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Serum 17-OH progesterone and free testosterone levels in women patients with Familial Mediterranean Fever: a pivotal study

K. DEVECI[1], A.K. GOKAKIN[2], A. BOZTOSUN[3], H. DEVECI[4], S. SENEL[5], A.U. USLU[6], E. SANCAKDAR[1]

[1]Departments of Biochemistry, Cumhuriyet University School of Medicine, Sivas, Turkey
[2]Department of General Surgery, Cumhuriyet University School of Medicine, Sivas, Turkey
[3]Departments of Obstetrics and Gynecology, Cumhuriyet University School of Medicine, Sivas, Turkey
[4]Department of Physical Medicine and Rehabilitation, Numune State Hospital, Sivas, Turkey
[5]Department of Rheumatology, Erciyes University School of Medicine, Kayseri, Turkey
[6]Departments of Internal Medicine, Cumhuriyet University School of Medicine, Sivas, Turkey

Abstract. – BACKGROUND: Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by short lived, febrile serosae inflammatory attacks. FMF has various effects in multiple systems and organs.

AIM: In the present study, our aim was to evaluate adrenal steroidogenesis in female FMF patients.

PATIENTS AND METHODS: There were 71 women in the study including 41 women with FMF and 30 women as healthy control group (HC group). Of 41 FMF patients, twenty were evaluated in attack period (AP-FMF group) whereas 21 of them were evaluated in attack-free period (AFP-FMF group). In all subjects; serum free testosterone, 17-OHP levels as hormones, IL-1 beta, TNF-alpha, IL-6, IL-18 as proinflammatory cytokines, CRP, fibrinogen, white blood cell (WBC) counts, and erythrocyte sedimentation rate (ESR) as acute phase reactants were measured in samples of venous blood taken in the morning before breakfast.

RESULTS: Serum 17-OHP levels in AP-FMF group and AFP-FMF group were higher than in HC group ($p < 0.001$). A positive correlation was detected between serum levels of 17-OHP and IL-1 beta in FMF patients ($p = 0.006$; r = 0.486). There was no difference between FMF patients and HC group in terms of free testosterone levels ($p > 0.05$).

CONCLUSIONS: Our results showed an increase in 17-OHP levels in FMF patients. These results may indicate that, regardless to the attack period adrenal steroidogenesis could be affected negatively in FMF patients.

Key Words:
Fungal peritonitis, Peritoneal dialysis, Bacterial peritonitis.

Introduction

Familial Mediterranean Fever (FMF) is a systemic relapsing autoinflammatory disease occurring in populations in the Mediterranean basin, mainly in Turks, Levantine Arabs, Sephardic Jews, Druze and Armenians[1,2]. The disease is characterized by recurrent attacks of fever, peritonitis, pleuritis, and arthritis, or erysipelas-like skin lesions[3]. The gene (MEFV) causing the disease maps to the short arm of chromosome 16, encodes a leukocyte- and monocyte-specific inflammatory regulator, and its mutations cause the autoinflammatory phenotype of FMF. The attacks are self-limited, lasting 1-3 days, and between episodes the individual is usually free of symptoms[4]. Acute attacks of FMF are accompanied by elevation of many serum markers of systemic inflammation. In the pathogenesis of the disease, adhesion molecules and cytokines play important roles in the inflammation of the serous membranes[5].

Since the first studies on immunomodulation of the endocrine system in 1970s[6], information has accumulated which demonstrates that the immune system influences the endocrine system and vice versa[7]. The adrenal gland is sensitive to the effects of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6)[8,9]. Because TNF and other cytokines are able to modulate different enzymes of steroid hormone cascades, altered TNF signaling in FMF patients may result in subsequent hormonal changes during the acute attack[10-14]. FMF is an auto inflammatory disease with episodic attacks. Both subclinic and clinically manifested inflam-

Corresponding Author: Köksal Deveci, MD; e-mail: drkdeveci@gmail.com.tr

mation play a key role in pathogenesis of FMF. However, the influence of inflammation on steroid hormone cascade remains unclear.

In the present study, we investigated the levels of serum proinflammatory cytokines, serum free testosterone, and 17-hydroxyprogesterone (17-OHP) in women with FMF to evaluate adrenal steroidogenesis.

Patients and Methods

The study was conducted in Cumhuriyet University Medical School, Departments of Internal Medicine-Rheumatology and Department of Biochemistry during 2010 December-2011 September. All women provided their written consent for the participation in the study which was approved by the local Ethics Committees and was in accordance with the Declaration of Helsinki. Forty-one female patients with FMF and 30 age-matched healthy female volunteers were enrolled into the study. None of the patients with FMF had an immunological or any other rheumatic disease. The clinical diagnosis of FMF was based on the Tel-Hashomer criteria[15].

Attack-free periods (defined as being free of attacks for at least 3 weeks) and acute phases were determined based on clinical (fever, abdominal pain, arthritis) and laboratory findings [high levels of fibrinogen, white blood cell (WBC) counts and erythrocyte sedimentation rate (ESR)]. Twenty out of 41 patients were evaluated only during an attack period, while 21 patients were evaluated only during an attack-free period. All patients were receiving colchicine during blood sampling. All FMF patients and age matched healthy controls (HC) were in age ranging from 25 to 35 years old. The HC group consisted of 30 individuals including women without a history of other potential health problems. Exclusion criteria were as follows: presence of systemic disease, including menstrual dysfunction, chronic renal failure, diabetes mellitus, ischemic heart disease, and malignancy; trauma; heavy exercise; and use of drugs with potential effects on biochemical parameters.

Totally, 8 ml sample of venous blood was taken in the morning before breakfast from each patient who applied during AP or AFP to Internal Medicine and Rheumatology Clinic. The blood samples of patients during FMF attack were obtained after 24-48 hours from the acute attack initiation. Venous blood was taken from HC, as same as in FMF patients. The serum samples were obtained by centrifuging blood samples at 3,000 rpm for 15 min at 4°C. Afterwards, serum samples were stored at –70°C until analysis.

Hs-CRP, ESR, fibrinogen and WBC count were measured on the same day of obtaining venous blood samples. Serum hs-CRP level was determined by the nephelometric method (Beckman Array 360 Protein System, Brea, MN, USA). The ESR was measured by Westergreen method, and ESR within one hour was recorded. Fibrinogen levels were measured by the clotting time method (Beckman Coulter, Inc., Fullerton, CA, USA), and leukocytes were determined with an automatic hematology analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). Free testosterone and 17-OHP levels were measured using an EISA kits (DiaMetra S.r.I., Segrate, Italy). Serum IL-1 beta, IL-18, IL-6 and TNF-alpha levels were measured using an ELISA kits (Boster Biological Technology Co., Ltd., Wuhan, China).

Statistical Analysis

Statistical analysis was performed using a Statistical Package for the Social Sciences (SPSS) 14.0 Package (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as median (min-max). For the tests of normality, we used the Kolmogorov-Smirnov test. Multiple comparisons were performed using the Kruskal-Wallis test. The differences between the two groups were evaluated by the Mann-Whitney U-test. To investigate the relations among the variables, we used the Spearman's rank correlation test. p-values ≤ 0.05 were evaluated as statistically significant.

Results

Some of the demographic features of our patients, the levels of acute-phase reactants and proinflammatory cytokines are presented in Table I. The ESR, hs-CRP and fibrinogen levels were significantly higher in FMF-AP than FMF-AFP and HC groups ($p < 0.001$). Serum IL-1 beta levels were found significantly higher ($p < 0.001$) in FMF-AP group and FMF-AFP comparing to HC group. Serum IL-18 and IL-6 levels were found significantly higher ($p < 0.001$) in FMF-AP group comparing to FMF-AP group and HC group. There was no difference in TNF-alpha levels between FMF-AP group and other two groups.

The 17-OH PRG levels were found significantly high in FMF-AP group and FMF-AFP group

Table I. Demographic features and levels of acute-phase reactants and proinflammatory cytokines of the groups.

	AP-FMF group (n=20) median (min-max)	AFP-FMF group (n=21) median (min-max)	HC group (n=30) median (min-max)
Age (yrs)	28 (19-36)	31 (21-37)	30 (25-35)
Disease duration (yrs)	5.50 (2.20-8.40)	5.60 (1.70-9.20)	NA
Number of attacks per month	1.94 (0.77-3.92)	1.86 (0.80-3.64)	NA
ESR (mm/hr)	25.10 (2-61)[b]	14.70 (2-23)	13.70 (2-21)
hs-CRP (mg/dl)	34.31 (0.81-120.07)[a,b]	2.22 (0.10-14.70)	2.81 (0.27-4.14)
Fibrinogen (mg/dl)	291.80 (220.01-408.11)	279.71 (189.01-331.12)[a]	272.7 (222.07-324.10)
Leukocytes $\times 10^3$ (/mm^3)	9.36 (4.92-15.06)[a,b]	7.80 (4.37-9.66)[a]	7.60 (4.21-9.63)
IL-1 beta (pg/ml)	32.81 (14.33-116.22)[a]	51.03 (13.11-182.81)[a]	18.78 (10.11-25.85)
IL-18 (ng/ml)	1.64 (0.41-3.44)[a,b]	0.66 (0.13-1.74)	0.69 (0.29-1.46)
IL-6 (ng/ml)	0.18 (0.34-1.24)[a,b]	0.06 (0.03-0.19)	0.07 (0.04-0.20)
TNF-alpha (ng/ml)	0.10 (0.06-0.13)	0.10 (0.05-0.18)	0.09 (0.06-0.13)
Free testosterone (pg/ml)	1.46 (0.20-2.90)	1.56 (0.50-3.40)	2.15 (0.50-3.40)
17-0H progesterone (ng/ml)	0.73 (0.10-3.80)[a]	1.20 (0.20-5.20)[a]	0.31 (0.01-1.10)

[a]Significantly different from HC at $p < 0.05$ level; [b]Significantly different from FMF-AFP at $p < 0.05$ level.

comparing to HC group ($p < 0.001$). There was no difference in free testosterone levels between FMF-AP group and other two groups ($p > 0.05$) (Figures 1, 2). In the evaluation of the relationship between the levels of serum proinflammatory cytokines and the levels of 17-OHP and free T, performed by correlation analysis, a positive correlation was found between IL-1 beta and 17-OHP and IL-1 beta ($p = 0.006$; r = 0.486) (Figure 3).

Discussion

To the best of our knowledge this is the first trial in English literature which investigates the levels of serum proinflammatory cytokines, serum free testosterone, and 17- hydroxyprogesterone (17-OHP) in women with FMF to evaluate adrenal steroidogenesis.

FMF is an auto inflammatory disease with episodic attacks[1-3]. Thus, the present study may lead future studies about other auto inflammatory diseases.

The results of this investigation clearly indicate that serum 17-OHP levels were significantly increased in both AP-FMF patients and AFP-FMF patients. Additionally, serum levels of IL-1 beta, IL-6, IL-18, CRP, fibrinogen, WBC, ESR were detected significantly high in AP-FMF patients as expected. Moreover, a positive correlation was found between IL-1 beta and 17-OHP levels, whereas no correlations were

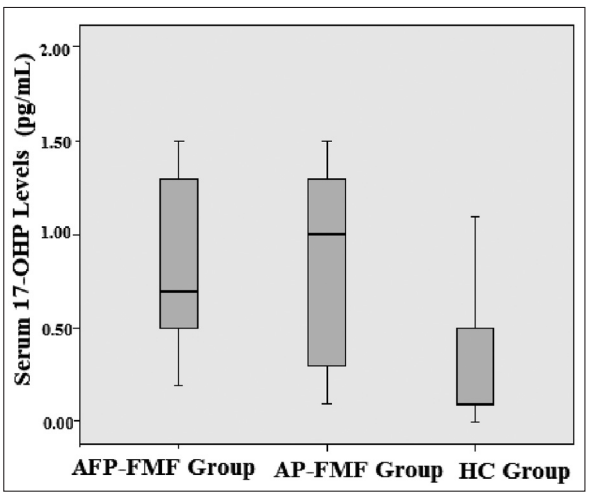

Figure 1. Serum 17-0H Progesterone levels of the groups.

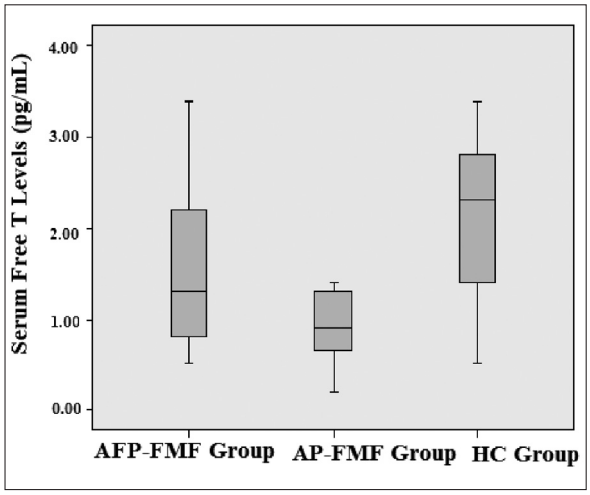

Figure 2. Serum free testosterone levels of the groups.

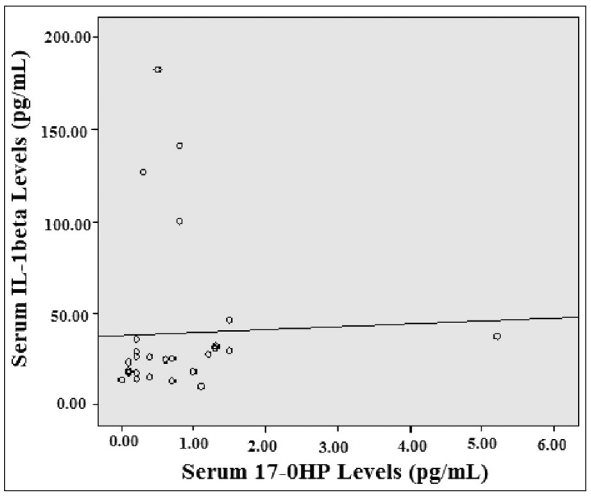

Figure 3. Association serum 17-0H Progesterone levels and serum IL-1 beta levels of the FMF patients (p = 0.006; R = 0.486).

detected between proinflammatory cytokine, acute phase reactants and 17-OHP levels . In a study performed by Straub et al[16] on a female with Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS), another autoinflammatory disease, it was reported that DHEA and 17-beta estradiol levels increased during the attack and there was no significant change in IL-6 and TNF levels. It has been postulated that this may be resulted from hormonal changes due to the local TNF effect in the adrenal glands and ovaries. Straub et al[14] have shown that anti-TNF antibody and IL-6 receptor monoclonal antibodies therapy improves adrenal androgen secretion due, most probably, to increased conversion of 17-hydroxyprogesterone (17-OHP) to androstenedione in patients with rheumatoid arthritis (RA).

The pharmaceutical suppression of endogenous testosterone production was shown to increase the inflammatory response[17]. Testosterone supplementation showed no effect on inflammatory markers including hsCRP, fibrinogen, or interleukin-6[18-20]. A decrease was detected in free T levels in patients with FMF in both attack and attack free periods, but this trend did not reach a statistically significance. This situation may be due to the insufficient number of patients in groups.

IL-1 beta, IL-6 and IL-18 levels were detected high in patients with FMF and its known that these proinflamatuary cytokines are also induce 17-OHP levels via inhibiting 17-hydroxylase and 17/20-lyase enzymes in adrenal steroidogenesis[8,9]. The increased levels of serum 17-OHP in patients with FMF may explained by this mechanism. Additionally, we found no difference between AP-FMF and AFP-FMF groups in terms of serum 17-OHP and free T levels. Also, a relationship was detected between IL-1 beta and 17-OHP levels. However, a similar relationship was not found between other proinflammatory cytokins and 17-OHP levels. Stroup et al[16], advocated that inhibition of adrenal steroidogenesis could be related to the local effect of TNF in patients with TRAPS (TNF-receptor-associated periodic syndrome). The results of this present study show that inhibition of adrenal steroidogenesis in FMF patients may be due to local effects of proinflammatory cytokine in adrenal glands.

Conclusions

Our results showed increased 17-OHP levels which lead us to assume that adrenal steroidogenesis could be affected negatively in both attack and attack free period in FMF patients. Future studies are required including larger group of patients in that subject.

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