Increased Asymmetric Dimethylarginine Levels in Young Men with Familial Mediterranean Fever (FMF): Is It Early Evidence of Interaction Between Inflammation and Endothelial Dysfunction in FMF?

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ABSTRACT. ***Objective.*** Unlike in many other chronic inflammatory rheumatic diseases, studies investigating endothelial dysfunction and atherosclerosis in familial Mediterranean fever (FMF) are limited, and the results are controversial. Asymmetric dimethylarginine (ADMA) is considered an indicator for endothelial dysfunction and a sensitive marker for cardiovascular risk. There have been no reports on serum ADMA levels in patients with FMF.

Methods. We aimed (1) to determine serum ADMA concentrations in 38 young male patients with FMF and 23 age- and body mass index-matched healthy volunteers; (2) to evaluate its correlations with *MEFV* mutations, C-reactive protein (CRP) levels, and lipid profile; and (3) to compare effects of colchicine on circulating ADMA concentrations.

Results. In patients with FMF, ADMA and CRP levels were higher than in healthy controls. The mean levels of ADMA and CRP were higher during acute attacks than in attack-free periods. Patients taking colchicine had lower serum ADMA levels than non-colchicine users. There was a positive strong correlation between ADMA and CRP in patients with FMF. Stepwise linear regression analysis in patients with FMF revealed that age and CRP levels were independently associated with serum ADMA levels.

Conclusion. Our data imply that higher serum ADMA levels in FMF may indicate inflammation-related "endothelial dysfunction." It seems likely that regular use of colchicine is effective in preventing the development of and reversing not only amyloidosis but also endothelial dysfunction in patients with FMF. (First Release Sept 1 2008; J Rheumatol 2008;35:2024–9)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER | ASYMMETRIC DIMETHYLARGININE | C-REACTIVE PROTEIN | INFLAMMATION | ENDOTHELIAL DYSFUNCTION | COLCHICINE | PYRIN MUTATIONS

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Familial Mediterranean fever (FMF) is an autosomal recessive inflammatory disorder of unknown etiology primarily found in populations originating from the Mediterranean basin, mostly Turks, Sephardic Jews, Levantine Arabs, Druze, and Armenians[1,2]. 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. *MEFV* codes for a 781-amino acid protein, termed pyrin or marenostrin. Pyrin is an antiinflammatory molecule, and it plays some intrinsic role in regulating leukocyte function[3,4]. The disease is characterized by self-limited acute attacks of fever and neutrophil-mediated recurrent serosal inflammation, and between episodes, the individual is usually free of symptoms. Severe inflammation occurs in FMF attacks, which subside usually in 3–4 days, but sub-

clinical inflammation continues during attack-free periods in patients with FMF even if they were taking regular colchicine treatment[1,4].

Endothelium-derived nitric oxide (NO) is a strong vasodilator that regulates vascular tone[5,6]. NO is not only an endogenous vasodilator but also possesses a variety of antiatherosclerotic biological activities such as regulation of tissue blood flow, and inhibition of platelet aggregation and leukocyte adhesion on the endothelial surface. Therefore, a decrease in NO bioavailability results in endothelial dysfunction[6,7]. Endothelial dysfunction, one of the earliest steps in the development of atherosclerosis, is a term that covers diminished production/availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors[8]. It is now well established that major risk factors for cardiovascular diseases affect endothelial function by decreasing NO bioavailability. In this context, NO is synthesized by stereospecific oxidation of the terminal guanidino-nitrogen of the amino acid l-arginine by a family of NO synthases (NOS)[9]. There are 2 types of NOS in vessels, endothelium derived NO synthase (eNOS) and inducible NO synthase (iNOS); the latter is induced by the inflammatory stimuli in the vascular wall[9]. Asymmetric dimethylarginine (ADMA) is an endogenous competitive NOS inhibitor that inhibits activities of both eNOS and iNOS[10-12].

In the body, ADMA derives from irreversible posttranslational methylation of guanidino-nitrogens of arginine residues. Proteolysis of these proteins containing methylarginine residues leads to the release of ADMA into the plasma[13]. Like NO, ADMA is synthesized and released by endothelial cells. Moreover, since vascular cells are thought to be a major source of ADMA, the doubling of plasma concentrations may reflect an even greater change within endothelial cells[14]. ADMA competitively inhibits NOS, thereby reducing the conversion of arginine to citrulline and limiting NO production[15]. ADMA stimulates many processes involved in atherogenesis such as monocyte adhesiveness[16], expression of proinflammatory and chemotactic factors[17], and accumulation of oxidatively modified low density lipoprotein (LDL) in macrophages[18]. ADMA is metabolized by the enzyme dimethylarginine-dimethylaminohydrolase (DDAH)[19], which degrades them to citruline and dimethylamine or monomethylamine, respectively. Two isoforms exist (DDAH1 and DDAH2), with distinct tissue distribution[20]. Pharmacological inhibition of DDAH increases ADMA concentrations and reduces NO production[14], whereas transgenic DDAH overexpression has the opposite effect[21].

It is widely accepted that ADMA is involved in inflammation-mediated NO production for human vascular diseases[22]. In experimental animals, overexpression of ADMA causes atherosclerosis[23]. Intraarterial infusion of ADMA causes endothelial dysfunction in humans[24]. Moreover, exogenous ADMA can induce apoptosis and inflammatory responses in endothelial cells[25,26]. High concentrations of ADMA are associated with risk factors for atherosclerosis, including chronic renal diseases, polycystic ovary syndrome, hypercholesterolemia, and hypertension[27-29]. It has recently been proposed that patients with ischemic heart disease, kidney dysfunction, and high risk-factor burden exhibit adverse cardiovascular outcomes, at least in part enhanced through ADMA-mediated NO depression[12]. Further, a close association has been found between plasma ADMA level and the severity of peripheral arterial occlusive diseases[30] or carotid intimal–medial thickness[5].

Systemic inflammation is now considered to have an increased risk of early structural vascular alterations and atherosclerosis. As FMF is a relapsing autoinflammatory disease with sustained subclinical inflammatory activity during attack-free periods, the relevance of inflammation as an accelerator of endothelial dysfunction and atherosclerosis in FMF is one of the main topics requiring in-depth clarification. Unlike in many other chronic inflammatory rheumatic diseases, studies investigating endothelial dysfunction and atherosclerosis in FMF are limited, and the results are controversial[31-35]. In contrast to a recent report[32], Akdogan, *et al* reported that common carotid artery intima-media thickness was increased significantly in patients with FMF compared to healthy controls[33]. In patients with FMF, impairment of coronary flow reserve, which reflects coronary microvascular dysfunction as an early manifestation of coronary atherosclerosis, has also been shown[35]. In a more recent report, Yilmaz, *et al* showed that ADMA levels are significantly higher in patients with FMF compared to patients with primary glomerular diseases, both having similar levels of proteinuria[36]. To our knowledge, there have been no reports on serum ADMA levels in uncomplicated patients with FMF. Therefore, we aimed (1) to determine serum ADMA concentrations in 38 young male patients with FMF and 23 age- and body mass index (BMI)-matched healthy volunteer controls; (2) to evaluate its correlations with *MEFV* mutations, C-reactive protein (CRP) levels, and lipid profile; and (3) to compare effects of colchicine on circulating ADMA concentrations.

MATERIALS AND METHODS

Patients. Thirty-eight men with FMF and 23 BMI- and age-matched healthy male controls were enrolled into the study. The clinical diagnosis of FMF was based on the Tel-Hashomer criteria[37]. All patients were evaluated during attack-free periods. In addition, 15 of the 38 patients gave an additional blood sample during an attack period (double donors). Attack-free periods (defined as being free of attacks for at least 3 wks) and acute phases were determined based on clinical (e.g., fever, abdominal pain, arthritis) and laboratory findings [e.g., high levels of fibrinogen, white blood cell (WBC) count, and erythrocyte sedimentation rate (ESR)]. *MEFV* gene mutations were available in only 16 patients with FMF. Serum ADMA levels of patients with active clinical presentations were measured within the first 2 days following the onset of attack.

All patients with FMF, except 10 newly diagnosed, were taking

colchicine. Subjects with a history of cardiovascular disease, hypertension, anemia, malignancies, hypothyroidism, any medication other than colchicine, current smoking, and chronic alcohol intake were excluded. Serum creatinine, hemoglobin, and WBC counts were within the normal limits in attack-free periods. Proteinuric patients were excluded from our study. Clinical and biochemical findings of controls were within normal limits. Each subject gave his informed consent to the study, which was previously approved by our local ethics committee and institutional review board.

Fasting blood samples were collected from patients and controls between 8:00 and 8:30 A.M. after overnight fasting. Blood samples were put on ice. Venous blood samples were centrifuged within 60 min. Plasma was stored at –80°C until measures were studied.

Plasma creatinine, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured on an Olympus AU-800 (Olympus, Tokyo, Japan) autoanalyzer by using its own commercial kits with enzymatic method. LDL-C was calculated by Friedewald's formula as LDL-C = (total cholesterol, mmol/l) – (TG, mmol/l)/2.2 – (HDL-C, mmol/l).

Serum ADMA concentrations were determined by ELISA method (Immundiagnostik AG, Bensheim, Germany). Detection limit of ADMA assay was 0.05 µmol/l.

Serum high-sensitivity CRP levels were measured by Immulite/Immulite 1000 high-sensitivity CRP kit according to solid-phase, chemiluminescent immunometric assay method using Roche Hitachi Cobas 6000 Analyzer (Hitachi High Technologies Corp., Tokyo, Japan).

Statistical analysis. All statistical analyses were performed with the SPSS 15.0 (SPSSFW, SPSS Inc., Chicago, IL, USA) statistical package. Descriptive statistics were given as arithmetic mean ± standard deviation (SD). According to the test results of normality tests (by K-S test), we used independent samples t-test or Mann-Whitney U-test. Correlations among the measures were investigated by Pearson correlation procedure. Stepwise linear regression analysis was used to show the effects of the other measures on ADMA. P values ≤ 0.05 were evaluated as statistically significant.

RESULTS

Comparisons of the clinical and laboratory characteristics of the attack-free patients with FMF and controls are given in Table 1. In patients with FMF, ADMA and CRP levels were higher than healthy controls. There was no difference between patients and controls with respect to age, BMI, and lipid profile.

The mean levels of ADMA and CRP were higher in the acute attack than in the attack-free period (Table 2). In addition, patients taking colchicine (n = 28) had lower serum ADMA levels than those not taking colchicine (n = 10) (0.51 ± 0.05 vs 0.62 ± 0.14 µmol/l; p = 0.008). When we compared all measures between patients with the M694V homozygote mutations and patients with any other heterozygote mutations, only serum ADMA levels were significantly higher in patients with the M694V homozygote mutations than in those with other genotypes (0.70 ± 0.13 vs 0.50 ± 0.06 µmol/l; p = 0.002).

There was a positive strong correlation between ADMA and CRP in patients with FMF (r = 0.736, p < 0.001; Figure 1). Stepwise linear regression analysis in patients with FMF revealed that age (t = 30.128, p < 0.001) and CRP levels (t = 8.887, p < 0.001) were independently associated with serum ADMA levels (adjusted R^2 = 0.955, p < 0.001).

DISCUSSION

Our results demonstrated that circulating levels of ADMA were elevated in young men with FMF in the attack-free period compared with healthy controls. Similarly, Yilmaz, *et al* showed that ADMA levels are significantly higher in patients with FMF compared to patients with primary glomerular diseases, both having similar levels of proteinuria[36]. We also found higher ADMA levels during the acute phase of the disease than in the attack-free period. It is well known that the endogenous NOS inhibitor ADMA is associated with reduced NO production[27]. Interestingly, it has been suggested that the levels of NO in the blood of patients with FMF significantly decreased during both inflammatory attacks and attack-free periods[38]. In this way, as a speculative comment, the reduced NO levels in the study of Panossian, *et al*[38] might be related to increased ADMA concentrations in FMF.

Table 1. Clinical and laboratory characteristics of patients with familial Mediterranean fever (FMF) and controls. Data are mean (± SD).

Characteristics	Patients with FMF, n = 38	Healthy Controls, n = 23	t*	p
Age, yrs	20.45 ± 0.50	20.48 ± 0.51	0.231	NS
Body mass index, kg/m^2	24.24 ± 0.97	24.30 ± 0.93	0.268	NS
Hemoglobin, mg/dl	14.59 ± 0.65	14.63 ± 0.53	0.245	NS
WBC counts mm^3	5855 ± 1825	6145 ± 1956	0.308	NS
Serum creatinine, µmol/l	84.86 ± 16.43	80.75 ± 13.02	0.401	NS
ADMA, µmol/l	0.54 ± 0.10	0.31 ± 0.07	9.365	< 0.001
CRP, mg/dl	4.86 ± 2.22	1.78 ± 0.49	6.523	< 0.001
Total cholesterol, mmol/l	4.02 ± 0.59	4.06 ± 0.59	0.259	NS
Triglyceride, mmol/l	0.88 ± 0.22	0.89 ± 0.20	0.172	NS
HDL-C, mmol/l	1.47 ± 0.25	1.49 ± 0.24	0.278	NS
LDL-C, mmol/l	2.14 ± 0.41	2.15 ± 0.41	0.159	NS

* Independent samples t-test. NS: nonsignificant; WBC: white blood cell; ADMA: asymmetric dimethylarginine; CRP: C-reactive protein; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 2. Comparisons between the attack-free period and attack period of FMF.

Measure	Attack-free Period, n = 15	Attack Period, n = 15	z*	p
ADMA, µmol/l	0.54 ± 0.08	0.68 ± 0.11	4.094	< 0.001
CRP, mg/dl	4.49 ± 1.77	30.56 ± 15.24	4.699	< 0.001

* Mann-Whitney U-test. NS: nonsignificant; ADMA: asymmetric dimethylarginine; CRP: C-reactive protein.

Hypothetically, increased ADMA levels in patients with FMF might be due, in part, to an existing inflammatory process. As DDAH, which degrades ADMA, is inhibited during an inflammatory response[39], this may cause plasma ADMA increments in FMF. In addition, acute inflammation due to attack of the disease is associated with a rise in serum ADMA levels. Such a possibility would also be supported by an observation showing a relationship between inflammation and increased ADMA levels[22]. Consistent with previous reports[40,41], CRP was an independent predictor of ADMA in our study, which indicates an important role of chronic subclinical inflammation on circulating ADMA concentrations. In this perspective, the interplay between inflammation and ADMA is a crucial issue because both factors, ADMA and CRP, have been implicated in endothelial dysfunction and the progression of atherosclerosis[5,42-44]. Meanwhile, although it has been shown that CRP can promote endothelial dysfunction by quenching the production of NO and diminishing its bioactivity by increasing oxidative stress[45], the mechanisms are not completely clear. On the other hand, it is widely accepted that inflammation is an integral feature of atherosclerosis. Determining the presence of any sign of preclinical atherosclerosis was not one of our aims. However, it has been suggested that carotid intima-media thickness values, a sign of preclinical atherosclerosis, in patients with FMF was statistically more significant than in age- and sex-matched healthy controls[45,46]. Moreover, decreased endothelium-dependent flow-mediated dilation of brachial artery, an indicator of endothelial dysfunction, was reported in patients with FMF compared with healthy controls[33]. Taken together, all the above information supports that patients with FMF, even in the younger age group, might have increased risk of endothelial dysfunction.

It is well known that homozygote M694V mutation is associated with a more severe disease course[2,46,47]. Moreover, Grimaldi, *et al*[48] have suggested that the M694V pyrin allele significantly predicted risk of acute myocardial infarction, after adjusting for coronary heart disease risk factors. Further, it has also been proposed that patients with homozygote M694V and homozygote 680I mutations might be at risk for premature development of coronary vascular events[35]. This possibility is in agreement with our findings that patients with homozygote M694V have higher serum ADMA concentrations than those of other genotypes. Other investigators, however, have failed to find differences in these measures in patients with homozygote M694V as compared with other mutation types[34]. In our study, CRP

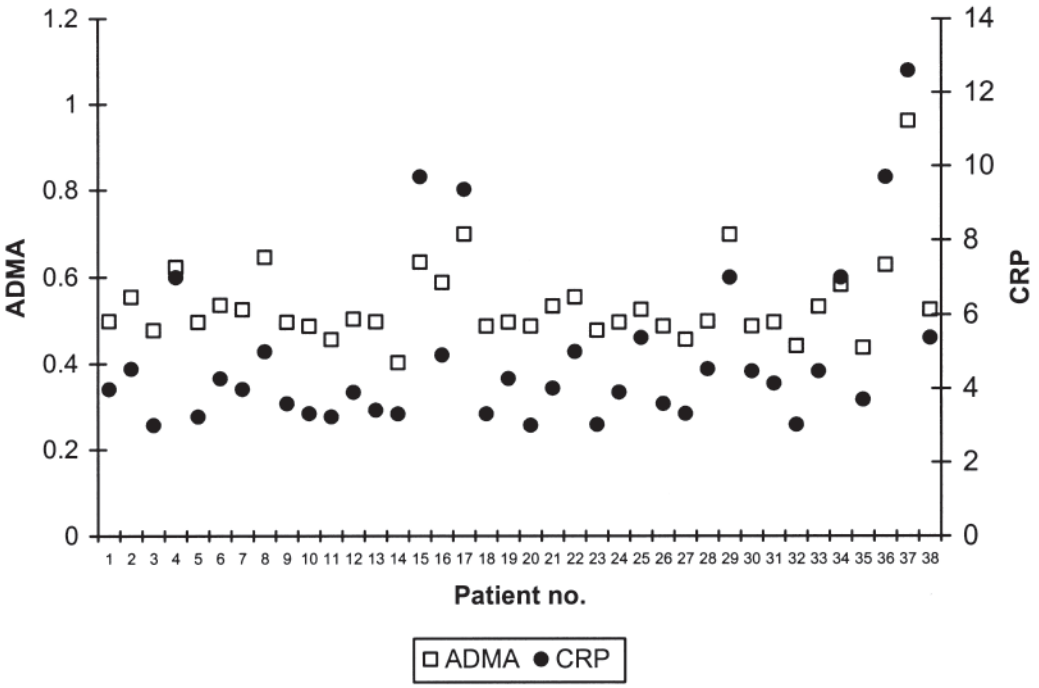

Figure 1. Correlation between asymmetric dimethylarginine (ADMA, µmol/l) and C-reactive protein (CRP, mg/dl) in patients with familial Mediterranean fever.

levels did not differ between the patients with and those without homozygote M694V. Notwithstanding, based on these observations, it is impossible to claim that only subclinical inflammation and related effects play a role in the pathogenesis of endothelial dysfunction only in patients with homozygote M694V. These effects and preventive strategies should be considered for all patients with FMF.

Recently, interactions of ADMA with cytokines have been shown to be important steps in the inflammatory processes of atherosclerosis[49]. It is known that the cytokine network is activated not only during attacks but also during the attack-free period of FMF[1,50-53]. Pyrin is implicated in the homeostatic control of inflammation through leukocyte apoptosis and interleukin 1ß and nuclear factor-κB (NF-κB) pathway activation[54]. Interestingly, in a more recent report, it has been suggested that exogenous ADMA increased the level of tumor necrosis factor-α, concomitantly with activation of NF–κB activity[49]. NF-κB is an important transcription factor involved in the initiation and resolution of inflammation through induction of proinflammatory gene products. Therefore, the authors also speculated that ADMA may have direct proinflammatory effects on cytokine production in monocytes[49]. Further studies are needed to clarify the role of pyrin mutations in the risk of developing endothelial dysfunction and atherosclerosis.

Classically, colchicine is very effective in treating FMF and is unique in preventing the development of amyloidosis[2]. In our study, patients taking colchicine had lower serum ADMA levels than those not taking colchicine. Hence, our findings support the previous opinion that colchicine might have played a role in the preserved endothelial function[32]. In contrast to these observations, Peru, *et al* proposed that regularly taking colchicine may not be sufficient to prevent the development of atherosclerosis[34]. Because CRP levels did not show any difference between the patients treated with colchicine or not, antiinflammatory effects of colchicine cannot be the single cause of this beneficial effect.

Our study has some limitations that have to be taken into consideration. This is a pilot study and provides evidence of association rather than of causation. The lack of assessment of endothelial function by dynamic testing and its correlation with circulating ADMA levels are other limitations of our study. Although we demonstrated a strong association between serum ADMA levels and CRP, it remains unclear whether ADMA is causally related to changes in vascular function or perfusion after changes in inflammatory status, and whether ADMA can serve as a marker of endothelial dysfunction in the presence of inflammation. Another limitation of our study is the small sample size, and a larger prospective study is necessary to confirm these findings. Only male patients with FMF were included; thus, whether these conclusions can be extended to women awaits confirmation in sex-matched studies.

Our data imply that higher serum ADMA levels in FMF may, in theory, indicate inflammation-related endothelial dysfunction. It seems likely that regular use of colchicine is effective in preventing the development of and reversing not only amyloidosis but also endothelial dysfunction in patients with FMF.

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