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Higher Pentraxin-3 Levels are Associated With Inflammation in Familial Mediterranean Fever

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Background: Circulating levels of Pentraxin-3 (PTX3) have been shown to increase in several inflammatory conditions. However, there is no information about the levels of PTX3 in patients with familial Mediterranean fever (FMF). This study was designed to evaluate the serum PTX3 levels in patients with FMF during attack and free-attack periods. *Methods:* Twenty FMF patients in attack and free-attack period, and 20 age-, sex-, and body mass index-matched healthy controls were included in the study. Blood samples were obtained within the first 24 h of the attack period and between attacks, and levels of white blood cell, erythrocyte sedimentation rate, Fibrinogen, high sensitive CRP, and PTX3 were determined. *Results:* PTX3 levels during the attack period were not significantly different from those in free-attack patients (4.9 ± 4.6 ng/ml vs. 2.8 ± 1.4 ng/ml, $P > 0.05$). However, both attack and free-attack patients had significantly higher PTX3 levels than healthy controls (4.9 ± 4.6 ng/ml vs. 1.8 ± 0.8 ng/ml, $P < 0.001$; 2.8 ± 1.4 ng/ml vs. 1.8 ± 0.8 ng/ml, $P < 0.025$, respectively). *Conclusions:* PTX3 levels were not markedly affected from FMF attacks, but high level of PTX3 in free-attack period of FMF patients shows ongoing subclinical inflammation. However, further studies are needed to determine its usefulness as a marker in clinical practice. J. Clin. Lab. Anal. **30**:978–981, 2016. © 2016 Wiley Periodicals, Inc.

Key words: attack; familial Mediterranean fever; pentraxin-3

INTRODUCTION

Familial Mediterranean fever (FMF), an autosomal recessive disease, is characterized by recurrent aseptic inflammation of serosal membranes. Although the Mediterranean fever (MEFV) gene has been identified, the diagnosis of this disease is predominantly based on clinical parameters (1, 2). Moreover, clinical attacks are accompanied by mild leukocytosis and increased acute-phase reactants such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, serum amyloid A, interleukin (IL)-6, and tumor necrosis factor (TNF) (1–3).

Pentraxin-3 (PTX3) is a multimeric inflammatory mediator secreted by especially leukocytes and endothelial cells. This protein is stored in neutrophils and increased rapidly in response to proinflammatory signals. PTX3 activates the classical pathway of the complement activation, modulates the phagocytic uptake of apoptotic cells by macrophages and dendritic cells, and also inhibits leukocyte recruitment by interacting with P-selectin (4–6).

High PTX3 levels have been shown to be associated with inflammatory conditions and autoimmune disorders in recently published studies (5), however role of PTX3 in the inflammatory response in FMF has not been investigated. This study was designed to evaluate the serum PTX3 levels

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in FMF disease during attack and attack-free periods, and to determine whether PTX3 could contribute to diagnosis of attack in FMF.

MATERIALS AND METHODS

This study was conducted in our outpatient nephrology unit. Forty patients previously diagnosed with FMF, who met the diagnostic criteria suggested by Livneh et al. (7), and 20 healthy volunteers of similar age and gender as the control group were included in the study. All participants were further evaluated in three groups—Group I: attack group ($n = 20$), Group II: attack-free group ($n = 20$), Group III: control ($n = 20$). The study was reviewed and approved by the ethics committee of our hospital, and an inform consent was obtained by all participants. Detailed medical history and physical examination of the participants were recorded. Patients with abnormal hepatic and renal functions, malignancy, rheumatologic disease, acute infection, chronic inflammation, and pregnant or breast-feeding patients were excluded. No drug was used other than colchicine (1–2 mg/day). All patients were investigated in terms of FMF gene mutation.

Blood samples were collected from the attack-free and control groups after 12 h of fasting. On follow-up, blood samples were taken from patients suffering from attack within 24 h after the attack started. All samples were stored at −80°C until assayed. High sensitive CRP (hsCRP) was measured by the immunonephelometric method, and in addition white blood cell (WBC) count, ESR, fibrinogen levels were determined by routine laboratory methods. Serum PTX-3 levels were measured by using a commercially available enzyme-linked immunosorbent assay kit (Human Pentraxin-3/TSG-14 Quantikine, R&D Systems).

All statistical analyses were performed by using the SPSS for Windows, version 15.0 (Chicago, IL). The data were presented as mean ± SD. The frequencies were calculated for each group, and comparisons were made for categorical variables using Chi-square test. Numerical data were compared by using Mann–Whitney U test. Pearson's correlation test was used to evaluate possible correlations between quantitative variables. P value of <0.05 was considered statistically significant.

RESULTS

The demographic and laboratory characteristics of the FMF and control groups are presented in Table 1. Fever (90%) and abdominal pain (80%) were the most common symptoms, followed by arthritis (65%) and chest pain (10%) in FMF attacks. Interestingly, skin lesions were observed in two patients (10%). Among the 40 patients with FMF, MEFV mutations were identified in 35 cases (87.5%) and the most common mutation was M694V (47.5%).

All acute-phase reactants and PTX3 levels were found increased significantly during the FMF attacks compared to the control group (PTX3; 4.9 ± 4.6 vs. 1.8 ± 0.8, $P = 0.001$, Fig. 1). In attack-free period, we found hsCRP, fibrinogen, and PTX3 levels higher than the control group (8.6 ± 10.2 vs. 2.9 ± 3.0, $P = 0.04$; 392.1 ± 78.9 vs. 329.1 ± 72.8, $P = 0.02$; 2.8 ± 1.4 vs.1.8 ± 0.8, $P = 0.025$, respectively, Fig. 1). However, ESR and WBC levels were similar between the attack-free patients and the healthy subjects ($P > 0.05$). The values of hsCRP, fibrinogen, and WBC were significantly higher in the attack group compared to the attack-free group (79.8 ± 54.8 vs. 8.6 ± 10.2, $P = 0.001$; 475.8 ± 118.1 vs. 392.1 ± 78.9, $P = 0.01$; $11.9 \times 10^3 \pm 6.1 \times 10^3$ vs. $7.8 \times 10^3 \pm 2.5 \times 10^3$, $P = 0.007$, respectively). Although serum PTX3 levels were increased to some extent during the attacks, this was not significant statistically (4.9 ± 4.6 vs. 2.8 ± 1.4, $P > 0.05$, Fig. 1). Additionally, there was positive correlation between the serum PTX3 levels and WBC, fibrinogen, hsCRP levels in attack period ($r = 0.628$, $r = 0.515$, $r = 0.504$, respectively, $P < 0.05$). There was no correlation between these parameters in the attack-free and control

TABLE 1. Characteristics of the Study Groups

	Attack group ($n = 20$)	Attack-free group ($n = 20$)	Control group ($n = 20$)
Sex, male/female	8/12	9/11	5/15
Age	29.55 ± 9.0	32.75 ± 10.12	32.85 ± 13.83
BMI (kg/m^2)	23.60 ± 3.53	23.75 ± 3.83	21.83 ± 1.07
WBC (mm^3)	$11.9 \times 10^3 \pm 6.1 \times 10^{3a,b}$	$7.8 \times 10^3 \pm 2.5 \times 10^3$	$7.2 \times 10^3 \pm 1.2 \times 10^3$
ESR (mm/h)	30.8 ± 22.1[a]	18.7 ± 13.7	11.9 ± 10.9
Fibrinojen (mg/dL)	475.8 ± 118.1[a,b]	392.1 ± 78.9[a]	329.1 ± 72.8
hsCRP (mg/dL)	79.8 ± 54.8[a,b]	8.6 ± 10.2[a]	2.9 ± 3.0
PTX3 (ng/ml)	4.9 ± 4.6[a]	2.8 ± 1.4[a]	1.8 ± 0.8

[a] $P < 0.05$ as compared with control.
[b] $P < 0.05$ as compared with FMF attack-free.
BMI, body mass index.

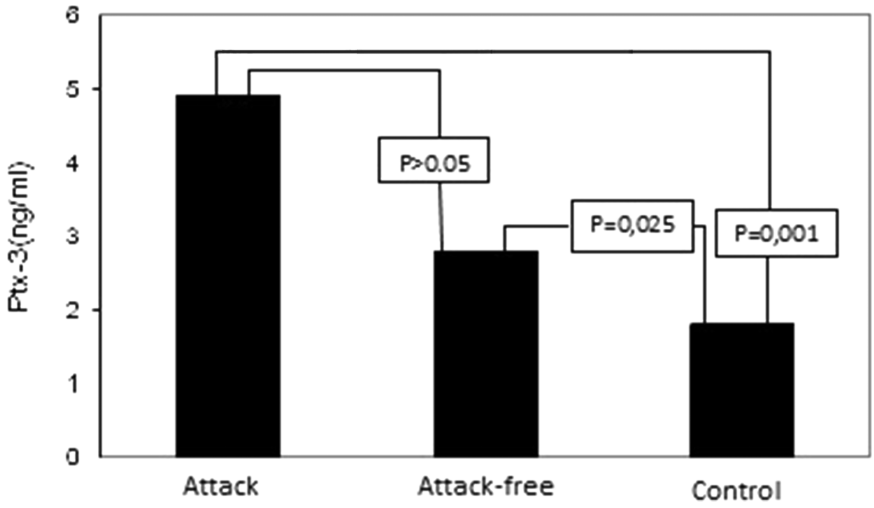

Fig. 1. Levels of PTX3 in patients with FMF and healthy controls.

groups. WBC, hsCRP, fibrinogen, ESR, and PTX3 levels did not show significant differences between the M694V and other mutations in FMF study groups.

DISCUSSION

In the present study, we aimed to evaluate the serum PTX3 levels in FMF disease during the attack and attack-free periods. We found that PTX3 level was significantly higher in both attack and attack-free patients than that of controls; however the increased PTX3 in the attack period was not statistically significant as compared to attack-free periods.

FMF is characterized by periodic febrile episodes and the attack limits itself rapidly. Although the genetic defect is known, the mechanisms of this defect caused by inflammatory attacks are largely unclear. Probably, mutated pyrin leads to uncontrolled inflammation by the production of IL-1 and to the inhibition of apoptosis of leukocytes (2). PTX3 production is induced by IL-1 and TNF-α, and it inhibits the clearance of late apoptotic neutrophils by macrophages. Therefore, it may be a candidate factor for the development of attacks and inhibition of apoptosis (5). However, our study could not indicate statistically significant increase in serum PTX3 levels during attack periods.

Serum PTX3 level increases rapidly in inflammatory conditions such as sepsis, myocardial infarction, and surgery. PTX3 reaches peak values after 6–8 h of any inflammatory condition, and the early phase of its elevation is due to rapid release of stored PTX3 by activated neutrophils (4, 5, 8). In our study, we were able to detect significant increase of serum PTX3 levels in the attack group as compared to the control. Based on these findings, it may be speculated that PTX3 can be considered as an early supporting marker for diagnosis in the inflammatory diseases such as FMF.

Colchicine is used to prevent recurrent attacks of FMF characterized by neutrophil infiltration. In addition, both regression of inflammation and improvement in daily activities are documented after colchicine usage during attack-free period (3, 9). This is probably due to suppressing effect of colchicine on leukocyte degranulation/chemotaxis and production of the inflammatory mediators such as CRP and IL-1, or on activity of the complement cascade (3, 10). In our study, we were unable to identify a significant increase in PTX3 levels during attacks. Therefore, we believe that this might be due to regular use of colchicine in the patients. Prospective studies comparing groups with and without colchicine treatment are necessary in order to determine the role of colchicine, but it is unethical to cease colchicine in these patients.

FMF is characterized by attacks of painful inflammation, but this presentation represents only the tip of the iceberg. In many patients with FMF, inflammation can persist in attack-free periods as shown by high levels of acute-phase proteins and cytokines (3). In our study, PTX3 level was found to be significantly higher in attack-free patients than that of controls. This result suggests that PTX3 may be an indicator of chronic inflammation in FMF patients or its high levels might contribute to subclinical inflammation in these patients.

Recently, various studies have reported the association between cardiovascular events and inflammation. CRP, a prototypic pentraxin, is an independent risk factor for cardiovascular events and atherosclerosis in healthy and high-risk subjects. Moreover, some studies suggest that strong PTX3 expression was found in macrophages and endothelial cells in advanced atherosclerotic lesions (5, 11). In few recent studies, it has been shown that

endothelial dysfunction, increased atherosclerosis, and activation of platelets accompany attack-free periods of FMF, and these conditions are usually characterized by subclinical inflammation with production of excessive acute-phase reactants (11, 12). Both PTX3 and hsCRP levels were elevated in attack-free periods in the present study, therefore higher PTX3 might be a marker for cardiovascular events and increased atherosclerosis in FMF disease. However, further studies are needed to define its role in atherosclerosis.

There are some limitations in our study. First, we had limited number in each group, which limits the power to detect changes in a marker as variable as PTX3. Second, inflammatory markers such as serum amyloid A, IL-1, IL-6, and TNF-α could be added to traditional acute-phase reactants, but they are not available in every laboratory.

In conclusion, PTX3 levels were not obviously affected from acute attacks and could not be used as a supportive marker to differentiate attacks from attack-free periods. However, high level of PTX3 in FMF disease shows ongoing subclinical inflammation. Further studies are needed to determine its usefulness as a marker in clinical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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