The Relationship Among the Level of Serum Amyloid A, High-Density Lipoprotein and Microalbuminuria in Patients With Familial Mediterranean Fever

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Background: Serum amyloid A (SAA), which is produced in the liver, acts as an apoprotein of high-density lipoprotein (HDL) accumulation in extracellular matrix of tissues and organs. SAA elevations play a significant role in the development of amyloidosis. Microalbuminuria (MAU) is the early period of amyloidosis in patients with familial Mediterranean fever (FMF). We assessed the association between SAA as an important factor for the development of amyloidosis in patients with FMF and cytokines, HDL, and MAU. **Methods:** A total of 40 FMF patients diagnosed with Tel-Hashomer criteria and making regular follow-up visits at the tertiary referral center from 2012 to 2013 were included in this study, besides 40 age- and sex-matched individuals as controls. **Results:** Compared with

controls, FMF patients had higher SAA (25.20 ± 45.78 vs. 1.68 ± 0.63 ng/ml; $P = 0.002$). Also, FMF patients had higher MAU than controls (23.20 ± 39.86 vs. 9.40 ± 5.32 mg/day; $P = 0.036$). HDL was significantly lower in the patient group than in controls (39.35 ± 10.45 vs. 47.82 ± 15.31 mg/dl; $P = 0.023$). Interleukin-1 beta (IL-1), IL-6, and tumor necrosis factor alpha (TNF- α) levels were higher in the FMF group than in controls ($P < 0.0001$, $P = 0.009$, $P = 0.003$, respectively). **Conclusions:** Our results suggest that IL-1, IL-6, TNF- α , SAA, and HDL may serve as markers of subclinical inflammation in FMF patients. Due to increased plasma HDL levels, antiinflammatory and antioxidant effects may elevate in FMF patients. *J. Clin. Lab. Anal.* **30**:1003–1008, 2016. © 2016 Wiley Periodicals, Inc.

Key words: familial Mediterranean fever; inflammation; serum amyloid A; high-density lipoprotein; microalbuminuria

INTRODUCTION

Familial Mediterranean fever (FMF) is an autoinflammatory autosomal-recessive disease characterized by recurrent attacks of fever, peritonitis, pleuritis, arthritis, and/or erysipelas-like skin lesions (1–3). The 781 amino acids long Mediterranean fever (*MEFV*) gene, responsible for the pathogenesis of the disease, is found in the short arm of chromosome 16 and codes the pyrin (marennostin) protein (4, 5). The deficiency in the amount or activity of this enzyme results in an inability to suppress and/or inhibit the inflammatory processes, leading to the development of attacks, which generally last between 1 and

3 days and are associated with leukocytosis, and increase in acute-phase reactants such as erythrocyte sedimentation rate, fibrinogen, C-reactive protein (CRP), and serum amyloid A (SAA) (4, 6–8). Development of secondary amyloidosis is the single most important determinant of

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the prognosis in FMF. Elevated SAA plays a significant role in the development of amyloidosis in the presence of chronic inflammation (6, 9, 10).

SAA is an acute-phase reactant with a molecular weight of 12–14 kDa that serves as the apoprotein of high-density lipoprotein (HDL). The plasma concentrations of SAA in acute inflammation may exhibit up to 1,000-fold increase under the effect of interleukin-1 (IL-1) beta, IL-6, and tumor necrosis factor (TNF) alpha (11, 12). In the presence of chronic inflammation, serum SAA elevations play a significant role in the development of amyloidosis. Amyloid fibrils are formed by the 76 amino acids (aa) long proteolytic N-terminal fragments produced by the removal of the C-terminal end of SAA. The pathology involved in the development of amyloidosis results from the presence of obstacles that lead to the accumulation of 76-aa intermediate form (7, 8).

In patients with FMF, the accumulation of amyloid A protein formed by the degradation of SAA in the extracellular matrix is responsible for the development of amyloidosis (13). Although multisystemic involvement may occur, renal involvement is more frequent and may manifest itself with early urinary albumin excretion before renal function loss. A urinary albumin excretion of 30 to 299 mg/day is defined as microalbuminuria (MAU), which is an indicator of renal prognosis (13, 14).

Studies examining SAA in patients with FMF suggest that it may be used as a marker of subclinical inflammation. Herein, our objective was to examine the association between SAA as an important factor for the development of amyloidosis in patients with FMF and inflammation or antiinflammatory markers such as HDL, cytokines, and MAU.

MATERIALS AND METHODS

A total of 40 FMF patients diagnosed with Tel-Hashomer criteria (15) and making regular follow-up visits at the Department of Internal Medicine, Medical Faculty of Cumhuriyet University, between April 2012 and April 2013 were included in this study. Also, 40 age- and sex-matched individuals served as controls. The study protocol was approved by the local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. The exclusion criteria included the presence of anemia, acute/chronic infections, diabetes, hypertension, chronic pulmonary disease, chronic liver disease, established malignancy, amyloidosis, and history of additional pharmacotherapy other than colchicine. All patients were attack-free at the time of study inclusion and were regularly being followed up. A period of at least 2 weeks from the termination of an FMF attack was described as attack-free period on the basis of the results of physical

examination; clinical symptoms; and acute-phase proteins such as CRP, fibrinogen, and leucocyte count.

Biochemical Analyses

Eight milliliters of fasting venous blood sample was obtained on the morning of analysis from all participants. The sera were separated by centrifugation of blood samples at $3,000 \times g$ for 15 min at 4°C. Afterward, serum samples were stored at –80°C until the time of analysis. Urinary samples were evaluated in the spot urine. Urinary albumin and creatine levels were estimated spectrophotometrically using a Beckman Coulter LX-20 (Beckman Coulter, Fullerton, CA), and the ratio of urinary albumin to urinary creatinine was calculated. HDL cholesterol, LDL cholesterol, total cholesterol, and triglycerides were measured using commercially available kits (Siemens Advia Chemistry) with a Siemens Advia 2400 auto-analyzer. Serum TNF- α and IL-6 levels were measured using ELISA kits (Boster Biological Technology, Wuhan, China). IL-1 β (IL-1 β EASIA Kit, DIA source) was measured by the enzyme-linked immunosorbent assay (ELISA) method. SAA levels were estimated using ELISA kits (Chem Well Awareness Technology, INC, CUSABIO).

Statistical Analyses

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) 14.0 Package (SPSS, Chicago, IL). Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. Continuous data were expressed as mean $\pm$ SD if not normally distributed and compared using independent samples *t*-test or Mann–Whitney *U* tests. Categorical variables were expressed as percentages and analyzed with the chi-square test. Relationships between variables were tested using Pearson's correlation analysis. Receiver operating characteristic (ROC) curve graphics were used in the comparison of sensitivity and specificity. *P*-values less than 0.05 were regarded as significant.

RESULTS

Of the study participants with FMF, 11 (27.5%) were male and 29 (72.5%) were female with a mean age of 30.2 ± 11.2 . Among the controls, 12 (30%) were male and 28 (70%) were female, with a mean age of 32.4 ± 9.8 . The two groups were not significantly different in terms of age and gender (*P* = NS and *P* = NS). The clinical characteristics, findings, and *MEFV* mutations in FMF patients are summarized in Table 1.

Compared with controls, FMF patients had higher SAA (25.20 ± 45.78 vs. 1.68 ± 0.63 ng/ml, respectively;

TABLE 1. Baseline Characteristics and *MEFV* Gene Mutations of Patients With FMF

	FMF patients (<i>n</i> = 40)
Age at diagnosis (years) ^a	24.2 ± 12.8
Duration of illness (years) ^a	5.9 ± 6.7
Dose of colchicine (mg/day) ^a	1.27 ± 0.46
Family history of FMF (%)	20 (50)
M694V/M694V <i>n</i> (%)	3 (7.5)
M694V/M680I <i>n</i> (%)	3 (7.5)
M680I/V726A <i>n</i> (%)	2 (5)
M694V/E148Q <i>n</i> (%)	2 (5)
M694V/V726A <i>n</i> (%)	1 (2.5)
E148Q/P369S <i>n</i> (%)	1 (2.5)
E148Q/V726A <i>n</i> (%)	1 (2.5)
E148Q/R761H <i>n</i> (%)	1 (2.5)
M680I/A744S <i>n</i> (%)	1 (2.5)
M694V <i>n</i> (%)	13 (32.5)
M680I <i>n</i> (%)	8 (20)
E148Q <i>n</i> (%)	2 (5)
V726A <i>n</i> (%)	1 (2.5)
No mutation <i>n</i> (%)	1 (2.5)

^aMean ± SD.

$P = 0.002$). Also, FMF patients had higher MAU than controls (23.20 ± 39.86 vs. 9.40 ± 5.32 mg/day; $P = 0.036$). HDL was significantly lower in the patient group than in controls (39.35 ± 10.45 vs. 47.82 ± 15.31 mg/dl, respectively; $P = 0.023$). Main laboratory parameters and proinflammatory cytokine status (IL-1 β , IL-6, TNF- α) of the patients and control subjects are shown in Table 2. Patients with and without MAU did not differ significantly with regard to SAA. No association between SAA and HDL, MAU, and proinflammatory cytokines was detected (Table 3).

As for the ROC curve analysis, the optimum IL-1 β cut-off point for patients with FMF was 22.18, with a sensitivity and specificity of 90 and 90%, respectively (area under curve [AUC]: 0.938, 95% confidence interval [CI]:

TABLE 3. Correlation Between HDL, MAU, and Proinflammatory Cytokines According to SAA

SAA (ng/ml)	<i>R</i>	<i>P</i> -value
IL-1 β (pg/ml)	0.068	0.713
IL-6 (pg/ml)	-0.080	0.626
TNF- α (pg/ml)	-0.193	0.233
HDL (mg/dl)	0.158	0.329
MAU (mg/day)	0.652	0.074

0.880–0.996, $P < 0.001$). In addition, for the optimum IL-6 cut-off point for patients with FMF was 61.50, with a sensitivity and specificity of 62.5 and 67.5 %, respectively (AUC: 0.660, 95% CI: 0.537–0.783, $P = 0.014$). Moreover, for the optimum TNF- α cut-off point for patients with FMF was 88.50, with a sensitivity and specificity of 65 and 62.5%, respectively (AUC: 0.663, 95% CI: 0.545–0.782, $P = 0.012$). Finally, for the optimum SAA cut-off point for patients with FMF was 1.70, with a sensitivity and specificity of 62.5 and 60%, respectively (AUC: 0.677, 95% CI: 0.560–0.794, $P = 0.006$; Fig. 1).

DISCUSSION

In the present study, we demonstrated higher levels of SAA, IL-1 β , IL-6, TNF- α , and MAU in attack-free FMF patients compared with controls. Also, HDL levels were lower in the patient group than in controls.

In patients with FMF, proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α induce the production of acute-phase proteins either directly and/or through their interaction in the presence of systemic inflammation (16, 17). Although acute-phase proteins are normal in approximately 30% of the attack-free FMF patients, inflammation persists, as supported by the results of the studies examining proinflammatory cytokines in this group of

TABLE 2. Baseline Laboratory Characteristics of Patients and Controls

	FMF patients (<i>n</i> = 40)	Controls (<i>n</i> = 40)	<i>P</i> -value
Leucocyte ($\times 10^9$ /l)	7.51 ± 1.88	6.90 ± 1.06	NS
ESR (mm/h)	8.94 ± 5.05	7.02 ± 4.26	NS
CRP (mg/l)	2.07 ± 2.16	2.17 ± 1.52	NS
Total cholesterol (mg/dl)	154.19 ± 50.79	170.55 ± 49.52	NS
Triglycerides (mg/dl)	166.75 ± 102.35	136.44 ± 69.71	NS
Low-density lipoprotein (mg/dl)	81.49 ± 34.07	99.74 ± 38.99	NS
HDL (mg/dl)	39.95 ± 10.45	47.82 ± 15.31	0.023
MAU (mg/day)	23.20 ± 39.86	9.40 ± 5.32	0.036
IL-1 β (pg/ml)	46.86 ± 40.41	17.31 ± 4.13	<0.0001
IL-6 (pg/ml)	78.58 ± 42.22	57.00 ± 27.51	0.009
TNF- α (pg/ml)	103.30 ± 33.72	83.55 ± 22.96	0.003
SAA (ng/ml)	25.50 ± 45.78	1.68 ± 0.63	0.002

All data are mean ± SD.

NS, not significant; ESR, erythrocyte sedimentation rate.

Fig. 1. ROC curve of the IL-1 β , IL-6, TNF- α , and SAA for predicting FMF patients.

patients (16, 18, 19). Among the cytokines, IL-1 β represents the one that is thought to provide strongest contribution to the pathogenesis of FMF. For instance, Yildirim et al. (20) observed higher IL-1 β levels in FMF patients during the attack-free period and suggested that this might be an indicator for the presence of subclinical inflammation. Kiraz et al. (21) also found higher IL-6 and TNF- α among attack-free FMF patients than in controls, concluding that this might reflect the chronic inflammation with a therapeutic effect of colchicine on these parameters. Higher levels of IL-1 β , IL-6, and TNF- α were found in FMF patients by Notarnicola et al. (18) as compared to controls, even during attack-free periods.

SAA is thought to play a significant role in immunomodulation due to its involvement in immunological processes such as the synthesis of cytokines, and stimulation of neutrophils and mast cells (22, 23). In the study by Düzova et al. (22), higher SAA levels were found in patients with homozygous or compound heterozygous mutations than in patients with single heterozygous mutations. Also, Berkun et al. (23) showed a 33% increase in SAA levels among FMF patients. Again in the same study, subjects with homozygous M694V carriers had the highest levels of SAA, those with compound mutations had higher levels as compared to those without mutation or with a single mutation, and a lower degree of elevation was present in FMF patients without mutation.

Lachman et al. (24) pointed out to the elevated SAA in FMF patients between attacks and in asymptomatic carriers of *MEFV* mutations, demonstrating the presence of subclinical inflammation.

An Apo AI mediated effect of circulatory HDL is thought to be responsible for the decrease in the interaction between T lymphocytes and monocytes, leading to a decline in the release of proinflammatory cytokines (IL-1 β , IL-6, TNF- α) (25, 26). HDL-associated Apo AI is a negative acute-phase protein that exhibits a 25% decrease with acute reactions. SAA may replace the position of Apo AI in HDL and has HDL-associated proinflammatory activity. Following this change, the antioxidant, antiinflammatory, and cholesterol-binding capacities of HDL are decreased (26, 27). Ugurlu et al. (28) in a study involving FMF patients found significantly lower levels of HDL in comparison with controls and suggested that this dyslipidemic change may arise from the alterations of the lipid metabolism within the enterohepatic circulation. In another study, Akdogan et al. (29) found significantly lower levels of HDL in FMF patients than in controls.

Amyloid nephropathy is one of the important conditions in FMF patients, which is classified into four stages, that is, preclinical stage, persistent proteinuria, nephrotic stage, and uremic stage (6, 30). The effect of cytokines on SAA and the resultant changes, in conjunction with vascular and glomerular effects, play a significant role on the

development and progression of renal injury (6, 13, 30). TNF leads to direct glomerular injury glomerulosclerosis and fibrosis via monocytes and macrophages, which may ultimately result in albuminuria (31, 32). In inflammation, reduced production of nitric oxide due to the inhibition of nitric oxide synthase causes endothelial dysfunction and impaired vasoreactivity. Ergüven et al. (33) found significantly higher MAU in FMF patients than in controls, suggesting that this parameter may be used as an early marker of renal injury, with a special emphasis on the colchicine dose and treatment adherence in patients receiving treatment.

In our study, attack-free FMF patients had higher IL-1 β , IL-6, TNF- α , and SAA as compared to controls; it might represent early markers of subclinical inflammation. Absence of an association between IL-1 β , IL-6, and TNF- α and SAA was probably due to the half-life of these proinflammatory cytokines. Patients had lower HDL levels than controls, and an increase in HDL might provide certain antiinflammatory and antioxidant effects. Also, MAU was more marked among patients than controls, suggesting that MAU may represent an early marker of renal injury that may be utilized for the monitoring of renal injury.

Limitations of the Present Study

The limitations of our study include the small sample size and absence of patients with amyloidosis. Larger prospective studies with inclusion of patients with amyloidosis will help better define the role of SAA in the development of amyloidosis and its utility in the therapeutic monitorization of patients.

CONCLUSIONS

It appears that IL-1 β , IL-6, TNF- α , and SAA may represent markers of subclinical inflammation in without-amyloidosis and attack-free FMF patients. Decrease in plasma HDL may also provide additional inflammatory and oxidant effects.

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