Sağlık Bilimleri Dergisi
Yüzüncü Yıl Üniversitesi

Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Dergisi, 6(1-2): 1-4, 2000

Lipoprotein (a), plasma cholinesterase and other risk factors in patients with type 2 diabetes mellitus*

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Abstract: The serum Lipoprotein (a) [Lp(a)] concentration is an independent risk factor for atherosclerosis. Atherosclerosis is the most common cause of death in diabetic patients. Thirty patients with type 2 diabetes mellitus (age: range 40-63 years; mean 51.9 years), and 30 healthy controls (age: range 40-58 years; mean 49.7 years) participated in this study. The levels of Lp (a), glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, apo A, apo B, HbAıc and cholinesterase activity in both groups were determined. Cholinesterase, apo B, triglyceride, glucose and Hb Aıc values of diabetic group were higher than those of the control group ($p<0.05$). There was no significant difference between the other parameters of both groups. Although no difference was observed in total cholesterol, LDL-cholesterol, HDL-cholesterol, apo A levels compared to the control groups; apo B, triglyceride, plasma cholinesterase levels were higher which indicate the risk of atherosclerosis for those patients. Additionally, Lp(a) did not show any significant variation between the both groups, suggesting that Lp(a) was an independent parameter from diabetes.

Key Words: Diabetes mellitus, Lp(a), cholinesterase.

Tip 2 diabetik hastalarda lipoprotein (a), plazma kolinesteraz ve diğer risk faktörleri

Özet: Serum Lipoprotein (a) [Lp(a)] konsantrasyonu ateroskleroz için bağımsız bir risk faktörü olarak kabul edilmektedir. Ateroskleroz da diabetik hastalarda ölümün en yaygın sebebidir. Bu çalışma 30 sağlıklı kontrol (ortalama yaş: 49.7 yıl) ile glisemik kontrol altında olan 30 tip 2 diabetes mellituslu hastada (ortalama yaş:51.9 yıl) gerçekleştirildi. Her iki grupta da Lp(a), glukoz, total kolesterol, HDL-kolesterol, LDL-kolesterol, trigliserid, Apo AI, Apo B ve HbA1C seviyeleri ile kolinesteraz aktivitesi saptandı. Diabetik grupta kolinesteraz, apo B, trigliserid, glukoz ve HbA1C seviyeleri kontrol grubundan yüksekti ($p<0.05$). Her iki grubun diğer parametreleri arasında anlamlı bir farklılık yoktu. Diabetik grupta kontrollere göre apo B, trigliserid ve plazma kolinesteraz seviyelerinin yüksek olması glisemik kontrol altında olsalar bile bu hastaların ateroskleroz açısından diabetik olmayanlardan daha yüksek bir riske sahip olduklarını göstermektedir. Ayrıca, Lp(a)' nın diabetik grupta değişmemiş olması bu parametrenin diabetten bağımsız bir parametre olduğu şeklindeki görüşleri desteklemektedir.

Anahtar Kelimeler: Diabetes mellitus, Lp(a), kolinesteraz.

INTRODUCTION

Diabetes mellitus is characterised by hyperglycaemia, definitive or relative insulin deficiency and certain complications. Diabetes leads to disorders of carbohydrate, lipid and protein metabolisms. The most widespread complication of diabetes mellitus is accelerated atherosclerosis (1).

This has been thought to be due to alterations in lipoprotein metabolism (2). Lp(a) was defined by Berg in 1965 that suggested as an independent risk factor in the pathogenesis of atherosclerosis. Lipoprotein(a) is a heterogeneous family of macromolecular particles consisting of an apolipoprotein (a) molecule joined by disulphide linkage to apolipoprotein B-100 which is solidly anchored in a lipid-rich LDL-like core (3). Apo(a) is a glycosylated protein with structural homology to plasminogen. Due to this homology, Lp(a) has both thrombotic and atherogenic activities. Recent studies have demonstrated that Lp(a) inhibited

* Presented at the " 8th Asian – Pacific Congress of Clinical Biochemistry" in Kuala Lumpur, Malaysia 1998.

fibrinolisis by competing with plasminogen for binding to fibrin. Higher plasma Lp(a) levels increase the risk of thrombosis (4, 5). Epidemiological studies reveal that, serum lipoprotein (a) {Lp(a)} concentration is an independent risk factor for atherosclerosis (6, 7).Therefore, recent studies have been directed towards the investigation of the role of Lp(a) in the pathogenesis of diabetes mellitus. Although some studies revealed high levels of Lp(a) in diabetes mellitus (8, 9), the others did not support these findings (10-12).

Plasma cholinesterase has been considered as a risk factor for coronary heart disease due to ability in affecting lipid and lipoprotein metabolisms (13-15). A recent study showed a relationship between a high level of cholinesterase and triglyceride (16).

In this study, we investigated serum Lp(a), plasma cholinesterase levels and some other risk factors in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

The present study included 30 patients with type 2 diabetes mellitus as patient group and 30 healthy subjects as control group. The characteristics of controls and patients with type 2 diabetes mellitus are given Table 1.

Table 1. Characteristics of patients and control subjects.

	Control Group (n: 30)	Patient Group (n: 30)
Mean age (ranges) Years	49,7 (28-67)	51,9 (32-70)
Sex ratio Male / female	13/17	11/19
Duration of diabetes (mean years)	----	13.4
Smoking (%)	46	43
Atherosclerosis of Lower limb (%)	----	10.2
Coronary artery Disease (%)	----	27.8

The frequency of coronary artery disease (CAD) and peripheral vascular disease (PVD) as an atherosclerotic complication were evaluated in patients with type 2 diabetes mellitus. The frequency of CAD and PVD were 27.8 % and 10.2 % in type 2 diabetes mellitus group, respectively. The diagnosis of CAD was based on history of proven myocardial infarction. Doppler sonography of the lower limbs was performed for the diagnosis of vascular disease. The patient group consisted of those from our outpatient clinic.

Blood samples were collected in tubes with anticoagulant and without in the morning by venipuncture after overnight fast. Serum and plasma samples were separated from blood. Each of the sera was divided into two aliquots. One of the aliquots was stored at -70 ^{0}C in plastic tubes until the analysis of Lp(a). The other analysed for lipids, apolipoproteins and glucose. Plasma was analysed for cholinesterase. An automated enzymatic colorimetric method was used for cholesterol and triglyceride determination. High density lipoprotein (HDL) cholesterol was measured after precipitation of other lipoprotein with phosphotungstic acid - magnesium acetate (cromatest kit). Low - density lipoprotein (LDL) cholesterol was calculated according to the method of friedewald et al. (17) formula.

Apolipoprotein AI and B levels were measured by an immunoturbidimetric assay (Sentinel CH kit, Italy). Lp(a) was measured using a commercially available antibody reagent set for Lp(a) kit (INCSTAR, SPQ Test System, Minnesota, USA). This assay permits the quantitative determination of human lipoprotein (a) by immunoprecipitin analysis. A calibration curve was generated by assaying a series of standards with known concentration of lipoprotein (a). Concentrations for the controls and samples were interpolated from the calibration curve.

Glucose levels were determined by the glucose oxidase method in serum (biotrol kit, France). Also plasma cholinesterase levels were determined by enzymatic colorimetric method (Ciba-Coming kit, USA). Glycosylated haemoglobin value (specific for Hb AIC) was assayed by cathion exchange chromatography method (Helana kit, USA).

Statistical analysis was performed using a non-parametric test (Mann-Whitney U test) for Lp(a). The student's test was used for evaluating other parameters.

RESULTS

Because it has been shown in previous studies that serum Lp(a) levels did not show differences according to sex, we did not divide the subgroups by male and female. All parameters of control and diabetic groups are shown in Table 2. Serum Lp(a), apo AI, total cholesterol, HDL-cholesterol and LDL-cholesterol levels showed no significant difference between control and diabetic groups. We found that serum apo B, glucose, triglyceride, plasma cholinesterase and HbAıc levels increased significantly in the diabetic group according to the control group.

Table 2. All parameter values of control and diabetic group.

Parameters	Control Group	Diabetic Group
Lp (a) (mg/dl)	20.4±15.1	13.7±14
Plasma Cholinesterase (U/L)	3808±649	4331±799*
Apo A (mg/dl)	135±30.8	138±33.1
Glucose (mg/dl)	78.05±9.73	186.4±90.7**
Triglyceride (mg/dl)	118.7±58.6	178±103 *
Total Cholesterol (mg/dl)	208.4±39.8	209.4±56.3
HDL-Cholesterol (mg/dl)	44.62±7.46	44.64+6.59
LDL-Cholesterol (mg/dl)	140.4±38.2	133.6±47.7
Hb Aıc (%)	5.33±0.37	9.37±3.39

*$p < 0.05$, ** $p < 0.001$, All values are expressed as mean ± SD.

DISCUSSION

Abnormalities in the concentrations of plasma lipoproteins in patients with type 2 diabetes include an increase in VLDL triglyceride concentration and a decrease in HDL cholesterol concentration (18). Increased plasma TG level in type 2 diabetes mellitus has been reported to be due to increase of hepatic VLDL-TG synthesis and secretion. The following mechanism of action has been used for explanation: The resistance to the insulin-dependent glucose uptake increases plasma concentration of insulin and this leads in turn to an increase in hepatic VLDL-TG synthesis and secretion (19, 20). This finding was supported by animal studies in which VLDL-TG secretion was shown to be directly related with insulin concentration in perfused rat liver (21). In humans this is also true; the resistance to insulin leads to hyperinsulinemia and hypertriglyceridemia may occur in patients with type 2 diabetes as a result of an increase in hepatic VLDL-TG synthesis and secretion (20).

In diabetic patients, total and LDL-cholesterol levels usually did not differ from those of non diabetics (22). However, in some studies LDL-cholesterol levels have been reported to increase in poorly controlled diabetic patients (23). We did not detect any significant differences regarding to serum total cholesterol, LDL-cholesterol and HDL-cholesterol levels compared to controls. Patients with type 2 diabetes are usually obese and dietary restrictions, exercise, weight lowering and good glycemic control may improve lipoprotein metabolism in these patients (22, 23). Therefore; normal HDL, LDL, and total cholesterol levels in our patients may be due to dietary restrictions and good glycemic control.

Studies on the alterations of Lp(a) levels have controversial results. Some studies revealed lower serum (a) levels compared to controls (24), while others found no statistically significant differences (12, 25). In addition some studies revealed higher serum Lp(a) levels in diabetics compared to controls (26, 27). In our study we found no statistically significant differences although Lp (a) levels in diabetics appeared lower than those of controls. Higher Lp (a) levels are explained to be due to hyperinsulinemia in those patients (26). Also, some studies reported that higher Lp(a) levels in uncontrolled diabetics might be reduced by increasing glycemic control (9). However, it should be emphasised that, plasma Lp(a) levels were under control of apo(a) gene and revealed inverse relation with apo(a) (28, 29). Therefore, studies, indicating that Lp(a) levels did not have alterations, pointed out that diabetes had no significant relationship with Lp (a) and that, Lp (a) was an independent parameter (30). Our results support these findings.

We found significant higher apo B levels while apo A1 levels in diabetics were not different significantly from that of the control group. As known, increases in apo B levels are consistent with a dismal prognosis whereas an increase in apo A1 is with a fair one. Increases in apo B levels are considered as a better indicator than LDL and HDL for the risk of atherosclerosis (31).

It has been suggested that plasma cholinesterase have some effects on lipid and lipoprotein metabolisms and also have close relationships with LDL, although the biological effects of plasma cholinesterase has not been fully understood (32, 33). Therefore, an increase in plasma cholinesterase levels is considered as a risk factor for atherosclerosis (14, 15). This is consistent with the findings of our study in which diabetics had higher plasma cholinesterase levels than controls. However one would expect that diabetics would have increased levels of LDL cholesterol as a result of increased plasma cholinesterase levels. Ragoobirsingh et al. (16) reported a close relationship between cholinesterase and triglyceride levels in diabetics.

In conclusion, patients with type 2 diabetes mellitus have various disorders associated with carbohydrate and lipid metabolisms. We also concluded that patients with higher levels of Apo B, triglyceride and plasma cholinesterase had higher risk for atherosclerosis although total cholesterol, LDL cholesterol, HDL cholesterol and apo A1 levels have been found to be unchanged compared to controls in patients with type 2 diabetes mellitus. Lp(a) levels which were statistically not different from the control

group supported that Lp(a) was an independent parameter from diabetes.

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