

## Research Paper

# Increased Levels of Macrophage Migration Inhibitory Factor in Patients with Familial Mediterranean Fever

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Received: 2013.02.21; Accepted: 2013.04.16; Published: 2013.04.30

## Abstract

**Objective:** To determine the level of macrophage migration inhibitory factor (MIF), its relationship with Mediterranean fever (MEFV) gene mutations and oxidative stress in familial Mediterranean fever (FMF).

**Methods:** Fifty one unrelated attack free FMF patients (24 M and 27 F, 32.8±8.7 years) and 30 healthy controls (16 M and 14 F, 32.7±7 years) were included in the study. Serum MIF, total oxidant status (TOS) and total anti-oxidant status (TAS) were studied.

**Results:** Age, sex distribution, anthropometrical indices, smoking status, serum lipids and TAS concentrations were similar between the patients and controls. However; erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), MIF, and TOS were significantly higher in the patients' group compared with healthy subjects. MIF, TOS and TAS levels were not different between patients with or without M694V mutations.

**Conclusion:** We found increased concentrations of MIF in patients with FMF. Increased MIF levels were significantly correlated with oxidative stress and in regression analysis MIF concentrations were independent from the inflammatory activity as assessed by ESR and CRP. M694V mutations seem no effect on MIF and oxidative stress.

Key words: Macrophage migration inhibitory factors; Familial Mediterranean fever; Oxidative stress; Inflammation.

## Introduction

Familial Mediterranean fever (FMF) is an auto-inflammatory disorder characterized by recurrent, inflammatory, self-limited episodes of fever and serositis [1]. The disease is caused by the mutations in the Mediterranean fever (MEFV) gene, which is located on the short arm of chromosome 16 [2]. It is suggested that mutated pyrin, a protein which is encoded by the MEFV gene, may cause uncontrolled inflammation [1]. The inflammatory episodes are mainly mediated by a massive influx of neutrophils into serous cavities and are accompanied by an eleva-

tion in the levels of acute-phase proteins and cytokines [3]. Neutrophils, one of the key players in FMF, are also an important source of free radicals which are implicated in the pathogenesis of various disorders [4]. Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine involved in several inflammatory processes including innate and adaptive immune responses [5, 6]. In addition, MIF has been shown to regulate trafficking of inflammatory cells to the sites of inflammation [6]. Because its association with innate immunity, leukocyte trafficking, and inflamma-

tion MIF may be considered as an attractive cytokine in the pathogenesis of FMF. In this study we aimed to investigate MIF levels, its relationship with M694V mutations and oxidative stress in patients with FMF.

## Methods

### Patients and controls

Fifty one unrelated attack free FMF patients (defined as at least 7 days free of any FMF symptom), diagnosed according to the Tel-Hashomer criteria [7] were recruited consecutively from the rheumatology outpatient clinic. Exclusion criteria were as follows: (1) history of chronic diseases including infections, lung diseases, renal or hepatic insufficiency, and diabetes mellitus, (2) evidence of acute infections at the time of the study, and (3) subjects who were treated with glucocorticoids during the past 3 months. Thirty healthy controls who have the same exclusion criteria with the patients and who don't have a first-degree relative with a diagnosis of FMF were recruited from the relatives of the health professionals and blood donors. Written informed consent was obtained from all subjects, and research protocols were approved by the Ethical Committee of our institution.

### Laboratory analysis

Serum MIF levels were measured by a specific sandwich cytokine ELISA from R&D (Abingdon, UK) specific for MIF. Serum total oxidant status (TOS) and total anti-oxidant status (TAS) were determined using a novel automated measurement method developed by Erel [8, 9]. Other laboratory tests were measured according to standard procedures. MEFV mutations were noted from the files of the patients retrospectively (Table 1).

### Statistical analysis

The Kolmogorov–Smirnov normality test was used to determine the distribution pattern of the variables. TAS, TOS and MIF showed normal distribution, and we used parametric tests for the statistical analysis. Continuous data are presented as mean  $\pm$  standard deviation (SD). Student's t test was used for comparisons between two groups of continuous variables. Fisher's exact test was performed for the comparison of categorical variables. The relationships between different variables were analyzed by the Pearson correlation test. Stepwise multiple linear regression analysis was used to identify factors associated with MIF levels. A double-tailed *P* value of  $< 0.05$  was considered as statistically significant. The statistical analysis was carried out by using Statistical Package of Social Science (SPSS), version 13.0 (Chicago, IL, USA).

**Table 1.** The distribution of MEFV variants in patients with FMF.

	MEFV sequence variations	The number of patients
Homozygous	M694V	9
	M680I	3
	F479L	1
	R761H	1
Heterozygous	M694V	6
	E148Q	1
	M680I	1
	V726A	1
Compound heterozygous	M680I/V726A	1
	M694V/M680I	6
	M694V/M694I	3
	M694V/V726A	8
	M694V/E148Q	2
	M694V/R761H	2
	F479L/V726A	1
Without mutation		5

## Results

There were 51 attack free (at least 7 days free of any FMF symptom) patients in the study (24 M and 27 F,  $32.8 \pm 8.7$  years). The disease duration of these patients were  $16.1 \pm 10.3$  years. None of the patients had proteinuria on urine dipstick testing. All patients were receiving colchicine and the mean drug dose was  $1.46 \pm 0.34$  mg/day.

There were 30 healthy subjects (16 M and 14 F,  $32.7 \pm 7$  years). Age, sex distribution, waist circumference, body mass index, smoking status, serum lipids and TAS were not different between the patient and control groups ( $P > 0.05$ , Table 2). On the other hand, the levels of standard CRP, ESR, MIF, and TOS were significantly higher in FMF patients compared to those of controls ( $P < 0.05$ ;  $4.7 \pm 7.1$  vs.  $1.8 \pm 2$  mg/L,  $15.8 \pm 17$  vs.  $8.3 \pm 5.2$  mm/h,  $30.1 \pm 18.8$  vs.  $9 \pm 4.4$  ng/mL, and  $62.2 \pm 13.4$  vs.  $22.3 \pm 9$   $\mu$ mol H<sub>2</sub>O<sub>2</sub> Eq/L respectively).

### Comparison of patients with and without M694V mutation

The allele frequency of M694V was 45 (44.1%). CRP, ESR, and TAS levels were significantly higher in patients carrying M694V mutations ( $P < 0.05$ ;  $7 \pm 9.5$  vs.  $2.8 \pm 3.3$  mg/L and  $20.7 \pm 22.2$  vs.  $11.9 \pm 9.7$  mm/h respectively). However, MIF, TOS and TAS concentrations were not different between patients with and without M694V mutations ( $P = 0.1$ ,  $33.5 \pm 19$  vs.  $27.6 \pm 18$  ng/mL;  $P = 0.08$ ,  $59.3 \pm 13.4$  vs.  $64.5 \pm 12.9$   $\mu$ mol H<sub>2</sub>O<sub>2</sub> Eq/L;  $P = 0.05$ ,  $1.51 \pm 0.4$  vs.  $1.35 \pm 0.4$   $\mu$ mol Trolox Eq/L respectively).

**Table 2.** Comparison of FMF patients and healthy controls regarding to their clinical and laboratory parameters.

	FMF (n=51)	Controls (n=30)	P value
Age (yr)	32.8±8.7	32.7±7	0.97
Sex (M/F)	24/27	16/14	0.64
BMI (kg/m <sup>2</sup> )	25.2±4.5	25.5±3.9	0.73
Smoking (%)	37.3	30	0.48
Waist circumference (cm)	82.9±12.7	82.8±12.4	0.96
ESR (mm/h)	15.8±17	8.3± 5.2	0.005
Standard CRP (mg/L)	4.7±7.1	1.8±2	0.009
Glucose (mg/dL)	85±7	84±7	0.5
Total cholesterol (mg/dL)	170±31	172±33	0.77
HDL cholesterol (mg/dL)	51±14	53±12	0.58
LDL cholesterol (mg/dL)	96±29	98±25	0.84
Triglyceride (mg/dL)	105±44	93±41	0.22
MIF (ng/mL)	30.1±18.8	9±4.4	<0.001
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eq/L)	62.2±13.4	22.3±9	<0.001
TAS (μmol Trolox Eq/L)	1.42±0.43	1.48±0.41	0.56

BMI= body mass index, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, MIF= macrophage migration inhibitory factor, TOS= serum total oxidant status, and TAS= total anti-oxidant status.

### Correlation analysis

MIF concentrations showed significant correlations with TOS, ESR and triglycerides ( $P < 0.05$ ,  $r = 0.4$ ,  $0.2$ , and  $0.3$  respectively). TAS levels were significantly correlated with BMI, waist circumference, and HDL cholesterol ( $P < 0.05$ ,  $r = 0.3$ ,  $0.4$  and  $-0.3$  respectively).

### Regression analysis

Regression analysis showed that none of the variables including disease duration, CRP, ESR, BMI, TAS, and TOS were predicting MIF concentrations ( $P > 0.05$ ).

### Discussion

In this study we showed that: (1) MIF concentrations were significantly higher in attack-free FMF patients compared to healthy subjects; (2) regression analysis showed that increased MIF levels were statistically independent from inflammatory activity (ESR and CRP); (3) M694V mutations had no impact on MIF concentrations, TOS and TAS levels; and (5) oxidative stress was positively correlated with MIF.

MIF is a pleiotropic pro-inflammatory cytokine which has a central role in both innate and adaptive immunity [10]. It is produced by a variety of cell types including neutrophils which are one of the key cells in the pathogenesis of FMF [11]. MIF has several inflammatory actions: (1) it acts like a positive acute phase reactant and its levels are increased during in-

flammation [5, 10]; and (2) MIF has a chemokine-like function and promotes the directed migration and recruitment of inflammatory cells into infectious and inflammatory sites [10, 12, 13]. It is well-known that mutations in the MEFV gene cause uncontrolled inflammation during acute attacks of FMF which is mainly mediated by neutrophils [1]. Thus, we sought whether there is a link between MIF and FMF. In the current study, we found increased MIF concentrations in FMF patients compared with healthy controls. Regression analysis showed that increased MIF levels were statistically independent from the inflammatory response. In literature, to the best of our knowledge, there is only one report investigated MIF in FMF. In that report Rigante et al. studied 22 patients (5 hyperimmunoglobulinaemia D syndrome and 17 FMF) and revealed that MIF-173\*C allele frequency and serum concentrations were significantly higher in patients compared to healthy controls [14]. In the current study, although we did not assess genomic DNA for MIF, we serologically confirmed the results of the previous study in a larger number of patients.

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and antioxidant systems which results in damage to cells or tissues [4]. In recent years, increasing attention has been focused on the role of ROS in the pathogenesis of inflammatory rheumatic diseases including FMF. Recent reports revealed that there is an increased oxidative stress both in remission and attack periods [15-19]. This finding is not surprising as neutrophils, one of the major sources of free radicals, play an important role in the FMF pathogenesis [19]. In the current study, in accordance with the former reports, we showed that TOS was increased in the remission phase of FMF. On the other hand, TAS was not changed between the patients and control groups. Oxidative stress arises as a result of an imbalance in the oxidative and anti-oxidative status [4]. Hence, it is important to take into account the complex interactions that occur between individual antioxidants in vivo. Therefore, unchanged TAS concentrations may reflect an increased effort of the protective anti-oxidative response. Herein we also showed that TOS was significantly and positively correlated with MIF concentrations. This finding may suggest oxidative stress may have an agonistic effect on MIF.

In this study, as some studies reported a severe clinical course in patients with M694V mutations [20], we performed a subgroup analysis regarding the presence of M694V mutations. In our group the allelic frequency of M694V was 44.1% which was in line with the reports of larger series in our country [20, 21]. The subgroup analysis of patients carrying M694V muta-

tions compared with non-M694V ones revealed that MIF, TOS and TAS levels were comparable between the groups. Thus, larger numbers of subjects are needed to determine whether genotype has an effect on MIF and oxidative stress.

In the current study, we did not include subjects who had chronic diseases such as diabetes mellitus, and hypertension to avoid the negative effects of these diseases on the results. Furthermore, the percentage of smokers and anthropometrical indices such as waist circumference and body mass index measurements were comparable between the groups which may also affect the results. For that reason, the finding of increased MIF and its relation with TOS is therefore noteworthy and supports the role of disease related factors from the condition. We acknowledge the limitations of our study: (1) we studied attack-free FMF patients, thus, including FMF patients with active disease state may also provide information regarding these biomarkers during the acute inflammation; and (2) including newly diagnosed patients who are not treated with colchicine may give additional information regarding colchicine and its effect on these molecules. In conclusion, FMF patients during inactive disease state are associated with increased MIF concentrations, and enhanced oxidative stress. Studies with a larger sample size and active FMF state are needed to confirm our results and the role of these biomarkers in the disease pathogenesis.

## Conflict of interest

There are no conflicts of interest for any of the authors. There were no funding sources or study sponsors for this article.

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