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Evaluation of MIF -173 G/C Polymorphism in Turkish Patients with Ankylosing Spondylitis

Çevik Gürel[1], Ahmet İnanır[2], Ayşe Feyda Nursal[3], Akın Tekcan[4], Aydın Rüstemoğlu[1], Serbülent Yigit[1]

[1]Department of Medical Biology, Gaziosmanpaşa University School of Medicine, Tokat, Turkey
[2]Department of Physical Medicine and Rehabilitation, Gaziosmanpaşa University School of Medicine, Tokat, Turkey
[3]Department of Medical Genetics, Giresun University, School of Medicine, Giresun, Turkey
[4]Ahi Evran University School of Health, Kırşehir, Turkey

Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease mainly affecting the spine and sacroiliac joints. Macrophage migration inhibitory *(MIF)* factor is a regulatory cytokine that inhibits random immune cell migration. MIF gene promoter polymorphisms play a role in the progression of several inflammatory disorders.

Aims: To investigate the relationship between the *MIF gene -173 G/C* single-nucleotide polymorphism (SNP) and AS.

Study Design: Cross-sectional study.

Methods: In this study, a total of 161 AS and 194 normal controls were recruited. The MIF gene -173 G/C SNP was analyzed by polymerase chain reaction using the restriction fragment length polymorphism method.

Results: There was no significant difference between groups in terms of genotype distribution ($p>0.05$). When wild-type G/G and G/C+C/C genotypes are compared in terms of clinical characteristics, there is a significant difference between the average age and the duration of disease in AS patients ($p<0.05$).

Conclusion: No significant relationship between AS disease and *MIF -173 G/C* polymorphism was found. *MIF -173 G/C* polymorphism (C allele) may affect the time of onset and the duration of disease in AS patients.

Keywords: Ankylosing spondylitis, macrophage migration inhibitory factor, polymorphism

Ankylosing spondylitis (AS) is a chronic inflammatory disease that affects the spine and sacroiliac joints. AS is characterized by sacroiliac joint inflammation, peripheral inflammatory arthropathy and a totally lack or low levels of rheumatoid factor (1,2). It influences EXTRA-ARTICULAR organs such as eye, skin and cardiovascular system organs less frequently. The prevalence of AS is about 0.1-1.4% (2). Most patients develop first symptoms in the second or third decade. Males are effected more frequently than females. AS is strongly associated with human leukocyte antigen B27 (HLA-B27) of the Major Histocompatibility Complex (MHC). First-degree relatives of patients with AS have approximately 10 times greater risk than the general population with HLA-B27-positive in terms of AS progression (3). The HLA-B27 occurring in 6 % of the US population but more than 90 % of patients with AS. The prevalence of AS in different populations around the world generally correlates with the prevalence of HLA-B27 (4). Although the environment, genes, gender, age and race may be related to this disease, the etiology and pathogenesis of AS remain unknown.

Cytokines are polypeptides that constitute a class of chemical mediator molecule. Macrophage migration inhibitory factor (*MIF*) is a multipotent molecule that regulates both in inbred and acquired immunity (5). It is known to be secreted T and B lymphocytes, neutrophils, dendritic cells, monocytes, basophiles and mast cells. *MIF* gene is localized on chromo-

Address for Correspondence: Dr.Akın Tekcan, Ahi Evran University School of Health, Kırşehir, Turkey
Phone: +90 505 571 9646 e-mail: akintekcan@hotmail.com
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some 22q11.2 and composed of 3 exons (6). It stated that the altered levels of *MIF* may be play a role in several inflammatory diseases such as rheumatoid arthritis (RA). It is known that the reactive oxygen species (ROS) are effective at the beginning to in inflammatory diseases including AS. Besides, the pro-inflammatory cytokines are responsible for the increased of nitric oxide (NO). *MIF* take a critical role in the progression of some immune system diseases with the production of NO. But it uncertain the associations between the progression of AS and commonly known oxidative stress markers (7). Therefore, we think that it should be investigate whether -173 G>C *MIF* gene polymorphism influence AS pathogenesis. It has been shown that *MIF* take a considerable role in the progression of inflammatory diseases (6). Two polymorphisms have been identified in the *MIF* gene promoter: these a silent polymorphism, *-173G>C* (rs755622), and a nucleotide repeat polymorphism $CATT_{5\text{-}8}$. These polymorphisms are related to the risk and intensity of the inflammatory disease (5). Based on these findings, in this study, we investigated the *MIF* promoter *-173 G/C* polymorphism in Turkish AS patients. Our study is the first study that analyzes *MIF* promoter polymorphism in AS.

MATERIALS AND METHODS

Subjects

Macrophage migration inhibitory gene promoter variation was analyzed in 161 AS patients and 194 healthy controls. Subjects included to the study were greater than 18 years. Patients were diagnosed in Physical Medicine and Rehabilitation clinics at our hospital. All patients fulfilled the New York criteria for AS and also, all participants had taken part in our previous study (3). The majority of AS patients has sacro-iliac involvement. The patients without sacro-iliac symptoms were diagnosed with HLA-B27 positivity and two other symptoms such as hip and other joint involvements. Clinical examinations were performed for all patients. The control group were chosen according to diagnosis criteria of AS. Data collection were performed according to some informations such as age, disease duration, smoking status, exercise habit, peripheral/extraarticular involvement and several clinical characteristics. Written consent was obtained from all patients and controls before the study, according to the ethical guidelines of the 2008 Declaration of Helsinki and the study was accepted by ethical committee of School of Medicine (13-KAEK-178). Both the groups were of white Caucasian origin, from the Blacksea region of Turkey. Bath Ankylosing spondylitis disease activity index (BASDAI) are the most widely used tools for the assessment of AS functional status and activity. The activity status of patients was evaluated by BASDAI (8). Clinical and laboratory findings were compared between the groups.

Genotype determination

DNA was extracted from 2 mL of venous blood according to Genomic DNA isolation kit procedure (Sigma-Aldrich; Taufkirchen, Germany) and stored at -20°C. The *MIF* gene genotyped with the PCR based-Restriction Fragment Length Polymorphism (RFLP) technique. The PCR primers were: sense, 5'-ACT-AAG-AAA-GAC CCG-AGG-C-3'; antisense, 5'-GGG-GCA-CGT-TGG-TGT-TTA-C-3'. These were brought to a final volume of 25 µL (9).

DNA was amplified for 30 cycles with denaturation at 95°C, annealing at 60°C and extension at 72°C for 30 cycles (5 min, 45 min, 45 min, 45 sec) and 72°C as for 7 minutes to finish the reaction. Final PCR products were digested in a 30 µL final volume using reaction buffer (2 µl) and *Alu* enzyme (1 µL) at 37°C 12 hour. The digested fragments were loaded to 2% agarose gel stained with ethidium bromide and visualized using UV transillumination (Quantum-ST4, Vilber Lourmat; Collégien, France).

Statistical analysis

Statistical analyses were performed using SPSS Statistical Package for Social Sciences Version 20.0 (IBM Corp.; Armonk, NY, USA) and the OpenEpi Info software package version 3.01 (10). The data were given as mean±SD (standard deviation) and (min-max). The allele frequencies and genotype distributions analyzed with chi square (x^2) test in patients and control groups. Odds ratio (OR) (OR) and 95% confidence interval (CI) were used for the assessment of risk factors. P values less than 0.05 were considered as significant.

RESULTS

Allele and genotype frequencies of SNPs were calculated and tested for departure from Hardy-Weinberg equilibrium using the Chi-square test. The demographic characteristics of the patients with AS and controls are shown in Table 1. There was no difference between patients and controls in terms of mean age ($p>0.05$). The mean age±standard deviation (SD) was 39.92±8.78 and 39.37±10.37 in in patients and control group, respectively. While gender rate was 84 female (52.2%) and 77 male (47.8%) in patient group, in the control group, it was 111 (56.42%) and 83 (42.8%), respectively. Females constituted the majority of cases in both patient and control groups (52.1% and 57.2%) respectively.

TABLE 1. Baseline demographic characteristics of patients with AS and healthy controls

Individual characteristic	Patients (n=161) (%)	Control (n=194) (%)	p
Average age	37.92±8.78	39.37±10.37	0.162
Gender			
Women	84 (52.2%)	111 (57.2%)	
Man	77 (47.8%)	83 (42.8%)	

TABLE 2. Distribution of MIF gene -173 polymorphism and allele frequencies between AS patients and controls

Genotype	Patients	Control	p
GG	116 (72.0%)	136 (70.1%)	
GC	42 (26.1%)	49 (25.3%)	0.356
CC	3 (1.9%)	9 (4.6%)	
Allel			
G	274 (85.0%)	321 (82.7%)	0.455
C	48 (15.0%)	67 (17.3%)	

TABLE 3. Comparison of some clinical paremeters between patients with GG allele and GC+CC alleles

	GG (n=116)	GC+CC (n=45)	p
Average age (years)	38.83±8.87	35.57±8.18	0.034
Diagnosis age mean (years)	32.62±8.77	30.73±7.73	0.208
Disease duration mean (years)	6.56±5.39	4.80±3.57	0.045
ESR (mm/hour)	16.82±13.64	15.02±13.60	0.443
Schober Test (cm)	4.07±1.58	4.36±1.34	0.290
BASDAI	2.80±1.34	2.53±0.91	0.212

ESR: erythrocyte sedimentation rate; BASDAI: BATH ankylosing spondylitis disease activity index

The distributions of *MIF -173 C/G* allele frequencies in patients with AS and controls are given in Table 2. We found no statistically significant difference in the genotype frequencies of the *MIF* gene polymorphism in patients and control groups ($p>0.05$). The *MIF* gene G allele was 85% in patients and 82.7% in the control group, while the C allele frequency was 15% in patients and 17.3% in the control group. In the combined analysis of the *MIF* gene, the GG/GC/CC combined genotype was no found to increase the risk of AS compared to the controls. It was found a significant difference between AS patients with homozygous G/G genotype and AS patients with heterozygous G/C and homozygous C/C genotype in terms of the average age and disease duration ($p<0.05$). Additionally, there was not any difference on patients in terms of diagnosis age, the results of erythrocyte sedimentation rate (ESR) measurements, Schober test measurements, the results of BASDAI ($p>0.05$) (Table 3). We found no association between *MIF* gene polymorphism and sacroiliac involvement, HLA-B27 positivity, new bone arrangement, ocular involvement, hip involvement, cervical involvement, oral aphthous or familial history ($p>0.05$) (Table 4).

DISCUSSION

Ankylosing spondylitis is associated with chronic inflammation of the sacroiliac and peripheral joints. Although the pathogenesis of AS is not known, genetic factors play a considerable role in its pathogenesis (11). *MIF* is an important cytokine as proinflammatory mediator related in the regulation of immunity. Besides, *MIF* is a key regulator in a variety of biological

TABLE 4. Comparison of disease charecteristics between patients with GG allele and GC+CC alleles

Characteristics	Status	Total n (%)	GG n (%)	GC+CC n (%)	p
Sacro-iliac involvement	Yes	130 (80.7)	94 (72.3)	36 (27.7)	>0.05
	No	31 (19.3)	22 (71.0)	9 (29.0)	
HLA-B27 positivity	Yes	109 (67.7)	38 (73.1)	14 (26.9)	>0.05
	No	52 (32.3)	78 (71.6)	31 (28.4)	
New bone arrangement	Yes	40 (24.9)	29 (72.5)	11 (27.5)	>0.05
	No	121 (75.1)	87 (71.9)	34 (28.1)	
Ocular involvement	Yes	24 (15)	15 (62.5)	9 (37.5)	0.324
	No	137 (85)	101(73.7)	36 (26.3)	
Hip involvement	Yes	51 (31.7)	38 (74.5)	13 (25.5)	0.708
	No	110 (68.3)	78 (70.9)	32 (29.1)	
Cervical involvement	Yes	19 (11.8)	13 (68.4)	6 (31.6)	0.786
	No	142 (88.2)	103 (72.5)	39 (27.5)	
Oral aphthous	Yes	38 (23.6)	27 (71.1)	11 (28.9)	>0.05
	No	123 (76.4)	89 (72.4)	34 (27.6)	
Familial history	Yes	37 (23)	130 (80.7)	29 (78.4)	0.406
	No	124 (77)	31 (19.3)	87 (70.2)	

AS: ankylosing spondylitis; HLA-B27: human leukocyte antigen-B27

functions including proinflammatory actions, immunological reactions and carcinogenesis. *MIF* expression anomalies are effective in the progression of chronic inflammatory diseases. We analyzed whether G/C polymorphism at the *MIF* gene promoter predisposes to AS disease in this paper. Also, this study is the first report to evaluate the *MIF* gene polymorphism among AS patients. Because of its relationship with inflammation, the *MIF* gene and its polymorphisms have been investigated in association with inflammatory rheumatic diseases. The *MIF*-173 C allele is obviously related to the disease as a result of their study that has been conducted with juvenile idiopathic arthritis (JIA) patients (11). The *MIF-173 C* allele affect a potential activator protein 4 (AP-4) that may affect *MIF* expression (12,13).

MIF levels are clearly high in patients with the *MIF* - 173 C allele, so researchers compared levels of the G/C and C alleles in the serum and synovial fluid of JIA patients (14). This research has supported that this can be predictive for the glucocorticoid treatment duration. Liu et al. (15) have examined the relationship between Chinese rheumatoid arthritis (RA) and *MIF*-173 polymorphism. They have not found a significant association between G/G, G/C and C/C genotype distributions whereas they have shown that the risk of RA disease is high in individuals who carry C/C genotype. In another study related to RA patients, it has been stated that C allele is related to the activity of RA disease (16). It was have determined that the *MIF* -173 C is related to inflammatory sensitivity of polyarthritis whereas it is not in association with the intensity of the disease (17). In another study, they have shown that particularly *MIF* -173 C allele leads to increased risk of early onset of RA disease (18). *MIF* gene polymorphism is studied in many different inflammatory diseases. The association between the *MIF -173 C/C* genotype and predisposition to ulcerative colitis was also found on the Chinese people (19). The C/C genotype is frequently seen in patients with pancolitis, although no genotype difference has been found to be associated with ulcerative colitis (20). Przybylowska et al. (21) have shown that there is no difference between patients with respect to their genotypes in inflammatory bowel disease but *MIF* -173 G/C polymorphism C allele leads to increase in the risk of ulcerative colitis disease. HLA-B27 is an important indicator on AS progression (3). The rate of HLA-B27 varies in different studies in Turkey. Gunal et al. (22) and Cinar et al. (23) reported that the proportion of HLA-B27 positivity was 70.0% and 66.7%. Although HLA-B27 rate was lower than expected and determined as 32.3% on patients. Various genetic and environmental factors may contribute to the decreased HLA-B27 frequency observed among Turkish AS patients. Sample size also plays a role (22).

It was determined that *MIF* polymorphisms may vary predictably for certain diseases, especially those related to the immune system. *MIF*-173 C allele and C/C genotype is high in asthmatic children (24). Also, it was reported that *MIF*-173 C gene polymorphisms are positively associated to psoriasis (25). *MIF* gene -173 C allele frequency is high in secondary sarcoidosis erythema nodosum patients (26). Additionally, C allele and C/C genotype are highly seen in systemic lupus patients (27). It was expressed that plasma MIF levels increase in Still's disease patients with C/C genotype (28). Previous studies in children with Henoch-Schönlein purpura disease (29) and cutaneous vasculitis patients have shown that there is no genotype-phenotype association or C allele difference in these patients (30).

In this study, we analyzed the relationship between AS disease, which is an inflammatory disease, and *MIF* polymorphism, which has a role in many inflammatory diseases. We have not determined the allele and genotype differences between AS patients and control group. We found a statistically significant difference in the average age of patients who carry the C allele and in the duration of disease. These findings show that the C allele can lead to early onset disease.

In conclusion, no previous study has examined the relationship between the AS disease and the *MIF* gene *-173 G/C* polymorphism in both Turkish. We have not found a relationship between AS disease and *MIF -173 G/C* polymorphism, but these results could change with further study if more patients and control samples are compared to each other with respect to age and gender. We have shown that the *MIF -173 G/C* polymorphism (C allele) can affect the time of onset and the duration of disease in AS patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziosmanpaşa University School of Medicine (Approval no: 13-KAEK-178).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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