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Subclinical Atherosclerosis and Oxidized LDL Levels in Familial Mediterranean Fever

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Research Article

History

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ABSTRACT

Objective: To investigate markers of carotid atherosclerosis and oxidized low density lipoprotein (OxLDL) levels in patients with Familial Mediterranean Fever (FMF) who have no risk factors for cardiovascular disease.

Method: It was included 44 patients (25 F/19 M; mean age: 33.5±7.5) with FMF in attack free period and gender and age matched 44 healthy subjects (25 F/19 M; mean age: 33.4±7.0). The patients with clinical coronary artery disease, chronic renal disease, diabetes mellitus, hypertension, history of myocardial infarction, angina pectoris, cerebrovascular disease, dyslipidemia, metabolic syndrome, active infection, those in postmenopausal period, and those using anti-lipid drugs were excluded. Carotid artery intima-media thickness (C-IMT) was measured and investigated atherosclerotic plaques in the carotids by using doppler ultrasound. Serum lipid and OxLDL levels were also assessed. Data were analyzed with the SPSS program and $p<0.05$ were accepted as significant.

Result: Mean disease duration of the FMF patients was 20±9 years. The mean C-IMT did not differ between the patient and control groups (0.52±0.10 mm, 0.53±0.06 mm, respectively, p=0.709). None of the patients or controls had atherosclerotic plaques. Total and LDL cholesterol levels were significantly lower in the patient group compared to the controls (total cholesterol: 157.07±34.18, 181.05±36.79, respectively, p=0.002; LDL cholesterol: 100.48±30.13, 126.25±34.05, respectively, p=0.001). However, we found that OxLDL levels were significantly higher in FMF patients compared to control group (337.48±438.56 ng/dl, 156.19±383.24 ng/dl, p=0.044). No correlation was found between C-IMT and OxLDL levels in both patients (p=0.324) and controls (p=0.246).

Conclusion: Our study provides further evidence for no increased atherosclerosis in FMF. As previously shown, FMF patients have lower cholesterol levels compared to healthy controls. On the other hand, increased OxLDL levels could be associated with increased subclinical inflammation.

Keywords: Atherosclerosis, Carotid arteries, Familial Mediterranean Fever, intima-media thickness, oxidized low density lipoprotein.

Ailevi Akdeniz Ateşi'nde Subklinik Ateroskleroz ve Okside LDL Değerleri

ÖZ

Amaç: Kardiyovasküler hastalık için risk faktörü olmayan Ailevi Akdeniz Ateşi (FMF) hastalarında karotid arter ateroskleroz belirteçleri ile okside düşük yoğunluklu lipoprotein (OxLDL) düzeylerinin araştırılması amaçlandı.

Metod: Ataksız dönemde FMF'li 44 hasta (25 K / 19 E; ort. Yaş: 33.5 ± 7.5) ve cinsiyet ve yaş uyumlu 44 sağlıklı birey (25 K / 19 E; ortalama yaş: 33.4 ± 7.0) incelendi. Dışlama kriterleri; klinik olarak gösterilmiş koroner arter hastalığı, kronik böbrek hastalığı, diabetes mellitus, hipertansiyon, miyokardiyal enfarktüs öyküsü, anjina pektoris, serebrovasküler hastalık, dislipidemi, metabolik sendrom veya aktif enfeksiyon ve menopoz sonrası dönemde olma veya anti-lipid ilaç kullanımıydı. Doppler ultrason yardımıyla karotid arter intima-media kalınlığı (C-IMT) ölçüldü ve aterosklerotik plaklar araştırıldı. Ayrıca serum lipid ve OxLDL düzeyleri de değerlendirildi. Veriler SPSS programı ile analiz edildi ve $p<0.05$ anlamlı kabul edildi.

Bulgular: FMF hastalarının ortalama hastalık süresi 20 ± 9 yıldı. Ortalama C-IMT hastalar ve kontroller arasında farklılık göstermedi (sırasıyla 0.52 ± 0.10 mm ve 0.53 ± 0.06 mm, p=0.709). Hasta veya kontrollerin hiçbirinde aterosklerotik plak yoktu. Total ve LDL kolesterol düzeyleri kontrollere kıyasla hastalar arasında anlamlı derecede düşüktü (Total kolesterol: sırasıyla 157.07 ± 34.18 ve 181.05 ± 36.79, p=0.002; LDL kolesterol: 100.48 ± 30.13 ve 126.25 ± 34.05, p=0.001). OxLDL seviyeleri FMF hastalarında (337.48 ± 438.56 ng/dl) kontrollere (156.19 ± 383.24 ng/dl) göre anlamlı olarak daha yüksekti (p=0.044). Hasta (p=0.324) ve kontrollerin (p=0.246) her ikisinde de C-IMT ve OxLDL seviyeleri arasında korelasyon bulunmadı (p>0.05).

Sonuç: Çalışmamız, FMF'de artmış ateroskleroz olmadığına dair kanıtları desteklemektedir. Daha önce gösterildiği gibi, FMF hastaları sağlıklı kontrollere kıyasla daha düşük kolesterol seviyelerine sahiptir. Öte yandan, artmış OxLDL seviyeleri, artmış sub-klinik inflamasyon ile ilişkili olabilir.

Anahtar sözcükler: Ailevi akdeniz ateşi, ateroskleroz, intima-media kalınlığı, karotid arterler, okside LDL.

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Introduction

Familial Mediterranean Fever (FMF) is the most common monogenic periodic fever syndrome. It's an autosomal recessive autoinflammatory disorder characterised by bouts of fever and recurrent inflammatory serositis [1]. The disease is associated with mutations in pyrin protein which is encoded by the Mediterranean fever gene (MEFV). Mutated production of Pyrin protein is responsible from uncontrolled interleukin-1 beta production, triggers the inappropriate inflammation and causes impaired immune response [2].

Although colchicine prophylaxy, which can be related to suppress some of the functions of neutrophils, T-cells, and endothelial cells, usually suppresses overt inflammatory attacks, chronic inflammation may persist in about one-third of patients, particularly in those refractory to treatment [3,4]. Chronic inflammation has been found to make a predisposition to increased onset and progression of atherosclerosis, development of acute coronary events or chronic ischemic heart disease [5-11].

Indeed, two fold increased risk for cardiovascular disease (CVD) has been reported for rheumatoid arthritis, psoriatic arthritis and other inflammatory disorders compared to the expected rate in the general population [12-15].

However, a similar increase in the risk has not still been shown in patients with FMF and there are conflicting results in a few studies in literature. In some studies higher risk for CVD was reported even in the absence of symptoms with SCORE or Framingham risk models [16]. Turkish FMF Study Group 2005 reported that patients with FMF are at a higher risk in terms of the development of early coronary artery events [17]. On the contrary, in other studies, the risk of developing CVD has been shown to be similar to that of the general population [17-19].

More data on this issue are needed, and different strategies to distinguish the role of subclinical inflammation on the occurrence of CVD in FMF should be employed. In this study we aimed to investigate the carotid atherosclerosis by using doppler ultrasound, and to measure serum oxidized low density lipoprotein (OxLDL) levels to determine whether it can be used as a non-invasive predictor of CVD in patients with FMF who have no risk factors for cardiovascular disease. A study investigating OxLDL levels in patients with FMF was not found in the literature.

Material and Method

Ethics Committee Approval

The local institutional review board approved this cross-sectional study, and it was supported by Cumhuriyet University Scientific Research Projects (CÜBAP) with project number T402.

Study Population and Design

A total of 75 patients, who admitted to the Rheumatology and Family Medicine outpatient clinics of Sivas Cumhuriyet University, Faculty of Medicine Application and Research Hospital (Sivas, Turkey) between March and April 2009 and diagnosed with FMF based on the Tel Hashomer criteria [20] were called for control by phone. Seven patients did not come due to transportation difficulties. The patients with coronary artery disease, cerebrovascular disease, diabetes, hypertension, metabolic syndrome, chronic kidney disease, dyslipidemia, active infections, those with a history of myocardial infarction and angina pectoris, those using lipid-lowering agents, those with serum creatinine level >1.4 mg/dl, non-responders to colchicine therapy, postmenopausal women, and individuals with clinical features that may affect the atherosclerotic process were excluded from the study. After the aim of the study was explained, an informed consent form was obtained from all participants. The study was completed with 44 patients (**Figure 1**). Clinical and laboratory assessment of all patients were performed during the attack-free period.

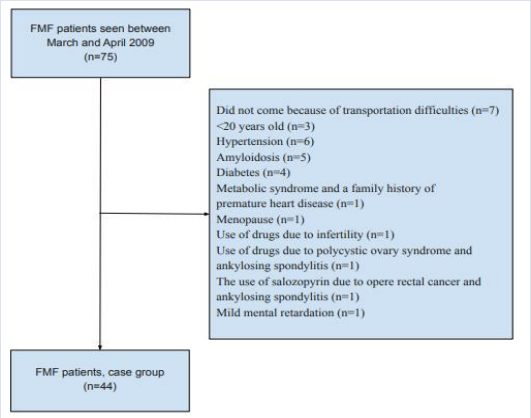

Figure 1. Flow diagram of patient selection for the study.

A questionnaire questioning the sociodemographic characteristics, FMF symptoms, the date of onset of symptoms, the date of diagnosis, the treatment used, family history, characteristics of the attacks, the presence of comorbid diseases that may accompany FMF, and smoking status was filled out by the same researcher through a face-to-face interview technique.

Before the physical examinations of all patients, blood pressure were measured by the same researcher from both brachial arteries using standard Erka brand

arm sphygmomanometer and the higher value was used for measurements. The measurements of height, weight, waist and hip circumference were made by using the same calibrated hospital instruments. Body mass indexes (BMI) were calculated. Mutation results were obtained from hospital file records and recorded.

An age and gender matching control group was formed from volunteer hospital staff, who declared that they did not have any health problems. The same procedures were applied to the control group.

Collection and Preparation of Blood Samples

After 10-12 hours of fasting, a 5 ml of blood sample was collected from peripheral veins into gel-lined vacuum tubes in the morning from all individuals included in the study. Hemogram, sedimentation, fibrinogen, biochemical tests (fasting blood glucose, lipid profile, creatinine, liver function tests), and CRP were examined in the relevant laboratories. Some part of the blood samples was centrifuged, and the serum was stored in a -80 C freezer in 1.5 ml eppendorf tubes with lids. After the formation of the groups was completed, the blood samples in the freezer were used to examine the OxLDL antibodies by the sandwich elisa method by using Immune Diagnostics (Belgium) brand commercial kits, in the Triturus (Spain) device.

Measurement of Carotid Intima Media Thickness (C-IMT)

C-IMT measurements were performed in supine position by using B-mode ultrasound device for assessment of carotid atherosclerosis by the same radiologist (E.G.) blinded to the clinical diagnoses in order to eliminate the margin of error. The right and left common carotid arteries, bulbs, the proximal internal and external carotid arteries were scanned for atherosclerotic plaques with TOSHIBA brand, 7.5 MHz high resolution probe. An atherosclerotic plaque was defined as a focal protrusion into the vessel lumen of >50% of the surrounding wall. Measurements were made at both the right and left sides of the near and distant (posterior) walls of the distal common carotids, the far wall of the carotid bulbs and the far wall of the internal carotid artery. After carotid bifurcation was found on transverse scanning, longitudinal images were taken by turning the transducer 90° and showing the front (near) and posterior (far) walls. C-IMT measurements were made from where bifurcation appeared the thickest on the back wall, at about 1.5 cm distal. The distance between lumen-intima and media-adventitia was accepted as intima-media thickness (**Figure 2**). Each measurement was recorded separately and the average value of six IMT readings was used in statistical analysis.

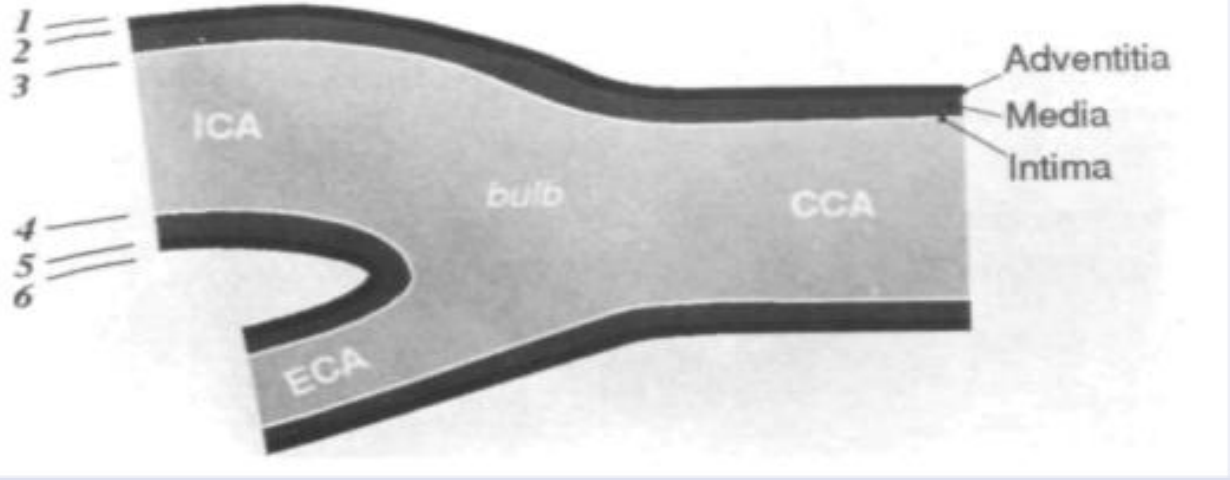

Figure 2. Schematic representation of carotid bifurcation (21): Intima-media thickness (IMT) is the distance between 2-3 and 4-5. IMT was measured from the posterior wall at the 1-1.5 cm distal in this study. (CCA: Common carotid artery, Bulb: Bulbus, ICA: Internal carotid artery, ECA: Eksternal carotid artery)

Statistical Analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) vers. 14.0 Package (SPSS Inc, Chicago, Illinois). Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were indicated as number (n) and percentage. Between groups, differences were assessed by the Student t test, while categorical variables were reviewed by the chi-square test. Relationships between variables were tested using Pearson's correlation. The statistical significance level was considered as $p < 0.05$.

Results

The mean age of the patients with FMF was 33.45±7.50 years (min-max: 20-47) and it was similarly 33.48±6.96 years (min-max: 20-48) in the control group ($p>0.05$). Of the individuals in the patient and control groups, 19 were male (43.2%) and 25 (56.8%) were female ($p>0.05$).

The individuals included in the study were compared in terms of risk factors of atherosclerosis. Although a family history of heart disease was higher in

individuals with FMF than in the control group, the difference was not statistically significant (p>0.05). There was no statistically significant difference between the two groups in terms of smoking (p>0.05). The mean of BMI, waist and hip circumference were significantly higher in the patient group compared to the control group (p<0.05) (**Table 1**).

Table 1. Comparison of anthropometric measurements.

	Control Group		Case Group		p
	Mean	SD	Mean	SD	
Ages	33.48	6.96	33.45	7.50	0.98
Weight (kg)	67.57	11.37	72.05	12.89	0.88
Height (cm)	167.50	7.57	167.07	11.02	0.83
BMI	24.02	3.30	25.86	4.38	0.03*
Hip circumference (cm)	98.80	8.77	105.00	9.34	0.00*
Waist circumference (cm)	89.00	10.68	94.39	12.47	0.03*
Systolic blood pressure (mm/Hg)	111.02	10.43	113.07	12.26	0.40
Diastolic blood pressure (mm/Hg)	70.00	8.35	72.27	9.37	0.23

*p<0.05; statistically significant. The Student's t-test was used for comparisons.

The most common three FMF symptoms in attack-free period were abdominal pain (93.2%), arthralgia (79.5%) and chest pain (77.3%), respectively. In the last six months, chest pain was observed in 52.3% (n=23), arthralgia in 52.3% (n=23), arthritis in 13.6% (n=6) and erysipelas-like lesions in 2.3% (n=1).

Whereas the mean time since the onset of complaints was 20.49±9.18 years, the mean of duration after diagnosis was 5.81±6.85 years. It was learned from the patients to be continue their complaints since 15.69±10.52 years for abdominal pain, 11.92±11.55 years for arthralgia, 9.74±11.72 years for chest pain, and 9.16±11.83 years for arthritis. The frequency of attacks were less than one per month in 65.1% (n=28) of patients, while it was one-two times monthly for 16.3% (n=7), and more than two times monthly for 18.6% (n=8).

Patients stated that 88.4% (n=38) were lying down during the attacks, and 81.4% (n=35) had a calf/leg pain between the attacks when they were standing or after exercise.

The mean duration of the attack was 2.7±0.89 days. It was ascertained that 90.91% (n=40) of the patients had been on colchicine therapy for an average of 5.28±6.38 years. Four patients have not used any medication, and four patients used their medications irregularly.

The most frequent three mutations were M694V, M680I and V726A. M694I mutation was not found in our patient population. The other rare mutations such as heterozygous A744S, heterozygous R761H, heterozygous P369S was revealed in 11.6% of the patients. In one of the patients was recorded no mutations detected with our genetic analysis; hovewer, it was thought to have one of the rare mutation that was not genetically analyzed in our hospital **(Table 2).**

Table 2. Some characteristics of patients with FMF.

Frequency of symptoms and signs observed during attacks in patients		**n**	**%**
Abdominal pain		41	93.2
Arthralgia		35	79.5
Chest pain		34	77.3
Arthritis		23	52.3
Erysipelas-like Erythema		6	13.6
Secondary infertility		2	4.5
Henoch Schönlein Purpura		1	2.3
Polyarteritis Nodosa		-	-
Colchicine treatment (n=40, 90.91%)		**n**	**%**
Irregular		4	10.0
Regularly 1mg/day		16	40.0
Regularly 1.5 mg/day		19	47.5
Regularly 2 mg/day		1	2.5
Duration of Complaints (years), mean ± SD		20.49±9.18	
Time after diagnosis (years), mean ± SD		5.81±6.85	
Duration of Colchicine Use (years), mean ± SD		5.28±6.38	
The Number of Subjects with FMF in Family, mean ± SD		3.37±4.56	
Observed MEFV Mutations (n=43)		**n**	**%**
M694V	Heterozygous mutations	8	18.6
	Homozygous mutations	5	11.6
M680I	Heterozygous mutations	2	4.7
	Homozygous mutations	3	7.0
E148Q	Heterozygous mutations	2	4.7
P369S	Heterozygous mutations	2	4.7
V726A	Heterozygous mutations	1	2.3
A744S	Heterozygous mutations	1	2.3
M694V – M680I (G/C)	Compound heterozygous¶	7	16.3
M694V – E148Q	Compound heterozygous	4	9.3
M694V – V726A	Compound heterozygous	3	7.0
M680I (G/C) – V726A	Compound heterozygous	2	4.7
M694V – R761H	Compound heterozygous	1	2.3
M680I (G/C) – R761H	Compound heterozygous	1	2.3
In one alleles M680I, in two alleles V726A	Compound heterozygous	1	2.3

¶ Compound heterozygous: Carrying different heterozygous mutations in two alleles

When the biochemical parameters were compared, it was observed that the mean values of mean corpuscular volume and platelet count were significantly differed between the groups. Mean total cholesterol and low-density lipoprotein levels were statistically significantly lower in the patient group compared to the controls ($p<0.05$). The mean OxLDL level in the patient group was 337.48±438.56, whereas in the control group was measured 156.19±383.24, and the difference was statistically significant ($p<0.05$) **(Table 3)**. When the association between OxLDL and laboratory parameters was examined, it was shown a statistically significant-positive correlation between fasting blood glucose and OxLDL levels in the control group ($p<0.05$) **(Table 4).**

Table 3. Comparison of the laboratory values of groups.

Laboratory Parameters	Control Group		Case Group		
	Mean	SD	Mean	SD	p
White Blood Cell Count(10^9/L)	6702.27	1294.98	6906.82	1823.67	0.546
Hemoglobin (Range: 12.5-16 g/dl)	14.60	1.50	14.00	1.82	0.092
Haematocrit (Range: %37-47)	42.93	3.96	41.75	4.84	0.216
MCV (Range: 78-100 fL)	87.58	4.42	82.47	6.67	0.000*
Platelet (10^9/L)	237.82	45.01	263.02	68.21	0.044*
ESR (Range: 0-15 mm/h)	7.84	5.35	10.45	8.75	0.097
CRP (Range: 0-5 mg/L)	3.10	3.51	6.71	13.19	0.084
Fibrinogen (Range: 193-412 mg/dl)	276.3	39.11	253.66	77.73	0.088
Fasting Blood Glucose (Range: 70-100 mg/dl)	90.02	10.60	91.82	8.10	0.375
Triglyceride (Range: 40-150 mg/dl)	104.39	65.82	104.68	71.63	0.984
Total cholesterol (Range: 100-200 mg/dl)	181.05	36.79	157.07	34.18	0.002*
HDL (Range: 50-85 mg/dl)	41.43	10.87	38.89	9.33	0.242
LDL (Range: 40-100 mg/dl)	126.25	34.05	100.48	30.13	0.000*
Creatine (Range: 0.5-0.9 mg/dl)	0.81	0.14	0.79	0.15	0.411
ALT (Range: 0-33 U/L)	21.27	11.42	27.52	21.29	0.090
AST (Range: 0-32 U/L)	20.36	5.65	24.70	10.30	0.016*
GGT (Range: 6-42 U/L)	20.48	14.06	17.11	8.28	0.175
Right C-IMT	0.53	0.06	0.52	0.11	0.626
Left C-IMT	0.53	0.06	0.53	0.11	0.808
OxLDL	156.19	383.24	337.48	438.56	0.044*

*p<0.05; statistically significant. The Student's t-test was used for comparisons.

Table 4. Correlation of OxLDL with anthropometric measurements and laboratory values.

	OxLDL					
	Control Group		Case Group		Totally	
	r	p	r	p	r	p
Age	0.226	0.140	-0.11	0.503	0.048	0.663
Weight	0.245	0.109	0.038	0.810	0.163	0.135
Height	0.213	0.164	0.064	0.688	0.109	0.316
BMI	0.147	0.340	0.003	0.986	0.108	0.321
Hip circumference	0.151	0.327	-0.06	0.710	0.104	0.340
Waist circumference	0.093	0.549	0.101	0.525	0.139	0.202
Systolic blood pressure	0.174	0.258	0.097	0.541	0.147	0.177
Diastolic blood pressure	0.247	0.106	-0.02	0.904	0.123	0.260
White Blood Cell Count	-0.069	0.657	0.227	0.147	0.128	0.241
Hemoglobin	0.149	0.335	0.086	0.588	0.072	0.509
Haematocrit	0.164	0.288	0.061	0.701	0.077	0.479
MCV	-0.139	0.367	-0.002	0.988	-0.137	0.207
Platelet	-0.341	0.023*	-0.103	0.515	-0.128	0.242
ESR	0.055	0.724	-0.016	0.921	0.045	0.684
CRP	0.012	0.938	0.071	0.656	0.094	0.391
Fibrinogen	-0.079	0.611	-0.062	0.696	-0.104	0.342
Fasting Blood Glucose	0.315	0.037*	0.111	0.483	0.237	0.028*
Triglyceride	0.131	0.397	-0.109	0.493	-0.002	0.983
Total cholesterol	0.004	0.982	0.019	0.903	-0.058	0.593
HDL	-0.295	0.052	0.167	0.292	-0.093	0.396
LDL	0.113	0.465	-0.123	0.439	-0.086	0.430
Creatine	0.168	0.276	-0.031	0.847	0.049	0.654
ALT	0.150	0.331	-0.083	0.600	0.032	0.770
AST	-0.096	0.537	0.006	0.968	0.033	0.762
GGT	-0.004	0.981	-0.058	0.715	-0.054	0.621

*p<0.05; statistically significant. The Pearson correlation coefficient (r) test was used.

In the measurements performed by ultrasound, there was no statistically significant difference in the mean value of both at right and left side of C-IMT between the groups. In addition, mean CCA intima-media thickness was measured 0.52±0.10 in FMF patients, and 0.53±0.06 in the control group (p>0.05) **(Table 3).**

A significant increase in CIMT in both groups was determined with ages. In addition, it was observed that the more increased at weight, BMI, waist and hip circumference in patients with FMF, C-IMT also increased. However, similar relation was not shown in the control group. In the case group, there was a positive correlation between fibrinogen, fasting blood glucose, triglycerides, ALT and AST levels and C-IMT levels **(Table 5).** Statistically significant correlation between OxLDL and C-IMT value was not found in our study (p>0.05) **(Table 6).**

Table 5. Correlation of C-IMT with anthropometric measurements and laboratory values.

	C-IMT					
	Control Group		Case Group		Totally	
	r	p	r	p	r	p
Age	0.584	0.000*	0.581	0.000*	0.567	0.000*
Weight	0.068	0.689	0.536	0.000*	0.371	0.001*
Height	-0.135	0.426	-0.084	0.586	-0.096	0.392
BMI	0.189	0.262	0.661	0.000*	0.506	0.000*
Hip circumference	0.172	0.310	0.516	0.000*	0.373	0.001*
Waist circumference	0.279	0.094	0.548	0.000*	0.452	0.000*
Systolic blood pressure	0.018	0.917	-0.040	0.796	-0.028	0.802
Diastolic blood pressure	0.024	0.886	0.151	0.329	0.105	0.353
White Blood Cell Count	0.115	0.499	0.123	0.426	0.117	0.300
Hemoglobin	0.017	0.919	-0.040	0.794	-0.018	0.875
Haematocrit	0.019	0.910	-0.045	0.771	-0.023	0.841
MCV	0.111	0.511	-0.107	0.487	-0.037	0.743
Platelet	-0.060	0.725	-0.216	0.159	-0.184	0.100
ESR	0.308	0.068	-0.006	0.970	0.057	0.617
CRP	0.142	0.401	0.012	0.938	0.019	0.868
Fibrinogen	0.144	0.396	0.356	0.018*	0.318	0.004*
Fasting Blood Glucose	0.163	0.336	0.414	0.005*	0.298	0.007*
Triglyceride	0.330	0.046	0.533	0.000*	0.456	0.000*
Total cholesterol	0.170	0.315	0.250	0.101	0.216	0.053
HDL cholesterol	-0.031	0.854	-0.246	0.108	-0.149	0.184
LDL cholesterol	-0.005	0.975	0.272	0.074	0.169	0.131
Creatine	-0.020	0.907	0.267	0.080	0.178	0.111
ALT	0.178	0.292	0.400	0.007*	0.343	0.002*
AST	0.039	0.820	0.360	0.016*	0.275	0.013*
GGT	0.191	0.258	0.236	0.124	0.187	0.095

*p<0.05; statistically significant. The Pearson correlation coefficient (r) test was used.

Table 6. Correlation between OxLDL and C-IMT.

	OxLDL					
	Control Group		Case Group		Totally	
	r	p	r	p	r	p
C-IMT	-0.196	0.246	-0.156	0.324	-0.164	0.150

The Pearson correlation coefficient (r) test was used.

Discussion

FMF is a chronic inflammatory disease characterized by episodes of attacks and attack-free periods. Subclinical inflammation can continue between the episodes [22]. It has been suggested that the inflammation in FMF is related to endothelial dysfunction, platelet hyperactivation, and increased atherosclerotic burden [23].

Due to chronic inflammation has been suggested to be an independent risk factor for the development of atherosclerotic heath disease [15,24], whether atherosclerosis is increased or not in FMF is still a controversial question. This possible relation is investigated in several studies. C-IMT a surrogate marker for subclinical atherosclerosis is found to be increased in a several studies, however this finding contrasts with the lack of increased frequency of atherosclerotic plaques [25-30]. In addition, population surveys do not indicate an increased prevalence of ischaemic heart disease in FMF patients (18). Therefore, in this study, we investigated subclinical atherosclerosis in FMF patients with no apparent atherosclerotic risk factors via OxLDL levels and C-IMT.

In the literature, increasing number of studies investigating atherosclerosis in FMF with carotid USG have been conducted [25-31]. Although the formation plaques have been more strongly associated with the development atherosclerosis, an increase in the formation of plaques was not reported by any of the studies. In these studies have been suggested that FMF patients are at a slightly higher risk of developing subclinical atherosclerosis and that its progression is not aggressive enough to result in a significant plaque. Therefore, this increased thickness in carotid arteries can be associated with endothelial dysfunction, platelet

hyperactivation, and increased inflammatory markers [23,29].

In the study conducted by Sarı et al., no increase in C-IMT was reported in FMF patients on regular daily colchicine treatment [28], however, in other five studies, the thickness of carotis wall was reported to be increased [25-27,29,30]. Our study results also supported the evidence showing no increase at atherosclerosis.

In the study conducted by Akdoğan et al. was included FMF patients who had previously known unresponsive to colchicine therapy. It was stated that atherosclerotic plaques was detected in two of the FMF patients, however, the ages of patients detected atherosclerotic plaques was not specified clearly in the study. A positive correlation was shown between the maximum C-IMT and age. In addition, acute-phase reactant levels were noted higher in FMF patients in comparison to control group [26]. Similarly, in the study investigated ischemia-modified albumin levels as an indicator of atherosclerosis, C-IMT was shown to be increased in patients with FMF compared to control group. Although the patients were selected in the attack-free period, the mean value of acute phase reactants were also higher than the controls [30]. In our study, there were no patients who did not responsive to colchicine treatment and acute-phase reactant levels were similar between groups. At this stage, the cause of the thickened IMT of carotid arteries should be investigated by further studies whether FMF disease itself or through increased acute phase reactants.

In the study of Uğurlu et al., C-IMT was found to be increased. However, in this study the mean age was greater and the duration of the disease was longer. In addition, the patients with risk factors such as diabetes, hypertension, and hyperlipidemia were included in that study [25]. On the other hand, in our study the mean age was smaller and the patients with atherosclerotic risk factors were excluded from the study. Considering the mean duration of the disease, we expected the development of early signs of atherosclerosis. However, we detected no increase neither in the frequency of the plaques nor in C-IMT compared with controls.

Two other studies which reported an increase in C-IMT were carried out on children with FMF [27,29]. In contrast to the results in children with FMF, Sari et al. did not find any difference among C-IMT measurements of adult patients with FMF and age and sex-matched healthy controls. The authors pointed out that regular colchicine treatment with its antiatherosclerotic characteristics may be a slowing factor of this process and may contribute to the lack of change C-IMT in the FMF patients [28]. Similarly, the lack of a significant difference between our study and control groups may be due to the fact that vast majority of our population have been using regular colchicine treatment. In addition, it may have also linked to the low degree of inflammation usually reported in FMF patients and a decrease in disease activity was indicated over the years.

Atherosclerosis is a multifactorial disease. It is accepted that the conversion of LDL to OxLDL by the action of free radicals is an important factor in the initiation and development of atherosclerosis [32]. There are studies, reporting that polyclonal antibodies (Ig-OxLDL) produced against epitopes of OxLDL may contribute to the formation of atherosclerosis (with a delay in LDL metabolism and an increase in hyperlipidemia) [33,34]. According to Salonen, there is a positive correlation between Ig-OxLDL and the development of atherosclerosis [35,36].

Another important finding of our study was that OxLDL antibodies were found to be significantly higher in individuals with FMF compared to the control group. However, these antibodies showed no correlation with C-IMT. OxLDL antibodies have been similarly investigated in patients with SLE and RA and its presence was found to be directly related to plaque and C-IMT [37-39]. The high levels of antibodies found in our study may be associated with inflammation. Similarly, OxLDL antibodies were investigated in Behçet's patients and it was shown that the levels of them were higher patients compared to control group [40,41].

In our study, total and LDL cholesterol levels were found to be low in FMF patients. Relationship between FMF and lipid levels in literature is still contradictory. It has been reported that cholesterol levels are significantly lower in FMF patients receiving colchicine compared to healthy controls [25,26,42]. The low levels of lipids may be due to the potential anti-atherogenic and lipid lowering effect of colchicine [25]. In the study conducted by Küçük et. al. was also observed lower cholesterol levels; however, this difference did not statistically significant [30]. In another study was not found significant difference in cholesterol levels, with the exception of HDL cholesterol [43]. Ugurlu et al. [44] demonstrated that the use of colchicine did not affect lipid levels in FMF patients. Similar lipid abnormalities may have been detected in other chronic inflammatory diseases. Therefore, these anomalies may be a different characteristic of ongoing inflammation.

On the other hand, this is a single-centered, case-control study with a limited number of subjects younger than other study populations; therefore, our results should be assessed cautiously. In addition, the patient population was comprised of only those who admitted to the outpatient clinic controls, and did not include asymptomatic or mildly severe patients who did not admitted for control. However, our study is still very important since OxLDL levels were studied for the first time in FMF patients.

Conclusion

FMF is a disease characterized by attacks; however, subclinical inflammation also continues in the attack-free periods. Inflammation has an important role in the development of atherosclerosis. Our study supports further evidence for no increased atherosclerosis which have been shown with ultrasound in FMF. Atherosclerotic plaques were not detected and C-IMT was similar to the values of the controls. In addition, examined OxLDL antibodies were found to be statistically significantly increased in patients with FMF,

no significant correlation was observed with C-IMT. Increased OxLDL levels could be associated with increased subclinical inflammation which could not triggered atherosclerosis. As previously shown, patients with FMF have low cholesterol levels when compared to healthy controls.

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