differ from the LDL of most healthy subjects by the ability to cause primary atherosclerotic changes it was shown that patient's LDL has a substantially lower content of sialic acid compared with the LDL of healtyhy subjects.

On the other hand, the role of hypercoagulability and fibrinolysis in cerebral infarction remains uncertain. It has been estimated that hematologic abnormalities account for 4% of all strokes. The recent development of immunochemical assays has allowed the detection of intermediate rate breakdown products of fibrin formation and fibrinolysis.

In the last decades the association of CAD and hemostasis attracted the attention of clinicians and studies have been directed to hemostatic parameters. Factor VII (F VII) has been one of the most frequently studied parameters for this purpose. In Northwick Pork Heart study, a significant relation has been observed between F VII and the development of coronary events. The authors have proposed F VII as an indepent risk factor for ischaemic heart disease.

PAI-2 is a fibrinolytic inhibitor produsedpredominatly by monocytes. tPA is thought to modulate vascular fibrinolysis, whereas urokinase type plasminogen activator (upa) is mainly involved in the fibrinolytic process occurring in tissue. PAI-2 was originalty identified in extracts of human plasenta and exists as both a secreted 60 kd and cytosolic 47 kd form. PAI-2 is a serine protease inhibitor that is spesific for urokinase and tissue type plasminogen activator. PAI-2 porticipate in fibrinolysis processes by regulating the formation of plasmin. Transcription of the PAI-2 gene and synthesis of PAI-2 are stimulated by endotoxin in peripheral blood menocytes. PAI-2 are stimulated by endotoxin in peripheral blood menocytes. PAI-2 was first isolated from human placenta as a single chain polypeptide of 47.000 daltons. The PAI-2 gene consists of 8 exons spanning 16.54b on the long arm of chromosome 18. Two RFLP polymorphisms (ECORE and BcII) have been described in the noncoding region polymorphisms two variants of the PAI-2 gene, variant A consists of Asn and Ser at positions 120, 404 and 413 respectively, and variant B consist of Asp, Ly and Cys at 120, 404 and 413 respectively. We report a simple PCR-RFLP method for distinguishing between the PAI-2 gene variants A and B in Turkish population who has the myocardial infarction.

MATERIALS and METHODS

Peripheral blood samples from patients with CAD were obtained from the department of cardiology subsequently the patients were angiographically determined. Normal peripherally blood samples were obtained from clinically healthy individuals in our medical school. The patients group for analysis of PF_4, BTG fibronectin, Lp(a), FPA, TAT, D-dimer, tPA and PAI levels consisted of 14 women and 16 men. The control group consisted of 5 women and 5 men without significant stenotic lesion of the coronary arteries, matched to the patient group in sex, and body mass index. The patient group ranged in age from 38 to 67 years and the control group from 30 to 62 years.

Peripheral blood samples were collected from all subjects in trisodium citrate only between 8.00 pm and 9.00 pm because diurnal variation has been described in the plasma levels of the inhibitor for the determination of hemostatic parameters. Blood was centrifuged at 3000q for 15 minutes at room temparature and plasma stored at -70°C until assayed PF_4 BTG, FPF, TAT, tPA, PAI-1, D-dimer and Lp(a) levels were determined by ELISA procedure and fibronectin levels was determined by the turbidometric immuno assay.

For the measurement sialidase and desialylated transferrin, our patients group consisted of 3 women and 28 men, ranging in age from 47 to 75. The control group consisted of 5 women and 5 men, ranging in age from 35 to 65. According to their angiography results, the patient group consisted of 4 subjects with single-vessel disease, 15 subjects with double-vessel disease and 12 subjects with triple-vessel disease. All of them were admitted with unstable angina pectoris, three of them also had myocardial infarction. Serum desialo transferrin was analysed by a double antibody RIA and sialidase levels was determined by using a coupled enzyme assay. We also investigated F VII levels as a risk factor for coronary atherosclerosis. Consecutive patients referred to coronary angiography were divided in three groups:

1. CAD group those with significant lesion in one or more coronary arteries (n: 155),

2. High risk group-patients with normal coronary arteries and with two or more risk factors (n: 54),

3. Controls patients with normal coronary arteries with no or one risk factor (n: 90).

FVII was measured using the automated one step clotting time method in coagulometry (ST4) using human F VII deficient plasma. Diagnostica stago, deficient F VII: France, catalogue number 00274 and rabbit calcified thromboplastin reagent.

We also worked with acute and chronic stroke group to evaluate the role of the coagulation and fibrinolysis abnormalities in the pathogenesis of ischemic stroke of undetermined etiology, we assayed plasma concentration of FPA, TAT and D-dimer. Fifty two adult patients, asdmited for ischemic stroke at Neorology clinic of Cerrahpaşa Medical Faculty in Istanbul.

Conventional methods were used for the calculation of means, student errors of the means and median values. The significane of differences between variables of the patient and control groups were determined by the Student's t-test. Significance p values equal or less them 0.05 were considered significant correlation analysis were determined by the pre arson correlation test.

In F VII experiments we used ANOVA and Krushal-Wallis methods. For statistical analysis of stroke groups, we used Shapiro-Wilk Lest, Kruskal Wallis and Dunn's Multiple comparisan tests.

RESULTS

The mean PF_4, BTG, Fibronectin and Lp(a) levels in patients with CAD and control group are shown in Table 1. In patients group, PF_4, BTG, fibronectin and Lp(a) levels were found to be significally higher from those the control group (Table 1).

The mean FPA, TAT, tPA and PAI-1 levels in patients with CAD and control group are presented in Table 2. FPA, TAT, D-dimer, t-PA and PAI-1 levels in patients with CAD were significantly higher then the control group (Table 2).

The mean total cholesterol, triglyceride, HDL, LDL and VLDL cholesterole levels in patients with coronary heart disease and control group are seen in (Table 3). In patients group, serum total cholesterol, triglyceride, LDL and VLDL cholesterol levels ($p< 0.00l$) and HDL-cholesterol levels ($p< 0.00l$) were found to be significantly different from those in control group.

Contains serum desialylated transferring levels and

sialidase activities in the patients with coronary heart disease and the control group (Table 4). Serum desialylated transferring levels ($p< 0.01$) and sialidase activity ($p< 0.00l$) in the patients with coronary heart disease were found to be significantly higher than the control group.

Shows sialidase activities and desialylated transferring levels in patients with single, double, triple vessel disease and the control group (Table 5). In patients with single-double vessel disease ($p< 0.01$) and triple-vessel disease ($p< 0.00l$) the mean serum sialidase activities were significantly different than those in the control group ($p= 0.967$). In patients with single-double vessel disease and triple vessel disease the mean serum desialylated transferring levels were significantly elevated compored with the control group ($p= 0.242$). There were not any correlation between the lipid parameters and the sialidase activity and desialylated transferring levels. F VII levels of the three groups and in one, two and three vessel disease were given in (Table 6). No difference could be found in F VII between the study groups. When CAD patients were investigated separately, mean level of F VII increased with the number of vessel involved. Mean F VII in three vessel disease was significantly higher than the patients with two, one and no vessel involvement ($p= 0.006$).

When we analyzed the results of stroke group, there were no significant differences between 32 patients with recent stroke, 20 patients with old stroke and 21 controls in terms of age, gender, or frequency of diabetes mellitus, smoking where as hypertension, hyperlipidemia and ischemic heart disease were significantly more frequent in bloth patient groups compared to the controls. The characteristics of patients are summarized in (Table 7). Shows FPA, TAT and D-dimer levels each group, FAP and TAT levels were not different in patients and controls, while D-dimer level was significantly higher in both acute and chronic patient groups ($p< 0.00l$ and $p< 0.005$, respectively) (Table 8). The results of the PAI-2 genotypes in patients and controls were shown in Table 9.

DISCUSSION

There are many factors associated with the development of CAD. Hypodysfunction of endothelial cells, hypercoagulability, hypofibrinoltic activity and hyperactivity of platelets are closely related to the progression of CAD. The pathologic and physiologic activation of the hemostatic process results in the generation of various defined markers of cellular and plasmatic origin.

The activation of plasminogen by tPA is enhanced in the presence of fibrin and also at the endothelial cell surface. The impairment of tPA release during fibrinolytic deficit in certain disorders results in thrombolytic complications. In addition, in various studies PAI-1

Table 1. PF_4, BTG, fibronectin and serum Lp(a) levels in patients with coronary artery disease and control group

Group	n	Age (years)	Sex (M/F)	PF_4(IU/mL) X ± SD	BTG(IU/mL) X ± SD	Fibronectin (mg/mL) X ± SD	Lp(a)(mg/dL) X ± SD
CAD	30	38-67	16/14	20.4 ± 9.1*	27.3 ± 20.8*	371.1 ± 89.8**	97.8 ± 77.9*
Control	10	30-62	5/5	4.6 ± 1.9	15.8 ± 8.7	320.7 ± 80.4	29.8 ± 25.0

* $p< 0.001$, ** $p< 0.05$.

Table 2. FPA, TAT, D-dimer, t-PA, PAI-1 levels in patients with coronary artery disease and control group

Group	n	Age (years)	Sex (M/F)	FPA (ng/mL) X ± SD	TAT (ng/mL) X ± SD	D-dimer (ng/mL) X ± SD	tPA (ng/mL) X ± SD	PAI-1 (ng/mL) X ± SD
CAD	30	38-67	16/14	37.0 ± 12.7*	3.1 ± 1.4**	751.3 ± 260.1*	8.1 ± 2.2*	2.8 ± 0.4*
Control	10	30-62	5/5	13.2 ± 6.5	2.6 ± 1.0	271.0 ± 124.5	2.6 ± 0.9	1.8 ± 0.8

* $p< 0.01$, ** $p< 0.001$.

levels were found significantly higher in may patients with fibrinolytic disorders.

On the other hand, it has been shown that tPA binds reversibly and saturably to surface-bound Lp(a) and that as a result of this binding the activation of plasminogen by tPA is inhibited. Lp(a) has been found to bind to soluble fibrin and to compete with plasminogen and tPA binding for soluble fibrin; as a result of this interaction in solution, Lp(a) inhibits the fibrin stimulation of plasminogen activation by tPA. Lp(a) acts specifically on the endothelial cell to increase the proportion of PAI to tPA at the endothelial cell surface as well as in the local enviroment our results show that tPA, PAI-1 and Lp(a) levels are elevated in patients with CAD. Also we found that fibrinectin levels were significantly higher than in the control group. The high fibronectin levels in circulating blood may cause increased interaction of fibronectin with Lp(a) on the blood vessel wall. D-dimer is a sensitive indicator of formation of fibrin and its digestion and PF_4 and BTG are spesific products of platelet activation. PF_4 and BTG are extremely sensitive markers of arterial thrombotic disorders such as thrombotic stroke, peripheral vascular disease or CAD. We demonstrated that D-dimer, PF_4 and BTAG levels in patients with CAD were higher than those in the control group. FPA and TAT are useful markers of thrombin mediated conversion of fibrinogen to fibrin. We found that TAT and FPA levels were significantly higher than in the control group. In this study; we found that serum sialidase levels in patients with single, double and triple-vessel disease were significantly higher than the control group. Also we found that serum desialylated transferring levels in patients with double and triple-vessel disease were significantly elevated compared with the control group. The elevated levels of serum sialidase may be caused by an increase in its activity. The increased levels of serum sialidase may be responsible for transferrin desialylation in coronary heart disease. We report that increased level of serum sialidase may be responsible for transferring desialylation and elevated desialylated transferring levels may play on important role in the pathogenesis and diagnosis of coronary heart disease. On the other hand we investigated the relation of F VII to the severity of CAD and tried to determine its association with coronary events. Our findings revealed a significant correlation between F VII and triglycerides, and after the adjusment for other parameters F VII could not be accepted as on independent risk factor for either the presence on the extent of coronary atherosclerosis. Neverheless, is spite of the result of logistic regression analysis, increased levels of F VII in patients with multi vessel disease and previous coronary events suggested that it is related to the thrombotic process of these syndromes.

For the stroke groups, in conclusion, our study suggest that prolapsed elevation of D-dimer connot be explained just by events initiated by cerebral infarction it self, but rather implicates a permanent hemostatic ab-

Table 3. Lipid parameters in atherosclerotic and control group

Group	n	Total cholesterol (mg/dL) X ± SD	Triglycerid (mg/dL) X ± SD	LDL Cholesterol (mg/dL) X ± SD	VLDL Cholesterol (mg/dL) X ± SD	HDL Cholesterol (mg/dL) X ± SD
CHD	31	222.36 ± 61.59*	177.16 ± 81.94*	149.58 ± 58.45*	35.43 ± 16.38	37.08 ± 10.62
Control	10	160.23 ± 34.81	97.81 ± 29.88	86.16 ± 26.59	19.56 ± 5.97	46.92 ± 6.15

* p< 0.01, p< 0.001.

Table 4. Desialylated transferrin levels and sialidase activities in atherosclerotic and control group

Group	n	Sialidase (U/L) X ± SD	Desialylated transferrin (U/L) X ± SD
CHD	31	72.73 ± 20.02*	62.93 ± 15.9**
Control	10	50.57 ± 5.1	48.20 ± 6.32

* p< 0.001; ** p< 0.01.

Table 5. Desialylated transferrin levels and sialidase activities in single-double-triple vessel disease and control group

Group	n	Sialidase (U/L) X ± SD	Desialylated transferrin (U/L) X ± SD
Single-vessel disease	4	75.52 ± 521.72*	49.75 ± 513.35**
Double-vessel disease	15	70.42 ± 21.08*	64.14 ± 513.72**
Triple-vessel disease	12	72.22 ± 516.98*	66.0 ± 519.14**
Control	10	50.57 ± 5.1	48.20 ± 6.32

* p= 0.967, **p = 0.242.

Table 6. FVII levels in the one vessel, two vessel, three vessel, total high risk and control groups

	CAD (n= 155)			Highrisk (n= 54)	Control (n= 90)
One-vessel (n= 60)	Two-vessel (n= 55)	Three-vessel (n= 40)	Total (n= 155)		
FVII 85 ± 20	92 ± 23	105 ± 23*	94 ± 23	91 ± 27	88 ± 22

* Difference between the vessel involvement, p= 0.006.
Difference between the CAD and the control group are not significaut.

Table 7. Patient characteristics of stroke groups

	Acute stroke group	Chronic stroke groups	Control
n	32	20	21
Mean age ± SD	62.2 ± 13.2	59.7 ± 6.8	56.2 ± 11.3
Range (years)	30-80	50-71	45-73
Male/female	16/16	10/10	9/12
Hypertension	24 (75.0%)*	10 (50.0%)**	2 (9.5%)
Diabetes mellitus	6 (18.7%)	2(10%)	1 (4.7%)
Hyperlipidemia	11 (34.3%)***	8 (40.0%)***	2 (9.5%)
Smoking	17 (53.1%)	11 (55.0%)	8 (38.0%)
Ischemic heart disease	16 (50.0%)****	7 (35.0%)****	0 (0.0%)

*p< 0.00001, **p< 0.05, ***p< 0.05, ****p< 0.05 v.s. control group.

Table 8. Hemostatic marker levels in stroke groups

	Acute stroke group	Chronic stroke group	Control
FPA (ng/mL)	11.7 ± 2.8	15.1 ± 1.7	12.1 ± 2.1
TAT (ng/mL)	10.1 ± 6.0	15.2 ± 10.9	13.4 ± 10.9
D-dimer (ng/mL)	668.0 ± 65.3	599.7 ± 65.2	333.9 ± 42.9

Table 9. PAI-2 patients and control (PAI-2 gene variants)

PAI-2 genotypes	Patients (n= 66)	Control (n= 20)
AA	33 (50%)	2 (10%)
AB	29 (44%)	18 (90%)
BB	4 (6%)	0
A	0.720	0.550
B	0.280	0.4

normality that may be the underlying factor in the pathogenesis of ischemic stroke. The measurement of D-dimer concentration may help to better decise the indications for therapy of the patients with ischemic stroke of undetermined etiology.

REFERENCES

1. Fareed J, Bick RL, Hoppensteadt DA, Bermez EW. Molecular markers of hemostatic activation: Applications in the diagnosis of thrombosis and vascular and thrombotic disorders. Clin App Thromb Hemost 1995;1:87-102.
2. Ulutin T, Sönmez H, Üç›ş›k N, Süer S, Bayram Ç, Kökoğlu E, Sultuybek G. The molecular markers of hemostatic activation on coronary artery disease. Thrombosis Research 1997;88:329-32.
3. Walenga JM, Fareed J, Messmore HL. Newer avenues in the monitoring of antithrombotic therapy: The role of automation. Semin Thromb Hemost 1983;9:346-54.
4. Messmore HL, Walenga JM, Fareed J. Molecular markers of platelet activation. Semin Thromb Hemost 1983;9:354-78.
5. Baver KA, Rosenberg RD. The pathophysiology of the prothrombotic state in humans: Insight gained from studies using markers of hemostatic system activation. Blood 1987;70:343-50.
6. Fareed J, Walenga JM. Changing trends in hemostatic testing In: Oukda K (ed). Automation and New Technology in the Clinical Laboratory. Oxford: Oxford Press, 1990:203-10.
7. Oida K, Maeda H, Naka T. Abnormal antithrombogenic function on endothelial cells in diabetes mellitus. In: King GL, Shigela Y, (eds). Endothelial Cell Dysfunction Diabetes. Japan: Churchill Livingstone; 1994:33-54.
8. Süer S, Ulutin T, Sönmez H, Kökoğlu E, Üç›ş›k N, Bayram Ç, Sultuybek G. Plazma Lp (a. and tPA-PAI-1 complex levels in coronary heart disease. Thromb Res 1996;83:77-85.
9. Loskutoff DJ, Sawdey M, Mimoro J. Type 1 plasminogen activator inhibitor. Prog Hemostas Thromb 1988;9:87-115.
10. Kostner GM, Avogaro P, Lazzolato G, Marth E, Bittolo BG, Qunici GB. Lipoprotein(a) and the risk for myocardial infarction. Atherosclerosis 1981;38:51-61.
11. Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL. Lp(a) as a risk factor for myocardial infarction. J Am Med Assoc 1986;256:2540-4.
12. Loscalzo J, Weinfeld M, Fless GM, Scann AM. Lipoprotein (a), fibrinbinding and plasminogen activation. Atherosclerosis 1990;10:241-5.
13. Balkuv-Ulutin Ş. Fibrinolytic system in atherosclerosis. Semin Thromb Hemost 1986;12:91-101.
14. Hadden JM, Haris PI, Srai KSJ, Chapman D. Conformational studies on human transferring. Biochem Soc Transact 1992;20:200.
15. Stibler H, Borg S. Evidence of a reduced sialic acid content in serum transferring in male alcoholics. Alcohol Clin Exp Res 1981;5:545.
16. Mukhin DN, Tertov VV, Kacharava AG, Orekhov AN. Desialylated lowdensity lipoproteins atherogenic lipoproteins occurring in blood of patients with coronary atherosclerosis. Biull Eksp Biol Med 1990; 110:138.
17. Tertov VV, Sobenin IA, Gevera KH, Morrisett DD, Orekkov AN. Carbohydrate composition of native and desialylated low density lipoproteins in the plasma of patients with coronary atherosclerosis. Kardiologica 1992;32:57.
18. Tertov VV, Sobenin IA, Tonevitsky AG, Orekhov AN, Simirnov VN. Isolation of atherogenic modified (desialylated) low density lipoprotein from blood of atherosclerotic patients: Separation from native lipoprotein by affinity chromatography. Biochem Biophys Res Commun 1990;167:1122.
19. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welinl, Tibblin G. Fibrinogen as a risk factor for strake and myocardial infarction. N Engl J Med 1984;311:501.
20. Salomaa V, Stinson V, Kark JD, Folsom AR, Davis CE, Wu KK. Association of fibrinoltic parameters with early atherosclerosis: The ARIC study. Circulation 1995;91:284-90.
21. Thompson SG, Kienastj, Pyke SD, Haverkate F, Van de Loo JC. Hemostatic factors and the risk of myocardial infarction and sudden death in patients with angina pec-

toris. N Engl J Med 1995;332:635-41.

22. Meade TW, Mellow S, Brozovic M, Miller GJ, Chakrabati RR, North WR. Hemostatic function and ischaemic heart disease. Principal results of Northwich Park Heart Study. Lancet 1986;533-57.
23. Gengni GF, Comeglio M, Colella A. Classical risk factors and emerging elements in the risk profile for coronary artery disease. Eur Heart J 1998;19:53-61.
24. Sönmez H, Öztürk ZG, Uultin T, Domaniç N, Kökoğlu E. Carbohydrate-deficient transferrin and sialidase levels in coronary heart disease. Thrombosis Reesearch 2000;99:311-5.
25. Domaniç N, Ural A, Vural VA, Gürel Ç, Ulutin T. Factor VII levels in patients undergoing coronary angiography. Factor VII and coronary artery disease. Journal of Cardiovascular Risk 2001;8:57-61.
26. İnce B, Bayram Ç, Harmanc› H, Ulutin T. Hemostatic markers in ischemic stroke of undetermined etiology. Thrombosis Research 1999;96:169-74.
27. Sakata Y, Eguchi Y, Mimura J, Matsudo MJ, Suni Y. Clot lysis induced by monoclonal antibody against a2 plasmin inhibitor. Blood 1989;74:2692-7.
28. Kruithof EK, Vasalli JD, Scleuning WD, Mattaliano RJ, Bachmann F. Purification and characterization of a plasminogen activator inhibitor from the histiocytic lymphoma cell line U-937. J Biol Chem 1986;261:11207-13.
29. Blasi F, Vasalli JD, Dano K. Urokinase-type plasminogen activator: Proenzyme, receptor, and inhibitors. J Biol Chem 1987;104: 801-4.
30. Chapman KA. Vaurin 2, nibs. JB Cell 1982;28: 653-62.
31. Kawano T, Morimoto K, Uemura Y. Urukinase inhibitor in human plasenta. Nature 1968; 217:253-4.
32. Webb AC, Collins KL, Snyder SE, Alexander SJ, Rosenwasser LJ, Eddy RL, Shows TB, Auron PE. Human monocyte Arg-Serpin cDNA Sequence, chromosomal assignment, and homology to plasminogen activator-inhibitor. J Exp-Med 1987;166:77-94.
33. Antalis TM, Clark MA, Barnes T, Lehrbach PR, Devine PL, Schevzov G, Goss NH, Stephens RW, Tolstoshev P. Cloning and expression of a cDNA coding for a human monocytederived plasminogen activator inhibitor. Proc Natl Acad Sci USA 1998;85:985-9.
34. Ye RD, Wun TC, Sadler JE. cDNA cloning and expression in *Escherichia coli* of a plasminogen activator inhibitor from human placenta. J Biol Chem 1987; 262:3718-25.
35. Sayhan N, Gürel ÇB, Y›lmaz Ş, Ulutin T, Ulutin ON. PCR-RFLP detection of PAI-2 gene variants and ACE gene polymorphism in myocardial infarction. Haemostasis 2000;30:1.
36. Chobanrian AV. Pathophysiology of atherosclerosis. Am J Cardiol 1992;70.
37. Metha J, Mehta P, Lawson P, Saldeen T. Plasma tissue plasminogen activator inhibitor levels in coronary artery disease: Correlation with age and serum triglyceride concentrations. JACC 1987;9:263-8.
38. Bono D. Significance of raised plasma concentrations of tissue-type plasminogen activator and plasminogen activator inhibitor in patients at risk from ischaemic heart disease. BR Heart J 1994;71:504-7.
39. Kruithoff EKO. Plasminogen activator inhibitor type 1 and its relation to thrombosis. Med Razgl 1990; 29:43-52.
40. Aillaud MF, Pignol F, Alessi MC, Harte JR, Escande M, Mongin M, Suhan-Vague I. İncrease in plasma concentration of plasminogen activator inhibitor fibrinogen, vonwillebrand factor, factor VIII: C and in erythrocyte sedimentation rate with age. Thromb Hemost 1986;55:330-2.
41. Simon DI, Fless GM, Scaro AM, Loscalzo J. Tissue type plasminogen activator binds to is inhibited by surface bound lipoprotein (a) and lowdensity lipoprotein. Biochemistry 1989;28:2370-4.
42. Edelberg JM, Gonzales GM, Pizzo SU. Lipoprotein (a) inhibits streptokinase-mediated activation of human plasminogen. Biochemistry 1989;28:2370-4.
43. Etingin OR, Hajjar DP, Hajjar KA, Harpez PO, Nachman RL. Lipoprotein (a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. J Biol Chme 1991;56:2459-65.
44. Ulutin O, Ulutin ŞB, Göker BB, Çizöeci G, Ferhanoğlu B, Özsoy Y, Uğur MŞ, Ulutin T, Yaman A, Yard›mc› T. Effect of defibrotide on platelet function. Seminars in Thrombosis and Hemostasis 1996; 22:21-4.
45. Ulutin Bayram Ç, Ohari, Özlük K, Tözün N, Ulutin O. The effect of endothelin-1 on venajugularis thrombus model in rabbits. Reviews in Clinical and Basic Pharmacology and Physiology 1995:6:295-302.
46. Ulutin O, Ulutin ŞB, Uğur MŞ, Ulutin T, Özsoy Y, Çizmeci G. The pharmacology and clinical pharmacology of defibrotide: A new profibrinolytic, antithrombotic and antiplatelet substance. In: Liu CY, Chein S (eds). Fibrinogen Thrombosis, loagulation and Fibrinolysis. New-York: Plenum. Priss, 1992; 429-38.
47. Sönmez H, Süer S, Ulutin T, Kökoğlu E, Üç›ş›k N. The relationship of various factors in the pathogenesis of atherosclerosis. Clin Appl Thromb Hemost 1998;4:105-10.
48. Süer S, Ulutin T, Sönmez H, Kökoğlu E, Üç›ş›k N, Bayram Ç, Sultuybek G. Plasma Lp(a) and tPA-PAI-1 complex levels in coronary heart disease. Thrombosis Research 1996:83:77-85.
49. Sönmez H, Süer S, Kökoğlu E, Dirican A, Ulutin T, Üç›ş›k N, Ulutin O. The importance of Lp(a)-fibronectin interaction in atherogenesis. Haematologica 1997;28:149-53.
50. Coull BM, Clark WM. Abnormalities of haemostasis in ischemic stroke. Medical Clinics of North America 1993;77:77-89.
51. Fon EA, Machey A, Cote R, Walfson C, Melbraith DM, Leclere J, Boungke F. Hemostatic marker in acute tran-

sient ›schemic attaks. Stroke 1994;25: 282-6.

52. France CL, Buscher MJJ, Van Wersh JWJ. Hemostasis and fibrinolysis after recent stroke. Cerebrovas Dis 1992;2:365-8.

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