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Ailesel Akdeniz Ateşinde prohepsidin seviyeleri: Demir metabolizmasının rolü var mı?

Prohepcidin levels in Familial Mediterranean Fever: Possible effect of iron metabolism?

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ÖZ

GİRİŞ ve AMAÇ: *Ailesel Akdeniz Ateşi sistemik otoinflamatuar bir hastalıktır. Hepsidin ise demir metabolizamasında rol oynayan inflamasyon ve enfeksiyonda yükselen bir peptiddir. Biz çalışmamızda Ailesel Akdeniz Ateşi ataklarında hepsidin düzeylerini ve hastalık patogenezinde hepsidinin olası rolünü araştırmayı hedefledik.*

YÖNTEM ve GEREÇLER: *Çalışmamıza 42 erkek hasta ve 28 erkek kontrol dahil edildi. Hastaların ataklı ve ataksız dönemlerinden ve kontrol hastalarından prohepsidin, interlökin-6, serum demiri, serum demir bağlama kapasitesi, ferritin, fibrinojen ve eritrosit sedimantasyon oranı değerleri analiz edildi.*

BULGULAR: *Hasta grubunun serum prohepsidin seviyeleri kontrol grubuna göre düşük iken, IL-6 seviyeleri hasta grubunda anlamlı yüksek saptandı ($p<0.05$). Serum prohepsidin ve IL-6 seviyeleri atak periyodunda anlamlı olarak yüksekti ($p<0.01$); atak sonrası dönemde IL-6 anlamlı olarak azalırken prohepsidinde anlamlı azalma gözlenmedi. Prohepsidin ve IL-6 seviyeleri arasında korelasyon saptanmadı.*

TARTIŞMA ve SONUÇ: *Demir metabolizmasının Ailesel Akdeniz Ateşi patogenezinde inflamatuar mekanizmalara katkısal rolü olabilir.*

Anahtar Kelimeler: *Ailesel Akdeniz Ateşi, inflamasyon, prohepsidin*

ABSTRACT

INTRODUCTION: *Familial Mediterranean Fever (FMF) is a systemic autoinflammatory disorder. Hepcidin is an acid peptide mainly involved in iron regulation that increases by inflammation and infection. In this study; we aimed to evaluate the relationship between the hepcidin levels and FMF attacks, and to determine the likely role of hepcidin in the diagnosis of the disease.*

METHODS: *42 male patients and 28 healthy male controls were included in the study. Prohepcidin, interleukin-6 (IL-6), serum iron, serum iron binding capacity, ferritin, fibrinogen and erythrocyte sedimentation rate were analyzed.*

RESULTS: *Prohepcidin levels was significantly lower in patient group than control group, wheras IL-6 levels was significantly higher ($p<0.05$). Serum prohepcidin and IL-6 levels had increased significantly ($p<0.01$) during the attack; however prohepcidin maintained after the attack but serum IL-6 level had significantly decreased. There was no correlation between prohepcidin and IL-6 levels.*

DISCUSSION and CONCLUSION: *Conclusions: Iron metabolism may contribute to the FMF pathogenesis in addition to inflammatory mechanisms.*

Keywords: *Familial Mediterranean Fever, inflammation, prohepcidin*

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INTRODUCTION

Familial Mediterranean Fever (FMF) is a disorder with an autosomal recessive trait, mainly seen in several ethnic groups originating from the Mediterranean area-Sephardic Jews, Armenians, Turks, Arabs, North Africans and less commonly Greeks and Italians. Clinical presentation is characterized by sporadic, paroxysmal attacks of fever, abdominal pain, pleuritic chest pain, arthritis and erysipelas-like skin lesions, lasting one to three days, and then resolving spontaneously. Patients are asymptomatic between the attacks. The diagnosis is usually made upon both the characteristic clinical features and the exclusion of other illnesses. Tel Hashomer's is the most frequently used diagnostic criteria (1,2). The FMF gene MEFV is located on the short arm of the chromosome 16 (3,4). Mutations on the FMF gene supports the diagnosis in a patient with typical clinical findings. Elevation in acute phase reactants such as white blood cells count, erythrocytes sedimentation rate, C-reactive protein, fibrinogen, haptoglobulin, complement components as C3-C4 and serum amyloid A also help for the diagnosis during the attack. Cytokins including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8) were found to be increased in several studies in FMF patients (5,6).

Hepcidin, a 25-amino acid peptide produced by hepatocytes, is involved in the regulation of iron hemostasis. It affects negatively the iron absorption in the small intestine, iron transport across the placenta, and iron release from the macrophages (7). Infection and inflammation prominently stimulates the synthesis of hepcidin (8,9). Among the cytokines, IL-1 and IL-6 strongly induces the production of hepcidin mRNA (9).

In this study; we aimed to evaluate the relationship between the hepcidin levels and FMF attacks, and to determine the likely role of hepcidin in the diagnosis of the disease; by analyzing serum prohepcidin (easily measurable precursor of hepcidin) and inflammatory markers in FMF patients.

MATERIALS AND METHODS

42 male patients with the diagnosis of FMF and 28 male healthy controls were enrolled into the study. All patients were diagnosed as FMF earlier and they were all on the colchicum therapy. Patients with hypertension, diabetes mellitus, chronic inflammatory or autoimmune disease, a body mass index of over 40 kg/m2, previous FMF attack within two weeks earlier and severe psychiatric disorders, and those denying to give informed consent were excluded.

At presentation, venous blood samples were obtained from all cases including control group for the measurements of complete blood count, erythrocyte sedimentation rate, serum iron, serum iron binding capacity, ferritin, fibrinogen, prohepcidin, IL-1 and IL-6. In FMF group, blood samples were obtained during and after the attack periods for subsequent measurements of prohepcidin, IL-1 and IL-6. Blood samples were preserved at -80 °C. Because the small size of hepcidin-25 and its highly cross-linked structure precludes to develop a reliable method of quantification of serum, we measured a high molecular weight precursor-prohepcidin by ELISA kit likewise the majority of the clinical researchers did (10,11).

Findings were evaluated statistically by the software SPSS (Statistical Package for Social Sciences) for Windows 15.0. Arithmetic mean, standart deviaton and Kolmogorov-Smirnov test for normal distribution width were calculated. Serum parameters of the patients measured at the presentation, i.e before the attack period, were used for comparisons with the control group. Independent- samples T test was used for comparison of FMF group and controls whereas paired-sample T test was applied to compare the data before, during and after the attacks. Bivariate two-tailed pearson correlation test was used for correlation analysis. $P<0,05$ was assumed as statistically significant at the confidence interval of 95%. Approvel from local ethic committee was obtained before the study.

RESULTS

42 male patients in the FMF group and 28 male healthy individuals in the control group were evaluated. Mean age was 21.47 ± 1,76 (range 19-27) and 21.71 ± 1.50 (range 20-27) in FMF group and controls, respectively (p>0,05).

Mean serum prohepcidin and IL-6 in control cases and in attack–free FMF patients were summarized in **table 1.** Mean serum prohepcidin level was significantly lower in patient group than control group whereas mean serum IL-6 level was significantly higher in patients than controls (p<0,05).

Table 1. Mean serum prohepcidin and IL-6 in control cases and in attack –free FMF patients

	Attack-free FMF patients	Control group	P
Prohepcidine (ng/ml)	160,71 ± 79,24	275,86 ± 86,67	**0,00001**
IL-6 (pg/dl)	**13,97 ± 11,95**	**6,93 ± 5,7**	**0,016**

Mean serum prohepcidin and IL-6 measurement in patient groups of 'before', 'during' and 'after' the attacks were shown in **figure 1.**

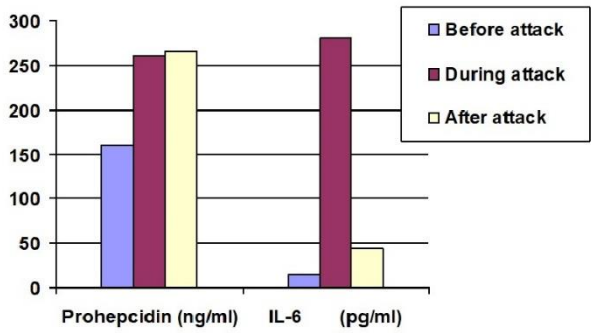

Figure 1. Mean serum prohepcidin and IL-6 measurement in patient group before, during and after the attacks.

Mean serum prohepcidin level was increased from 160,71 ± 79,24 ng/ml to 261,81 ± 68,11 ng/ml (p<0,01) during the attack and maintained approximately the same level (265,0 ± 54,28 ng/ml) after the attack (p>0,05). Mean serum IL-6 level was strikingly increased from 13,97 ± 11,95 pg/ml to 280,38 ± 167,93 pg/ml during the attack when compared to baseline (p<0,01) and showed significant decrease to 42,91 ± 86,26 pg/ml at after the attack period. (p<0,01). Mean serum IL-1 level was decreased from 20,88 ± 7,51 pg/ml at baseline to 17,33 ± 6,94 pg/ml after the attack period (p<0,05). Also there was no significant difference between the attack period and post attack period of IL-1 levels of the FMF patients (p>0,05). There was no correlation between prohepcidin and IL-6 levels before, during and after the attacks. Mean values of serum fibrinogen, ferritin and C-reactive protein levels and sedimentation rate were significantly higher in patient group than controls **(table 2).** On the other hand mean serum iron level was significantly lower in patient group than controls.

Table 2. Mean values of serum iron, fibrinogen, ferritin and C-reactive protein levels and sedimentation rate in attack-free FMF patients and controls.

	Attack-free FMF patients	Control group	P
Iron, µgr/dl	56,86 ± 31,68	85,21 ± 40,77	**0,0032**
Fibrinogen, mg/dl	551,0 ± 170,4	279,07 ± 51,88	**0,00001**
Ferritin, ng/ml	115,93 ± 62,73	85,21 ± 42,6	**0,0268**
C-reactive protein mg/dl	27,12 ± 18,07	2,61 ± 1,33	**0,0002**
Erythrocyte Sedimentation rate mm/hr	**15,52 ± 14,18**	**3,71 ± 3,93**	**0,00001**

DISCUSSION

FMF inflammation is mediated by a massive influx of polymorhponuclear leucocytes into the affected tissues, neutrophilia and a rapid acute phase response (12). The onset of symtoms usually begins before the age of 20 years (13). A typical attack usually lasts 12-96 hours, with the peak intensity occuring within the first 12 hours. The time interval between attacks ranges from days to months or even longer. Patients are generally asymptomatic between attacks but subclinical inflammation may persist (14,15).

The exact pathogenesis of FMF is still unknown. Supposedly, an event which induces a mild inflammation in a normal situtation might cause a severe inflammatory response in FMF patients. The MEFV gene produces a protein called pyrin, expressed mostly in neutrophils in serosal cells (16). The pyrin protein probably act as an inhibitor of a chemotactic factor (C5a, IL-8 or supressor cells) (15).

Several studies suggested that IL-6 levels in FMF patiens was increased during the bouts of serositis, when compared to those during the remission period (17-20). In addition, IL-6 level was found to be more

elevated in attack-free FMF patients than in controls, implying the persistance of subclinical inflammatory process (19,21). Our study showed slight but statistically significant elevation of IL-6 level in patients with FMF remission as compared to healthy controls, indicative of ongoing subclinical inflammation as previous studies suggested. During the period of attacks, IL-6 levels had a steep elevation, suggesting a severe inflammatory response. Our results were in accordance with the studies about IL-6 levels in FMF patients.

The functional protein product of MEFV, pyrin, is not yet fully understood but have a subtle role of leukocyte function. At its N-terminal, the protein include a pyrin death domain (PYD). Proteins with PYDs are involved in modulation of IL-1β production, apoptosis and NF-κB signalling and have been implicated in FMF (22). On the other hand, the most critical and life-threatening complication of FMF is AA amyloidosis. The amyloid fibrils are derived from rupturing fragments of the circulating acute phase reactant, serum amyloid A (SAA) protein, which is synthesized by hepatocytes under the transcriptional regulation of IL-1, IL-6 and TNF (23). In the light of these facts we evaluated IL-1β levels of FMF patients and healty controls, but there was no significant difference between the attack period and post attack period of IL-1 levels of the FMF patients as previous studies (17).

Hepcidin is a peptide isolated from human urine, taking its name based on its site of synthesis (the liver, hep-) and antibacterial properties in vitro (-cidin) (24). Afterwards the same peptide was isolated from plasma ultrafiltrate and named as LEAP-1 (liver expressed antimicrobial peptide (25). Pigeon et al (26) pointed out the connection between hepcidin and iron metabolism, showing hepcidin mRNA induced by not only dietary or parenteral iron overload but also by immune stimuli. Hepcidin was first proposed as an iron regulatory hormone possibly responsible for anemia of inflammation (27). Nemeth et al revealed that hepcidin mRNA production was stimulated by lipopolysaccharides and by monokines from monocytes exposed to lipopolysaccharides. Among cytokines, IL-6, which is produced by macrophages, including Kuppfer cells, was found to strongly induce hepcidin mRNA production (9). It was postulated that, kuppfer cells, or perhaps sinusoidal cells could sense iron and give signals to hepatocytes to regulate hepcidin production (28). In our study, prohepcidin level was significantly increased during the attack period, suggesting an induced inflammatory process as stated previous studies. As opposed to be expected in a subclinical ongoing inflammatory state, we found prohepcidin level to be lower in attack-free FMF patients than in controls, which might partly be explained by significant lower level of serum iron in these patients. Moreover, there as no correlation between serum prohepcidin and IL-6 levels during the attack period although both were increased. After the attack, serum IL-6 decreased significantly while serum prohepcidin remained nearly at the same level. These findings suggest elevation of prohepcidin might be related to mechanisms other than inflammatory process, such as iron metabolism during and after the attacks. We hypothesized the explanation of this result that, in the attack-free period, a trigger factor related to iron metabolism may cause an increase in serum iron levels which may induces not only inflammatory cascade but also hepcidin production. This assumption should be verified by further studies in which serial iron measurements are made before the attacks of FMF patients.

In conclusion, our study indicates prohepcidin level is lower in attack-free period in FMF patients when compared to controls but increases during the attacks. Mechanisms other than inflammatory pathways including iron metabolism may contribute to the elevation of prohepcidin in FMF attacks, but further studies are needed to clarify this theory.

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