Turkish Journal of Endocrinology and Metabolism, (1999) 2 : 53-63 **ORIGINAL ARTICLE**

Diabetes Mellitus and the Natural Anticoagulants

Kevser Onbaşı* Belgin Efe Aysen Akalın** Nur Kebapçı** Esat Erenoğlu***

Osmangazi University, Eskişehir

** Department of Internal Medicine*

*** Department of Endocrinology and Metabolism*

Diabetes Mellitus is associated with disturbances of the hemostatic system, which might contribute to the development of diabetic vascular disease. The purpo se of this study was to clarify whether the levels of natural anticoagulants are changed in diabetics and whether biochemical changes, especially hyperlipidemia and hyperglycemia could affect the levels of the natural anticoagulants. We investigated also the effect of the therapy type; smoking, hypertension, ischemiac ECG findings, BMI status, retinopathy, neuropathy and nephropathy status on the natural anticoagulants. These parameters were studied in 84 type 2 and 18 type 1 diabetic patients and compared with 15 healthy control subjects for type 2 patients and 11 healthy control subjects for type 1 patients matched for age, sex and BMI. The natural anticoagulants protein C, protein S and antithrombin III were correlated to triglyceride and the protein C was correlated with total cholesterol in type 2 patients. The protein C and protein S levels were correlated to prothrombin time and activated partial thromboplastin time in type 1 diabetics. Protein C levels were correlated to fibrinogen levels in type 1 patients. Antithrombin III levels were also correlated to HbA_1C, fasting glucose and triglyceride levels in type I diabetics. The levels of protein S were found to be lower in type 1 patients in comparison to type 2 patients. The levels of protein C were found to be lower in type 2 patients when compared with the control group. The type 2 patients had significantly higher fibrinogen levels than the control group. From these results, we have concluded that there is a thrombotic tendency or at least an imbalance between the hemostatic and thrombosis protecting system in type 2 diabetic patients, especially in patients with hyperlipidemia; hyperglycemia and hyperfibrinogenemia.

KEY WORDS Diabetes Mellitus, natural anticoagulants, hypercoagulability

Introduction

Disturbances of hemostatic and fibrinolytic mechanisms have been frequently described in patients with diabetes mellitus and have been associated with the development of diabetic complications and the increased incidence of cardiovascular events in diabetic subjects (1). Thrombosis and related complications are the major causes of morbidity and mortality in diabetic patients. The causes of hypercoagulability in diabetics can be listed as follows:

Correspondence address:

Kevser Onbaşı, M.D.
Cumhuriyet Mah. Sakarya Cad. Güven Apt. 51/3, Eskişehir
Tel : 0 432 214 78 36
Fax: 0 432 214 78 36
E-mail: kevseronbasi@yahoo.com

Platelet Function In Diabetics:

Diabetic patients are hypersensitive to aggregating stimuli. Hyperaggregalibility of the platelets and an increase in plasma levels of von Willebrand factor in diabetic subjects, which is important in the adhesion of platelets to subendotelial cells, have been reported. In diabetic patients, elevation of levels of β-thromboglobulin and platelet factor 4 released from α-granules of platelets when platelets are activated have also been described. Also enhanced biosynthesis of thromboxane in platelets, which enhances thrombotic tendency, has been reported (2,3,4,5).

Coagulation Factors in Diabetic Patients:

Factor VII,VIII, vWF, fibrinojen are raised in diabetic patients. Also during hyperglycemia the

function of antithrombin III decreases and because antithrombin III is the natural inhibitor of factor X, the levels of factor X might be increased, too (1,4).

Physiological Inhibitors of Blood Coagulation in Diabetic Patients:

Regarding physiological inhibitors of blood coagulation in plasma; there have been conflicting reports so far. It has been suggested that glycosylation of these coagulation inhibitors in diabetic patients may impair the function of these inhibitors. The most important natural inhibitors are protein C, protein S, antithrombin III and TFPI(Tissue Factor Pathway Inhibitor)

Eritrocytes:

Eritrocytes in diabetics are prone to injury and when the eritrocytes are damaged, the result is the reduction of half-life of eritrocytes. Polycytemia and increased blood volume are factors which may cause blood hyperviscosity with a consequent increase in thrombotic risk (6).

Fibrinolysis:

Decreased levels of tissue plasminogen activator (t PA),which triggers activation of the fibrinolytic system, have been reported in diabetic patients. The inhibitor of tissue plasminogen activator 1 (PAI-1) is reported to be high in diabetics (1,2,3).

Patients and Methods

Eighty-four type 2 diabetic patients (66 female; 18 male) and 18 type 1 diabetic patients (11 female; 7 male)admitted to the Endocrinology and Metabolism Department at the Osmangazi University were included in our study. The control group for type 2 patients consisted of 8 female and 7 male patients and the control group for type 1 patients consisted of 8 female and 3 male healthy voluntary persons, matched for sex, age and body mass index (BMI), recruited from hospital staff and relatives. None of the patients or control subjects had any clinical or laboratory signs of liver or renal dysfunction, malign diseases, a history of thromboembolic events, pregnancy, thyroid disease, autoimmune disease or myleproliferative disease. None of the subjects were treated with drugs known to influence the hemostatic system or metabolic parameters.

For the determination of hemostatic and coagulation parameters blood was collected after an overnight fasting period by venipuncture with plastic syringes.

For the biochemical parameters the samples were collected in glass tubes and fasting glucose, BUN, creatinin, total cholesterol, triglyceride and HDL-Cholesterol were measured with a Boehringer Mannheim (BM) 747 autoanalyser with BM reagants. Also HbA_1C and urinary creatinin were measured with the same autoanalyser.

For the platelet counts the blood samples were taken into Becton Dickenson Vacutainer tubes, which were ready for use and prepared with anticoagulants. The platelet count was measured with the Cell-Dyn 3500 R Automatic Blood Counter. The platelet count was measured in heparinized tubes that do not contain EDTA. For prothrombin time, activated thromboplastin time and fibrinogen the blood was taken into tubes containing 0,38 % Na_3-citrate and measured with the neoplastin, CK-prest and fibriprest reagants in the Stago St_4 semi-automatic coagulameter. For protein C, protein S, antithrombin III the blood was taken also into tubes containing 0,38 %Na_3-citrate and centrifuged for 20 min at 3500 x g. The supernatant plasma was aliquoted and stored at -7O°C until measuring time. Protein C and protein S levels were measured with the thrombonostica PC and thrombonostica PS reagants with the Organon Technica TEK Time Microwell System. A III was measured with Beckmann Array 360 nephelometry system and with reagants of Beckmann. The natural anticoagulant's antigenic levels were measured.

To determine whether nephropathy exists creatinin clearance (CCr) and 24 hour proteinuria values were calculated. Patients with ketoacidosis, bacteriuria and heavy exercise were excluded. Proteinuria was researched with the turbidometric analyzer. The patients were divided into three groups for the degree of proteinuria. Group 1 included patients with proteinuria less than 30 mg/day; group 2 included patients with proteinuria values between 30-300 mg/day and the last group included

the patients with proteinuria above 300 mg/day. Patients with proteinuria above 1500 mg/day were exclu-ded from the study.

The patients were divided into three groups according to their CCr values. Group 1 included the patients with CCr values less than 50 ml/minute. Group 2 included the patients with CCr values between 50-80 ml/min, Group 3 included the patients with values above 80 ml/min. Patients with CCr values below 30 ml/min were excluded from the study.

In searching for retinopathy direct and indirect ophtalmoscopy and flourescein angiographic methods were used. The patients were classified for retinopathy as follows: Group 0: normal fundoscopy, Group 1: background retinopathy, Group 2: proliferative retinopathy.

While searching for neuropathy causes for neuropathy other than diabetes were excluded. Patients with subjective complaints of peripheral neuropathy were examined for Romberg's sign and superficial and deep sensory neurological findings. Peripheral neuropathy was evaluated based on ENMG (Electroneuromyography) and the patients with abnormal ENMG results were determined to have peripheral neuropathy. Abnormalities in cardiovascular, gastrointestinal, urogenital, thermoregulatory, sudomotor autonomic function were researched and in each case resting heart rate, and heart rate response to deep breathing, heart rate and blood pressure response to upright position were investigated. In patients with complaints regarding gastrointestinal autonomic neuropathy, esophagus and gastric motility tests were performed scintigraphically. If two or more of the blood pressure and heart rate tests regarding autonomic neuropathy were positive, then the patient was included in the autonomic neuropathy group. The patients with gastrointestinal autonomic neuropathy were included in the autonomic neuropathy group if at least one of the blood pressure and heart rate tests were positive.

Statistically findings were defined with mean ± and with standard error values. The student t-test, Pearson correlation test and variance analysis were used.

Results

Table 1. Comparison of the basic characteristics of the diabetics.

	Type II	Type I	p
Patient number (n)	84 (82,35%)	18 (17,65%)	
Sex			
Female	66 (78,57%)	11 (%61,11)	
Male	18 (21,43%)	7 (38,89%)	
Age (year)	56,35±1,01	30,11±1,62	p<0,001
Mean diabetes Duration (year)	6,58±0,64	8,11±1,44	NS
Body mass index (kg/m^2)	28,92±0,55	23,36±0,87	p<0,001

NS: Nonsignificant

84 type II and 18 type I patients were included in the study. Type II patients were older and had higher BMI values.

Table 2. Comparison of the biochemical parameters, BMI values, age and sedimentation rate between type II patients and the control group.

	Type II (n=84)	Control group (n=15)	P
Age (year)	56,35±1,01	54,20±2,13	NS
BMI (kg/m^2)	28.92±0,55	25,36±0,83	P<0.01
Sedimentation (mm/h)	24,34±2,27	19,86±1,53	NS
Fasting glucose (mg/dl)	204,01±8,96	95,60±3,72	P<0,001
HbA_1C (%)	9,15±0,37	5,56±0,09	P<0,001
Triglyceride (mg/dl)	234±14,60	142±11,41	P<0,001
Total cholesterol (mg/dl)	216,63±4,88	173,73±5,40	P<0,001
HDL cholesterol (mg/dl)	45,17±1,15	50,20±1,90	NS

Type II diabetic patients had higher BMI values than the control group. Also fasting glucose, HbA1C, triglyceride and total cholesterol levels were higher than in the control group. Age, sedimentation rates and HDL cholesterol levels were similar in both groups.

Table 3. Comparison of the biochemical parameters, BMI values, age and sedimentation rates between type I diabetic patients and the control group.

	Type I (n=18)	Control Group (n=11)	P
Age (year)	30,11±1,62	28,45±1,75	NS
BMI (kg/m^2)	23,36±0,87	23,30±0,92	NS
Sedimentation (mm/h)	14,823±5.33	13,72±4,39	NS
Fasting glucose(mg/dl)	240,50±25,34	89,00±3.03	P<0.001
HbA_1C (%)	11,56±0,81	5,04±0,09	P<0.001
Triglyceride (mg/dl)	115,05±17,64	128,36±17,85	NS
Total cholesterol (mg/dl)	172,55±7,99	168,8±13,18	NS
HDL (mg/dl)	56,22±2,44	49,90±2,72	NS

In spite of being diabetic (only fasting glucose and $HbA_1$1C were not similar) type I diabetic patients shared the same parameters with the control group.

Table 4. Comparison of the biochemical parameters between type II and type I patients.

	Type II	Type I	P
Fasting glucose (mg/dl)	204,01±8,96	240,50±25,34	NS
HbA_1C (%)	9,15±0,37	11,56±0,81	p<0,01
Triglyceride (mg/dL)	234,00±14,60	115,05±17,64	p<0,001
Total cholesterol (mg/dl)	216,63±4,88	172,55±7,99	p<0,001
HDL cholesterol (mg/dl)	45,17±1,15	56,22±2,44	p<0,001

Fasting glucose and HbA_1C levels of the type I patients were higher than those of the type II patients, but statistically only HbA_1C levels were found to be higher. Type II patients had higher triglyceride and cholesterol levels and lower HDL cholesterol levels compared to type I patients.

Table 5. Natural anticoagulants of type II and type I patients.

	Type II (n=84)	Type I (n=18)	P
Protein C levels (IU/ml)	1,05±0,39	1,12±0,41	NS
Protein S levels (%)	117,91±4,98	86,22±9,72	p<0,01
Antithrombin III Levels (mg/dl)	27,38±0,43	26,88±0,70	NS

The protein S levels in type I patients were lower than in the type II patients. Protein C levels were lower in type II patients, but this difference was not statistically important. Antithrombin III levels were similar in both groups.

Table 6. Comparison of the hemostatic parameters of diabetic patients.

	Type II (n=84)	Type I (n=18)	P
pt prolongation (sec)	0,40±0,08	0,05±0,005	p<0,001
aptt prolongation (sec)	0,42±0.09	0,11±0,04	NS
Fibrinogen levels (mg / dL)	404±12,02	354±37,12	NS
Platelet number (x/mm^3)	274917±10265	259444±13404	NS
Fibrinojen/AIII	15,60±0,93	13,21±1,31	NS

The prothrombin time of type II patients was longer than that of type I patients.

Table 7. Comparison of the natural anticoagulants of type II patients with the control group.

	Type II (n=84)	Control Group (n=15)	Comparison
Protein C levels (IU/ml)	1,05±0,04	1,25±0,06	P< 0,05
Protein S levels (%)	117,91±4,98	123,66±9,36	NS
Antithrombin III Levels (mg/dl)	27,38±0,43	27,90±0,57	NS

Protein C levels of type II patients were lower than in the control group. Protein S and antithrombin III

levels were lower than in the control group, too, but this difference was statistically nonsignificant.

Table 8. Comparison of the natural anticoagulants of type I patients with the control group.

	Type I patients (n=18)	Control Group (n=11)	Comparison
Protein C levels (IU/ml)	1,12±0,09	0,94±0,06	NS
Protein S levels (%)	86,22±9,72	94,36±6,35	NS
Antithrombin III levels (mg/dl)	26,83±0,70	24,59±0,57	P<0,05

There was no difference between the type I diabetic patients and the control group for protein C and protein S levels, but antithrombin III levels were surprisingly lower in the control group.

The fibrinogen levels of the type II diabetic patients were prominently higher than those of the control group. The pt and aptt and platelet number of type II patients were similar wo the control group. The fibrinogen/AIII levels were higher in the type II group than in the control group, but there was no difference in the type I group. The hemostatic parameters of the type I group were not different from the control group.

Table 9. Comparison of the hemostatic parameters between the diabetic patients and the control group.

	Diabetic patients	Control group	P	
pt prolongation (sec)	0,40±0,08	0,13±0,01	NS	
aptt prolongation (sec)	0,42±0,09	0,20±0,14	NS	Type II Patients (n=84)
Fibrinogen (mg/dl)	404,75±12,02	295,46±9,51	P<0,001	Control group (n=15)
Platelet Number (x/mm^3)	274197±10265	240966±17283	NS	
Fibrinogen/AIII	15,60±0,93	10,66±0,42	P<0,001	
pt prolongation (sec)	0,05±0,005	0,0±0	NS	
aptt prolongation (sec)	0,11±0,01	0,0±0	NS	Type I patients (n=18)
Fibrinogen (mg/dl)	354±37,10	284±17,13	NS	Control group (n=11)
Platelet number (x/mm^3)	259444±13404	256272±24423	NS	
Fibrinojen/AIII	13,21±1,31	11,70±0,84	NS	

Table 10. Parameters which show positive correlation with the natural antioagulants in type II diabetic patients.

	Protein C	Protein S	Antithrombin III	Fibrinogen/AIII
Triglyceride	r=0,32 p<0,01 (n=84)	r=0,25 p<0,05 (n=84)	R-0,24 P<0,05 (n=84)	
Cholesterol	r=0,50 p<0,001 (n=84)			
Fibrinogen				R=0,48 p<0,001 (n=84)
Sedimentation				R=0,33 p<0,001 (n=84)

Table 11. Parameters which show positive correlation with the natural anticoagulants in type I diabetic patients.

	Protein C	Protein S	Antithrombin III	Fibrinogen/AIII ratio
Pt	r=0,52 p<0,05 (n=18)	r=0,48 p<0,05 (n=18)		
Aptt	r=0,52 P<0,05 (n=18)	r=0,53 p<0,05 (n=18)		r=0,74 P<0,01 (n=18)
Fibrinogen	r=0,54 p<0,05 (n=18)			r=0,96 p<0,001 (n=18)
HbA_1C			r=0,57 P<0,05 (n=18)	
Triglyceride			R=0,48 P<0,05 (n=18)	
Fasting glucose			R=0,49 P<0,05 (n=18)	
Sedimentation				r=0,89 p<0,001 (n=18)

Triglyceride values were positively correlated to protein S, protein C and antithrombin III in type II diabetics. Cholesterol levels were correlated only to protein C in type II diabetic patients. Triglyceride values correlated to antithrombin III levels in type I diabetics. The prothrombin time and activated partial thromboplastin time were in positive correlation with protein S and protein C. Also fibrinogen levels were correlated to protein C in type I patients. Fasting glucose and HbA_1C were correlated to antithrombin III levels in type I diabetics. The acute phase reactants fibrinogen and sedimentation correlated to fibrinogen/A III ratio in type I diabetics and also in type II diabetics,as presented in table 10 and 11.

Table 12. Parameters which show positive correlation with the natural anticoagulants and fibrinogen/AIII ratio in the control group.

	Protein C	Protein S	Antitrombin III	Fibrinogen/AIII
Age	r=0,47 p<0,05 (n=26)	r=0,49 p<0,05 (n=26)	r=0,64 p<0,001 (n=26)	
HbA_1C	r=0,59 p<0,01 (n=26)		r=0,43 p<0,05 (n=26)	
Triglyceride	r=0,43 p<0,05 (n=26)	r=0,44 p<0,05 (n=26)		
Cholesterol		r=0,42 p<0,05 (n=26)		
Fasting glucose			r=0,43 p<0,05 (n=26)	
Fibrinogen				r=0,87 p<0,001 (n=26)

Age was found to be in positive correlation with the natural anticoagulants. Antithrombin III level was higher in the patients with higher fasting glucose and HbA_1C levels. Protein C was correlated to triglyceride and HbA_1C levels. Protein S was correlated to triglyceride and cholesterol. The control group in this table includes control subjects for type II and type I diabetic patients together.

Table 13. Comparison of the natural anticoagulant levels between the metabolically well controlled ($HbA_1C<8$) and the metabolically poorly controlled group ($HbA_1C>8$).

	$HbA_1C<8$ (n=66)	$HbA_1C\geq8$ (n=62)	P
Protein C Levels (IU/ml)	1,01±0,04	1,13±0,05	NS
Protein S Levels (%)	113,48±5,32	110,64±5,72	NS
Antithrombin III Levels (mg/dl)	26,97±0,37	27,28±0,53	NS
Fibrinogen/AIII	13,05±0,37	15,70±1,28	NS

There was no difference in the natural anticoagulants between the metabolically well controlled and metabolically poorly controlled group.

The natural anticoagulants and fibrinogen/AIII levels were investigated in order to answer the question as to whether the therapy type had any influence on the natural anticoagulants and fibrinogen/AIII ratio. There was no difference regarding the types of therapy (diet, oral antidiabetics and insulin).

In order to answer the question as to whether the natural anticoagulants change when diabetic complications such as retinopathy, neuropathy and nephropathy exist, the patients were subdivided into three groups for retinopathy degrees: background retinopathy group, proliferative retinopathy group and the group without retinopathy. There was no change in the natural anticoagulants when each group was compared. Also no difference was observed when the patients were investigated for proteinuria and CCr values. The patients were subdivided into three groups as normoalbuminuria group, microalbuminuria group and macroalbuminuria group. For detection of nephropathy the patients were divided into three groups as follows CCr values below 50 ml/min, CCr values between 50-80 ml/min, CCr values above 80 ml/min Patients with CCr values below 30 ml/min were excluded from the study. The patients were divided into two groups according to whether they had peripheral neuropathy or not; and whether they had autonomic neuropathy or not. Neuropathy did not affect natural anticoagulant levels.

Also the effects of hypertension, smoking, BMI status and ischemiac ECG findings were investigated. No change was observed in natural anticoagulant levels and fibrinogen/AIII levels for these groups.

Discussion

If diabetic patients are compared with the normal population there is a thrombotic tendency in diabetics. Early atherosclerotic complications are more frequent in diabetic patients than in healthy subjects of the same age class. Diabetes is frequently associated with hypertension, lipid abnormalities and vascular changes. It should also be reminded that hypercoagulability and defects of the fibrinolytic system have been recognised to be important factors in the pathogenesis of atherosclerosis and its complications (7). In patients with diabetes, vascular complications such as myocardial infarction and cerebral infarction are important. Hypercoagulability in a broad sense, including enhanced platelet function and impaired fibrinolysis, may play a critical role in the pathogenesis of atherogenesis and thrombosis in diabetic patients (8). Disturbances of hemostatic and fibrinolytic mechanisms have been frequently described in patients with diabetes mellitus and have been associated with the development of diabetic complications and the increased incidence of cardiovascular events in diabetic subjects (8). Thrombosis of the veins and arteries, together with complicating embolic phenomena, is perhaps the most important cause of morbidity and death in the developed countries of the world at the present time. It is of far greater overall clinical importance than all of the hemorragic disorders (9). The purpose of this study was to clarify whether hypercoagulability exists in patients with diabetes.

Abnormalities in both lipid metabolism and the plasma coagulation system have been considered to contribute to the development of atherosclerotic changes and thrombotic events in diabetic patients. In order to investigate the relationship between lipid levels and the natural anticoagulants we compared the levels of protein C, protein S and AIII in plasma to cholesterol, triglyceride and HDL cholesterol. Triglyceride was in positive correlation with protein C, protein S and AIII in type II patients. Total cholesterol levels were correlated to protein C. Fibrinogen/AIII ratio was in positive correlation with fibrinogen and sedimentation rate. Saito et al has reported significant positive correlations between levels of protein C or protein S with serum cholesterol levels in type II patients (10). Also Moccia et al showed a positive correlation between protein S and hypertriglyceridemia in type II diabetic patients (11).

The fibrinogen/AIII ratio was in positive correlation with fibrinogen and sedimentation rate. The level of fibrinogen was correlated to thrombin formation and therefore increased fibrinogen level may suggest a tendency for atherosclerosis (12). Increased fibrinogen level is known as a cardiovascular risk factor and it is correlated to cholesterol and triglyceride levels (13). Also increased fibrinogen/AIII is a marker for hypercoagulability (14). This is supported by studies which reported an increased ratio between fibrinogen and ATIII due to an increase in ATIII (14). In our study this ratio was increased in type II patients and we think that an increase in this ratio is seen because fibrinogen levels in type II patients are elevated, while AIII levels are not. We have also studied sedimentation rates and thrombocyte and fibrinogen levels in order to investigate whether these acute phase reactants were elevated in diabetic patients. Increased fibrinogen levels in diabetic patients may result in part from adhesion of monocytes to damaged endothelial cells, leading to release of interleukin-6, which increases hepatic synthesis of fibrinogen and other acute phase proteins (15).

In type I patients we observed more positive correlations explained below: Protein C and protein S were correlated to prothrombin time (pt) and activated partial thromboplastin time (aptt). Protein C was correlated to fibrinogen. HbA_1C and fasting glucose and triglyceride levels were correlated to AIII. Fibrinogen/AIII ratio was correlated to fibrinogen and sedimentation rate.

Schernthaner et al reported correlation between protein C and pt, aptt and fibrinogen levels. Also they reported correlation of protein C and protein S with cholesterol and triglyceride. Protein S levels were reported to be in correlation with all glycaemiac parameters. HbA_1C and fasting glucose levels were correlated with antithrombin III (16).

Carmassi et al reported correlation of thrombin-antithrombin and fibrinogen with HbA_1C and fasting glucose (7). All these findings show that metabolically poorly controlled diabetics have a tendency for thrombosis. Hyperglycemia may induce the glycosylation of the anticoagulants and their function may be impaired.

In order to answer the question whether haemostatic abnormalities change with glycaemiac improvement Knöbl et al scannned 61 type II patients and researched the levels of the natural anticoagulants before and after metabolic improvement. They did not observe prominent change with glycaemiac improvement (4).

In our study we could not find elevated fibrinogen and fibrinogen /AIII levels in type I patients as in type II patients. Also the natural anticoagulant protein C was not lower in the type I diabetic patients group when compared with the control group. We can say that in type I patients we could not find any sign of hypercoagulability. But these findings may depend on the facfact that we could only scan total anticoagulant antigenic level and that we could not search for free and bound protein S fractions and protein C activity.

In the control group all the natural anticoagulant levels were in positive correlation with age. Also AIII levels were in positive correlation with fasting glucose and HbA_1C levels. Also protein C was correlated with HbA_1C. Total cholesterol levels were correlated with protein S and triglyceride levels were correlated with protein C and protein S.

In order to correct hypercoagulability, the lipid profile and hyperglycaemia must be improved.

Type II diabetic patients' mean age values were higher than those of type I diabetic patients in our study group. Also BMI values were higher and female prominence was observed. In type II diabetic patients total cholesterol levels and triglyceride levels were higher than in type I patients. HDL cholesterol levels were higher in the type I group as expected. Surprisingly HDL cholesterol levels were found to be higher in type I diabetics, when compared with the control group but statistically this was found to be nonsignificant. In spite of their high levels of HbA_1C the HDL levels were not very low in type I diabetics. Some studies have reported lower levels of VLDL and LDL and higher levels of HDL in type I diabetics (17-20).

Type I diabetic patients differed from the control group regarding the parameters fasting glucose and HbA_1C, but type II patients differ from the control group with lipid profile, BMI, fibrinogen, Fibrinogen/AIII, HbA_1C and fasting glucose levels. The natural anticoagulant levels were not higher in the type II group if it is taken into consideration that these natural anticoagulants were in positive correlation with triglyceride and cholesterol levels. Higher anticoagulant levels should have been expected when this correlation was observed, but the protein C levels were lower in the type II group when compared with type I group. Protein S levels were lower in the type I group and this may depend on the factors given below:

- Triglyceride levels are in the normal range in the type I group and protein S levels are not as high as in the type II group because triglyceride levels are higher in the type II group and protein S is correlated with triglyceride. Therefore higher protein S levels in type II patients are expected.
- Diabetes duration is longer in type I patients and therefore it is expected that type I patients have more prominent vasculopathy, and low protein S levels may depend on the fact that a consumption of this protein can be observed due to reactive activation of the coagulation cascade.
- Diabetes duration is longer in type I patients and more prominent proteinuria is expected and therefore protein S is excreted with urine.
- Glycaemiac parameters are not good in type I patients and therefore protein S levels are lower.
- The number of type I patients that we included in this study is insufficient.

Surprisingly AIII levels of the type I diabetics were higher than those of the control group, but we did not take this into consideration because the AIII levels in each group were in the normal range.

The fibrinogen/AIII ratio is markedly high in type II group when compared with the control group, but there is not much difference between type II and type I patients for this ratio. High fibrinogen, high fibrinogen/AIII and low protein C levels in type II patients show a thrombotic tendency.

Natural anticoagulant levels were similar in the metabolically poorly controlled group ($HbA_1c>8$) and metabolically well controlled group ($HbA_1C<8$). Knöbl et al reported that some hemostatic abnormalities persisted in spite of glycaemiac improvement (4).

We investigated the effect of different therapy types and we could not find any change in the natural anticoagulant and fibrinogen/AIII levels. Veglio et al did not observe any change in the hemostatic parameters with different therapy types, either (21).

Patients were divided into three categories regarding the degree of retinopathy: normal fundoscopic findings, background retinopathy and proliferative retinopathy. Statistically no change could be found in these groups in natural anticoagulants and fibrinogen/AIII levels.

There was no change in these hemostatic parameters in different proteinuria and CCr groups. We excluded patients with CCr below 30 ml/min and patients with proteinuria above 1500 mg/day. Also there was no change in the natural anticoagulant levels in different neuropathy types, such as autonomic neuropathy or peripheral neuropathy. Smoking behavior and hypertension did not affect the natural anticoagulant levels, either.

Patients were divided into two groups for ECG findings: normal ECG findings and ischemiac ECG findings and protein S levels were lower in

the ischemiac group, but this was statistically non-significant.

Fibrinolytic activity is impaired in obese patients. High BMI and high fasting glucose occur together with impaired fibrinolysis and high plasminogen activator inhibitor -1 levels. A defect in fibrinolytic activity is seen together with microangiopathy (22).

In order to investigate the effects of BMI on natural anticoagulants and on fibrinogen/AIII ratio we subdivided the patients into three groups: the first group had BMI values below 20, the second group had BMI values between 20-25 and the last group had BMI values above 25. There was no difference in the natural anticoagulant levels and fibrinogen/AIII ratio.

Fibrinogen and fibrinogen/AIII levels did not change in the case of neuropathy or retinopathy, either. Knöbl et al reported a positive correlation between fibrinogen and retinopathy in type I patients (2). Efe et al reported elevated fibrinogen levels in diabetes with advanced degrees of retinopathy and neuropathy (23).

In summary we can say that in type II patients there is a thrombotic tendency, but in type I patients we could not find any sign of thrombotic tendency. In type II patients protein C levels are lower and fibrinogen and fibrinogen/AIII levels are higher than in normal patients. These findings may be markers for hypercoagulability in type II patients.

References

1. Chait A. Bierman EL. Pathogenesis of Macrovascular Disease in Diabetes. In Joslin's Diabetes Mellitus. Thirteenth edition ed. Lea and Febiger. Philadelphia. 1994; 648-662.
2. Sherwin RS. Diabetes mellitus. In Bennet and Plum Eds. Cecil Textbook of medicine. 20th edition. WB Saunders Company. USA. 1996, 1258-1277.
3. Tüzün M. Diabetes mellitus. Kabalak T, Yılmaz C, Tüzün M editörlüğündeki Endokrinoloji el kitabı. Ege Üniversitesi Basımevi. İzmir. 1995; 419-526.
4. Knöbl P, Schernthaner G, Schnack C, Pietschmann P, Proid S, Prager R, Vukovich T. Haemostatic abnormalities persist despite glycaemic improvement by insulin therapy in lean type 2 diabetic patients. *Thromb Haemost* **71:** 692-697, 1994.
5. Schwarz HP, Scherntaner G, Griffin JH. Decreased plasma levels of protein S in well-controlled type I diabetes mellitus. *Thrombosis and Haemostasis* 57(2): 240, 1987.
6. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GDO, Small M.Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. *Diabetes* **41:** 909-913, 1992.
7. Carmassi F, Morale M, Puccetti R, De Negri F, Monzani F, Mariani N and G. Coagulation and fibrinolytic system impairment in insulin dependent diabetes mellitus. *Thromb Research* **67:** 643-654, 1992.
8. Matsuda T, Morishita E, Jokaji H, Asakura H, Saito M. Yoshida T, Takemoto K. Mechanism on disorders of coagulation and Fibrinolysis in Diabetes. *Diabetes* **45:** 109-110, 1996.
9. Bithell TC. Thrombosis and antithrombotic therapy.In Wintrobeís ed Clinical Haematology.Ninth edition. Lea & Febiger. Philadelphia 1994;1525-1530.
10. Saito M, Kumabashiri I, Jokaji H, Asakura H, Uotani C. Otsuka M, Hamada M, Tatsumura M, Morigana K, Matsuda T. The levels of protein C and protein S in plasma in patients with type II diabetes mellitus. *Thrombosis Research* **52:** 479-489, 1988.
11. Moccia F, Colla G, Castelli F, Greco GM. Evaluation of the coagulation and fibrinolytic system in 54 patients with type 2 diabetes mellitus: correlations with lipid metabolism and blood glucose controls. *Clin Ter* **147** (1-2): 37-46, 1996.
12. Iwashima Y, Sato T Knöbl P, Schernthaner G, Schnack C, Pietschmann P, Proid S, Prager R, Vukovich T. Haemostatic abnormalities persist despite glycaemic improvement by insulin therapy in lean type 2 diabetic patients. *Thromb Haemost* **71:** 692-697, 1994.
13. Greaves M, Malia RG, Goodfellow K, Mattock M, Stevens LK, Stephenson JM, Fuller JH and EURODIAB IDDM Complications study. Fibrinogen and von Willebrand factor in IDDM: relationships to lipid vascular risk factors, blood pressure, glycaemic control and urinary albumin excretion rate: the EURODIAB IDDM complications study. *Diabetologia* **40:** 698-705, 1997.
14. Ceriello A. Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects. *Diabetologia* 1119-1125, 1993.
15. Knöbl P, Schernthaner G, Schnack C, Pietschmann P. Griesmacher A, Prager R, Müller M. Thrombogenic factors are related to urinary albumin excretion rate in type I and type II diabetic patients. *Diabetologica* **36:** 1045-1050, 1993.
16. Schernthaner G, Vukovich T, Knöbl P, Hay U, Müller M. M. The effect of near-normoglycaemic control on plasma levels of coagulation factor VII and the anticoagulant proteins C and S in insulin-dependent diabetic patients. *British Journal of haematology* **73:** 356-359, 1989.

17. Sosenko JM, Breslow JL, Miettinen OS et al. Hyperglycaemia and plasma lipid levels: a prospective study of young insulin dependent diabetic patients. *N Engl J Med* **302:** 650-4, 1980.

18. Nikkila EA. Plasma lipid and lipoprotein abnormalities in diabetes. Jarret J ed. Diabetes and Heart Disease. New York: Elsevier, **1084:** 133-66.

19. Strobl W, Widhalm K, Schober E et al. Apolipoproteins and lipoproteins in children with type I diabetes: relation to glycosylated serum protein and HbA1. *Acta Pediatr Scand* **74:** 966-71, 1985.

20. Gibbons GF. Hyperlipidemia of diabetes. *Clin Sci* **71:** 477-86, 1986.

21. Veglio M, Gruden G, Marmilet A, Girotto M, Rosetto P. D'Este P, Cavallo -Pein P. Anticoagulant protein C activity in non-insulin-dependent patients with normoalbuminuria and microalbuminuria. *Acta Diabetologia* **32**(2):106-9, 1995.

22. Vague P, Raccah D, Juhan-Vague. Hemobiology, Vascular Disease and Diabetes with special reference to impaired fibrinolysis. *Metabolism* **42:** 2-6, 1992.

23. Efe B, Harmancı A, Erenoğlu E. Diabetes Mellitus ve sedimentasyon yüksekliği. *Endokrinolojide Yönelişler* 1992; **5:** 14-18, 1992.