Neuroendocrine immune system in familial Mediterranean fever

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Familial Mediterranean fever (FMF) is an autoinflammatory disorder and is characterized by self-limited attacks of inflammation. Although mutations in the gene coding for pyrin are responsible for the inflammation seen in attacks, the question of whether the failure to mount an appropriate cortisol response to inflammation has any additive effects allowed us to plan this study. The aim was to determine the interactions between the neuroendocrine and immune system in patients with FMF and investigate the role of the neuroendocrine system in the acute inflammation process. Demographic characteristics, disease activity, mutation analysis, and duration of the disease were defined in 15 FMF patients (7 female, 8 male; mean age ± SD: 9.1 ± 4.2 years). The diagnosis was based on Tel-Hashomer criteria. Ten healthy volunteers and 21 active juvenile idiopathic arthritis (JIA) patients formed the control groups. Furthermore, 10 of these 15 patients with FMF were also studied during the attack-free period. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, adrenocorticotropic hormone (ACTH), cortisol, insulin-like growth factor-1 (IGF)-1, IGF binding protein (BP)-3, urinary cortisol levels, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were evaluated in FMF patients with attack and during the attack-free period. Although the median levels of ACTH (12.7 pg/ml) and cortisol (12 ug/dl) at 08:00 a.m. were lower in FMF patients during attack than in the attack-free period, these differences did not reach statistical significance. On the other hand, the median levels of ACTH were significantly lower during attack than in the healthy control group ($p<0.05$). Median levels of IGF-1 (118.5 ng/ml) were significantly lower during FMF attack than in the attack-free period ($p<0.05$). There was a negative correlation between IGF-1 and CRP ($r = -0.47$). The median level of IL-6 was 18.1 pg/dl during FMF attack and was significantly higher than in the attack-free period and in the healthy control group ($p<0.05$). There was a negative correlation between cortisol level at 08:00 am and IL-6 ($r = -0.45$). When we compared JIA with FMF patients during attack, inappropriately low secretion of adrenal cortisol and ACTH and low urine cortisol levels were more pronounced in JIA than FMF. Although it is more prominent in chronic inflammation, the neuroendocrine immune system seems to be impaired in relation to acute inflammation in FMF.

Key words: familial Mediterranean fever, cortisol, insulin-like growth factor-1, interleukin-6.

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever accompanied by abdominal, chest or joint pain, myalgia, and erysipelas-like skin lesions. The disease is more common in populations originating from the

Mediterranean region - Jews, Armenians, Turks and Arabs[1,2].

The gene responsible for FMF, MEFV, encodes a protein of 781 amino acids termed pyrin or marenostrin, which is mainly expressed in mature granulocytes and is supposed to play a part in the down regulation of the inflammatory mediators. Mutations in the MEFV gene are thought to lead to uncontrolled neutrophil activation and inflammation[3,4].

However, the trigger that starts the inflammation has not been clarified yet. It has been shown that FMF attacks are accompanied with an outburst of acute phase inflammatory products and cytokines. There are some data about the cytokines in FMF, and the information available in the literature points to activation of the cytokine network[5-7]. On the other hand, in one study, Notarnicola et al.[8] documented enhanced cytokine mRNA levels not during attack but in attack-free patients with FMF. These findings of cytokine regulation support that these patients may have subclinical inflammation between attacks.

Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β and IL-6 are also potent activators of the hypothalamic-pituitary-adrenal axis (HPA axis) through increased production and secretion of corticotrophin releasing hormone (CRH) that leads to increased adrenocorticotropic hormone (ACTH) and cortisol levels. On the other hand, glucocorticoids, end products of HPA axis activation, are the most potent endogenous inhibitors of immune and inflammatory processes, including proinflammatory cytokine production[9,10].

Based on these facts, activation of the HPA axis in inflammatory processes is tentatively expected. In contrast to these expectations, recent reports suggested the presence of impaired HPA axis in juvenile idiopathic arthritis (JIA), which may contribute to the development or persistence of chronic inflammation[9]. Furthermore, there is a possible association of inflammatory cytokines interfering with the synthesis of insulin-like growth factor-1 (IGF-1) and other proteins necessary for growth[9,11].

Growth is also influenced by stress system activation. In prepubertal children, epiphyseal growth of long bones is largely determined by growth hormone (GH). This has some direct effect on target cells, but mainly acts through a mediator, IGF-1. IGF-1 is bound to specific carrier proteins that may affect its availability. These include IGF binding protein-1 (IGFBP-1) and IGFBP-3[12].

We undertook the present study to investigate the HPA axis function in FMF along with IGF-1, IGFBP-3 and urinary cortisol levels in patients with FMF both during the active and remission periods.

Table I. Clinical Characteristics of FMF and Control Groups

Variable	FMF (n:15)	Disease-Control JIA (n:21)	Healthy Control (n:10)
Age (years) Mean ± SD	9.1 ± 4.2	10.5 ± 4.1	9.4 ± 3.5
Sex (F/M)	9/6	11/10	5/5
Height (cm) Mean ± SD	136.7 ± 12.9	137.61 ± 20.7	132 ± 10.4
HSDS* Median (min-max)	0.6 (-1.3-2.1)	0.21 (-1.91-2.18)	0.44 (-0.5-1.4)
Age at diagnosis (years) Mean ± SD	6.5 ± 2.8	7.7 ± 3.9	
Disease duration (months) Median (min-max)	46 (12-10)	34 (6-132)	
Mutational analysis			
M694V / M694V	10 (66%)		
M694V / M680I	3 (20%)		
M694V / E148Q	2 (14%)		

FMF: Familial Mediterranean fever. HSDS: Height standard deviation scores.

Material and Methods

Patients

The study group was composed of 15 patients who were followed in the Pediatric Nephrology and Rheumatology Department of Hacettepe University Faculty of Medicine. Ten age- and sex-matched healthy children and 21 children with JIA served as control groups. Furthermore, 10 of these 15 patients with FMF were also studied during the attack-free period. The diagnosis of FMF was made according to Tel-Hashomer criteria[12].

Active disease was defined by the presence of fever and abdominal pain and/or arthritis and/or pleurisy lasting at least 12 hours and elevated acute phase reactants (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP])[12]. The diagnosis of JIA was made according to the International League against Rheumatism (ILAR) classification criteria[13]. The clinical data including age, gender, history of the disease, age at onset, age at diagnosis, presence of fever, abdominal pain, pleurisy, joint pain, and frequency of attacks were obtained. Standing height was measured by a Harpenden stadiometer. Mean of three measurements was recorded as height. All patients and control groups were prepubertal clinically.

All patients enrolled into the study were seen by the same physician in the active and attack-free periods of the disease. All patients were receiving colchicine at a dose of 1-1.5 mg/day depending on body weight. None of the patients had amyloidosis.

Methods

Blood was drawn after an overnight fast at 08:00 a.m. to analyze the acute phase reactants (ESR, CRP) along with ACTH, cortisol, IGF-1, IGFBP-3, cytokine, and urine cortisol levels both in the active and attack-free periods. ACTH and cortisol levels were also evaluated at 8:00 p.m. Laboratory variables relevant to disease activity were measured by routine methods.

Plasma ACTH levels were measured by chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, USA). Serum cortisol levels were measured by competitive chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corporation, USA). IGF-1 and IGFBP-3 levels were measured by Coated-Tube Immunoradiometric Assay (DSL-6600 ACTIVE, Diagnostic Systems Laboratories, USA).

Twenty-four hour urinary cortisol was measured by I125 mediated radioimmunoassay (RIA) in accordance with the manufacturer's instructions, and expressed in mmoles/24 hours.

Aliquots of serum samples for cytokine level measurements were stored at -80°C until use. IL-1β, TNF- α and IL-6 were measured using commercial enzyme immunoassay (EIA) kits (Accutyte; Cytimmune, USA).

Statistical Analysis

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) 11.0 and expressed as median (minimum-maximum) for data not showing normal distribution and as mean ± standard deviation (SD) for data showing normal distribution. One way ANOVA test was used to compare the data for normal distribution and Kruskal-Wallis test for the data not showing normal distribution. Wilcoxon test was used to compare the data between the active and remission periods. Values of $p<0.05$ were considered statistically significant.

Results

The baseline characteristics of the study population are presented in Table I. There were no significant differences between groups in regards to age and sex. Median ESR (49 mm/hr) and CRP levels (4 mg/dl) were significantly higher in FMF patients during attack than in the attack-free period (11 mm/hr, 0.2 mg/dl) and healthy controls (12 mm/hr, 0.3 mg/dl), respectively ($p<0.05$).

Although the median levels of ACTH (12.7 pg/ml) and cortisol (12.3 ug/dl) at 08:00 a.m. were lower in FMF patients during attack than in the attack-free period (14.05 pg/ml, 12.6 ug/dl), these differences did not reach statistical significance. The median ACTH level was significantly lower in patients during attack than in healthy controls (17.6 pg/ml). There was no statistical difference in median ACTH and cortisol levels at 08:00 p.m. between FMF patients during attack (12.2 pg/ml, 10.4 ug/dl) and in the attack-free period (11.6 pg/ml, 9.5 ug/dl) and in healthy controls (11.1 pg/ml, 8.7

Table II. Median Levels of ACTH, Cortisol and Urinary Cortisol

	FMF attack	FMF attack-free	Healthy control	JIA active
Cortisol at 08:00 a.m. (μg/dl) (min-max)	12.3* (1.2-30.5)	12.6 (6.86-30)	12.7 (7.31-33.4)	9.3* (1.2-20)
ACTH at 08:00 a.m. (pg/ml) (min-max)	12.7** (8.15-32)	14.05 (5.93-22)	17.6** (9.03-26)	12.25 (3.1-31.6)
Urinary cortisol (μg/day) (min-max)	23.3*** (5.75-89.78)	23.96*** (18.5-34)	24,89*** (15.79-43)	15.07*** (4.97-46.53)

ACTH: Adrenocorticotropic hormone.

*p<0.05 – FMF attack versus JIA active

**p<0.05 – FMF attack versus healthy control

***p<0.05 – JIA active versus FMF attack, healthy control and FMF attack-free

ug/dl), respectively. The median level of urinary cortisol was 23.3 μg/day in FMF patients during attack and there was no significant difference compared to the attack-free period (23.96 μg/day) and the healthy control group (24.89 μg/day) (Table II).

There was no statistical difference in height and height deviation standard score between FMF patients and healthy controls (p>0.05). The median level of IGF-1 (118.5 ng/ml) was significantly lower in FMF patients during attack than in the attack-free period (265.9 ng/ml) (p<0.05) (Table III). There was a negative correlation between IGF-1 and CRP (r = -0.47, p=0.025) and IGFBP-3 and CRP (r = -0.571, p=0.026) (Figs. 1, 2).

The median level of IL-6 was 18.1 pg/dl during FMF attack, and this was significantly higher than in the attack-free period and the healthy control group (p<0.05). Furthermore, there was a negative correlation between serum cortisol levels at 08:00 a.m. and IL-6 (r = -0.45, p=0.0024) (Fig. 3).

With regards to comparing FMF patients with the control group with JIA, the median levels of morning cortisol (9.3 ug/dl) and urinary cortisol (15.07 μg/day) were significantly lower in active JIA patients than in the FMF patients with attack (p<0.05) (Table II).

Discussion

Familial Mediterranean Fever (FMF) is a periodic inflammatory disease characterized

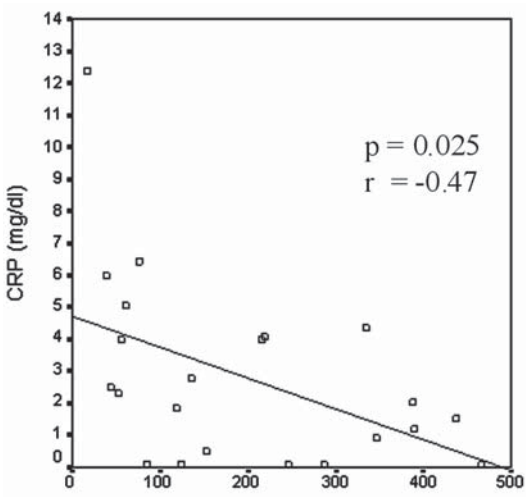

Fig.1. Correlation between CRP and IGF-1 in FMF patients with attack.

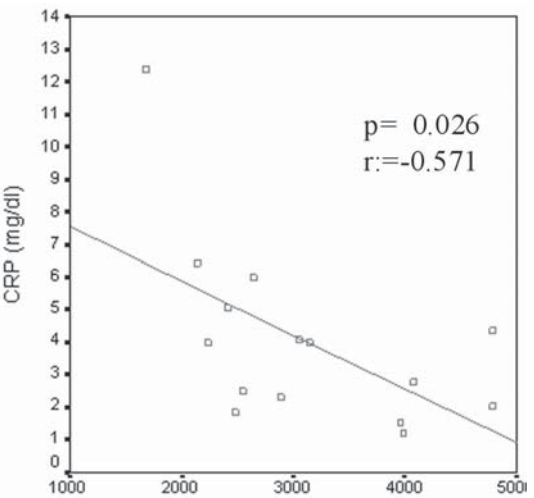

Fig.2. Correlation between CRP and IGFBP-3 in FMF patients with attack.

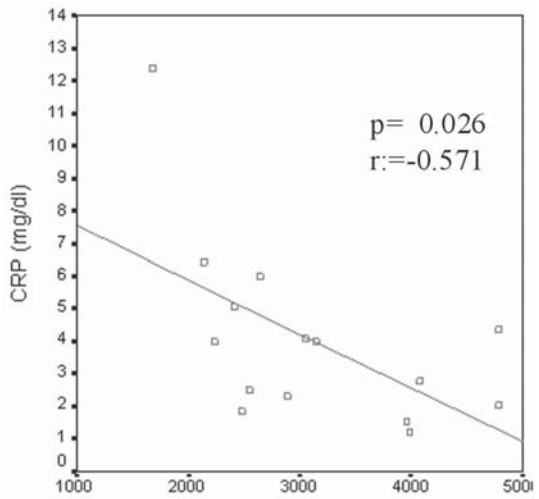

Fig. 3. Correlation between IL-6 and basal cortisol levels in FMF patients with attack.

by a very intense acute phase response. Many studies have been carried out in an attempt to determine the mechanism of the inflammatory attacks in FMF[15,16].

Gang et al. (7) examined the cytokine network during attacks of FMF and found that IL-6 is a major mediator in the pathogenesis of the attacks and that the cytokine network activation occurs during the attack. Similar to these findings, we found in our study high levels of IL-6 in FMF patients compared to the remission period and the healthy control group. IL-6, IL-1β and TNF-α are the proinflammatory cytokines that increase the production of acute phase reactants and also stimulate the CRH secretion. IL-6 can also stimulate the adrenal gland directly[9,17]. Activation of the HPA axis in response to stress results in secretion of glucocorticoids by the adrenals. Glucocorticoids exert powerful anti-inflammatory actions, inhibiting inflammatory mediators including cytokines, phospholipid products, proteases, and oxygen metabolites. Glucocorticoids, while down regulating IL-1, IL-2, IFN-γ, IL-3, and TNF-α, increase the production of IL-4, IL-10 and transforming growth factor (TGF)-β. This is consistent with their imparting a selective bias towards Th2 immune interaction[2,3,18].

The role played by defective neuroendocrine-immune interactions in the pathophysiology of autoimmune inflammatory disorders in children is not well characterized, and only limited data are available. It is difficult to conduct studies in children because hormone profiles change as children grow. Our study is the first conducted to assess the HPA axis in children with FMF. In our study, despite high levels of IL-6, an expected increment in cortisol level at 8:00 a.m. was not observed in FMF patients during attack. This finding may suggest the impairment of the HPA axis in these patients.

There is one study reporting that the HPA axis is activated in an FMF attack in adult patients. In that study, the response of cortisol to 250 μg ACTH stimulation was significant during attack when compared with the attack-free period, and the authors claimed that the HPA axis is stimulated by cytokines and that the axis is regulated normally in FMF attacks[19]. These results are in disagreement with our findings, while on the other hand, the results and the interpretation of the study by Korkmaz et al.[20] are in agreement with our results. Korkmaz et al.[20] showed early blunted cortisol response to insulin-induced hypoglycemia in FMF patients, and they claimed that this may suggest defective behavior of the HPA axis and cortisol response in FMF.

Although it has been reported that GH and IGF-1 levels of FMF patients did not differ from healthy children, patients with inflammatory conditions have unchanged levels of GH and reduced levels of IGF-1[21-23]. It has been shown in both experimental and prospective studies

Table III. Median Levels of IGF-1 and IGFBP-3

	FMF attack	FMF attack-free	Healthy control
IGF-1 (ng/ml) (min-max)	118.5* (17.2-437.4)	265.9* (84.6-466.93)	141.18 (60-481.5)
IGFBP-3 (ng/ml) (min-max)	2882 (1682-4783)	3345 (1700-3837)	2993 (2127-3915)

IGF: Insulin-like growth factor. BP: Binding protein.
*p< 0.05: FMF attack versus FMF attack-free period.

that proinflammatory cytokines, IL-1β, TNF-α and IL-6 overexpression result in reduced IGF-1 and IGFBP-3 levels. There is also evidence that IL-1β stimulates IGFBP-1 protein expression, which will inhibit IGF-1 activity[24]. In our study, we showed significantly lower IGF-1 levels during attack and a negative correlation between CRP and IGF-1 and IGFBP-3 levels. Our study is consistent with the close relation of GH, IGF-1 axis and inflammation.

When we compared JIA with FMF patients during attack, inappropriately low secretion of adrenal cortisol and ACTH and low urinary cortisol were more pronounced in JIA than FMF. Although it is more prominent in chronic inflammation, the neuroendocrine immune system seems to be impaired in relation to acute inflammation in FMF.

There are not enough studies in the literature about the function of the HPA axis in FMF patients for one to obtain genuine data as yet, but the results of the few studies and of ours could lead one to think of the presence of a defective HPA axis in FMF patients. Further studies are necessary to clarify this point.

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