

Paraoxonase 1 and arylesterase levels in children with familial mediterranean fever

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Abstract. – BACKGROUND: The pathogenesis of Familial Mediterranean Fever (FMF) is not clearly elucidated. It emerges as a result of triggering of the several environmental factors at the people who are genetically vulnerable.

OBJECTIVES: To evaluate the anti-oxidant enzymes at the remission period of familial mediterranean fever (FMF).

MATERIALS AND METHODS: Study group is consisted of 80 patients between the age of 2 and 16 years old who are routinely followed up. The control group is consisted of 80 healthy children whose physical examination is normal, and whose demographic findings are similar to the study group. Paraoxonase 1 (PON1) and arylesterase (ARE) levels are measured at both study and control group.

RESULTS: The difference between the levels of ARE and PON1 are statistically significant between the FMF and control group ($p = 0.007$, $p = 0.001$). According to the weight scoring, ARE and PON1 levels of light cases are higher versus the levels of moderate cases ($p < 0.01$).

CONCLUSIONS: Endogenous anti-oxidants Paraoxonase 1 and arylesterase levels are important in evaluating the inflammation at the remission period of FMF.

Key Words:

Familial Mediterranean Fever, Anti-oxidants, Paraoxonase, Arylesterase.

Introduction

Familial Mediterranean Fever (FMF) is an auto-inflammatory disease that is characterized with exacerbations of non-infectious painful inflammation of serous membranes with accompanying fever. Although it is thought to be related with the environmental factor based on the genetic vulnerability, the pathogenesis of the disease is not clearly elucidated¹.

It is reported that the free oxygen radicals that are generated after the passing of the activated inflammatory cells to the tissue is an important fac-

tor in the pathophysiology of FMF. During the FMF exacerbation, levels of proinflammatory cytokines like interleukin-8 (IL-8), interleukin-6 (IL-6) and tumour necrosis factor- α (TNF- α) increase^{2,3}. Reactive oxygen species (ROS) are produced by the polymorphonuclear leukocytes (PMNL) that are activated by the stimulation of cytokines. Increased ROS activates the NF- κ B which is a transcription factor and a vicious circle emerges between the ROS and cytokines^{4,5}. The increased inflammation, cytokines and generated ROS at FMF causes oxidant burden at the body. The oxidative stress generated is balanced by the anti-oxidants at the body⁴⁻⁷. During several inflammatory diseases like (systemic lupus erythematosus) SLE, (rheumatoid arthritis) RA and Behcet's disease at which the oxidative stress emerges and ROS increases, the endogenous anti-oxidant enzymes paraoxonase 1 (PON₁) and arylesterase (ARE) levels are shown to be decreased⁸⁻¹⁰.

At this study the aim is to evaluate the PON₁ and ARE which are antioxidant serum enzymes as an indicator of oxidative stress at the FMF patients during the remission period.

Materials and Methods

The patient group of the study is created amongst the patients that are followed up and treated at the Pediatric Nephrology Division of Pediatric Clinic of Ataturk University, Faculty of Medicine with the diagnosis of FMF. The study is conducted in compatible with the principles of Helsinki Declaration. Parents of the all of the patients and children in the control group that are enrolled in the study are informed and written informed consents were taken.

The study group is generated from 80 children between the age of 2 and 16 years old, whose diagnosis are done with Tel Hashomer Criteria, who are being followed up routinely and using colchicum treatment.

The control group is generated from 80 healthy children with similar demographic features whose physical examination are normal, who don't have any sign or symptom of active infection and chronic disease and also who don't carry FMF gene mutation and don't have family history of FMF. An interview with all of the parents of the control group individually is done and the parents were questioned for the presence of complaints like recurrent stomach pain with unknown nature, fever, pain of chest and articulations and rash that can indicate FMF exacerbations. The children that have symptoms that can indicate FMF exacerbations are excluded from the control group. Venous blood samples of the children at both the study and control group are taken. The samples are centrifuged and serum parts of them are separated. They are stored at -80°C degrees until they are analyzed.

Serum PON_1 activity is measured with spectrophotometric method at which diethyl-p-nitrophenylphosphate (Sigma Aldrich Co, Gillingham, Dorset, SP84XT, UK) is used as substrate. For the activity measurement of ARE, phenylacetate (Sigma Aldrich, Co, UK) is used as substrate and ARE activity is detected by the measurement of absorbance of the produced phenol at 270 nm. Molar absorptivity coefficients ($17100 \text{ M}^{-1} \text{ cm}^{-1}$ and $1310 \text{ M}^{-1} \text{ cm}^{-1}$, respectively) are used for calculation of activities of PON_1 and ARE. One unit for PON_1 activity is defined as 1 nmol 4 nitro-phenol/mL serum/min, and for ARE activity as 1 nmol phenol/mL serum/min.

Adequate number of cases are calculate according to the result measurements of PON_1 and ARE. As more cases are needed for ARE, during the sample calculation ARE level is taken as base. Mean ARE levels measured at previous studies are $21.4 \pm 4.3 \text{ U/mL}$ for healthy subjects and $14.8 \pm 6.1 \text{ U/mL}$ for patients¹⁶. When alfa bias rate 5%, standard deviation 6 U/mL, importance degree difference between means 4 U/mL is accepted, in order to calculate the ARE levels at healthy children and patients with FMF with 90% power the needed case number is 49 at each study and control group (totally 98).

Serum ARE and PON_1 levels are studied at patient and control group. Patients and children that are in the control group were compared in terms age, gender, weight, height, PON_1 and ARE levels.

Scores of the patients that are in the FMF group are calculated via assessing the measures (early beginning of the symptoms of the patients, frequency and severity of joint involvement, presence of erythema like erysipelas and colchicum dose needed to

control the symptoms and presence of amiloidosis) that are previously defined by Pras¹¹.

Serum PON_1 and ARE levels and correlations are evaluated according to the weight scoring of the patients with FMF diagnosis. Although 80 patient and healthy subjects were enrolled, as hemolized samples are excluded from the study, 77 patient and 70 healthy subjects were included in the study.

Statistical Analysis

Data analyses were done at SPSS for Windows 15 software (SPSS Inc., Chicago, IL, USA). Definitive statistics are done as mean $\pm$ standard deviation for continuous variables and as case number and (%) for categorical variables. Categorical variables are assessed with Pearson's Chi-Square test. It is assessed with Pearson's correlation test, whether there is any significant correlation between the continuous variables or not. For $p < 0.01$, the results are accepted as statistically significant.

Results

Mean age for the FMF patients is 10.7 ± 3.1 years, while the control groups' is 8 ± 3 years. In the FMF group 41 of the patients (53.2%) were female while 36 of them (46.8%) were male. At the control group 30 of the patients (42.9%) were female while 40 of them (57.1%) were male. There wasn't any statistically significant differences between the FMF and control group in terms of gender ($p = 0.208$).

Mean PON_1 and ARE levels of the patients at FMF and control group are shown at Table I. Differences between the ARE and PON_1 levels of both group were statistically significant.

When both groups are assessed together, while the mean ARE level is $38.2 \pm 18.5 \text{ U/ml}$ at female subjects, it is $39.9 \pm 17.3 \text{ U/ml}$ at male subjects. And while the mean PON_1 level is $96.8 \pm 62.1 \text{ U/ml}$ at female subjects, it is $100.4 \pm 64.7 \text{ U/ml}$ at male subjects. There isn't any statistically significant difference between the ARE and PON_1 levels between the female and male subjects amongst all of the patients (for ARE $p = 0.439$; and for PON_1 $p = 0.827$). At the correlation analyses there is a positive way correlation between the ARE and PON_1 levels. (Correlation coefficient $r = 0.27$).

Mean ARE level at male patients at FMF group was $32 \pm 12.4 \text{ U/ml}$, and mean PON_1 level was $81.7 \pm 52.9 \text{ U/ml}$. And mean ARE level at female patients at FMF group was 33.9 ± 16.9

Table I. Comparison of ARE and PON₁ levels of the cases of the FMF and control group.

	FMF (n: 77)	Control (n: 70)	p value
ARE (U/ml)	33 ± 14.9	45.8 ± 18.5	0.001*
PON ₁ (U/ml)	85.7 ± 54.7	112.9 ± 69.1	0.007*

Note: Pearson Chi-Square test was held. Values are given as Mean ± Standard deviation. FMF: Familial Mediterranean Fever; n: Patient number; ARE: arylesterase; PON₁: paraoxonase 1. **p* < 0.01 is accepted as significant.

U/ml, and mean PON₁ level was 89.3 ± 56.7 U/ml. Between the female and male subjects in the FMF patients group, there wasn't any statistically significant differences between the levels of ARE (*p* = 0.585) and PON₁ (*p* = 0.561).

Mean ARE level at male patients at control group was 47.1 ± 18.1 U/ml, while it was 44 ± 19.2 U/ml at the female subjects (*p* = 0.355). Mean PON₁ levels at female and male subjects were 117.3 ± 70.1 U/ml and 107 ± 68.5 U/ml, respectively (*p* = 0.390). ARE and PON₁ levels were not statistically significant between the female and male subjects at the control group.

There was a positive way correlation between the ARE and PON₁ levels at the patients with FMF (*r* = 0.24). ARE levels were increasing with the increase of PON₁ at the patients with FMF. This finding was statistically significant (*p* < 0.05).

Cases with FMF are classified according to the Pras weight scoring. According to this classification, while 41 patients have mild disease according to weight scoring, the score of 36 patients were found to be moderate. No patient with severe disease is detected. ARE and PON₁ levels of mild cases according to the weight scoring are higher than the cases at the moderate group. While there was statistically significant difference between the groups in terms of ARE levels (*p* < 0.01), there wasn't statistically significant differences in terms of PON₁ levels (*p* > 0.01) (Table II).

Discussion

Although the MEFV gene which is responsible from the Familial Mediterranean Fever is

identified, the etiopathogenesis is not clearly elucidated. Any mutation that emerges at the MEFV gene causes abnormal pyrine protein synthesis and this leads to deterioration of the suppression of the inflammation at the body. The most important suggested pathogenic mechanisms at FMF are this inflammation and oxidative stress^{6,7,12}.

Although the FMF is a chronic inflammatory disease, until this day adequate studies is not held about the levels of anti-oxidant enzymes. Especially, at the recent years the serum levels of PON₁ and ARE enzymes are accepted as an important indicator of inflammation⁸⁻¹⁰. At our study the levels of serum PON₁ and ARE levels are found to be lower at the patients with FMF diagnosis when compared with healthy subjects.

Clinical heterogeneity in FMF is related with the severity of inflammation that goes during the remission period. Most of the reports have shown that the inflammatory indicators are high even in the remission period^{13,14}. The low levels of PON₁ and ARE even at the remission period can be due to this sub-clinical inflammation that goes on in the body. Oxidative blasting at monocytes and neutrophils causes oxidative stress at FMF patients at remission periods. The increased oxidative stress in the body is buffered with the anti-oxidant system which is known to be balanced with oxidant system and this causes decreasing of enzymes of anti-oxidant stress^{6,15,16}. Besides PON₁ and ARE lipoproteins having protective effect from the oxidation, it is shown that the enzyme activity is sensitive to oxidative stress and inactivated with the increasing oxidants and also PON₁ enzyme activity decreases with the events that increases the oxidative

Table II. Comparison of ARE and PON₁ levels of the FMF cases according to the weight scoring.

	Mild (n: 41)	Moderate (n: 36)	p value
ARE (U/ml)	36.3 ± 14.3	29.3 ± 14.9	0.008*
PON ₁ (U/ml)	90.6 ± 54.6	80.2 ± 55.1	0.184

Note: Pearson Chi-Square test was held. Values are given as Mean ± Standard deviation. n: Patient number; ARE: arylesterase; PON₁: paraoxonase 1. **p* < 0.01 is accepted as significant.

stress^{17,18}. In the literature there are studies about the paraoxonase and arylesterase levels at the diseases at which's pathogenesis the inflammation and oxidative stress are blamed like Behcet, RA and SLE. At these studies, at these diseases with chronic inflammation, PON₁ and ARE enzyme levels are found to be decreased and shown that PON₁ and ARE enzyme levels increase after anti-inflammatory treatment. As a result of these investigations, it is found the endogenous anti-oxidants PON₁ and ARE decrease due to the increase of oxidative stress which plays an important role at the pathogenesis of the disease^{8-10,19}. At our study, any correlation between the age and gender and levels of PON₁ and ARE at the control and FMF group is not detected. Geldmacher et al²⁰ in a multicenter trial showed that there isn't any correlation between the age and gender and PON₁ activity, and PON₁ activity is not effected from the age and gender. When the relationship between PON₁ and ARE levels and severity score of the disease, ARE and PON₁ levels of mild cases were higher when compared with the moderate cases. This situation shows that, there is a correlation between the enzyme levels and severity of inflammation.

As a result, the decrease of serum PON₁ and ARE enzyme levels during the remission period of FMF shows the subclinical inflammation and can be parameter to be used at the evaluation of severity of inflammation.

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