Arch Rheumatol 2016;31(2):112-120
doi: 10.5606/ArchRheumatol.2016.5628

ORIGINAL ARTICLE

Glutathione-S-Transferase Variants are not Associated With Increased Carotid Intima-Media Thickness in Turkish Familial Mediterranean Fever Patients

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ABSTRACT

Objectives: This study aims to evaluate the carotid intima-media thickness (CIMT) in patients diagnosed with familial Mediterranean fever (FMF) and investigate whether there is a relationship between glutathione-S-transferase (GST) gene polymorphisms and CIMT.

Patients and methods: Sixty FMF patients (17 males, 43 females; mean age: 31.43±11.36 years; range 18 to 45 years) and 60 healthy controls (22 males, 38 females; mean age: 29.8±5.82 years; range 18 to 40 years) were enrolled in this study. Polymerase chain reaction-restriction fragment length polymorphism methods were carried out to assess GST polymorphisms. CIMT was measured by carotid ultrasonography. Biochemical parameters were also evaluated using biochemical methods.

Results: Right and left CIMT of FMF patients were statistically significantly higher than that of control group (CIMT right p=0.001 and CIMT left: p=0.033). There was no significant association in terms of GST polymorphisms between FMF and control groups. No significant association was observed between GST polymorphisms and CIMT. Low density lipoprotein, erythrocyte sedimentation rate, and fibrinogen levels were significantly higher in the patient group (p<0.05). The difference between groups was not significant in terms of other biochemical parameters (p>0.05).

Conclusion: Although no significant association was observed between GST polymorphisms and CIMT in FMF patients and controls, CIMT was statistically significantly higher in FMF patients compared to controls.

Keywords: Carotid intima-media thickness; familial Mediterranean fever; glutathione-S-transferase; polymorphism.

Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disease, characterized by recurrent self-limited episodes of fever and unprovoked inflammation resulting in pain which is localized in the abdomen, chest and joints, which last one to three days.[1-3] Mutations in the Mediterranean Fever gene, encoding pyrin-marenostrin protein, cause FMF disease.[4,5] Pyrin protein acts as a regulator of the inflammatory response in innate immune system. Although the nature of this regulation is not revealed precisely, pyrin, within a complex called inflammasome, serves as a regulator of the conversion of pro-interleukin (IL)-1β to mature IL-1β.[6,7]

During inflammation episodes, neutrophils migrate into serous cavities and the levels of acute-phase inflammatory products, including serum amyloid A and C reactive protein,[8] and cytokines including IL-6,[9,10] IL-8,[10] IL-17[11] and IL-18[11,12] increase. Besides these findings, it has been reported that substantial subclinical inflammation occurs during attack-free periods in patients with FMF and oxidative stress and oxidant/antioxidant

Received: April 03, 2015 ***Accepted:*** October 05, 2015 ***Published online:*** November 02, 2015

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imbalance might play a role in this persistent subclinical inflammation.[8-12]

The relationship between inflammation and atherosclerosis has been investigated in several studies in chronic inflammatory diseases such as inflammatory bowel disease[13] and coeliac disease.[14] Chronic inflammation and immune dysregulation play a major role in both initiation and the development of atherosclerosis.[15,16] Studies have shown that IL-1β, which is the main determinant of immune response in FMF, is an efficient pro-inflammatory and atherogenic cytokine.[17,18] IL-1β up-regulates the expression of various effectors through the induction of IL-1 receptors and the nuclear factor-κB pathway. Stimulated acute-phase inflammatory products, cytokines, and the other inflammatory mediators are also involved in coronary artery syndromes. Moreover, there is a link between cholesterol metabolism and inflammation in atherosclerotic lesions.[19] FMF is a disease of recurrent chronic inflammation and increased carotid artery intima-media thickness (CIMT) might reflect the continuous systemic inflammation in this disease.

Oxidative stress has an important role in the pathogenesis of atherosclerosis.[20] In the innate immune response, macrophages play a major role through the generation of reactive oxygen species (ROS) like superoxide, nitric oxide, hydroxyl radical, hydrogen peroxide, hydrochlorous acid, and peroxynitrite.[21] Chronic inflammation may lead to cell damage or cellular hyperplasia and inflammatory cells produce excess amounts of ROS. The overproduction of free radicals and decreased antioxidant enzyme production are responsible for cellular adverse effects of chronic inflammation including atherosclerosis.[22]

In addition to their ability of conjugation of non-polar compounds carrying electrophilic centers (i.e; xenobiotics, drugs, pesticides) to glutathione, glutathione-S-transferases (GSTs) contribute to the detoxification of endogenously produced ROS via their glutathione peroxidase activity.[23] Glutathione acts on eliminating ROS through the action of glutathione peroxidase and (GST).[24] Although there are many polymorphisms in GST genes, studies generally focused on the GSTT1, GSTM1, and GSTP1 polymorphisms.[25]

Therefore, in this study, we aimed to evaluate the CIMT in patients diagnosed with FMF and investigate whether is there a relationship between GST gene polymorphisms and CIMT.

PATIENTS AND METHODS

Our case-control study protocol was approved by Cumhuriyet University Institutional Ethics Committee and performed in accordance with the Helsinki declaration between 2012 and 2013. Sixty FMF patients (17 males, 43 females; mean age 31.43±11.36 years; range 18 to 45 years) who applied to Department of Internal Medicine, Faculty of Medicine, Cumhuriyet University were included. All patients satisfied the Tel-Hashomer FMF diagnosis criteria and were on regular colchicine treatment. The control group contained 60 healthy controls (22 males, 38 females; mean age 29.8±5.82 years; range 18 to 40 years). An informed consent and a standardized questionnaire regarding presence of diabetes mellitus, hypertension, and smoking were obtained from all subjects.

Blood samples were collected after overnight fasting and levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, total cholesterol, D-dimer, fibrinogen, erythrocyte sedimentation rate (ESR), C reactive protein, and ferritin were measured biochemically from FMF and control groups.

To measure CIMT, both FMF patients and controls were studied under standardized conditions. Each subject was fitted with measurement devices for ultrasonography and then rested in supine position for about 15 to 20 minutes. Then, carotid artery values of both patients and controls were measured. CIMT measurements were performed with TOSHIBA ultrasonography device using 7.5 MHz high-resolution probe from the right and left main carotid artery. IMT measurements taken from both common carotid arteries. Average CIMT was calculated as the mean of three measurements from both carotid arteries.

Peripheral blood samples obtained both from FMF patients and controls were collected in tubes with K3 ethylenediaminetetraacetic acid and stored at -20 °C until the extraction time. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral venous blood from all subjects using spin

column-based nucleic acid extraction technique. QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction according to the manufacturers' instructions.

To detect GSTM1 and GSTT1 genotypes in the genomic DNA sample, multiplex polymerase chain reaction (PCR) was used. Albumin gene was used as an internal control. The multiplex PCR amplification was performed with the following primers: GSTT1 forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and GSTT1 reverse 5'-TCA CCG GAT CAT GGC CAG CA-3', and GSTM1 forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and GSTM1 reverse 5'-GTT GGG CTC AAA TAT ACG GTG G-3', additionally, for internal control albumin gene forward 5'-GCC CTC TGC TAA CAA GTC CTA C-3', albumin reverse 5'-GCC CTA AAA AGA AAA TCC CCA ATC-3'. The PCR mixture was prepared in a total volume of 25 µL containing 50-100 ng DNA, 2.5 mmol/L deoxynucleotides, 0.8 mmol/L GSTT1 primers, 0.4 mmol/L GSTM1 primers, 0.8 mmol/L albumin prime, 5 µL of 10x PCR buffer, 2 mmol/L MgCl2, and 5 U DNA Taq polymerase (Vivantis, Technologies, Selangor, Malaysia). The PCR condition was consisted of an initial denaturation step of 10 minutes at 94 °C, 35 cycles of one minute at 94 °C, one minute at 58 °C, and one minute at 72 °C, followed by a last elongation step of five minutes at 72 °C. Genotypes were determined by electrophoresis in 2% agarose gel with added ethidium bromide. For GSTT1, GSTM1 and albumin genes, 459 bp, 219 bp, and 350 bp PCR fragments were seen in the agarose gel electrophoresis, respectively.

Genotypes of the GSTP1 gene exon 5 (rs947894, Ile105Val) and 6 (rs1799811, Ala114Val) were determined by the PCR-restriction fragment length polymorphism method. For determination of exon 5 Ile105Val and exon 6 Ala114Val polymorphisms, a PCR reaction was performed in a 25 µl volume which contained 10 µl PCR buffer, 2,5 mmol/L deoxynucleotides, 3 mmol/L MgCl2, 5 U DNA Taq polymerase (Vivantis, Malaysia), and 0.3 mmol/L of primers GSTP1 forward (5'-GTA GTT TGC CCA AGG TCA AG-3') and GSTP1 reverse (5'-AGC CAC CTG AGG GGT AAG-3') for exon 5 and GSTP1 forward (5'-GGG AGC AAG CAG AGG AGA AT-3'), GSTP1 reverse (5'-CAG GTT GTA GTC AGC GAA GGA G-3') for exon 6. PCR conditions were established as initial denaturation step of 5 minutes at 94 °C, followed by 5 cycles in which the annealing temperature decreased by 1 °C each cycle (cycle 1: 30 seconds at 94 °C, 30 seconds at 64 °C, and 30 seconds at 72 °C) and followed by another 25 cycles, 30 seconds at 94 °C, 30 seconds at 59 °C, and 30 seconds at 72 °C, with a final elongation step of 5 minutes at 72 °C. A 433bp and 420bp DNA fragment was amplified for I105V and A114V polymorphisms, respectively.[26] I105V and A114V PCR products were digested with 5U BsmAI (Fermentas, Lithuania) and 5U AciI (Fermentas, Lithuania) restriction endonuclease at 37 °C for 16 hours, respectively. The digestion products were visualized in 3% agarose gel. For genotyping I105V polymorphism, the wild type genotype (Ile/Ile) showed 328 and 105 bp fragments, while the mutant genotype (Val/Val) showed 222, 106, and 105 bp fragments. For A114V polymorphism, the wild type Ala/Ala genotype, the digestion yielded bands at 246bp, 116bp, and 58bp; for Val/Val mutant genotype, digestion yielded bands at 362bp and 58bp. Homozygous mutant genotype was not detected in our FMF and control groups for A114V polymorphism.

Statistical analysis

All statistical analyses were performed by SPSS version 22.0 (SPSS IBM, Armonk, NY, USA). For the comparison of independent samples, independent samples T test was used if the data were normally distributed, whereas the Mann-Whitney U test was used if the data were not normally distributed. The chi-square test or Fisher's exact test was used in the comparison of categorical data between FMF patients and controls. All demographic and quantitative data were expressed as mean ± standard deviation. *P* values of less than 0.05 were regarded as significant and all results were expressed with a 95% confidence interval.

RESULTS

There were no significant differences between the FMF and control groups in terms of mean age (p=0.324) and sex (p=0.823). Rates for smoking were 20% and 15% in the FMF and control groups, respectively and the difference between two

groups was not statistically significant (p=0.471). Number of attacks per month was ≥3 for two patients (3.3%), 1 for 24 patients (40.0%), and <1 for remaining 34 patients (56.7%). Demographic characteristics of FMF patients and controls are shown in Table 1.

Biochemical parameters including LDL, HDL, triglycerides, total cholesterol, D-dimer, fibrinogen, ESR, C reactive protein, and ferritin levels of FMF and control groups are shown in Table 1. Levels of LDL (p=0.004), fibrinogen (p=0.033), and ESR (p=0.007) were statistically significantly higher in FMF patients compared to the controls. Other biochemical parameters were not statistically significantly different.

Carotid intima-media thickness was measured at intervals 0.4-0.9 mm in the FMF patients and 0.3-0.7 mm in the controls. Right and left CIMT of patients were statistically significantly higher than that of controls (CIMT right: p=0.001; CIMT left: p=0.033) (Table 1).

Subjects in the FMF and control groups were evaluated in terms of the GSTT1, GSTM1 and GSTP1 I105V, and A114V gene polymorphisms. In the FMF and control groups, GSTT1 null allele frequencies were 18.3% (n=11) and 13.3% (n=8), respectively. GSTM1 null allele frequencies in the patients and controls were detected as 50% (n=30) and 56.7% (n=34), respectively. The difference between two groups was not statistically significant in terms of GSTT1 and GSTM1 gene polymorphisms (p>0.05). GSTP1 Ile105Val polymorphism frequencies were 55% (n=33), 36.7% (n=22), and 8.3% (n=5) for Ile/Ile wild type, Ile/Val heterozygous, and Val/Val mutant homozygous in FMF patients, respectively. Frequencies of Ile/Ile, Ile/Val, and Val/Val genotypes were 63.3% (n=38), 33.3% (n=20), and 3.3% (n=2) in controls, respectively. No homozygous mutant genotype was detected for Ala114Val polymorphism. Ala/Val heterozygous genotype was detected in eight of FMF patients and seven of control subjects. Distribution of allele frequencies of two GSTP1 polymorphisms was also not statistically significant (Table 2).

The relationship between CIMT values and GSTT1/M1 genotypes was not statistically significant (p>0.05). Additionally, both GSTP1 I105V and GSTP1 A114V polymorphisms did not show any significant association with CIMT values (p>0.05) (Table 3).

Combined genotype analysis was performed for GSTT1/M1 and two polymorphisms of

Table 1. Comparison of demographic and laboratory findings of familial Mediterranean fever and control groups

Variables	FMF Patients (n=60)			Controls (n=60)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			31.43±11.36			29.8±5.82	0.324
Gender							0.823
Male	17			22			
Female	43			38			
Diabetes mellitus	2	3.3		0	0		–
Hypertension	6	10		0	0		–
Smoking	12	20		9	15		0.471
Number of attacks per month							
≥3	2	3.3		–	–		–
1-2	24	40		–	–		–
<1	34	56.7		–	–		–
Low-Density Lipoprotein (mg/dL)			100.2±31.5			85.6±22.6	0.004*
High-Density Lipoprotein (mg/dL)			41.6±10.1			42.6±9.2	0.570
Triglycerides (mg/dL)			114.4±59.6			111.3±50.0	0.757
Total cholesterol (mg/dL)			166.6±43.8			154.9±31.7	0.097
D-dimer (ng/mL)			220.0±211.3			163.9±94.7	0.063
Fibrinogen (mg/dL)			283.7±43.7			265.8±37.7	0.018*
Erythrocyte sedimentation rate (mm/h)			14.0±13.7			8.6±6.4	0.007*
C-reactive protein (mg/L)			9.7±19.3			7.8±13.1	0.532
Ferritin (ng/mL)			50.9±95.8			41.1±39.9	0.466
Carotis Intima-Media Thickness right (mm)			0.53±0.11			0.46±0.08	0.001*
Carotis Intima-Media Thickness left (mm)			0.53±0.12			0.49±0.10	0.033*

FMF: Familial Mediterranean fever; SD: Standard deviation; * Statistically significant.

Table 2. Frequencies of glutathione-S-transferase T1, glutathione-S-transferase M1 and glutathione-S-transferase P1 polymorphisms in familial Mediterranean fever patients and controls

GST genotype	Patients (n=60)		Control (n=60)		χ^2	p
	n	%	n	%		
GST T1						
Active	49	81.7	52	86.7	0.56	0.45
Null	1	18.3	8	13.3		
GST M1						
Active	30	50	26	43.3	0.53	0.46
Null	30	50	34	56.7		
GSTP1 I105V						
Ile/Ile	33	55	38	63.3	1.73	0.42
Ile/Val	22	36.7	20	33.3		
Val/Val	5	8.3	2	3.3		
Allele						
Ile	88	73.33	96	80	1.49	0.22
Val	32	26.67	24	20		
GSTP1 A114V						
Ala/Ala	52	86.7	53	88.3	0.076	0.78
Ala/Val	8	13.3	7	11.7		
Val/Val	0	0				
Allele						
Ala	112	93.3	114	95	0.30	0.58
Val	8	6.7	6	5		

GST: Glutathione-S-transferase.

GSTP1 gene. No significant association was detected between combined GST genotypes and subjects (p>0.05). Combined mutation analysis results are shown in Table 4. The possible association between combined genotypes and CIMT, and triple genotype analysis was not investigated because of the possibility of low statistical power.

DISCUSSION

Familial Mediterranean fever is a genetic based autoinflammatory disease, prevalent among the eastern Mediterranean region, particularly Armenians, Jews, Arabs, and Turks.[27] The hallmark of the disease is recurrent, self-limiting febrile episodes of serosal and synovial inflammation and

Table 3. Glutathione-S-transferase genotypes and carotid intima-media thickness in familial Mediterranean fever patients and controls*

Genotypes	CIMT right			CIMT left		
	Patients	Control		Case	Control	
	Mean±SD	Mean±SD	p	Mean±SD	Mean±SD	p
GSTT1						
Active	0.5±0.1	0.5±0.1	>0.05	0.4±0.0	0.5±0.1	>0.05
Null	0.5±0.1	0.5±0.1		0.5±0.1	0.5±0.1	
GSTM1						
Active	0.5±0.1	0.5±0.1	>0.05	0.5±0.1	0.5±0.1	>0.05
Null	0.5±0.1	0.6±0.1		0.5±0.1	0.5±0.1	
GSTP1 I105V						
Ile/Ile	0.5±0.1	0.5±0.1	>0.05	0.5±0.1	0.5±0.1	>0.05
Ile/Val	0.6±0.1	0.5±0.1		0.6±0.1	0.5±0.1	
Val/Val	0.5±0.1	0.4±0.0		0.5±0.1	0.5±0.1	
GSTP1 A114V						
Ala/Ala	0.5±0.1	0.5±0.0	>0.05	0.5±0.1	0.5±0.1	>0.05
Ala/Val	0.5±0.1	0.5±0.1		0.5±0.1	0.5±0.1	
Val/Val	–	–		–	–	

CIMT: Carotis Intima-Media Thickness; SD: Standard deviation; * Mann Whitney-U test was used for statistical analysis; GST: Glutathione-S-transferase.

Table 4. Combined genotype analysis of glutathione-S-transferase polymorphism in familial Mediterranean fever patients and controls*

Combined genotypes	Patients (n=60)		Controls (n=60)		
	n	%	n	%	*p*
GSTP1 I105V/A114V					
II/AA	31	51.67	36	60	>0.05
II/AV	2	3.33	2	3.33	>0.05
IV/AA	17	28.33	16	26.66	>0.05
IV/AV	5	8.33	4	6.67	>0.05
VV/AA	4	6.67	1	1.67	>0.05
VV/AV	1	1.67	1	1.67	>0.05
GSTT1/GSTM1					
T1(+)/M1(+)	25	41.67	21	35	>0.05
T1(+)/(M1(-)	24	40	31	51.67	>0.05
T1(-)/M1(+)	5	8.33	5	8.33	>0.05
T1(-)/M1(-)	6	10	3	5	>0.05

* The Chi-square test or Fisher's exact test was used for statistical analysis; GST: Glutathione-S-transferase.

attack-free periods. Although precise mechanism of the disease has not yet been determined, mutations of pyrin protein which is encoded by Mediterranean Fever gene taking part in regulation of innate immune system is responsible for this disease.[1]

During inflammatory episodes, high number of abnormally activated polymorphonuclear neutrophils and monocytes circulate and influx into the affected sites.[28,29] Neutrophils are responsible for endothelial dysfunction, oxidative stress, and over production of adhesion molecules.[30] Moreover, attack-free periods of FMF are characterized by subclinical inflammation.[10,11] It was shown that serum IL-17 and IL-18 levels of FMF patients are higher than healthy controls both during acute phase and attack-free period.[11] IL-8 has proatherogenic effect via inflammasome activation in paracrine proinflammatory pathway.[31]

Several studies have revealed that atherogenesis is highly accelerated by upregulated inflammation and autoimmune attacks in high-grade inflammatory disorders such as rheumatoid arthritis and SLE.[32,33] Relationship between atherogenesis and low-grade inflammatory conditions such as ankylosing spondylitis and FMF has been relatively recently suggested and studies about this relationship build up over time.[34-36] However, studies investigating carotid atherosclerosis in FMF found less aggressive course of atherogenesis than atherogenesis in high-grade inflammatory disorders.[37] CIMT represents an indicator of atherosclerosis and increased CIMT has been accepted to be associated with severity of coronary atherosclerosis. Increased CIMT is considered as a marker in early phase of atherosclerosis.[38]

In our study, CIMT was significantly higher in patients with FMF. This finding supports the existence of subclinical inflammation in FMF patients. To our knowledge, all of the studies investigating the relationship between FMF and CIMT were conducted in Turkey. In a study which has been carried out in the western part of Turkey, Sari et al.[39] have found no differences in the values of right, left, and averaged CIMT between 61 adult patients with FMF on regular daily colchicine treatment and controls. In another study conducted in the central part of Turkey, Akdogan et al.[40] studied 43 adult FMF patients and found that CIMT was increased in FMF patients compared to healthy controls. However, they demonstrated no difference between colchicine responder and non-responder patient groups in terms of maximum CIMT values. Also, Ugurlu et al.[41] have reported increased CIMT and dyslipidemia in 100 adult FMF patients. The discrepancy between these studies probably results from regional and socioeconomic differences of central and western part of Turkey.

In one of two studies conducted on children with FMF in Turkey, Peru et al.[42] have investigated the premature atherosclerosis risk and possible association between mutation types and atherosclerosis. Although they have found significantly increased CIMT in children with FMF, they detected no correlation between CIMT and mutation subgroups. Bilginer et al.[34] have suggested that increased CIMT is an early predictor of atherosclerosis and may be associated with subclinical inflammation in children with FMF. The authors have also detected high ESR and fibrinogen levels in FMF patients similar to our results.

In many disorders including atherosclerosis, cancer, allergy, and autoimmune diseases, toxic oxidative stress is caused by free radicals.[43] GSTs are well known phase II detoxification enzymes which catalyze reactions between reduced glutathione and electrophilic substrates.[23] Miller et al.[44] have suggested that certain GST genotypes,

particularly GSTM1 and GSTT1, are associated with inflammation, hemostasis, and endothelial function by reducing the oxidative damage. Studies evaluating the relationship between GST gene polymorphisms, inflammation, and carotid atherosclerosis have generally focused on smoking.[44] It has been shown that smokers carrying the GSTM1 and/or T1 null genotype have higher risk of developing atherosclerosis and coronary heart disease.[45,46] Additionally, in a study conducted in Taiwan, Wang et al.[47] have shown that GSTP1 polymorphism increased the risk of carotid atherosclerosis.

Despite presence of studies on CIMT values of patients with FMF in the literature, we found no studies investigating the associations of GST polymorphisms and CIMT in FMF patients. To our knowledge, our study is the first on this issue. Guzel et al.[48] measured the levels of certain antioxidant markers including GST and showed increased oxidative stress and acute phase response during the attacks in FMF patients. Ediz et al.[49] have measured the levels of some antioxidant markers in serum and whole blood of FMF patients in FMF-attack period and FMF-attack-free period and suggested that patients in FMF-attack period has increased level of oxidative stress.

Erden et al.[50] showed that colchicine which is used in treatment of knee osteoarthritis increases the total antioxidant capacity and decreases malondialdehyde levels. In *in vitro* studies, especially those performed with the multidrug resistant cell lines, it has been demonstrated that colchicine increases the glutathione level and GST activity.[51-53] Therefore, colchicine therapy may be responsible for less aggressive course of atherogenesis in most studies on FMF.[37]

In this study; LDL, ESR, and fibrinogen levels were statistically significantly higher in the patient group. The difference between groups was not significant in terms of other biochemical parameters. There are different results about LDL and HDL levels of FMF patients in the literature. In the studies which showed higher CIMT value in FMF patients, Ugurlu et al.[41] have found statistically significantly lower HDL and LDL levels and Bilginer et al.[34] found statistically insignificant LDL and significantly higher HDL in FMF patients compared to healthy controls. Acay et al.[36] have detected higher LDL level in FMF patients but the difference between patients and controls was not statistically significant. In their study, HDL cholesterol was statistically significantly lower in FMF patients. Although they did not measure CIMT in the study group, they found statistically significantly higher atherogenic values in FMF patients. In a study which showed no difference in measurements of common CIMT between FMF patients and healthy controls, no statistically different results were obtained in terms of LDL and HDL levels between FMF patients and controls.[39] Due to the lipid lowering effect of colchicine,[54] further investigations are needed to explain impact of LDL and HDL levels on atherogenesis in FMF patients.

Our study has some limitations. Firstly, our FMF patients have received colchicine treatment regularly. Therefore, we were unable to compare results of colchicine responders and non-responders within FMF patients according to the GST genotypes. In addition, colchicine treatment probably enhances oxidative stress markers such as glutathione and GST, and protects the FMF patients from the harmful effects of inflammation. We did not measure the levels of glutathione or other oxidative stress markers and GST activity.

In conclusion, right and left CIMT of FMF patients were statistically significantly higher than that of control group. There were no statistically significant differences in terms of GST polymorphisms between FMF patients and controls. Moreover, no significant difference was observed between GST polymorphisms and CIMT. In line with other studies, our study has indicated that there are cardiovascular results of subclinical inflammation in FMF. Large and homogeneous cohorts are required for clarifying the explicit relationship between oxidative stress caused by subclinical inflammation and its cardiovascular effect in FMF patients.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

This work is supported by the Scientific Research Project Fund of Cumhuriyet University under the project number "T-515".

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