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# Serum lipids and Lp-PLA2 activity in multiple sclerosis

## [Multipl sklerozda serum lipitleri ve Lp-PLA2 aktivitesi]

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**ABSTRACT**

**Objective:** Multiple sclerosis (MS) is an inflammatory demyelinating disease arising from unknown origin. Dyslipidemia, T-lymphocyte mediated inflammation, lipoprotein associated oxidative activity and lipoprotein associated phospholipase A2 (Lp-PLA2) activity include the pathogenesis of MS. In the current study, we evaluated the effect of Lp-PLA2 activity and serum lipids in MS patients with different clinical stages to understand the effect these parameters on MS progression.

**Methods:** The study included a total of 30 patients and 30 controls. Lp-PLA2 activity, total cholesterol, triglyceride, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), atherogenic index of plasma (AIP), non HDL cholesterol (non-HDL-C) and total cholesterol to HDL-C ratio (TCHOL/HDL-C) were compared between controls and patients. Additionally, patients were grouped according to their clinical stage and compared each other in terms of serum Lp-PLA2 activity, LDL-C, HDL-C, total cholesterol and triglyceride concentrations.

**Results:** There were no statistically significant differences between patients and controls in terms of Lp-PLA2 activity, total cholesterol, triglyceride, HDL-C, LDL-C, AIP, non-HDL-C and TCHOL/HDL-C. Additionally, no significant difference was observed in terms of Lp-PLA2 activity, total cholesterol, triglyceride, HDL-C and LDL-C concentrations among patients divided according to clinical stage.

**Conclusion:** Serum lipids concentrations and Lp-PLA2 activity are not seem to be involved in MS and its progression. Therefore, we believe that lipids and Lp-PLA2 reducing treatment strategies are not expected to be hopeful in preventing disease occurrence and vasculary events.

**Key Words:** Multiple sclerosis, lipoprotein associated phospholipase A2, lipid

**Conflict of Interest:** Authors have no conflict of interest.

**ÖZET**

**Amaç:** Multipl skleroz (MS) nedeni bilinmeyen inflamatuvar demiyelizan bir hastalıktır. Dislipidemi, T lenfosit aracılı inflamasyon, lipoprotein aracılı oksidatif aktivite ve lipoprotein ilişkili fosfolipaz A2 (Lp-PLA2) aktivitesi hastalığın patogenezinde rol almaktadır. Bu çalışmada farklı klinik dönemlerde olan MS hastalarında Lp-PLA2 aktivitesi ve serum lipit düzeyleri incelenerek hastalığın ilerleyişi üzerindeki etkilerinin ortaya konması amaçlanmıştır.

**Metod:** Çalışmaya 30 hasta ve 30 kontrol dahil edildi. Kontrol ve hasta grupları Lp-PLA2 aktivitesi, total kolesterol, trigliserid, HDL kolesterol (HDL-K), LDL kolesterol (LDL-K), plazma aterojenik indeks (PAİ), non HDL-K ve total kolesterol/HDL-K düzeyleri yönünden karşılaştırıldı. Ayrıca hastalar klinik durumlarına göre gruplandırıldı ve gruplar Lp-PLA2 aktivitesi, total kolesterol, trigliserid, LDL-K ve HDL-K düzeyleri yönünden karşılaştırıldı.

**Bulgular:** Hasta ve kontrol grupları arasında Lp-PLA2 aktivitesi, total kolesterol, trigliserid, HDL-K, LDL-K, PAİ, non-HDL-K ve total kolesterol/HDL-K düzeyi yönünden herhangi bir farka rastlanmadı. Buna ilave olarak klinik durumlarına göre gruplandırılan hastalar arasında Lp-PLA2 aktivitesi, total kolesterol, trigliserid, HDL-K ve LDL-K düzeyleri yönünden anlamlı herhangi bir fark yoktu.

**Sonuç:** Serum lipit düzeyleri ve Lp-PLA2 aktivitesinin MS hastalığında ve hastalığın ilerleyişinde rol oynamadığı düşünülmektedir. Bu nedenle serum lipid ve LP-PLA2 düzeylerini düşürücü tedavi stratejilerinin hastalığın oluşumu, ilerleyişi ve vasküler olayların önlenmesinde önemli bir katkı sağlamayacağı düşünülmektedir.

**Anahtar Kelimeler:** Multipl skleroz, lipoprotein ilişkili fosfolipaz A2, lipit

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## Introduction

Multiple sclerosis (MS) is the most common autoimmune inflammatory demyelinating disease of the central nervous system (CNS) [1]. Although the main cause of the disease is still unknown, it is proposed that multiple possible mechanisms including higher production of proinflammatory cytokines, activated macrophage, T lymphocyte and reactive oxygen species contribute to MS pathology [2,3]. Axon degeneration and loss of oligodendrocytes are the major pathologic mechanisms that cause the clinical manifestations [4].

The categorization of MS is a subset of that published in 1996 [5]. The study made by Lublin et al. [5] categorized the common patterns of MS as relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS). RRMS is characterized by clearly defined relapses with full recovery or with squeal and residual deficit upon recovery, while SPMS is an initial RRMS disease course followed by progression with or without occasional relapses, minor remissions, and plateaus. The third pattern, PRMS, is characterized by progressive disease from onset, with clear acute relapses, with or without full recovery. The treatment of MS varies depending upon these patterns [6]. In addition to these patterns, clinically isolated syndrome (CIS) is described as a first neurologic episode that lasts at least 24 hours, and is caused by inflammation and demyelization in CNS. Brain lesions associated with a clinically isolated syndrome may be the indicative of multiple sclerosis [7].

Lipoprotein associated phospholipase A2 (Lp-PLA2), which is produced as an active form by T lymphocytes and monocyte-derived macrophages, is a $Ca^{+2}$ independent protein which belongs to secretory phospholipase A2 group VII. Lp-PLA2, which has both antioxidant and inflammatory activity, hydrolyses bioactive lipids [8].

Impaired cholesterol homeostasis in serum and brain has been linked to chronic neurodegenerative disorders including Alzheimer's disease, Huntington's disease, Parkinson's disease, Niemann-Pick type C disease and Smith-Lemli Opitz syndrome [9]. There is now emerging evidence indicating that dyslipidaemia is associated with MS disease progression. Comorbidities linked to dyslipidemia were reported to be associated with increased risk for MS disability progression [8,10]. It has been reported that lipids and Lp-PLA2 activity has modest effects on MS [8,10-12]. However the data in this field is limited to reach an exact conclusion.

In the present study, we investigated the role of lipid profile and Lp-PLA2 activity in MS and its clinical progression. The study was conducted in MS patients with different clinical patterns. As our best knowledge, this is the first study that compares AIP, non-HDL cholesterol (non-HDL-C) and total cholesterol/HDL-C (TCHOL/HDL-C) values between MS patients and healthy controls. Identifying the role of these parameters on MS might allow the development of therapeutic strategies on MS disease.

## Material and Patients

### *Study population*

The study was conducted in the Department of Clinical Biochemistry, Ankara Numune Training and Research Hospital. A total of 30 patients [9 males and 21 females, aged 20–59 years (mean age: 35.75±9.96)] and 30 healthy controls, [9 males and 21 females, aged 27–53 (34.34±9.86)] were included in this study. All patients had clinically or laboratory-supported definite MS according to Mcdonald et al. [13] Exclusion criteria included the history of thyroid disease with or without treatment (medical, surgical, radio iodine, etc.), presence of endocrine disorders, pregnancy, patients receiving any medication that may alter serum lipid concentrations, patients with liver disease, renal disease and acute infection. Samples were sent by physicians from various medical inpatient and outpatient clinics of the Neurology Department at Ankara Numune Education and Training and Hospital. The protocol was approved by local ethical committee.

### *Biochemical analysis*

### *Measurement of Lp-PLA2 activity*

Blood samples were collected in evacuated red top tube containing gel (BD Vacutainer®, Becton Dickinson, UK) and centrifuged at 1300 g for 10 minutes. Sera were frozen in aliquots and stored at -70° C for advanced analysis. Lp-PLA2 activity was measured spectrophotometrically with an enzyme assay (PLAC®, diaDEXUS, South San Francisco, USA) by Roche Modular Analytics (Roche Diagnostics, Germany). The limit of detection for the test was 1.4 µmol/L. The intra-assay, inter-assay and total precisions were 2.9%, 3.0% and 3.8%, respectively.

### *Determination of Serum Lipids and Lipoproteins*

Lipid and lipoprotein determinations were performed with serum samples collected after an overnight fasting period. Total cholesterol, HDL-C and triglyceride concentrations were determined using enzymatic colorimetric method (Roche Diagnostics, Mannheim, Germany). The concentrations of LDL-C cholesterol were estimated using the Friedewald formula [(Total cholesterol – (triglyceride / 5+HDL-C)] for samples with triglyceride concentrations of <400 mg/dL [14]. LDL-C concentration was directly determined by enzymatic colorimetric method for samples with triglyceride concentrations of ≥400 mg/dL. Non-HDL-C was calculated as the difference between total cholesterol and HDL-C. AIP were calculated by using the formula; Log (triglyceride/HDL-C) [14,15]

### *Statistical analyses*

Histogram and q-q plots were plotted to assess data normality obtained from Shapiro-Wilk's test. Because our data set was normally distributed, Student's t test was performed to compare the differences in Lp-PLA2 activity,

**Table 1.** Comparison of lipids and Lp-PLA2 activity between controls and patients

| | Patients (n=30) | Controls (n=30) | *p* |
|---|---|---|---|
| Total cholesterol (mg/dL) | 179±47 | 200±32 | 0.094 |
| Triglyceride (mg/dL) | 128±60 | 146±75 | 0.367 |
| HDL-C (mg/dL) | 56±16 | 50±9 | 0,125 |
| LDL-C (mg/dL) | 107±37 | 13±30 | 0.162 |
| Non-HDL-C (mg/dL) | 124±52 | 148±35 | 0.052 |
| AIP | 0.43±0.23 | 0.33±0.21 | 0.137 |
| TCHOL/HDL-C | 4±1 | 3±1 | 0.051 |
| Lp-PLA2 (µmol/L) | 154±27 | 155±23 | 0.821 |

AIP: Atherogenic index of plasma, TCHOL: Total cholesterol. Results are expressed as mean ± SD with 95% confidence intervals. p indicates the significance between patients and controls. To get from mg/dL to SI unit (mmol/L) multiple by 0.0259 for HDL-C, LDL-C and total cholesterol and multiple by 0.0113 for triglyceride.

total cholesterol, triglyceride, HDL-C, LDL-C, AIP, non-HDL-C and TCHOL/ HDL-C concentrations between patients and healthy control group. Kruskal- Wallis H test was used to compare Lp-PLA2 activity, total cholesterol, triglyceride, HDL-C and LDL-C between patients with different clinical stages. Analysis were performed using IBM SPSS software (release 20.0, IBM, SPSS Inc., Chicago, IL, USA) with considering $p < 0.05$ as statistically significant.

## Results

A total of 60 subjects (30 patients and 30 controls) were included in analyses. Mean concentrations of Lp-PLA2 activity, total cholesterol, triglyceride, HDL-C, LDL-C, non-HDL-C, AIP and TCHOL/HDL-C concentrations and differences between patients and controls in terms of these parameters were given in Table 1. Patients had an expanded disability status scale (EDSS) of 1.00 (0.5 - 3.38). From a total of 30 patients, 17 (56.67%), 6 (20%), and 7 (23.33%) had RRMS, SPMS CIS, respectively. No difference was observed between groups in terms of Lp-PLA2 activity, total cholesterol, triglyceride, HDL-C and LDL-C concentrations (Table 2).

## Discussion

In this study, we compared Lp-PLA2 activity, serum total cholesterol, triglyceride, HDL-C, LDL-C, API, TCHOL/ HDL-C and non-HDL-C concentrations between MS patients and controls. We also investigated the changes of Lp-PLA2 activity, serum total cholesterol, triglyceride, HDL-C, LDL-C concentrations in different clinical stages. It is shown that Lp-PLA2 activity and serum lipoprotein metabolisms are not seem to be involved in MS.

MS is an inflammatory autoimmune disease. Variety of symptoms including impaired motor and mental function are observed in MS patients. Recent evidence demonstrated that alterations in lipid metabolism are involved in the pathogenesis of neurodegenerative diseases [8,16]. In our study, no significant differences were observed between patients and controls in terms of serum HDL-C, LDL-C, triglyceride and total cholesterol concentrations (Table 1). Additionally, no statistically significant difference was observed among clinical stages in terms of these parameters (Table 2). Our data do not support previous studies [11,17,18]. The differences among studies are likely to depending on the genetic, environmental and nutritional factors. The study made by Weinstock et al. [12] showed that no associations of LDL-C and total cholesterol with disability changes in patients with high risk clinically isolated syndromes after the first demyelinating event. It was also reported that lipid metabolism in the CNS is regulated independently and almost all cholesterol present in

**Table 2.** Comparison of serum Lp-PLA2 activity and lipids between clinical stages

| | RRMS (n=15) | SPMS (n=6 ) | CIS (n=9) | *p* |
|---|---|---|---|---|
| LDL-C (mg/dL) | 150.00 (126.50 - 188.15) | 126.50 (93.50 -155.75) | 86.00 (67.7 - 116.5) | 0.065 |
| HDL-C (mg/dL) | 51.00 (46.50 -75.00) | 49.50 (46.00-56.00) | 48.00 (44.00 - 67.50) | 0.931 |
| Total cholesterol (mg/dL) | 177.00 (150.50 - 216.50) | 189.50 (142.00-251.00) | 161.00 (144.50 -192.00) | 0.137 |
| Triglyceride (mg/dL) | 98.00 (85.00 -145.50) | 115.00 (75.50-210.75) | 124.00 (80.00 -200.00) | 0.942 |
| Lp-PLA2 (µmol/L) | 150,00 (126.50 - 188.15) | 158.95 (149.07-190.47) | 148.52 (126.50 -160.90) | 0.619 |

Results are expressed as median (25 th-75 th percentil) with 95% confidence intervals. p indicates the significance between patients and controls. To get from mg/dL to SI unit (mmol/L) multiple by 0.0259 for HDL-C, LDL-C and total cholesterol and multiple by 0.0113 for triglyceride.

the brain is synthesized in situ by CNS cells [16]. It was reasonable to hypothesize that serum lipid concentrations had not primary effect on disease and did not increase risk for disability progression in MS when these findings had been taken together .

Vascular inflammation is known to play a key role in the development of MS [19]. Non-HDL-C, TCHOL/HDL-C and AIP are reliable and early predictors of vascular events associated with dyslipidemia [14,15,20]. In the current study, no differences were observed between controls and patients in terms of non-HDL-C, TCHOL/HDL-C and AIP (Table 1). These data might demonstrate that these markers were not useful to evaluate the vascular events in MS patients.

Lp-PLA2 activity was investigated in MS patients. We found no significant difference between patients and controls in terms of Lp-PLA2 activity (Table 1). This result is in accordance with the study made by Sternberg et al. [8]. We also did not find any statistically difference among clinical stages. These results might indicate that Lp-PLA2 activity was not involved in MS and disease progression. It was reported that there were differential associations of Lp-PLA2 mass and activity in vascular events [21]. In our study we did not measure Lp-PLA2 mass which was one of the limitations of this study. To assess the effect of Lp-PLA2 in MS future studies are warranted to investigate both Lp-PLA2 mass and activity measurements.

The weak point of this study is the limited sample size. Additionally the measurement of other relevant and already established strong risk predictors, such as small dense LDL, HDL subfractions, ApoA and Apo B might increase the power of the future studies about the role of lipids in MS.

In conclusion; Lp-PLA2 and serum lipoprotein metabolisms are not seem to be involved in MS. Therefore, we believe that lipids and Lp-PLA2 reducing treatment strategies are not expected to be hopeful in preventing disease occurrence and vasculary events. Future studies should aim towards measuring plasma Lp- PLA2 both activity and mass in a larger cohort including RRMS, SPMS, and PPMS, as well as in MS patients in clinical relapse.

### *Conflict of Interest*

There are no conflicts of interest among the authors.

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