Relationship of paraoxonase-1, malondialdehyde and mean platelet volume with markers of atherosclerosis in familial Mediterranean fever: an observational study

Ailevi Akdeniz ateşinde paraoksonaz-1, malondialdehit ve ortalama trombosit hacmi ile ateroskleroz belirteçlerinin ilişkisi: Gözlemsel bir çalışma

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ABSTRACT

Objective: There are many studies demonstrating deteriorated ventricle and endothelium functions in familial Mediterranean fever (FMF) patients. As FMF is an autoinflammatory disease with an ongoing inflammatory activity and inflammation plays an important role in the development and progression of atherosclerosis in some of the rheumatic diseases, we aimed to investigate the early markers of atherosclerosis in patients with FMF by the measurements of serum paraoxonase-1 (PON-1) activity, mean platelet volume (MPV) and malondialdehyde (MDA) level.

Methods: This study is a cross-sectional, observational study. Forty consecutive patients with FMF and twenty healthy volunteers were selected to form the study population. The diagnosis of FMF was based on Tel-Hashomer criteria. Serum PON-1 activity, MPV and MDA level were determined to examine their association with FMF. Student's t-test, Mann-Whitney U test, Pearson correlation analysis were used for statistical analysis.

Results: The mean PON-1 activity in FMF patients was significantly lower than in the healthy population (141.46±38.29 vs. 179.62±10.73 U/l, p<0.01). Serum MDA levels were the same between the groups (1.08±0.66 vs. 1.08±0.33 nmol/mL, p=0.99). MPV was higher in FMF patients than in the control l group (8.87±0.99 vs. 8.22±0.45 fl, p=0.04). PON, MPV and MDA levels were the same in FMF patients with acute attack and attack -free period.

Conclusion: Our results show that PON-1 activity is lower in patients with FMF. Reduced PON-1 activity and increased MPV, independent of the oxidative stress status of these patients, may lead to increased atherosclerotic propensity in FMF. *(Anadolu Kardiyol Derg 2013; 13: 357-62)*

Key words: Familial Mediterranean fever, atherosclerosis, paraoxonase, mean platelet volume, malondialdehyde

ÖZET

Amaç: Ailevi Akdeniz Ateşi (AAA) hastalarında bozulmuş ventrikül ve endotel fonksiyonlarını gösteren pek çok çalışma bulunmaktadır. AAA devamlı enflamatuvar aktivite ile seyreden bir otoenflamatuvar hastalık olduğundan ve bazı romatizmal hastalıklarda enflamasyon ateroskleroz gelişimi ve ilerlemesinde önemli rol oynadığından, AAA hastalarında serum paraoksonaz-1 (PON-1) aktivitesi, ortalama trombosit hacmi (OTH) ve malondialdehit (MDA) seviyesi ölçerek aterosklerozun erken belirteçlerini araştırmayı amaçladık.

Yöntem: Bu çalışma kesitsel ve gözlemsel bir çalışmadır. Kırk AAA'lı hasta ve yirmi sağlıklı gönüllü çalışma topluluğunu oluşturmak için seçildi. AAA tanısı Tel-Hashomer kriterlerine göre konuldu. AAA, MDA seviyesi, OTH ve PON-1 aktivitesi arasındaki ilişkiyi araştırmak için serum PON-1 aktivitesi ve MDA seviyesi ve OTH ölçüldü. İstatistiksel analiz için Student's t-testi, Mann-Whitney U testi, Pearson korelasyon analizi kullanıldı.

Bulgular: AAA hastalarında ortalama PON-1 aktivitesi normal bireylere göre önemli olarak daha düşüktü (141.46±38.29 karşın 179.62±10.73 U/L, p<0.01). Gruplar arasında serum MDA seviyeleri aynıydı (1.08±0.66 karşın 1.08±0.33 nmol/mL, p=0.99). OTH normal bireylere göre önemli olarak daha yüksekti (8.87±0.99 vs. 8.22±0.45 fl, p=0.04). AAA hastalarında akut atak sırasında ve ataksız dönemlerde PON-1 aktivitesi, MDA seviyeleri ve OTH aynıydı.

Sonuç: Sonuçlar göstermektedir ki AAA hastalarında PON-1 aktivitesi düşüktür. Azalmış PON-1 aktivitesi ve artmış OTH bu hastalarda, oksidatif stres durumundan bağımsız olarak, AAA'de artmış ateroskleroz yatkınlığına işaret ediyor olabilir. *(Anadolu Kardiyol Derg 2013; 13: 357-62)*

Anahtar kelimeler: Ailevi Akdeniz Ateşi, ateroskleroz, paraoksonaz, ortalama trombosit hacmi, malondialdehit

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Accepted Date/Kabul Tarihi: 21.11.2012 **Available Online Date/Çevrimiçi Yayın Tarihi:** 26.03.2013

doi:10.5152/akd.2013.103

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease manifested by recurrent attacks of serositis (peritonitis, pleuritis, pericarditis, synovitis/arthritis), fever and characterized by clinical, histological and laboratory evidence of inflammation. The prevalence reaches 1 in 200 individuals in non-Ashkenazi Jews and 1 in 1.073 in the Turkish population (1-3). Although FMF presents with exacerbations and attack free periods, it has been demonstrated that there is sustained inflammation during attack-free periods of FMF patients (4, 5). There have been many studies demonstrating the cardiovascular involvement in patients with FMF. Most familiar of these is pericarditis in a ratio of 1.4%. It has been demonstrated that left ventricle diastolic functions, heart rate recovery index, coronary flow reserve in other words coronary microvascular function are impaired in FMF patients (6-8). Carotid artery intima-media thickness is found to be increased in many studies. Hypercoagulability, increased asymmetric dimethylarginine (ADMA) and lipoprotein (a) levels, increased platelet activation and QT dispersion are the other manifestations of the cardiovascular system disorder (9-14). In spite of these atherosclerotic risk markers there is no conclusive data showing increased atherosclerotic heart disease prevalence at FMF patients.

An increased level of high- density lipoproteins (HDL) has been reported to be associated with decreased risk for coronary artery disease (CAD). This protective effect of HDL against atherosclerosis is attributed to the enzymes associated with HDL metabolism. One of these enzymes is paraoxonase (PON). The PON-1 is synthesized in the liver and transported to the HDL in the plasma (15). It is a protein of 354 amino acids with a molecular weight of 43 kDa. Studies have shown that HDL-associated PON-1 inhibits lipid peroxidation or degrades biologically active oxidized lipids in low density lipoprotein (LDL). The PON-1 is recruited with breakdown of lipid peroxides before they can accumulate on LDL (16, 17). The PON-1 can hydrolyze lipid peroxides in oxidized lipoproteins (18). The PON-1 over-expression protects mouse from atherosclerosis (19). The PON activity decreases in many diseases such as slow coronary flow , cardiac syndrome X, coronary artery disease, diabetes mellitus, myocardial infarction and acute phase response (20-25). Several studies have demonstrated that there is a significant relationship between PON-1 activity and concentration and severity of CAD (26-28).

Reactive oxygen species are highly reactive molecules that, when present in excess, overwhelm the protective systems and results in cell damage and lipid peroxidation. Lipid peroxidation product measurement is used to evaluate the effects of increased oxidative injury. Lipid peroxidation leads to the formation of highly reactive lipid hydroperoxides and their subsequent decomposition causes to the formation of various products; one of the best known being malondialdehyde (MDA) (29). MDA is confirmed to be a useful oxidative stress marker.

Mean platelet volume (MPV) is believed to be a marker of increased thrombogenicity and increased in patients with acute myocardial infarction, stroke, diabetes mellitus, congestive heart failure and hypertensive patients with evidence of target organ damage (30, 31).

No studies have previously evaluated the serum PON activity at FMF.

As FMF is an autoinflammatory disease with an ongoing inflammatory activity and as inflammation plays an important role in the development and progression of atherosclerosis in some of the rheumatic diseases, we aimed to investigate the early markers of atherosclerosis in patients with FMF by the measurements of serum PON-1 activity, MPV and MDA level. PON-1 activity was measured to search the atherosclerosis propensity at these patients. MDA level known as a precious marker for the oxidative status of FMF patients and MPV was included in the study to determine the thrombogenic status.

Methods

Study design

This study is a cross-sectional, observational study.

Study population

This study was performed at Ministry of Health Dışkapı Education and Training Hospital on 2009-2010. Forty consecutive patients with FMF and age and sex matched twenty healthy volunteers were selected to form the study population. FMF patients were selected from the patients admitted to the rheumatology outpatient clinic. Healthy volunteers were selected from the people admitted to the internal medicine outpatient clinic with various complaints and found to be disease free.

Subjects who had myeloproliferative diseases, hypertension, diabetes mellitus, congestive heart failure, malignancies, renal, hepatic and thyroid diseases, immunological diseases, hematocrit <0.30 or >0.52, platelet count <100 000/mm^3, patients with acute coronary syndromes, coronary heart disease and those with severe valvular heart diseases were excluded from the study.

The permission of a research ethics committee and written informed consent was obtained from all patients before study.

Study protocol and definitions

The diagnosis of FMF was based on Tel-Hashomer Criteria. Attack was defined when following criteria were fulfilled: 1) admission at first 72 hours with clinical symptoms (fever, abdominal pain, chest pain, arthralgia, serositis, pleuritis, pericarditis, arthritis, peritonitis, myalgia, erysipelas-like erythema) 2) exclusion of other fever etiologies 3) fever should be ≥37.5°C and continue at least 12 hours 4) laboratory findings: sedimentation rate ≥30 mm/hour, C - reactive protein (CRP) ≥5 mg/dL, fibrinogen ≥350 mg/dL, white blood cell (WBC) count ≥10.000/mm^3. Attack-free period was defined as being free of attacks for at

least 3 weeks. Study participants were checked for the cardiovascular system involvement. Blood pressure measurement, electrocardiogram and echocardiography were performed to all study participants. None of the study participants had cardiovascular complaints. Study participants were examined every week during one month.

Study variables

Age, sex, age of disease onset, list of medications, symptoms, mean WBC, hemoglobin, platelet count, total cholesterol, HDL, triglyceride (TG), LDL levels, sedimentation rate, fibrinogen and CRP level of study participants were recorded as baseline variables. PON, MDA and MPV levels of the study participants were measured as outcome variables. Presence of FMF was accepted as a predictor variable.

Blood sampling protocol

Peripheral venous blood samples were obtained following an overnight fasting period. The serum was separated from the cells by centrifugation at 3000 rpm for 10 min and stored at-78°C until measurement. Blood glucose, lipid parameters, liver function tests were measured by P800 Roche Hitachi (Germany) and Olympus AU 5200 (Australia) automated analyzers. LDL cholesterol was calculated using Friedewald formula [LDL=Total Cholesterol- (HDL)+Triglyceride (TG)/5]. Complete Blood Count was completed by ROCHE Sysmex SE 9000 (Germany) automated analyzer. MPV was measured with ROCHE Sysmex SE 9000 (Germany) automated analyzer. Normal value of MPV was 6.9-10.1 fl. The serum PON activity was measured using the synthetic substrate paraoxon (diethyl-p-nitrophenol, PS610, SUPELCO, USA). The rate of paraoxon hydrolysis was measured by monitoring the increase of absorbency at 415 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 16 900 M-1cm-1 (32). Paraoxonase activity was expressed as U/l. Serum MDA levels were measured by the thiobarbituric acid reactive substances (TBARS) method (33). MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. For this purpose, 0.1 ml of serum was suspended in 1 ml of phosphate buffered saline (pH 6, 100 mmol/l) and then 1 ml 20% trichloroacetic acid, 1 ml ethyl alcohol (95%) and 1 ml thiobarbituric acid solution (2%) were added. After keeping it in boiling water for 30 min the tube's content was removed and absorbance's were read at 532 nm. MDA concentrations were calculated by comparing the absorbance values of the samples with those of standard MDA solutions. The results were expressed as nmol/ml.

Statistical analysis

Data were analyzed with the software SPSS version 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Continuous variables are presented as mean±SD and categorical variables as frequency and percentage. Student's t-test was used to compare normally distributed continuous variables and the Mann-Whitney U test - for variables without normal distribution. The Chi-square test was used to compare categorical variables. Spearman and Pearson correlation analysis was used for correlation analysis. A p value of < 0.05 was considered significant.

Results

Overall, 13 male (32.5%), 27 female (67.5%) totally 40 FMF patients, 8 male (40%), 12 female (60%) totally 20 normal healthy volunteers were enrolled in the study. Clinical and demographic variables of the groups were summarized in Table 1. Three patients (7.5%) were not under colchicine treatment, 75% of patients were taking 1.0-1.5 mg/day colchicine. Mean WBC, hemoglobin, platelet count, total cholesterol, HDL, TG, LDL levels were similar in control and FMF groups. Sedimentation rate and CRP were higher in the FMF group compared to the controls (Table 1).

The mean PON-1 activity in FMF patients was significantly lower than in healthy population (141.46±38.29 vs. 179.62±10.73 U/l, $p<0.01$) (Fig. 1). The mean platelet volume (MPV) was significantly higher in FMF group than in control group (8.87±0.99 vs. 8.22±0.45 fl, p=0.04). Serum MDA levels were the same between the groups (1.08±0.66 vs. 1.08±0.33 nmol/mL, p=0.99)

In patients with FMF, disease activity status did not affect the PON activity and MDA levels (PON; 141.38±37.90 vs. 141.68±41.00 U/l, p=0.90, MDA; 1.11±0.71 vs. 0.99±0.52 nmol/mL, p=0.50, MPV; 9.00±1.01 vs. 8.53±0.91, p=0.19, in attack free patients and patients with attack respectively). However, as have been expected WBC, sedimentation rate, CRP level and age of disease onset was different between attack free patients and patients with acute attack. WBC, sedimentation rate, CRP

Table 1. Clinical and demographic variables of groups

Variables	FMF patients (n=40)	Normal healthy individuals (n=20)	*p
Male, n (%)	13 (32.5)	8 (40)	0.18
Age, years	30.79±12.23	29.18±9.97	0.65
Mean age of disease onset, years	17.10±9.60	-	
White blood cell count, cell/µl	7253±1900	6172±1008	0.80
Hemoglobin, g/dL	13.45±1.87	13±1.26	0.45
Platelet count, cell/µL	259564±82319	251818±32501	0.75
Sedimentation rate, mm/hour*	21.67±14.80	12.36±2.50	0.04
C-reactive protein level*, mg/L	19.78 (3-241)	2.81 (2-4)	0.02
Total cholesterol, mg/dL	163.18±26.48	167.61±41.60	0.67
High- density lipoprotein, mg/dL	45.27 ±7.59	46.60±11.98	0.65
Triglyceride, mg/dL	116.36 ±28.02	119.76±58.80	0.78
Low- density lipoprotein, mg/dL	108.90±20.02	101±33.64	0.36

Data are presented as mean±SD, median (range) and number (percentage)
*Student's t-test, Mann-Whitney U and Chi-square tests

level and age of disease onset were higher in the active disease group compared to the attack free group (Table 2).

In the correlation analysis PON and MDA were not found to be related to any clinical and laboratory parameters.

Discussion

In our study, we found that mean PON-1 activity was significantly lower in FMF patients compared to the normal healthy individuals. Also, mean platelet volume was significantly higher in the FMF group. But MDA levels were the same between groups. In the subgroup analysis of FMF patients there was not any difference at PON activity, MPV and MDA levels between patients with acute attack and attack free period. In other words PON and MDA do not seem as good markers to identify disease activity.

PON activity was found to be decreased in systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (34, 35). Beside this, PON1 activity was lower in lupus patients suffering from atherothrombotic clinical events than lupus patients without atherothrombotic complications (35). Serum MDA level was found to be higher and PON 1 activity lower in the rheumatoid arthritis (RA) patient group than the controls (36). There was not any difference at serum MDA levels and PON activity between active and inactive RA patient subgroups. One of the explanations of these findings is thought to be effect of proinflammatory cytokines on liver. Proinflammatory cytokines including tumor necrosis factor (TNF) and interleukine-1 (IL-1), have previously been shown to suppress and interleukine-6 (IL-6) has been shown to induce the hepatic synthesis of PON- 1 (37, 38). Popa et al. (39) reported that in RA patients therapy with infliximab causes sustained increases in PON and arylesterase activities of PON-1. RA patients treated with anti-TNF therapy were recently reported to show a decreased incidence of cardiovascular events compared with those on other anti-rheumatic medication.

Table 2. Comparison of clinical variables between patients with attack and attack free

Variables	Patients with acute attack (n=11)	Attack-free patients (n=29)	*p
Mean age of disease onset, years	25.2±13.1	14.1±5.8	<0.01
White blood cell count, cell/μL	8581±2489	6732±1337	<0.01
Sedimentation rate, mm/hour	35.7±17.9	16.4±9.5	<0.01
CRP level, mg/L	53.9 (3-241)	6.35 (3-36)	<0.01
Fibrinogen level, mg/dL	455±205	328±88	<0.01
PON activity, U/L	141.6±41	141.3±37.9	0.90
MDA level, nmol/mL	0.9±0.5	1.1±0.7	0.50

Data are presented as mean±SD and median (range)
*Student's t-test, Mann-Whitney U test
CRP - C-reactive protein, MDA - malondialdehyde, PON - paraoxonase

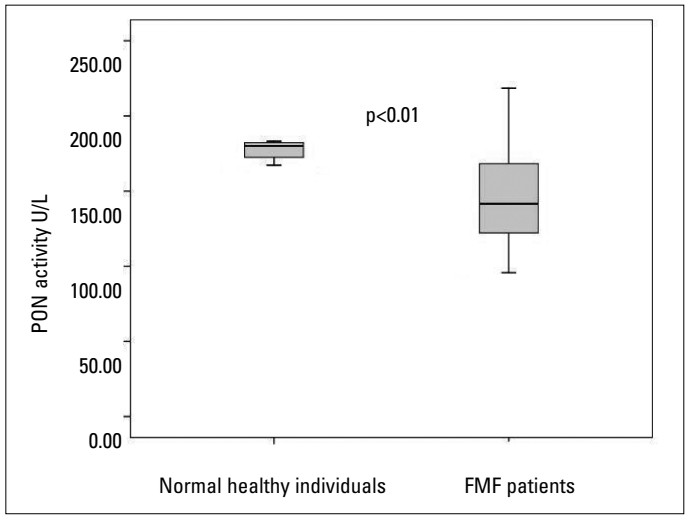

Figure 1. PON activity in normal healthy individuals and FMF patients, p<0.01
PON - paroxonase

It has been shown that higher disease activity representing higher inflammatory burden is associated with increased morbidity and mortality from cardiovascular disorders in patients with RA and SLE (40, 41). However, there are conflicting results about cardiovascular risk in FMF. Although FMF presents with exacerbations and attack free periods, it has been demonstrated that there is sustained inflammation during attack-free periods of FMF patients (4, 42). Neutrophils of patients with FMF remain hyperactive during attack free period due to the sustained overproduction of interleukins and these interleukins exert proatherogenic effects.

There are many findings about the effects of sustained inflammation on cardiovascular system at FMF. Increased carotid artery intima media thickness and decreased endothelium dependent flow-mediated dilation of brachial artery have been found in FMF patients in many studies (9-11). Also as an expected finding Bilginer et al. (10) showed a positive correlation between serum amyloidal A, fibrinogen level, erythrocyte sedimentation rate and carotid intima media thickness. This result supports the idea that acute phase response and increased inflammation begets atherosclerotic lesion development. Although preclinical atherosclerosis is more prevalent in FMF, clinical atherosclerotic heart disease prevalence was reported to be significantly lower than the normal controls in Israel (43). This unexpected finding was interpreted as a consequence of colchicine treatment by Langevitz et al. (43). Coronary flow reserve was found to be significantly lower in the FMF patients. Decreased coronary flow reserve at this disease is indicator of impaired coronary microvascular function. Interestingly deterioration in coronary flow reserve correlated with hs-CRP level, supporting the importance of inflammation on cardiac involvement (6). Elevated levels of ADMA in FMF is thought to be another index of endothelial dysfunction. Besides ADMA levels were higher in the attack period than the attack free period, telling us the inflammation mediated impairment of

the endothelial functions during attack (12). Shortened prothrombin time and thrombin time, decreased protein C activity and elevation of prothrombin fragment F1+2 are found in FMF patients. These changes in hemostatic parameters points to the hypercoagulable state in FMF even at the attack-free period (13). Increased platelet activity is one of the mostly accused mechanisms of atherosclerosis pathogenesis. MPV is a parameter of platelet size. Large platelets that contain more dense granules are metabolically more active than small platelets and they are more thrombogenic than the smaller ones (44). MPV is increased in patients with acute myocardial infarction , stroke, diabetes mellitus, congestive heart failure and hypertensive patients with evidence of target organ damage (30, 31).

Our study supports the idea of increased atherosclerotic burden and thrombogenic activity in FMF patients. Both decreased PON activity and increased MPV confirm these findings. On the other hand, these results cannot solely be attributed to the increased oxidative stress in the disease status. Because an oxidative stress marker; MDA was the same between normal individuals and FMF patients. There are many possible explanations. One of these is decreased PON production from liver during the inflammatory process. Inflammatory cytokines suppress the PON production in liver. Another mechanism may be the altered molecular conformation of PON on HDL and decreased activity of PON due to this conformational change. Interestingly, according to our study disease activity does not cause any difference at PON activity. It may be concluded that even the ongoing subclinical inflammation during attack free periods is enough for the altered PON activity and any increase in the inflammation cannot further reduce PON activity. FMF may be thought as a risk factor for atherosclerosis.

Study limitations

Small patient number is the most important limitation of the study. Carotid artery intima media thickness, ankle-brachial index, forearm flow mediated dilatation, myocardial perfusion scintigraphy could be performed to the participants to correlate the biochemical markers to the clinical variables. This is one of the most important limitations of the study. Relative young age of the patients is another limitation of the study. Further studies may be planned with older and greater patient population.

Conclusion

According to our results, PON-1 decreased and MPV increased in FMF patients independent of the attack status. FMF may be thought as a risk factor for atherosclerosis. This relationship seems to be independent of the oxidative stress. Even in the young patient population as in our study, deteriorated atherosclerosis biomarkers should alarm the clinicians. Strict risk factor modification can be managed to prevent the atherosclerotic disease.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - Ö.K.A., K.Ü.; Design - M.S.; Supervision - N.Y.; Resource - E.E., İ.E.; Materials - Ö.K.A., K.Ü., M.S., N.Y., E.E., İ.E.; Data collection&/or Processing - Ö.K.A., K.Ü., M.S., N.Y., E.E., İ. E.; Analysis &/or interpretation - Ö.K.A., K.Ü., M.S., N.Y., E.E., İ.E.; Literature search - Ö.K.A., K.Ü., M.S., N.Y., E.E., İ. E.; Writing - Ö.K.A., K.Ü., M.S., N.Y., E.E., İ. E.; Critical review- Ö.K.A., K.Ü., M.S., N.Y., E.E., İ.E.

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