Genomic Particularities of the Thrombophilic Status in Type 2 Diabetic Patients

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Abstract

Type 2 diabetes mellitus (T2DM) is a very common disease, which is characterized by a prothrombotic status, very frequently leading to severe complications.The thrombophillic status results from chronic activation of the coagulation system, associated with a decrease in the endogenous fibrinolytic capacity. The objective of this study was to evaluate the thrombotic status in type 2 diabetic patients and to establish if the genomic polymorphisms of the haemostatic factors favor the increase of thrombosis in insulin resistance statuses. The research was done on a group of 60 patients with T2DM.Metabolic parameters such as serum glucose, total cholesterol, LDL, HDL, triglycerides, glycated hemoglobin and renal function indicators were assessed after 12 hours of fasting. The haemostasis was investigated through the levels of fibrinogen, thrombocytes count and morphology, the antithrombin III plasmatic concentration, the presence of different PAI-1 and coagulation factor XIII genotypes. The study revealed that PAI-14G/4G genotype was associated with high glycated hemoglobin levels and with increased total and LDL cholesterol, while the other PAI-1 genotypes had no such associations. Our research also showed that the mutant WT+MT factor XIII heterozygote was associated with high total and LDL cholesterol levels and with increased CRP concentrations.

Key words: PAI-1, factor XIII, antithrombine III

1.Introduction

Diabetes mellitus type 2 is a very common disease, which is characterized by hypercoagulability, that very frequently causes severe complications. Even more, despite optimal secondary prevention therapy following acute thrombotic events, a recurrence of this type of complications is more frequent in patients with type 2 diabetes mellitus (T2DM) (HAGRACY & al [1]; PAOLETTI & al [2]). Patients with type 2 diabetes mellitus (T2DM) display a predisposition for accelerated atherosclerosis, the studies comparing the prevalence of coronary artery disease in diabetic and nondiabetic patients having shown a threefold higher incidence of atherosclerosis, with an earlier clinical onset and a twofold higher cardiovascular risk when diabetes is associated (LISAK & al [3]; IKEDA & al [4]).The thrombophillic status in type 2 diabetic patients results from both chronic activation of the coagulation system, associated with a decrease in the endogenous fibrinolytic capacity (JETSY & al [5]; MOHAMMAD & al [6]). It is well known that the vascular complications in patients with T2DM have a multifactorial etiology, being influenced by the changes in the thrombotic and thrombolytic balance, consisting mostly in the activation of the thrombotic process, thus leading to ischemic complications (GENTILE & al [7]; CARR & al [8]). Diabetic patients seem to develop abnormalities of the haemostatic process, such as

alterations of the thrombocytic function, modifications of the coagulation and of the fibrinolysis that lead to a thrombophilic status. Different studies have shown that the thrombotic complications that appear in type 2 diabetic patients may be caused by an increase of different coagulation factors. Plasmatic fibrinogen is an important cardiovascular risk factor. It was proven that increased levels of Interleukin 6, which is present in type 2 diabetic patients, may lead to an increased hepatic synthesis of fibrinogen, thus representing an important link between inflammation and thrombophilic status (BOGDANOV & al [9]; WHALEN & al [10]). Thrombin or FIIa is a serinprotease which has an important clotting effect. The importance of thrombin in the clotting anomalies observed in type 2 diabetic patients is rather disputed. Some studies have shown that the concentration of thrombin in diabetic patients is normal and that it doesn't represent a predicting factor for vascular complications, while other researchers have stipulated that thrombin is one of the most important factors responsible for the thrombotic complications (CALLES-ESCANDON & al [11]; KOH & al [12]). Nevertheless, increased levels of thrombin are associated with the presence of denser thrombus structures, with a diminished permeability, which is resistant to fibrinolysis.Beside the anomalies of the thrombocytic function and of the clotting system that may be present in type 2 diabetic patients, studies have suggested that the thrombolytic function is modified as well in hyperglycemic states. The fibrinolysis is initiated by the conversion of plasminogen in plasmin, a process which is mediated mainly by the tisular plasminogen activator (tPA). The plasminogen activator inhibitor type 1 (PAI-1) is the primary inhibitor of the fibrinolysis, which acts by forming a complex with tPA. A long-term study, which covered 18 years, proved that the levels of glycated hemoglobin was positively correlated with PAI-1 and had a negative correlation with tPA levels. This result suggests that hyperglycemia leads to increased PAI-1 levels. Even more, hyperinsulinemia has a role in increasing PAI-1 levels, thus explaining the high concentrations of PAI-1 in insulin resistance states (YARMOLINSKY & al [13]). PAI-1 gene has its location on the 7 th chromosome and is structured in 9 exons and 8 introns. The 4G/5G polymorphism is a major genetic determinant of PAI-1 levels. Some authors suggested that the association of the 4G allele with other thrombophilic defects (such as factor V Leiden, prothrombin gene mutation, hyperhomocysteinemia, diminished protein C and protein S activity) increases the risk of thrombosis. In diabetic patients with coronary artery disease, the presence of 4G/4G genotype was associated with an increased risk of sudden death (YARMOLINSKY & al [13]; ELMAHGOUB & al [14]; MADAN & al [15]). The objective of this study was to evaluate the thrombotic status in type 2 diabetic patients and to establish if the genomic polymorphisms of the hemostatic factors may influence the risk of developing type 2 diabetes mellitus, promoting the atherothrombotic complications found in insulin resistance statuses.

2. Materials and Methods

The study was done on a group of 60 patients having attended the diabetes outpatient service of the hospital of the Medical University of Iasi (Romania) aged between 51 and73 years, with an average age of 63 years, having a diagnostic of type 2 diabetes, controlled with diet, or with oral antidiabetics, but not with insulin. Inclusion criteria were (a) type 2 diabetes mellitus; (b) the duration of diabetes between 5 and 15 years; (c) not having a treatment that might interfere with the prothrombotic status. Only patients who freely gave informed consent were included in the study.

Clinical Evaluation

The following data were evaluated: gender (men or women), age, duration of diabetes, height (cm, measured with a stadiometer), weight (kg). The BMI (kg/m^2) was calculated and

included in a classification of the degree of obesity. Obese patients were defined as having BMI index superior to 30 and were divided into three grades: class I: BMI 30-34.9, class II: BMI 35-39.9, class III: BMI>40.

Laboratory Evaluation

Metabolic parameters such as serum glucose, total cholesterol, LDL, HDL, triglycerides (enzymatic colorimetric method), glycated hemoglobin and renal function indicators like creatinine, creatinine clearance (MDRD), uric acid, natremia and potassium levels were assessed after 12 hours of fasting. The hemostasis was investigated through the levels of fibrinogen, thrombocytes count and morphology, the antithrombin III plasmatic concentration, the presence of different PAI-1 and coagulation factor XIII genotypes. Factor XIII genotyping was realized by Real-Time PCR. We used a Bosphore FXIII Detection kit that detects Factor XIII mutation: a point mutation (Guanine base replacement by Thymine) found in the 2nd exon of FXIII,which leads to conversion of Valine amino acid to Leucine within codon 34 (FXIIIVal34Leu) in human biological samples. Wild-type FXIII allele was amplified, and fluorescence detection was realized during FAM filter. The polymorphism of PAI-1 gene was realized through Real-Time PCR, using the Bosphore PAI 1 4G/5G detection kit that detects sequence length polymorphism, which is formed by guanosine insertion/deletion variation at 675 bp upstream in PAI-1 gene. PAI-1 4G allele was amplified, and fluorescence detection was accomplished using the FAM filter. PAI-1 5G allele was amplified and detection was accomplished using the Cy5 filter.

Statistical Analysis

Statistical analysis was carried out using SPSS version 18. ANOVA test was done in order to analyze the disperssion of the dependent variable: intra and intergroup. When assessing the significant difference between two or more groups, we used for the quantitative variables: the t-student test and the F test (ANOVA). To compare clinical and laboratory biochemical and physiological parameters in relation to the studied SNPs and nutritional status, the Kruskal-Wallis and Pearson correlation coefficient were done. Significance was considered to be $p= 0.05$. We realized the ROC (Receiver Operator Characteristic) curve, in which on the abscise was the false positive level (specificity) and on the ordinate the true positive level (sensitivity) in order to evaluate the sensitivity/ specificity balance.

3. Results and discussion

The investigated group consisted of 60 patients, 30 being men and 30 being women, having type 2 diabetes. The age of the patients varied between 51 and 73 years, with an average age of 63 years. Regarding the BMI index, the results showed a preponderance of patients with obesity class I(80%), from which more than half were women (56.2%). The history of type 2 diabetes varied between 5 and 15 years, the statistical analysis having shown an increased duration of diabetes in the younger age group (<65 years): 9.25 years vs. 8.13years, p=0.044 and also in obese patients: 9.75 vs. 8.25 years, p=0.049. There were no significant differences between the duration of diabetes between women and men. Patients having both type 2 diabetes and coronary artery disease had a slightly increased duration of diabetes (8.93 vs. 8.50 years).

In the studied group, we evaluated the polymorphism of PAI-1 gene. 65% of patients had the 4G/4G genotype, 15% had a combination of genotypes 4G/5G, and 20% had the 5G/5G genotype (Table 1). The 4G/4G genotype was more frequent in men compared to women and had a higher incidence in older patients (p = 0.01).

Table 1.The presence of PAI-1 polymorphism in the studied sample

PAI-1	Case number	%	Male patients		Aged > 65 years	
			n	%	n	%
4G/4G	39	65.0	23	57.7	21	53.8
5G/5G	12	20.0	3	25.0	3	25.0
4G/5G	9	15.0	3	33.3	-	-
Kruskal Wallis test (p values)			0.102		0.011	

With respect to the factor XIII genotyping, 25% of the patients had mutant heterozygotes associating a wild allele with a mutant one (WT+MT), the incidence being much higher in men (80%) when compared to women (20%) and in older patients (60%) (Table 2). 30.8% of patients with PAI-1 4G/4G genotype and 25% of the ones with PAI-1 5G/5G genotype associated mutant factor XIII heterozygotes (WT+MT) (Fig.1). Combined PAI-1 genotypes 4G/5G were associated only with factor XIII wild type (WT) genotype (p=0.540).

Table 2. The presence of FXIII polymorphism in the studied sample

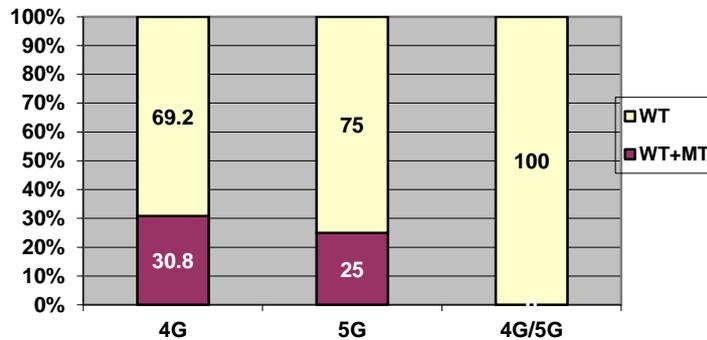| FXIII	Case number	%	Male patients		Aged > 65 years	
			n	%	n	%
WT	45	75.0	18	40.0	15	33.3
WT+MT	15	25.0	10	70.0	9	60.0
Chi2 test (p values)			0.104		0.598	

Fig1. PAI-1 and FXIII genotype correlation: association between the different types of PAI-1 and FXIII polymorphisms in the studied sample; $1_{/3}$ of patients with PAI-1 4G genotype and ¼ of patients with PAI-1 5G had mutant FXIII genotypes

Antithrombin III had values between 88-132%, 35% from the individual values being higher than normal (Fig.2).
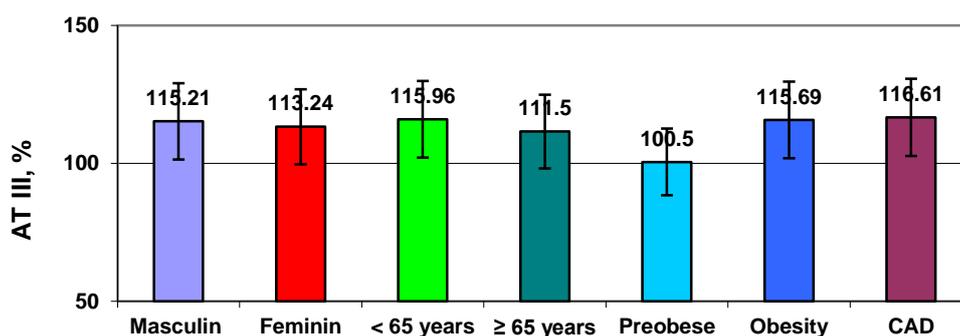
Fig 2.Mean antithrombin III levels in the different epidemiological groups

The mean antithrombin III level was gender independent (115.21% vs 113.24%), but was significantly higher in obese patients (115.69% *vs* 100.50% years; p=0.004) and in the group with coronary artery disease (116.61% *vs* 108.50% years; p=0.02). Antithrombine III plasmatic level wasn't influenced by the duration of diabetes(r= +0.041; R^2= 0.0017; p=0.803).Antithrombine III concentrations were slightly higher in patients with 4G/5G PAI-1 genotype (114.38% *vs* 118.67%; p=0.426) and in the ones with factor XIII WT genome (114.73% *vs* 112.50%; p=0.557)(Table 3).

Table 3.Descriptive antithrombine III (%) indicators in the different epidemiological groups

Parameter	N	Mean	Std. Deviation	Std. Error	Confidence interval 95%		Min	Max	Test t-Student p
					-95%CI	+95%CI			
Gender									
Male	29	115.21	9.28	2.13	110.74	119.68	96	132	0.550
Female	31	113.24	11.19	2.44	108.14	118.33	88	128	
Age									
< 65 years	36	115.96	10.99	2.24	111.32	120.60	88	132	0.181
≥ 65 years	24	111.50	8.66	2.16	106.89	116.11	96	122	
Weight status									
Preobese	6	100.50	13.40	6.70	79.17	121.83	88	114	**0.004**
Obesity	54	115.69	8.82	1.47	112.71	118.68	96	132	
CAD									
YES	42	116.61	9.55	1.80	112.91	120.31	96	132	**0.020**
NO	18	108.50	9.91	2.86	102.20	114.80	88	120	

The metabolic parameters showed increased levels of the glycated hemoglobin (HbA1C) in all patients, the mean value being significantly smaller in women than in men (7.06%*vs.* 7.69%, p=0.042), and being higher in obese patients (7.46% *vs.* 6.45%, p=0.05). The diabetic

patients associating coronary artery disease also had higher levels of HbA1C (7.46% *vs.* 7.11%, p=0.295). HbA1C was directly correlated with antithrombin III levels (r= +0.473; R^2= 0.2235; p=0.002). The cholesterol levels varied between 131 and 265 mg/dl, the mean cholesterol level being slightly elevated in men compared to women (194.84vs 192.62 mg/dl, p=0.838). The total cholesterol level was indirectly correlated with antithrombin III(r= -0.335; R^2= 0.1193; p=0.029): 33.5% of the patients associated high total cholesterol levels with reduced antithrombin III concentrations. Mean cholesterol levels were increased in patients who had PAI-1 4G/4G or 5G/5G genotype compared to the 4G/5G polymorphism (199; 200.38 *vs*161.67 mg/dL; p=0.037), and in patients who were mutant heterozygotes for factor XIII (210.90vs 184.93 mg/dL; p=0.05). HDL-cholesterol levels varied between 30 and 58 mg/dl, 65% of patients having diminished levels. Mean value were significantly diminished in men group compared to women(36.37 *vs* 41.76 mg/dL; p=0.028) and in patients associating coronary artery disease (37 *vs* 44.33 mg/dL; p=0.005). There was no correlation between HDL-cholesterol and antithrombin III (r= +0.045; R^2= 0.002; p=0.783). HDL-cholesterol levels were slightly lower in the 4G/4Ggenomegroup(40.63 *vs* 42.50 mg/dL; p=0.392)and in patients having mutant WT+MT heterozygotes(39.83 *vs* 37.30 mg/dL; p=0.385).LDL-cholesterol varied between 80 and 215 mg/dl, the mean values being slightly higher in men(152.79vs140.33 mg/dL; p=0.342). LDL-cholesterol and antithrombin III had a slight indirect correlation(r= -0.356; R^2= 0.127; p=0.024). LDL-cholesterol levels were higher in patients having PAI-1 4G/4Ggenotype(153.69; 146.25 *vs* 114.0 mg/dL; p=0.098) and in patients with mutant WT+MT factor XIII(139.43 *vs*166.70 mg/dL; p=0.067). Plasmatic triglycerides varied between 92 and 289 mg/dl, with values higher in men(174.58 *vs* 161.48 mg/dL; p=0.364). The triglycerides levels and antithrombin III plasmatic concentration had a slight indirect correlation (r=-0.247, R^2=0.0611, p=0.124). Mean values of triglycerides had no significant variations regarding the PAI-1 or factor XIII polymorphism.The inflammatory and clotting parameter: the fibrinogen had plasmatic values between 3.81 and 7.32 g/l, 72.5% of patients having high individual levels. The plasmatic level of fibrinogen was gender independent and didn't vary due to age or nutritional status. The fibrinogen concentration seemed to have a minor direct correlation with antithrombin III (r= +0.268; R^2= 0.0717; p=0.095)and with the duration of the diabetes(r= +0.402; R^2= 0.1617; p=0.01). The fibrinogen levels were slightly increased in patients with 4G/5G PAI-1 genotype (6.42 vs. 5.59 g/l, p=0.037) and in the mutant heterozygote WT+MT factor XIII group (5.75 vs. 5.56 g/L, p=0.06). The other inflammatory parameter, the C-reactive protein (CRP) had values between 3.7 and 7 mg/dl, 62.5 % of the patients having increased levels. The CRP values were gender independent and weren't influenced by nutritional status or by the presence of coronary artery disease. The CRP levels were directly correlated with antithrombin III levels (r=+0.642, R^2=0.4126, p=0.001). CRP values were independent of factor XIII or PAI-1 polymorphism. To summarize, PAI-1 4G genotype was associated with high glycated hemoglobin levels and with increased total and LDL cholesterol, while the other PAI-1 genotypes had no such associations. Regarding the coagulating factor XIII genotype, our research showed that mutant WT+MT heterozygote was associated with high total and LDL cholesterol levels and with increased CRP concentrations, while the other genotype hadn't any similar correlations.The most frequent atherosclerotic complication of type 2 diabetes: the coronary artery disease (CAD) was present at 70% of the patients. In the study group, we found a preponderance of 3 vessel coronary artery disease (52%), the majority consisting of men and older patients. The duration of type 2 diabetes was slightly increased in patients with 3 vessel CAD (9.10 vs. 8.29 years, p=0.331). All patients with coronary artery disease were obese. Antithrombin III levels were slightly higher in the 3 vessel CAD group than in the 2 vessel

CAD group (116.57 *vs* 114.50%, p=0.625). The metabolic parameters showed no significant differences regarding the severity of coronary artery lesions.

As recent studies demonstrated that both endogenous fibrinolytic parameters and clotting activity are changed in type 2 diabetic patients with increased cardiovascular risk, we investigated some of the proteomic and genomic anomalies of the haemostatic system that may be responsible for the thrombotic complications seen in type 2 diabetes. The metabolic alterations that cause a change in rheology, platelet activity, haemostasis and endogenous fibrinolysis found in diabetic patients favor the occurrence of a thrombophilic status. Even more, the haemostatic alterations seen in diabetic patients seem to be responsible for the microvascular and multiple vessel localization ofthe coronary atherosclerosis [15].The metabolic changes found in the study group were less common in women than in men, the atherogenicdyslipidemia with low HDL cholesterol and increased LDL cholesterol level being preponderant in male subjects. Regarding the lipidic profile, it seemed that women had a higher predisposition for hypertriglyceridemia, which was associated with slightly lower glycated hemoglobin levels. The association of increased LDL-cholesterol levels, abnormal triglycerides levels, small and dense LDL-cholesterol particles and diminished HDL-cholesterol levels leads to the atherogeniclipoproteic phenotype, which is correlated with an early onset of coronary arterydiseaseandextensive atherosclerotic process in type 2 diabetic patients. Moreover, the existing data in medical literature suggests that small and dense LDL particles seem to be a marker for a series of anomalies including the decrease of HDL-cholesterol levels, the increase in apoB concentrations, diminished insulin sensitivity, procoagulant anomalies (some data implying a deficit in antithrombin III) and diminished thrombolytic capacity (some studies showing increased PAI-1 levels) (YARMOLINSKY & al [13]; ELMAHGOUB & al [14]; MADAN & al [15]). Our research showed that diabetic patients had normal or higher antithrombin III levels, that were gender independent and didn't vary with the BMI index. Even more antithrombin III wasn't significantly correlated with the duration of diabetes. Regarding the lipidic parameters, there was no correlation between HDL-cholesterol and antithrombin III. LDL-cholesterol and triglycerides had a slight indirect correlation with antithrombin III that wasn't statistically significant. The insulin resistance and metabolic changes seen in type 2 diabetes frequently lead to an inflammatory status. The inflammatory markers such as CRP and fibrinogen were directly correlated with the concentration of antithrombin III. While the correlation between CRP and antithrombin III was evident, the fibrinogen levels weren'tcorrelated with the plasmatic concentration of antithrombin or with the duration of diabetes.There were no differences regarding the inflammatory status due to gender or BMI index.Diabetic patients seem to have a diminished fibrinolysis, possibly due to anomalies related to PAI-1 plasmatic levels and PAI-1 genomic modifications. Up to now, the results regarding the link between the presence of type 2 diabetes mellitus and the levels of PAI-1 are inconclusive. A recent systematic review done by Yarmolinsky et al. proved that both type 1 diabetes and type 2 diabetes are associated with PAI-1 anomalies, thus suggesting that PAI-1 may become a potential, yet frequently over-looked risk factor for diabetes. This meta-analysis showed that patients with higher plasmatic PAI-1 levels had a 67% increased risk of developing type 2 diabetes at a median follow-up of 5.7 years. The adipose tissue is responsible for the highest amount of PAI-1 synthesis, visceral fat being able to produce more PAI-1 than the subcutaneous one. Along with PAI-1 synthesis, the adipose tissue secretes inflammatory adipocytokines that lead to insulin resistance but may also increase PAI-1 levels. As a result, it 's hard to conclude if PAI-1 is directly linked to the ethiopathogeny of diabetes, or if it is just another inflammatory marker. Some researches proved that increased PAI-1 concentrations promote insulin resistance, while others postulated that high insulin levels stimulate PAI-1 production. Thus the link between PAI-1

and type 2 diabetes may be dual, each of them being alternatively the cause and the effect (YARMOLINSKY & al [13]).The phenotypical manifestation of PAI-1 derives from its genetic expression.The most studied polymorphism is represented by the guanosine deletion at position -675 nucleotides relative to the transcriptional start site, leading to PAI-1 4G allele. This allele has been shown to determine a higher plasmatic concentration of PAI-1compared to PAI-1 5G allele. The PAI-1 -675 4G/5G polymorphism contains an additional binding site for a DNA-binding protein that may act as a repressor during transcription and exert a strong impact on the plasmatic concentration of PAI-1 (XU & al [16]).

Some studies regarding the correlation between the polymorphism of PAI-1 and the development of type 2 diabetes mellitus have proved a link between the presence of 4G allele of PAI-1 and the onset of diabetes mellitus in Pima Indians. This population is characterized by an increased incidence of noninsulin-dependent diabetes (NAGI & al [17]). In opposition, some other studies that were done on the Caucasian population failed to prove a correlation between PAI-1 polymorphism and diabetes mellitus (BROCH & al [18]; DE COSMO & al [19]; PETROVIC & al [20]). A subsequent case-control study that included 856 Tunisian patients who were suffering of type 2 diabetes mellitus proved that the incidence of 4G/4G genotype was high. Therefore the results suggested that the PAI-1 4G/4G genotype may increase the thrombotic risk in diabetic patients (EZZIDI & al [21]). A meta-analysis was done by Xu et al. in 2013 including 20 published articles researched the link between PAI-1 polymorphism and the ethiopathogeny of diabetes. The authors assessed all types of genetic inheritance of PAI polymorphism: allelic comparison, dominant, co-dominant and recessive and concluded that there is no association between PAI-1 4G/5G polymorphism and the diabetes risk. The PAI-1 4G seemed to have a weak-association when measured in the recessive model, but the overall analysis of this polymorphism failed to prove a consistent relationship between the risk of diabetes and the presence of this genomic variant [16]. A systematic review realized by Zhao in 2013 on 14 case-control studies contradicted the previous results. The analysis of the included studies showed a recessive genetic pattern. Therefore the authors assessed the PAI-1 4G/4G, 4G/5G and 5G/5G polymorphisms. The results proved that PAI-1 4G/4G polymorphism is associated with an increased risk of type 2 diabetes (ZHAO & al [22]). This result could be explained by the fact that the activity of the 4G allele promoter is higher than that of 5G in a cytokine-stimulated state, while the 5G allele binds a transcription repressor protein determining a low PAI-1 expression (ERIKSSON & al [23]).Our research supports a link between PAI-1 polymorphism and the risk of type 2 diabetes. The results proved that the majority of patients had the PAI-1 4G/4G genotype which was associated with high glycated hemoglobin levels and with increased total and LDL cholesterol concentrations, while the other PAI-1 genotypes (5G and 4G/5G) had no such associations. The 4G/4G genotype was more frequent in men compared to women and had a higher incidence in older patients. Furthermore, PAI-1 4G/4G genotype was not only frequently associated with atherogenic dyslipidemia, but also had a minor direct correlation with the inflammatory parameters, suggesting that diabetic patients having this genomic variant are prone to develop more frequent atherothrombotic complications.Coagulation factor XIII, which is involved in hemostasis, fibrinolysis, vascular remodeling, and tissue repair, represents a candidate gene for assessing the prothrombotic status present in type 2 diabetes.It is a pro-transglutaminase of tetrameric structure (A2B2) consisting of 2 potentially active A (FXIII-A) and 2 inhibitory/protective B (FXIII-B) subunits (REINER & al [24]). Val34Leu polymorphism of the A subunit of coagulation factor XIII (FXIII-A) is located in the activation peptide (AP) just 3 amino acids away from the thrombin cleavage site. The activation of mutant FXIII by thrombin is faster and requires less thrombin than that of the wild-type variant, thus having a prothrombotic effect (ISTVAN & al [25]). Regarding the

coagulating factor XIII genotype, our research showed that the mutant WT+MT heterozygotes were associated with high total and LDL cholesterol levels and with increased CRP concentrations, while the other genotypes hadn't any similar correlations. 25% of the patients had mutant heterozygotes (WT+MT), the incidence being much higher in men than in women and in older patients. One third of the patients with PAI-1 4G genotype and 25% from the ones with PAI-1 5G genotype associated mutant factor XIII heterozygotes (WT+MT), while combined PAI-1 genotypes 4G/5G were associated only with factor XIII wild-type genotype.

4. Conclusion

This clinical study showed that there might be a link between the genomic polymorphisms of PAI-1 and factor XIII and the ethiopathogeny of type 2 diabetes mellitus. These polymorphisms are associated with a more severe atherogenic dyslipidemia and an increased inflammatory status, that frequently lead to a more extensive atherosclerotic process. PAI-1 4G/4G polymorphisms and mutant factor XIII may become genetic risk factors for type 2 diabetes mellitus.

5.Acknowledgment

Conflict of interests
The authors declare that they have no competing interests.

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