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Lipoprotein-Associated Phospholipase A2: A Risk Factor for Ischemic Stroke?

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ABSTRACT

Aim: The aim of the study is to investigate whether there is an association between lipoprotein associated phospholipase A2 (Lp-PLA2) and stroke occurrence.

Methods: Fifty-two patients with acute ischemic stroke within the 24 hours of the event, and age and gender matched 42 control subjects were enrolled to the study. This is a randomised, case-controlled, observational study, involving patients and control subjects admitted to the Neurology Department of Ankara Numune Education and Research Hospital between December 2009 and May 2010.

Results: The mean Lp-PLA2 level was 207.3±107.3 ng/ml in the patient group, and 128.6±10.3 ng/ml in controls. The difference between patients and control groups were statistically significant ($p<0.01$).

Conclusion: The results of our study revealed that Lp-PLA2 plays a role in ischemic stroke and can be considered as an early indicator for atherosclerosis. There is still need for comprehensive studies with larger patient groups in order to clarify an accurate deduction.

Keywords: Atherosclerosis, ischemic stroke, lipoprotein-associated phospholipase A2, risk factors

Lipoprotein-İlişkili Fosfolipaz A2: İskemik İnme için bir Risk Faktörü mü?

ÖZ

Amaç: Çalışmanın amacı, lipoprotein ile ilişkili fosfolipaz A2 (Lp-PLA2) ile inme oluşumu arasında bir ilişki olup olmadığını araştırmaktır.

Yöntem: Çalışma kapsamında 24 saatiçerisinde akut iskemik inme teşhisi konmuş 53 hasta ile hasta grubu yaş ve cinsiyetine uygun 42 kontrol denek incelendi. Bu araştırma Aralık 2009 ve Mayıs 2010 tarihleri arasında Ankara Numune Eğitim ve Araştırma Hastanesi Nöroloji Bölümüne başvuran hastaları ve kontrol konularını içeren randomize, vaka kontrollü, gözlemsel bir çalışmadır.

Bulgular: Çalışmamızda, Lp-PLA2'nin ortalama düzeylerinin hasta grubunda 207.3 ± 107.3 ng/ml, kontrol grubunda ise 128.6 ± 10.3 ng/ml olduğu, hasta ve kontrol grubu arasındaki farkın ise istatistiksel olarak anlamlı olduğu bulunmuştur ($p <0.01$).

Sonuç: Çalışmamızın sonuçları, Lp-PLA2'nin iskemik inmede rol oynadığını ve ateroskleroz için erken bir gösterge olarak değerlendirilebileceğini gösterdi. Kesin bir sonuca ulaşabilmek için daha geniş hasta grupları ile kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Ateroskleroz, iskemik inme, lipoproteinle ilişkili fosfolipaz A2, risk faktörleri

1. Introduction

Cerebrovascular diseases (CVD) is the second common cause of death and the most common cause of disability worldwide. Preventive treatment depends on the identification and treatment of risk factors. Therefore, the identification of the unknown risk factors and a better understanding of known risk factors are of extreme importance [1]. Recently, many parameters associated with lipid metabolism have been determined. One of these is the lipoprotein associated phospholipase A2 (Lp-PLA2).

Lp-PLA2 has been defined as an independent inflammatory marker. The Lp-PLA2 is a member of the intercellular and secretory phospholipase enzymes family, capable of hydrolyzing the sn-2 ester bond of the cell membrane and the phospholipids of the lipoproteins [2,3]. At the same time, Lp-PLA2 is a calcium-independent serine lipase [4] of 45 kDa, consisting of 441 amino acids, which is also known as the platelet-activating factor acetylhydrolase (PAF-AH). It is expressed by macrophages in atherosclerotic plaques and the fibrous lesions prone to rupture [5]. In *in vitro*, next to its hydrolyzing effects on the platelet-activating factor, Lp-PLA2 hydrolyzes the modified phospholipids in the oxidized LDL, which is produced around the arterial wall by LDL oxidation [3, 6, 7]. Due to the effect of the Lp-PLA2 on the oxidized LDL particles in the vessel walls, vascular inflammation is initiated, which in turn stimulates the collection of the monocytes in the vessel wall and the apoptosis in the plaque [8].

In regards to the previous studies mentioned here, we aimed to investigate whether there is an association between this inflammatory markers and occurance of ischemic stroke. We also aimed to evaluate whether the Lp-PLA2 is a risk factor for this disease.

2. Materials and Methods

2.1. Materials

Fifty-two patients with the diagnosis of acute ischemic stroke within the first 24-hours in the Neurology Department of Ankara Numune Education and Research Hospital, and 42 healthy age and gender matche control subjects of similar demographic characteristics were enrolled to the study. Exclusion criteria included the following: presence of hemorrhagia in cranial imaging; kidney and liver diseases; rheumatologic or connective tissue diseases; diagnosis of cancer; fever and/or clinical signs of infection; who underwent vascular surgery within the last three months; and using drugs –that could- affect the levels of Lp-PLA2.

The diagnosis of acute ischemic stroke was confirmed according to history, neurological examination and cranial MRI findings.

2.2. Methods

In the patient and the control groups, in addition to the serum Lp-PLA2 levels, total cholesterol, LDL, and serum C-reactive protein (CRP) levels were determined. Blood samples were taken from the patients on their first day of admission and they were put into 10 mm tubes with red caps not containing gel (BD Vacutainer). After a at least 30 minutes of incubation, the specimene were centrifuged at 1500 x g for 10 minutes, and lipid profiles and CRP levels were determined using commercial kits (Beckman Coulter DXC 800).

The serum levels of Lp-PLA2 were determined by the PLAC (Diadexus) test. The PLAC test is used for the quantitative determination of serum levels of Lp-PLA2, and works with the turbidimetric immunoassay method. This testing procedure was performed with the Beckman autoanalyzer. The serum levels of Lp-PLA2 were measured in both groups. The measurement range of the method was 7-500 ng/ml. Statistical analyses were performed with SPSS for Windows (version 13.0).

The Shapiro Wilk and Kolmogorov- Smirnov tests were used to examine whether or not the data had normal distribution. For the statistical analysis of the groups the Mann-Whitney U test, Student's t test, Shapiro Wilk test, Wilcoxon test, Spearman's rho coefficient and Pearson' correlation test is used_and a p value of P<0.01 is considered to be statistically significant.

3.Results

Fifty-two patients and 42 controls were enrolled to the study. There were 30 women (57.7%) and 22 men (42.3%) in the patient group and 31 (64.6%) women and 17 men (35.4%) in the control group. The mean age of the patient group was 71.5 ± 12 (Range=51-90) years, whereas it was 60,55 ± 8,294 (range=48-88) years in the control group. There was no statistically significant difference in terms of age and gender between the patient and control groups (p> 0.05). Twelve patients (23.07%) had no concomitant systemic disease. The demographical characteristics and con-comitant diseases of the patients and controls are shown in Table 1.

Table.1: The demographic characteristics and concomitant diseases of the patient and controls

		Patient		Control	
		N	%	N	%
Gender	F	30	57.7	31	64.6
	M	22	42.3	17	35.4
HT	+	37	71.1	11	26.1
	–	15	28.9	31	72.9
DM	+	14	26.9	11	26.1
	–	38	69.1	31	72.9
AF	+	13	25	10	23.8
	–	39	75	32	76.2
Smoking	+	13	25	–	
	–	39	75		
W/O Disease*	–			10	23.8

*Control group members without any systemic disease

The average value of serum Lp-PLA2 levels was 207.3 ± 107.3 ng/ml in the patient group, whereas it was 128.6 ±10.3 ng /ml in the control group. The difference was statistically significant (p<0.01) (Figure1).

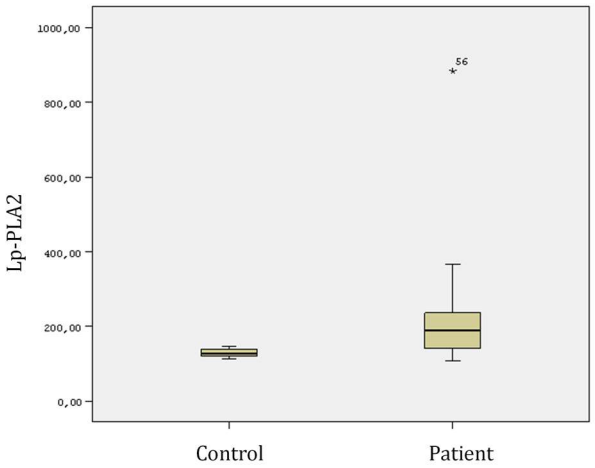

Figure.1: The comparison of the serum Lp-PLA2 levels in the patient and the control groups.

No statistical difference was determined between the genders in terms of Lp-PLA2 (p>0.05).
The average value of total cholesterol levels were 185.0±43.7 mg/dl and 188.9±39.5 mg/dl, respectively, in the patient and the control group (Figure.2).

The average value of the LDL levels in the patient group was 120.5±36.2, and that of the control group was 118.0±29.9 (Figure. 3).

There was no statistically significant difference between the LDL levels in the patient and the control group difference (p>0.05). Also no relation was found between the LDL and LP-PLA2 levels (Figure 4.A and 4.B) between the study and control groups (p>0.05).

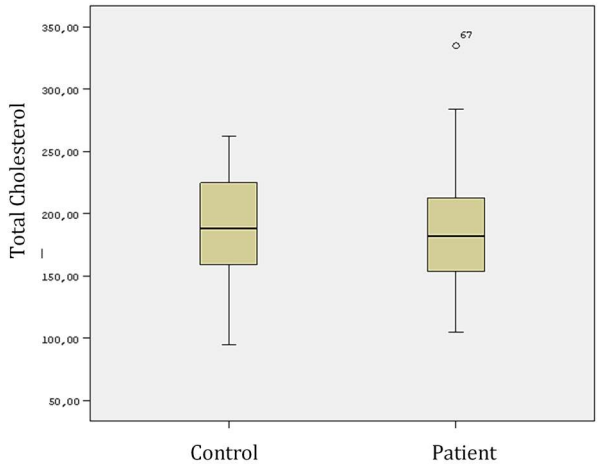

Figure.2: The comparison of the serum Total Cholesterol levels in the patient and the control groups.

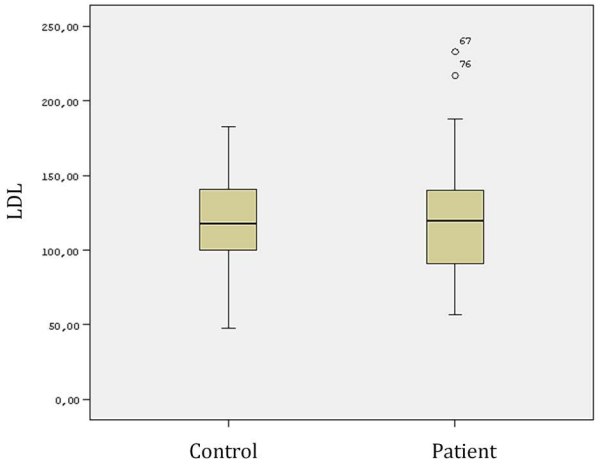

Figure.3: The comparison of the serum LDL levels in the patient and the control groups.

When we compare the two groups in terms of coronary heart disease related to Lp-PLA2 levels no statistically significant was found between the groups (p>0.05). There was a statistically significant difference between the patient and control groups in terms of CRP (Figure.5). It was 1.47 ± 2.4 mg/dl in the patient group, whereas it was 0.56 ± 0.16 mg/dl in the control group (p<0.01).

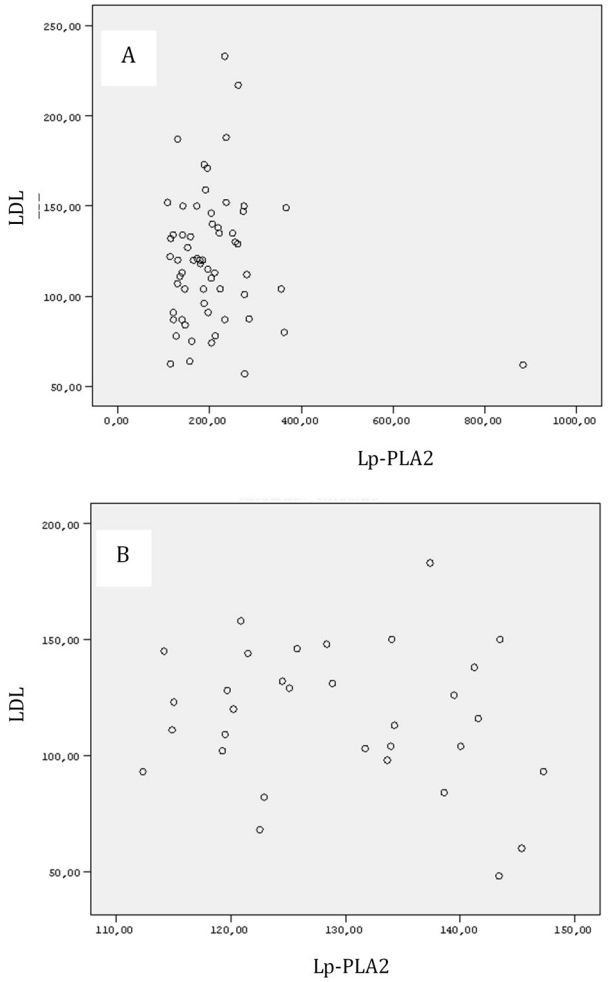

Figure.4: The comparison of the serum LDL and LP-PLA2 levels in (A) the patient and (B) the control groups

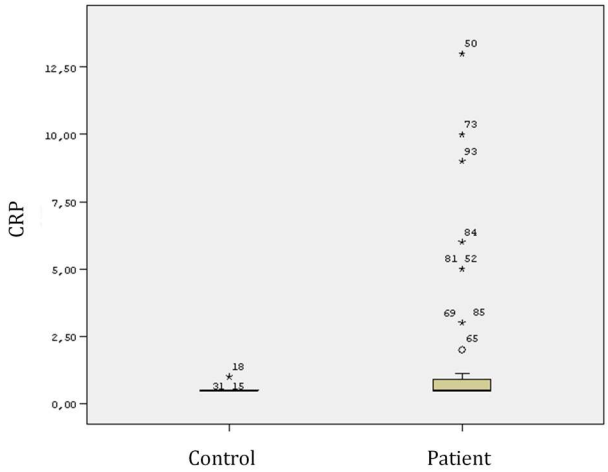

Figure.5: The comparison of the serum CRP levels in the patient and the control groups.

4.Discussion and Conclusion

There are various studies on the epidemiology and pathogenesis of atherosclerosis, which plays an important role in ischemic stroke, as well as its early diagnosis and prevention. Atherosclerosis plays a role in the development of many diseases and leads to high morbidity and mortality. This has increased the interest in the topic, especially in the prevention of the complications of atherosclerosis in the asymptomatic phase using easily applicable diagnostic tools.

In recent studies, high levels of the Lp-PLA2 has been demonstrated to be an important risk factor in the development of atherosclerosis [9]. In our study, we have determined the Lp-PLA2 levels to evaluate, which may be a risk factor in the pathogenesis of atherosclerosis and a determinant of the plaque that is prone to rupture, in patients with acute ischemic stroke.

Today, inflammation and oxidative stress are both considered to be key components of atherosclerosis. The markers of inflammation are CRP, TNF-alpha, cellular adhesion molecules, and the markers of oxidative stress such as oxidized LDL and Lp-PLA2. Lp-PLA2 plays a role as a marker in the biology of plaque[10]. Lp-PLA2 is secreted by many cells such as, monocyte-macrophages, and mast cells, which are involved in the inflammatory / immune response [11].

The Lp-PLA2 is a circulating enzyme, which is released by macrophages and has a close relationship with LDL cholesterol. The Lp-PLA2 is an indicator of inflammation and plays an important role in plaque rupture and in the LDL oxidation cascade. It has been demonstrated to be released from the necrotic core of atherosclerotic plaque. This suggests that it may be an indicator or a marker of the unstable plaque. High Lp-PLA2 levels were found to be associated with a high risk of ischemic stroke [12]. This indicates that elevated Lp-PLA2 levels may be an independent risk factor for ischemic stroke.

In the ARIC study, it was found that inflammation plays an important role in cerebrovascular diseases as well as the cardiovascular diseases, and the elevated serum CRP and Lp-PLA2 levels were found to be associated with an increased risk of ischemic stroke [13].

In our study, in the patient group, the average value of serum Lp-PLA2 levels was 207.3 ± 107.3 ng/ml, whereas in the control group, it was 128.6 ±10.3 ng/ml. The difference was statistically significant ($p<0.01$). The significantly high Lp-PLA2 levels in our study may suggest that elevated Lp-PLA2 levels are associated with an increased risk of ischemic stroke.

CRP levels were significantly higher in ischemic stroke patients compared to the subjects with no history of ischemic stroke in our study. The high levels of CRP are responsible for an increased synthesis of adhesion molecules and chemokines, and in the initiation of vascular inflammation. Elkind et al. have determined significantly high levels of both CRP and Lp-PLA2 in patients with acute ischemic stroke.

The Lp-PLA2 levels were not associated with the severity of stroke. In epidemiological studies, the increased levels of Lp-PLA2 were found to be associated with ischemic cerebrovascular disease and cardiac diseases. Compared to CRP, Lp-PLA2 was found to be a more specific determinant of the vascular damage [14, 15].

Additionally, factors such as Lp-PLA2, CRP, hypertension, diabetes, and smoking all initiate endothelial dysfunction and inflammation, triggering intimal proliferation in the great arteries, and the proliferation of adventitia and fibrosis in the small cerebral arteries. As a result,

the selective inhibition of Lp-PLA2 and/or CRP may reduce the risk of the ischemic stroke. Furthermore, the fibrates and the statins can be used for more effective prevention of ischemic stroke in patients with high levels of Lp-PLA2 [13]. We excluded the patients taking drugs to lower the blood lipids. Using this method, our results were not influenced by the effects of these drugs.

Similar to previous studies, the ARIC study reported that the Lp-PLA2 was positively correlated with LDL levels and negatively correlated with total cholesterol levels [13]. Lp-PLA2 is an enzyme with a close relationship with LDL cholesterol and a pro-inflammation indicator, which plays a role in the LDL oxidative cascade. When the relationship between LDL and Lp-PLA2 levels were compared between the patient and control groups, no statistically significant difference was found ($p>0.05$) in our study. Some other similar small sized studies also showed no relationship between Lp-PLA2 and LDL levels. Therefore, Lp-PLA2 can be assessed as a risk factor independent from the LDL levels [13, 16-18].

The Lp-PLA2 levels were determined to be a marker for recurrent ischemic stroke. Lp-PLA2 is a more significant marker in patients with LDL levels under 130 mg/dl than in the patients with LDL levels of 130 mg/dl or more. But we did not find a difference between the groups in terms of LDL levels. This led us to think that Lp-PLA2 may be elevated independent of LDL in ischemic stroke.

The Rotterdam study is the first general population-based prospective study; in which it was shown that the Lp-PLA2 is an independent marker for ischemic stroke and coronary artery disease [9]. In the small study group, Lp-PLA2 activity was higher in patients with ischemic stroke, compared to the healthy controls. Our results were in concordance with the Rotterdam study in terms of results related to Lp-PLA2.

The Lp-PLA2 is still not used routinely in daily practice. There are various clinical studies indicating its importance in clinical follow-up in terms of detecting the risk factors and diagnosis. However, these studies have been conducted in different countries in certain groups of patients.

In current study, we aimed to evaluate whether the Lp-PLA2 is a risk factor for ischemic stroke. The Lp-PLA2 levels in our study were significantly higher in the patient group. Elkind et al. suggested an association between Lp-PLA 2 activity levels and stroke recurrence [14]. Also, in a study by Massot et al. Lp-PLA2 activity has been suggested as a potential new tool to predict ischemic cerebrovascular events [19].

Numerous epidemiological studies consistently demonstrate that an elevated plasma level of Lp-PLA2 is independently associated with the risk of coronary heart disease (CHD) and ischemic stroke[20]. In a meta-analysis published in 2010 also indicated a modest association between Lp-PLA2 and ischaemic stroke [21].

To conclude the results of our study which are in concordence with the results of previous studies revealed that Lp-PLA2 can be a potential new risk factor and may have a utility as a biomarker among the patients with symptomatic intracranial atherosclerotic disease [15, 22].

Further studies with large number of patients are needed to make an accurate deduction and randomized trials of potent reversible pharmacological inhibitors of Lp-PLA2 activity may help to establish whether modification of Lp-PLA2 can reverse vascular risk [8].

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