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Anti-prothrombin antibodies in type 1 Diabetes Mellitus

[Tip 1 Diabetes Mellitus'ta Anti-protrombin antikorları]

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ABSTRACT

Aim: Type 1 diabetes mellitus (T1DM) is an autoimmune disease, and autoimmunity related prothrombotic factors might contribute to the hypercoagulable state seen in T1DM. The aim of this study is to investigate the presence of anti-prothrombin (aPT) antibodies as a prothrombotic risk factor in patients with T1DM.

Materials and Methods: The study was performed with 121 type 1 diabetic patients, who were admitted to the Endocrinology and Metabolism outpatient clinic, and 92 healthy volunteers without history of any vascular, thromboembolic or hemorrhagic disease were included as controls. The quantitative determination of aPT IgG, IgM and anti-β2-glycoprotein-1 (anti-β2GP1) IgG antibodies in plasma samples were performed using commercially available ELISA kits according to manufacturer's instructions.

Results: aPT IgG antibodies present for the type 1 diabetic and control groups were 52.06% and 2.16% respectively. The circulating levels of aPT IgG was significantly higher in type 1 diabetics (20.17±8.28 AU/ml) when compared to controls (4.47±2.01 AU/ml, $p<0.05$). 88.42% of type 1 diabetic patients showed elevated aPT IgM levels (16.12±6.01 AU/ml) that were significantly higher than the control group (4.86±2.62 AU/ml, $p<0.05$). Similarly, 69.42% of type 1 diabetic patients were positive for anti-β2GP1 IgG antibodies with significantly higher levels than the control group (7.72±1.11 U/ml, 0.81±0.32 U/ml, respectively).

Conclusion: aPT antibody levels are significantly increased in patients with T1DM when compared to controls. Similarly, the plasma levels of anti-β2GP1 IgG antibodies, an important marker of coagulation cascade, were found significantly elevated in type 1 diabetic group. These data suggest that the higher levels of aPT antibodies seen in type 1 diabetic patients may contribute to the development of hypercoagulability in T1DM.

Key Words: Type 1 diabetes, thrombosis, anti-prothrombin, anti-β2GP1

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Tip 1 Diabetes Mellitus (T1DM) otoimmün bir hastalıktır ve otoimmünite ile ilişkili protrombotik faktörler T1DM'de görülen koagülasyona yatkınlığa katkıda bulunabilirler. Bu çalışmanın amacı, Tip 1 diyabet hastalarında bir protrombotik risk faktörü olarak anti-protrombin (aPT) antikorlarının düzeyinin araştırılmasıdır.

Gereç ve Yöntemler: Bu çalışma, Endokrinoloji ve Metabolizma Kliniği'ne gelen tip 1 diyabet tanısı almış 121 gönüllü hasta ile gerçekleştirilmiştir. Herhangi bir vasküler, tromboembolik ya da hemorajik hastalığı olmayan 92 gönüllü ise kontrol grubunu oluşturmuştur. Plazma örneklerindeki aPT IgG, IgM ve anti-β2-glikoprotein-1 (anti-β2GP1) IgG antikorları ELISA kitleri kullanılarak saptanmıştır.

Bulgular: aPT IgG antikorları tip 1 diyabetik grupta %52.06 iken, kontrol grubunda %2.16 bulunmuştur. Dolaşımdaki aPT IgG düzeyi, tip 1 diyabetik grupta (20.17±8.28 AU/ml), kontrole göre (4.47±2.01 AU/ml) anlamlı olarak yüksek bulunmuştur ($p<0.05$). Tip 1 diyabetik hastaların %88.42'sinde, saptanan artmış aPT IgM düzeyleri (16.12±6.01 AU/ml), kontrol grubuna göre (4.86±2.62 AU/ml) anlamlı olarak yüksek bulunmuştur ($p<0.05$). Tip 1 diyabetik hastaların %69.42'si anti-β2GP1 IgG pozitif olarak saptanmıştır ve plazma düzeyleri kontrol grubuna göre anlamlı olarak yüksektir (7.72±1.11 U/ml, 0.81±0.32 U/ml, sırasıyla).

Sonuç: aPT antikorları, kontrol grubu ile karşılaştırıldığında, T1DM hastalarında anlamlı olarak yüksek bulunmuştur. Benzer şekilde koagülasyon yolağında önemli bir belirteç olan anti-β2GP1 antikor düzeyinin de tip 1 diyabetik grupta anlamlı olarak yüksek olduğu saptanmıştır. Elde edilen bulgular, tip 1 diyabet hastalarında gözlenen yüksek aPT antikor düzeylerinin, T1DM'de hiperkoagülasyon gelişimine katkıda bulunabileceğini göstermektedir.

Anahtar Kelimeler: Tip 1 diyabet, tromboz, anti-protrombin, anti-β2GP1

Çıkar Çatışması: Yazarlar çıkar çatışması bulunmadığını beyan ederler.

Introduction

In the last decade, anti-phospholipid (aPL) antibodies have been identified in various autoimmune diseases including anti-phospholipid syndrome (APS) and systemic lupus erythematosus [1-3]. Anti-phospholipid antibodies may recognize a variety of antigens such as phospholipid binding proteins and protein-phospholipid complexes [4-6]. Of these plasma proteins, prothrombin (PT) is identified as one of the major antigenic targets for those antibodies [7]. After the presence of anti-prothrombin antibodies (aPT) in the plasma of lupus anticoagulant positive patients was shown [8], their role in development of thrombosis has also been reported in several autoimmune diseases [9-12].

The prothrombotic factors and hypercoagulable state affect the rate of morbidity and mortality in type 1 diabetes mellitus [13,14]. Vascular inflammation, endothelial dysfunction, impaired fibrinolysis and abnormal platelet function contribute to the increased thromboembolic events in diabetic patients [15].

β2GP1, also known as apolipoprotein H, is an abundant phospholipid-binding protein which circulates in the plasma [16]. Recent studies have demonstrated that β2GP1 may interact directly with the components of the plasminogen activator system and affect the generation of cell surface and plasma fibrinolytic activity [17,18].

Although T1DM is an autoimmune disease, there is no study evaluating the presence of aPT antibodies in this disease. Therefore in the present study we aimed to evaluate the presence of aPT antibodies in patients with T1DM.

Materials and Methods

Subjects

125 patients, who were previously diagnosed with type 1 diabetes, were evaluated for this study. 121 of those patients, including 57 women and 64 men with a mean age of 29±9 years, who were admitted to the Endocrinology and Metabolism outpatient clinic at Ankara University School of Medicine were included. The exclusion criteria of the study were: personal history of any thromboembolic or hemorrhagic disease, use of any medication or substance that effects hemostasis, patients with any kind of diagnosed malignancy. Ninety-two volunteers with normal fasting plasma glucose levels and no history of any vascular, thromboembolic or hemorrhagic diseases were included in control group. The study was approved by the local ethics committee, and informed consent was obtained from each participant before enrollment. Venous blood samples were collected into vacutainer serum separating tubes (Greiner bio-one, Germany). Samples were immediately centrifuged at 1000 x g for 10 minutes at room temperature, and separated sera were stored frozen at -80°C until assayed.

Biochemical measurements

Glycated hemoglobin (HbA1c) levels were analyzed in an ADAMS A1c HA-8160 analyzer (Arkray Inc., Kyoto, Japan) by high performance liquid chromatography (HPLC).

Anti-prothrombin antibodies

The quantitative determination of anti-prothrombin (aPT) IgG antibodies was performed using commercially available ELISA kits according to manufacturer's instructions (American Diagnostica Inc., Stamford, CT, United States). Results are expressed in units relative to the calibrator as Arbitrary Unit (AU) /ml as follows: negative < 10 AU/ml, 'grey zone' 10-20 AU/ml, low positive ≥20 - <50 AU/ml, moderate positive ≥50 - <100 AU/ml and high positive ≥100 AU/ml. Arbitrary unit is a relative unit of measurement to show the ratio of amount of substance, intensity or other quantities to a predetermined reference measurement. The calculated intra- and inter-assay coefficients of variation were 4.2% and 5.2%, respectively.

Anti-prothrombin IgM antibody levels were detected by anti-Prothrombin IgM ELISA kit (DRG Instruments, GmbH, Germany, Marburg). The analytical sensitivity was 1.0 U/ml. The calculated overall intra-assay and inter-assay coefficients of variation were 4.2% and 5.9%, respectively. aPT IgM antibody levels are expressed as follows: 'normal' <10 AU/ml and 'elevated' ≥10 AU/ml.

Anti-β(2)-glycoprotein 1 IgG antibodies

The quantitative determination of anti- β2GP1 IgG antibodies was performed using commercially available ELISA kits according to manufacturer's instructions (Orgentec Diagnostica GmbH, Mainz, Germany). Results are expressed in units relative to the calibrator (U/ml) as follows: negative < 5 U/ml and positive > 8 U/ml. Functional sensitivity was 0.5 U/ml. The calculated overall intra-assay and inter-assay coefficients of variation were 7.9% and 2.1%, respectively.

Statistical analyses

The data for anti-prothrombin and anti-β2GP1 antibody levels were expressed as mean±SD and compared using Student's t test. ROC curve analysis was applied and the areas under the curve (AUCs) were calculated. Correlation analyses were performed within the Pearson correlation test. Statistical analyses were performed by SPSS (version 15; SPSS Inc., Chicago, IL) and p-values of less than 0.05 were considered as statistically significant.

Results

The demographic and clinical characteristics of the study groups are shown in Table 1. Age, body mass index and smoking habits were similar between patients with T1DM and control subjects. The mean HbA1c level of diabetic patients (9.56%) was significantly higher than the control group (5.03%, Table 1).

aPT IgG antibodies were present in 63/121 (52.06%) of the type 1 diabetic group whereas only two controls were positive for aPT IgG antibodies (Table 2). The circulating levels of aPT IgG was significantly higher in type 1 diabetics (20.17±8.28 AU/ml) when compared to controls (4.47±2.01 AU/ml, $p<0.05$) (Figure 1, Table 3). 88.42% of type 1 diabetic patients had elevated aPT IgM and anti-β2GP1 IgG levels, and the mean aPT IgM and anti-β2GP1 IgG levels was significantly higher than the control group (Table 3).

To investigate the diagnostic value of aPT IgG, IgM and anti-β2GP1 IgG, the ROC curves were produced and the area under the curve (AUC) calculated (Figure 2). The results of ROC analysis and the corresponding diagnostic indices are summarized in Table 4. The areas under the respective ROC curves were 1.00 (aPT IgG), 0.97 (aPT IgM) and 0.99 (anti-β2GP1 IgG).

The correlation analysis showed that there was no significant correlation between aPT IgG / IgM and anti-β2GP1 IgG antibodies.

Discussion

In this study, we demonstrated that aPT IgG and IgM antibody levels were significantly higher in type 1 diabetic patients when compared to healthy controls.

It was previously reported that high aPT antibody levels were a risk factor for the development of myocardial infarction, cardiac death or any sort of thrombotic events in middle-aged men [7]. Although, some studies [19,20] have reported that there was no significant correlation between aPT IgG/IgM antibodies and thrombosis in lupus anticoagulant positive patients, the presence of anti-phosphatidylserine/anti-prothrombin was correlated with increased thrombin formation in patients with APS [21].

Antiphospholipid antibodies are known to bind to plasma proteins with an affinity for phospholipids' surfaces [22-25]. Prothrombin is another important autoantigen recognized by anti-phospholipid antibodies [26,27]. The role of the anti-prothrombin (aPT) antibodies in the development of thrombosis has been evaluated

Table 1. Demographic and clinical characteristics of the study groups. Statistical comparisons were performed between type 1 diabetic patients (T1DM) and controls.

Variable	Case group (n=121)	Control Group (n=92)	p value
Age, mean±SD	29±9 (17-59 year)	30±9 (18-54 year)	NS
Female, n (%)	57 (47.1%)	35 (38%)	NS
Male, n (%)	64 (52.9%)	57 (62%)	NS
BMI (kg/m^2)	23.12±4.49	22.78±2.12	NS
Duration of diabetes (year)	11±9.5	-	NA
Smoker (%)	19	16	NS
HbA1c , % (mmol/mol)	9.56±2.77 (81±9)	5.03±0.69 (32±7)	<0.001*

BMI: Body mass index; HbA1c: Glycated hemoglobin. NS: non-significant; NA: not-applicable; SD: standard deviation. The HbA1c levels between 4.5 -6 % were accepted normal. *p <0.05 was considered as statistically significant.

Table 2. Distribution of aPT IgG, IgM and anti-β2GP1 IgG according to groups.

	Case group (n=121)	Control group (n=92)	p value
aPT IgG, n (%)			
Antibody-negative	10 (8.27%)	86 (93.5%)	0.001
Grey zone	48 (39.67%)	4 (4.34%)	0.001
Antibody-positive	63 (52.06%)	2 (2.16%)	0.001
aPT IgM, n (%)			
Normal	14 (11.58%)	87 (94.6%)	0.001
Elevated	107 (88.42%)	5 (5.4%)	0.001
anti-β2GP1 IgG, n (%)			
Negative	37 (30.58%)	92 (100%)	0.001
Borderline	-	-	NA
Positive	(69.42%)	- (0%)	0.001

aPT IgG results are expressed in units relative to the calibrator, Arbitrary Unit (AU) /ml, as follows: negative < 10 AU/ml, 'grey zone' 10-20 AU/ml, positive ≥20 AU/ml. aPT IgM antibody levels are expressed as follows: 'normal' <10 AU/ml and 'elevated' ≥10 AU/ml. Anti-β2GP1 IgG results are expressed in units relative to the calibrator (U/ml) as follows: negative < 5 U/ml, borderline 5-8 U/ml, positive > 8 U/ml. $p<0.05$ is considered as statistically significant. aPT: anti-prothrombin; β2GP1: β-2-glycoprotein 1, NA; non-applicable.

Table 3. Circulating levels of aPT IgG, IgM and anti-β2GP1 IgG antibodies among type 1 diabetic patients and controls.

	Case group (n=121)	Control group (n=92)	p value
aPT IgG (AU/ml)	20.17±8.28	4.47±2.01	0.001*
aPT IgM (AU/ml)	16.12±6.01	4.86±2.62	0.001*
anti-β2GP1 IgG (U/ml)	7.72±1.11	0.81±0.32	0.001*

The results are expressed as mean ± standard deviation. Statistical comparisons were performed between type 1 diabetic patients and controls. *p <0.05 between patients and controls. aPT: antiprothrombin; β2GP1: β-2-glycoprotein 1; AU: Arbitrary Unit.

Table 4. Summary of the receiver operating characteristic curve analysis for aPT IgG, IgM and aβ2GP1 IgG antibody levels.

	aPT IgG	aPT IgM	aβ2GP1 IgG
AUC	1.00 ± 0.00	0.977 ± 0.02	0.993 ±0.01
p value	<0.001	<0.001	<0.001
Cut-off	20 AU/ml	10 AU/ml	8 U/ml
95% CI	1.000 : 1.000	0.932 : 1.000	0.976 : 1.000
Sensitivity	100.0%	93.3%	75.0%
Specificity	41.2%	84.2%	100.0%
LR (+)	1.70	5.91	∞
LR (-)	0	0.079	0.25

,The areas under the curves were expressed as AUC±standard error. The cutoff values for sensitivity and specificity analysis were 20 AU/ml for aPT IgG, 10 AU/ml for aPT IgM and 8 U/ml for aβ2GP1 IgG. AUC: Area under curve. AUC: area under curve; CI: confidence interval; LR: likelihood ratio.

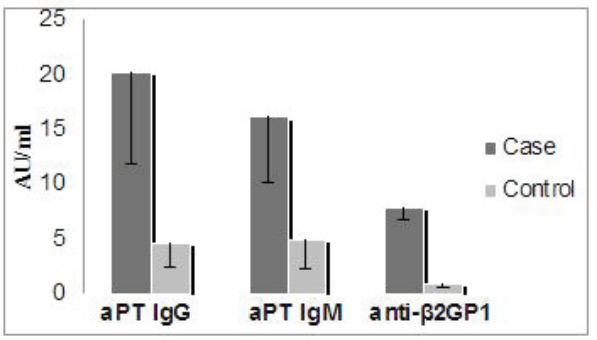

Figure 1. aPT IgG, IgM and anti-β2GP1 IgG levels between type 1 diabetic and control groups. The results are expresssed as mean ± SD.

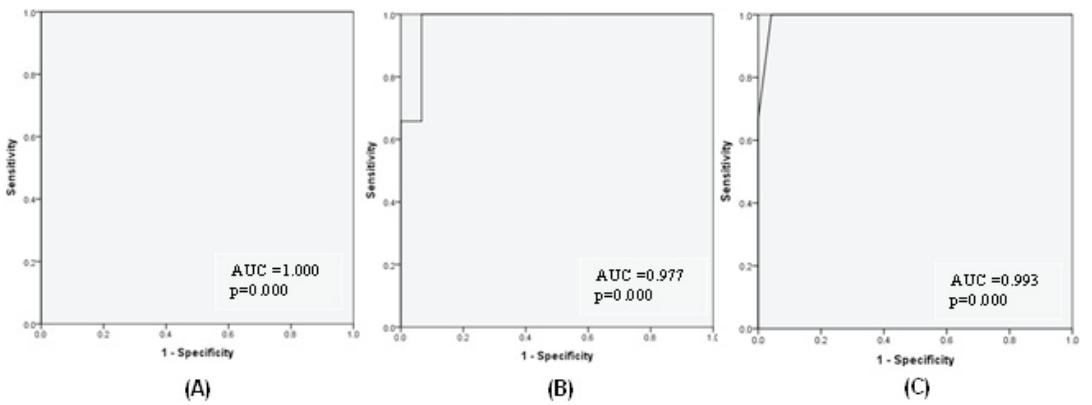

Figure 2. Receiver operating characteristic curves. The sensitivity and 1-specificity were plotted for (A) aPT IgG, (B) aPT IgM and (C) anti-β2GP1 IgG. The areas under the curve (AUCs) and the *p* values were indicated.

and significant associations have been reported in several autoimmune diseases [28-36]. Yang et al. [26] demonstrated the aPT antibodies with prothrombinase activity and suggested that these catalytic aPT antibodies might contribute to thrombosis observed in APS patients. The present study also showed that aPT antibodies might be one of the prothrombotic risk factors for type 1 diabetic patients.

β2GP1 is identified as a major target antigen in patients with APS as well [37]. It has been suggested that β2GP1 and β2GP1-reactive antibodies may have direct interactions with the fibrinolytic system [38-39]. In our study, we found elevated anti-β2GP1 IgG antibodies in the plasma samples of type 1 diabetic patients. Bu et al. [40] identified the role of β2GP1 in the regulation of fibrinolysis by showing that β2GP1 interacts with tissue plasminogen activator (tPA) and induces tPA-dependent plasminogen activation in the fluid phase, and that this process is inhibited by β2GP1 antibodies.

By preliminary analysis, aPT IgG had the best diagnostic value with an AUC 100%, (p=0.000) and anti-β2GP1 IgG with an AUC over 99%. As with the cutoff value of 20 AU/ml, aPT IgG had the best ROC curve. The statistical analysis also showed that aPT IgG has the best likelihood ratio with the lowest LR(-) value.

In conclusion, this is the first study evaluating the plasma levels of aPT antibodies in type 1 diabetic patients. When compared with healthy controls, aPT antibodies were significantly increased in patients with T1DM. We have also determined that the levels of anti-β2GP1, which is known to inhibit the fibrinolytic activity, were significantly higher in the case group. These data suggest that the higher levels of aPT IgG and IgM determined in type 1 diabetic patients may contribute to the development of hypercoagulability in T1DM. Further studies are necessary to elucidate the clinical impact of these changes in type 1 diabetes.

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Conflict of Interest: Authors have no conflict of interest.

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