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Comparison of Serum Anticardiolipin Antibodies and Carotid Intima Media Thickness in Diabetic Patients

Diyabetik Hastalarda Serum Antikardiyolipin Antikorları ile Karotis İntima Media Kalınlığının Karşılaştırılması

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SUMMARY

Objectives: *Decreases in fibrinolytic activity and thrombocyte activation changes are seen in diabetic patients, which leads to a procoagulant state. In the presence of hyperglycemia, the role of anticardiolipin antibodies (ACA) on phospholipids from the degraded endothelium and the relation between these antibodies and microvascular and macrovascular complications in a diabetic survey were investigated. In our study, we aimed to determine the relation between carotid intima media thickness (CIMT) and serum ACA levels and other routine parameters in type I and type II diabetic patients.*

Methods: *Patients were divided into two groups, as Group I: Type I DM (n=15) and Group II: Type II DM (n=45). Serum levels of ACA IgG were measured by using ELISA (Trinity Biotech, USA). Mann-Whitney U test was used in the statistical analysis.*

Results: *The groups were similar with respect to the duration of diabetes (p=0.261). There was no significant difference in the ACA levels between the two groups. Spot urine microalbumin levels and CIMT were statistically higher in Group II (p=0.0001, p=0.001, respectively). No correlation was found between ACA and CIMT (p=0.258).*

Conclusion: *Since the patients were older in the second group and had hypertension and metabolic syndrome, microalbuminuria and CIMT values were higher in this group as expected because of endothelial dysfunction. Large population–based prospective studies are needed to provide stronger evidence about the relation of serum ACA and CIMT in patients with DM.*

Key words: *Anticardiolipin antibodies; diabetes mellitus; carotid intima media thickness.*

ÖZET

Amaç: Diyabetik hastalarda fibrinolitik aktivitede azalma ve trombosit aktivitesinde değişiklikler olur ve bunlar prokoagülan bir durum yaratır. Hiperglisemi varlığında degrade endotelden kaynaklanan antikardiyolipin antikorların (ACA) fosfolipidler üzerine etkisi ve bu antikorlarla diyabetin mikro ve makrovasküler komplikasyonlarının ilişkileri araştırılmıştır. Çalışmamızda tip 1 ve tip 2 diyabetik hastalarda serum ACA düzeyleriyle karotis intima media kalınlığının (CIMT) ilişkisi incelenmiştir.

Gereç ve Yöntem: Hastalar iki gruba ayrıldı: Grup I: Tip I diyabetes mellitus (DM) (n=15) ve Grup II: Tip II DM (n=45). Serum ACA IgG düzeyleri ELISA yöntemiyle (Trinity Biotech, USA) ölçüldü. İstatistiksel analiz için Mann-Whitney U testi kullanıldı.

Bulgular: Gruplar diyabetin süresi açısından benzerdi (p=0,261). İki grup arasında ACA düzeyleri bakımından fark yoktu. Spot idrarda mikroalbümin seviyeleri ve CIMT grup II'de istatistiksel olarak belirgin yüksekti (sırasıyla p=0,0001, p=0,001). ACA ile CIMT arasında korelasyon bulunmadı (p=0,258).

Sonuç: İkinci gruptaki hastalar daha yaşlı olup hipertansiyon ve metabolik sendromları olduğundan, beklendiği gibi endotel disfonksiyonundan dolayı mikroalbüminuri ve CIMT değerleri bu grupta daha yüksekti. DM hastalarında ACA ve CIMT ilişkisi konusunda delil sağlamak için büyük popülasyonlu çalışmalar gereklidir.

Anahtar sözcükler: Antikardiolipin antikorları; diabetes mellitus; karotis intima media kalınlığı.

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INTRODUCTION

Coagulation abnormalities occur in the course of diabetes mellitus through an imbalance between thrombus formation and dissolution which results in a state of thrombophilia.[1] Vascular damage and endothelial dysfunction occur early in the course of diabetic angiopathy. Antibodies to cell surface antigens initiate vascular injury, and antiphospholipid antibodies target phospholipid binding proteins on cellular membranes. Anticardiolipin anticors which belong to this family may promote ischemia and thrombosis by different pathways such as alterations of protein C and antithrombin III, impaired fibrinolysis, inhibition of prostacyclin activity, platelet aggregation and complement activation.[2] Anticardiolipin anticors are a subgroup of antiphospholipid antibodies, and IgM and IgG izotypes are the most important. Previous data reflect controversy about the prevalance of these antibodies in diabetes mellitus and the possible implications.[3] It has been suggested that antiphospholipid antibodies directed against endothelial antigens could be responsible for vascular injury. There is a pathological shift from an antithrombotic to a thrombotic state even in the early phases of diabetes.[4]

Most patients with diabetes mellitus die of atherosclerosis and its complications. The abnormal lipoprotein profile associated with insulin resistance accounts for part of the elevated cardiovascular risk with type 2 diabetes. These patients have more atherogenic LDL particles.[5] Type 1 diabetic young adults have also detectable vessel wall abnormalities even if they do not have any signs of disease complications suggestive of hyperglycaemia-related early endothelial dysfunction.[6]

Carotid intima-media thickness (IMT) is a measure of subclinical atherosclerosis and is predictive of future vascular events;[7,8] and abdominal obesity (WC), hypertension, high insulin and LDL-C levels are associated with IMT.[9]

In this study we aimed to measure anticardiolipin antibodies (ACA) in type 1 and type 2 diabetics; and to find out if there is a correlation between these antibodies and carotid IMT as an indicator of macrovascular complication and microalbuminuria as a reflection of microvascular complication.

MATERIALS AND METHODS

Sixty patients who were followed up at the Outpatient Clinic of Diabetes were included in our study. The groups consisted of 15 type 1 and 45 type 2 diabetic patients. Subjects with established diabetic micro or macro vascular complications, malign hypertension and collagen disease were excluded. Body weight, weight circumference (WC) were measured. Body mass index (BMI) was calculated according to the formula: weight/height m^2.

Serum glucose, urea, creatinine, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, and blood HbA1c and microalbuminuria levels were measured by Aeroset 2.0 (Abbott Diagnostics) in Clinical Biochemistry Laboratory, after overnight fasting. Post-prandial glucose measurements were done after two hours of meal.

Blood samples for serum anticardiolipin IgG tests were taken with plain tube and centrifuged in 30 minutes at 5000 rpm for 10 minutes. Serum samples were kept in -80˚C and ELISA method (Captia Cardiolipin IgG Trenecity Blodech -Ireland- cat no: 2338400) was used. Cut off level was determined with multiplying standart deviation value of healthy individuals by 5 and expressed as GPL/mL (immunglobulin G fosfolipid unit/mL).

Carotid artery color Doppler ultrasonography by the high- resolution B- mode ultrasonography (GE Logic 9, Milwaukee, United States) examination was performed using 10 MHz linear transducer. Examinations performed in the supine position after at least 15 minutes rest by a single radiologist. The right and left carotid artery measurements were made according to CIMT values. Values of bilateral carotid arteries were measured from proximal 3 cm. from the bulbus. Although up today there is no standardized value, in our study CIMT over 1.2 mm diameter accepted as pathological.

Written consent was obtained from patients, and the study was approved by our Hospital's Ethical Board. Our study was conducted in accordance with

Helsinki Declaration. Statistical analysis was performed using the NCSS 2007 programme. The data were evaluated with descriptive statistical methods, Mann Whitney-U test and chi-square test.

RESULTS

When the two groups were considered, there was not a significant difference with regard to gender (p=0.456) and the duration of diabetes (p=0.261). Clinical and biochemical features of the subjects are shown on the tables (Table 1, Table 2).

Type 2 diabetic patients had higher values of triglyceride, microalbuminuria and CIMT. There was not any correlation between ACA and age (p=0.703), duration of diabetes (p=0.807), BMI (p=0.103), SBP (p=0.489), DBP (p=0.379), HbA1c (p=0.103), microalbuminuria (p=0.161) and CIMT (p=0.258). With regard to CIMT, there was not any relation with age (p=0.74), duration of diabetes (p=0.581), HbA1c (p=0.229), LDL-cholesterol (p=0.13), HDL-cholesterol (p=0.90), triglyceride (p=0.45), microalbuminuria (p=0.46), systolic (p=0.20) and diastolic blood pressure (p=0.93); and there was a slight corelation with BMI (p=0.033).

DISCUSSION

We compared the age, the duration of diabetes, antropomorphic charecteristics, systolic and diastolic blood pressures, biochemical values and CIMT in type 1 and type 2 diabetic patients.

Obesity is known as a risk factor for diabetes and 80% of patients with type 2 diabetes mellitus are obese. [10] As expected mean weight and BMI were higher in type 2 diabetic group than type 1 diabetes mellitus patients (p=0.005 and p=0.0001 respectively).

Type 2 diabetic patients were significantly older (p=0.0001), and they had higher systolic (p=0.027) and diastolic (p=0.034) blood pressure. There was

Table 1. Clinical and demographic features of patients

	Type 1 DM (n=15)	Type 2 DM (n=45)	p
Mean age (year)	26.2±6.3	52.5±9.1	0.0001
Duration of DM (year)	4,69±4,52	5,58±4,18	0,261
Weight (kg)	65,8±11,21	75,93±9,96	0,005
Height (cm)	169,87±10,02	166,82±6,17	0,493
BMI (kg/m^2)	22,7±2,5	27,29±3,36	0,0001
Systolic blood pressure (mmHg)	119,33±15,34	130,56±16	0,027
Diastolic blood pressure (mm/hg)	76±9,86	82,44±9,51	0,034

Mean age, weight, BMI, systolic and diastolic blood pressure were higher in type 2 diabetic patients.

Table 2. Biochemical values of patients

	Type 1 DM (n=15)	Type 2 DM (n=45)	p
FBG (mg/dl)	188,53±93,57	184,84±72,08	0,701
PPBG (mg/dl)	250,87±103,49	253,49±81,43	0,442
Urea (mg/dl)	30,6±12,71	33,44±10,7	0,318
Creatinine (mg/dl)	1,19±1,34	0,89±0,23	0,910
T. Cholesterol (mg/dl) (mg/dl)	196,53±53,41	210,11±50,01	0,225
LDL (mg/dl)	120,27±39,87	125,64±38,76	0,544
HDL (mg/dl)	46,2±14,23	39,62±8,21	0,149
Triglyceride (mg/dl)	145,53±112,67	231,04±199,89	0,013
HbA1c (mg/dl)	8,33±2,42	7,6±1,59	0,335
Microalbuminuria (ml/min)	32,27±28,58	163,89±159,18	0,0001
CIMT	1,23±2,16	1,88±3,8	0,001
ACA (GPL/ml)	4,22±1,01	4,25±1,02	0,258

not any significant correlation between BMI and ACL antibody titers (p=0.103). We could not find ant report about the relationship of obesity and ACL antibody levels.

Prevalence of hypertension occurs twice more frequent in type 2 diabetes mellitus patients.[11] Hypertension and type 2 diabetes mellitus are highly prevalent in adults and the elderly. Their association is even more frequent than can be surmised based on statistical considerations because the haemodynamic and neurohumoral alterations accompanying hypertension favour the appearance and progression of insulin resistance, a well-known precursor of type 2 diabetes, has a number of effects (e.g. sympathetic activation and sodium and water retention) that lead to an increase in blood pressure.[12] Insulin resistance and diabetes can precipitate hypertension by stimulating the sympathetic nervous system and the renin-angiotensin system, and promoting sodium retention. Diabetes is also associated with increased proliferation of vascular smooth muscle cells. High blood glucose and elevated blood pressure can impair vascular endothelial cells, leading to increased oxidative stress. Patients with diabetes also have increased vascular reactivity.[13]

We found significantly higher levels of both systolic and diastolic blood pressures (p=0.0001) in type 2 diabetic subjects; but we could not find any association with ACL antibodies and systolic (p=0.489) and diastolic (p=0.379) blood pressures.

Factors such as genetic properties, age, hypertension, hyperlipidemia, smoking, obesity, stress and inadequate physical activity are important risk factors for diabetic complications. Alterations in the hemostatic system may also be related to the development of these complications. There may be an activation of intrinsic coagulation system, decreased fibrinolytic activity and alterations in platelet function in diabetic subjects.[2] The abnormalities observed in diabetes seem to be caused by the hyperglycemia which determines the abnormality of thrombus formation through three mechanisms. These mechanisms are nonenzymatic glycation, increased oxydative stress and decreased heparan sulphate. Glucose directly alter coagulation system; such as affecting both thrombus formation and its inhibition, fibrinolysis, platelet and endothelial function.[1]

Several studies reported there is a tendency to venous and arterial thrombosis in the presence of antiphospholipid antibodies. These antibodies may play a role in the impairment of thromboresistant property of vascular endothelium and enhancement of platelet activation. All of these factors may play a role in the pathogenesis and progression of diabetic complications.[2,14] ACL may play a role in platelet aggregation and may promote ischemia and thrombosis.[15] IgA and IgG antibodies for cardiolipin and/or phosphatidin were detected in type 2 diabetic patients with the highest prevalance (86%) with macrovascular complications.[14]

Studies point out there is a relation between ACL antibodies, endothelial dysfunction and hemostatic alterations in diabetic individuals which give rise to micro and macrovascular complications. Ciarla et al.[16] reported high levels of IgG-ACL in well-controlled uncomplicated insulin dependent diabetes mellitus patients. They suggested there was a synergism between generation of these antibodies and endothelial activation in the early phases of IDDM vascular disease. Janardhan et al.[17] reported elevated ACL concentration, independently of other cardiovascular risk factors, predicted risk of stroke and TIA in women but not in men. Another study of Alagözlü at al.[15] suggested that high ACL antibody levels may be associated with diabetic foot.

There are some studies with conflicting results. According to Eber a higher ACL antibody level is not a marker for recurrent cardiovascular events.[18] Many investigators also reported there was no significant correlation between vascular complications and antiphosphopholipid antibodies.[2,19] Vinik found significantly high antiphosphatidyl antibodies with diabetic neuropathy. But no correlation was observed with ACL and microalbuminuria or macrovascular disease.[20] According to the study of Galtier-Dereure et al.,[21] uncomplicated diabetes was not associated with APL antibodies; whereas phospholipid- binding antibodies correlated with macroangiopathy or nephropathy. Palomo et al.[22] and Calvo-Romero et al.[3] observed that these antibodies in diabetic pa-

tients were not correlated with vasccular complications. Mohammed et al.[23] reported higher anticardiolipin IgG and IgM antibodies in type 1 diabetic children, especially recent-onset diabetes, compared to healthy controls. There was a negative correlation betweeen the level of antibodies and the duration of diabetes.[24] In our study we measured ACA IgG levels in type 1 and type 2 diabetic subjects. We found mean GPL/ml 4.22 in type 1 diabetic and 4.25 in type 2 diabetic patients which were both below the critical level of 10 GPL/ml. Duration of diabetes was 4.6 vs 5.5 years in type 1 and type 2 diabetic patients respectively. HbA1c levels in our uncomplicated patients were high indicating uncontrolled diabetes. But HbA1c (p=0.103) was not associated with ACL antibody levels.

We know that complications are already present when diabetes mellitus first have been diagnosed. But when we created the groups we excluded the patients with known complications. So our study design was not able to demostrate the relationship of ACA levels and the types of complications. Microalbuminuria levels as an indicator of microvascular complication and nephropathy was significantly higher in type 2 diabetic patients (p=0.0001). As we can see from Table 1 duration of diabetes was longer in type 2 diabetes. But there was not any meaningful relation between ACL and microalbuminuria (p=0.161) or duration of diabetes (p=0.807) in our groups. None of the metabolic variables were related to ACL.

Diabetes mellitus is responsible for atherogenic vascular diseases which arise from endothelial dysfunction, oxidation, inflammation and remodelling. [24] Atherosclerosis is charecterized by diffuse thickening of the artery wall due to progressive vascular smooth muscle cellular proliferation and accumulation of ground matrix.[25] Quantitative measurement of IMT of the distal common carotid artery is regarded as an indicator of atherosclerosis. High resolution B-mode ultrasound has been used for non-invasive assesment of CIMT is increasingly used as a surrogate marker for cardiovascular morbidity and mortality.[25-27] IMT is a predictor of vascular complications in both type 1 and type 2 diabetes.[28-30] CIMT is increased in diabetes and it is a predictor of ischemic stroke but its role in microangiopathy needs further investigation.[29] Early endothelial dysfunction and atherosclerosis related changes can also be detected using ultrasonography in children and young type 1 diabetes; and in this population IMT correlated with HbA1c.[31] Subjects with type 1 diabetes from a Mediterranean area without additional cardiovascular risk factors displayed signs of increased and accelerated atherosclerotic process shown by carotid IMT.[32]

In our study IMT values were 1.23 mm in type 1 diabetic and 1.88 mm in type 2 diabetic subjects. Both were higher than the accepted limit of IMT 1.2 mm. IMT of type 2 diabetic individuals were significantly higher than the values of type 1 diabetic patients (p=0.001). This may be explained with higher BMI, systolic and diastolic blood pressure and older age that promote the atherosclerosis.

Müller et al.[33] and Kotsis et al.[34] showed mean IMT of carotid arteries increased in obese subjects compared with normal ones. Kotsis showed this association after the groups were matched for age, sex and bloods pressures. Mean IMT of the carotid arteries increased from the lowest to the highest quartile of BMI. Fasting serum glucose was independently associated with CIMT. They concluded that obesity induced hyperglycemia is an important predictor of carotid atherosclerosis. Hyperleptinemia, the proinflammatory state of obesity and adipocytokines may affect vascular system and carotid endothelium.[34] Some studies showed an association between CIMT and duration of diabetes, BMI, HDL-cholesterol,[35] HbA1c[36] and hypertension.[9] We did not find a relation with respect to IMT and obesity, HbA1c, cholesterol levels and blood pressure.

Evaluation of flow mediated dilation (FMD) may indicate that disturbed endothelial function preceeds clinical or preclinical atherosclerosis which is shown by CIMT.[37] Recent studies reported aortic IMT is an earlier marker than CIMT for preclinical atherosclerosis in children.[28] We did not evaluate FMD or aortic IMT as an indicator of endothelial dysfunction in our patients.

There are some limitations of our study. First the groups are small. Second wre did not have a healthy control group. Moreover we were not able to dem-

onstrate the lack of complications. We excluded the patients with established vascular complications on the basis of history and records of ECG, urinalaysis, peripheral pulse examination and ophtalmologic examination. We did not perform EMG, stress test or syntigraphy, peripheral artery Doppler USG and florescein angiography.

CONCLUSION

Endothelial injury and alterations of hemostatic system lets to a procoagulant state in diabetes mellitus. The studies concerning relation between ACL antibodies and microvascular and macrovascular complications in diabetic subjects are conflicting. In our study comparing type 1 and type 2 diabetic patients, there was no significant difference between ACL levels of two groups. Systolic and diastolic blood pressure, triglyceride, microalbuminuria and CIMT were higher in type 2 diabetic individuals regardless of duration of diabetes. Large population based prospective studies are needed to provide stronger evidence about the relation of serum ACL antibodies and CIMT in patients with DM.

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