ORIGINAL ARTICLE

Macrophage migration inhibitory factor *(MIF)* gene -173 G>C polymorphism and its relationship to coronary artery disease and type 2 diabetes

Makrofaj migrasyon inhibitör faktör *(MIF)* geni -173 G>C polimorfizminin koroner arter hastalığı ve tip 2 diyabet ile ilişkisi

Neslihan Çoban, PhD.,[1] Aycan Fahri Erkan, M.D.,[2] Berkay Ekici, M.D.,[2] Maide Kaşit,[1] Nihan Erginel Ünaltuna, PhD.,[1] Eren Vurgun M.D.[1]

[1]Department of Genetics, Aziz Sancar Institute For Experimental Medicine, İstanbul University, İstanbul, Turkey
[2]Department of Cardiology, Ufuk University Faculty of Medicine, Ankara, Turkey

ABSTRACT

Objective: Recent studies indicate that macrophage migration inhibitory factor (MIF) is a potent proinflammatory cytokine which mediates the inflammatory process during atherosclerosis. The purpose of the study was to investigate an association between *MIF* gene polymorphism and type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD) in the Turkish population.

Methods: A total of 139 unselected Turkish patients with significant CAD (coronary lesion with 50–100% stenosis) and 120 control participants (coronary lesion with <30% stenosis) were genotyped for *MIF* rs755622 polymorphisms using hybridization probes in a Roche LightCycler 480 Real-Time Polymerase Chain Reaction 480 device. Blood samples were drawn before coronary angiography. Gensini and SYNTAX scores were used to determine the angiographic extent and severity of CAD.

Results: When the groups were stratified according to T2DM, polymorphism of *MIF* was not associated with T2DM in CAD patients (p>0.05). In the same subgroups, carriers of the *MIF* common allele in the control group demonstrated a protection against developing T2DM compared with noncarriers (p<0.05). In addition, *MIF* C allele carriage was associated with higher glycated hemoglobin (HbA1c) in the T2DM group (p=0.038).

Conclusion: The *MIF* rs755622 polymorphism was associated with HbA1c. This result suggests that the *MIF* gene variant may contribute to CAD risk through diabetes in the Turkish population.

ÖZET

Amaç: Yapılan son çalışmalar, makrofaj migrasyon inhibitör faktörünün (MIF) ateroskleroz sırasında enflamatuvar sürece aracılık eden güçlü bir proenflamatuvar sitokin olduğunu göstermektedir. Bu çalışmadaki amacımız, koroner arter hastalığı (KAH) olan ve KAH olmayan bireylerde *MIF* gen polimorfizmi ve tip 2 diyabetes mellitus (T2DM) arasındaki ilişkiyi araştırmaktır.

Yöntemler: Seçilmemiş 259 Türk hasta, anlamlı KAH (%50–100 stenoz bulunan koroner lezyon) ve kontrol (<%30 stenozlu koroner lezyon) grupları, Real-Time PCR LightCycler 480 cihazında hibridizasyon probları kullanılarak *MIF* rs755622 polimorfizmleri için genotiplendi. Koroner anjiyografiden önce kan örnekleri alındı ve sonrasında hesaplanan Gensini ve SYNTAX skorlarına göre KAH anjiyografik yaygınlık ve ciddiyet dereceleri belirlendi.

Bulgular: Hasta ve kontrol grupları T2DM hastalığına göre gruplandırıldığında, *MIF* gen polimorfizminin KAH grubunda T2DM ile ilişkisi bulunmadı (p>0.05). Buna karşılık, aynı alt gruplarda, taşıyıcı olmayanlar ile karşılaştırıldığında, *MIF* geni rs755622 polimorfizmi yaygın allel taşıyıcıları, kontrol grubundaki T2DM hastalığını geliştirmeye karşı bir koruma göstermektedir (p<0.05). Buna ek olarak, T2DM grubunda *MIF* C alel taşıyıcılığının yüksek HbA1c ile ilişkili olduğu belirlendi (p=0.038).

Sonuç: *MIF* geni rs755622 polimorfizmi HbA1c ile ilişkili olarak belirlendi. Bu sonuç, *MIF* gen varyantının, Türk popülasyonunda diyabet yoluyla KAH riskine katkıda bulunabileceğini düşündürmektedir.

Received: July 26, 2018 *Accepted:* September 18, 2018

Correspondence: Dr. Neslihan Çoban. İstanbul Üniversitesi Aziz Sancar Deneysel Tıp Araştırma Enstitüsü, Genetik Anabilim Dalı, Vakıf Gureba Cad., 34280 İstanbul, Turkey
Tel: +90 212 - 414 20 00 e-mail: neslic@istanbul.edu.tr

The macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine of natural immunity. It represents an important component of antimicrobial and stress responses.[1] The *MIF* gene located at the 22q11.2 chromosomal region is a small gene consisting of three exons.[2] *MIF* gene is expressed in many cell types including monocytes, macrophages, vascular smooth muscle cells and cardiomyocytes.[3–5] Clinical studies have shown that MIF protein can be used as a biomarker for different diseases with an inflammatory component.[6] Recruiting cytokines and inflammatory cells, including monocytes and T lymphocytes to the vascular wound site through the action of chemokines, is the initial phase of atherosclerosis.[7]

MIF protein, a strong proinflammatory cytokine, plays an important role in this process.[8] The relevant findings obtained indicate that MIF protein plays a role in the development of early atheromatous plaque and the progression of atherosclerotic lesions.[9] Thus, elevation of MIF protein levels may lead to the development of coronary artery disease (CAD).[8]

A total of four polymorphisms, including single nucleotide polymorphisms (SNP) and -794CATT$_{5-8}$ microsatellite polymorphisms, have been reported in the human *MIF* gene as -173 (rs755622), +254 (rs2096525) and +656 (rs2070766). While rs2096525 and rs2070766 are found in introns, rs755622 and -794CATT$_{5-8}$ are located in the promoter region.[10] The polymorphism at the G-173C point in the promoter region has been associated with an increase in MIF protein production in parallel with the increase in the transcription activity of the *MIF* gene.[10]

In MONICA/KORA study, a relationship between *MIF* gene rs755622 and rs2070766 SNPs and corresponding haplotypes and CAD in women was reported.[11] In addition, it was shown that the polymorphism of the *MIF* gene at the -173 position was statistically correlated with CAD.[12] High levels of MIF protein are expressed in complications of type 2 diabetes mellitus (T2DM) such as myocardial injury,[13] CAD,[14] diabetic retinopathy,[15] obesity[16] and metabolic syndrome.[17] The MIF protein plays an indirect role in the development of T2DM by promoting the production of proinflammatory cytokines and adipocytokines that have a role in insulin receptor signaling which leads to the development of insulin resistance.[18]

Several clinical studies have shown that serum MIF levels increase in T2DM patients.[19] In 2006, it has been suggested that before the onset of T2DM, blood MIF levels elevate.[20] Herder et al.[21] reported a significant increase in circulating levels of MIF, C-reactive protein (CRP) and interleukin-6 (IL-6) in T2DM patients compared to normoglycemic controls. The relationship between T2DM and MIF seems to be stronger than other proteins such as CRP and IL-6.[20]

Abbreviations:

CAD	*Coronary artery disease*
CRP	*C-reactive protein*
GDM	*Gestational diabetes mellitus*
HDL	*High-density lipoprotein*
IGT	*Impaired glucose tolerance*
IL-6	*Interleukin-6*
LDL	*Low-density lipoprotein*
MIF	*Macrophage migration inhibitor factor*
PCR	*Polymerase chain reaction*
RT	*Real-time*
SNP	*Single nucleotide polymorphism*
T2DM	*Type 2 diabetes mellitus*

In a recent large cohort study, increased serum MIF levels were associated with a higher risk of T2DM in women than in men (HR 1.74).[21] Another factor that can explain the variability of *MIF* gene expression in populations is the detection of a series of different *MIF* promoter genotypes.[2,22] The relationship between high plasma levels of MIF protein and long-term adverse outcomes in patients with stable CAD and T2DM has been reported in a prospective case-cohort study performed in a Japanese population.[14]

T2DM disease has been known for many years as an independent risk factor for atherosclerosis. However, in recent years, better understanding of the relationships between T2DM and the cardiovascular system, and in particular based on accumulated epidemiological evidence, atherosclerosis has been referred as a 'cardiovascular disease equivalent' in the risk assessment for T2DM.[23] Starting from this information, we aimed to investigate the contribution of *MIF* gene polymorphism to the coexistence of these two diseases in CAD and T2DM patients.

METHODS

Coronary artery disease and selection of controls

The patients who were detected as having significant CAD based on findings of coronary angiography (50–100% stenotic coronary lesion, n=139) performed according to the clinical findings and results of noninvasive ischemia tests, and as controls 259 individuals with normal coronary arteries or minimal atherosclе-

rotic lesions were included in the study. Blood samples were taken from these individuals for DNA extraction and a CAD DNA bank was established.

Then, 259 individuals were included in the first subgroup according to their diabetes status as those with (n=112) and without T2DM (n=147). In our study, the second subgroup consisted of patients with both CAD and T2DM (n=74) and controls (n=80). The *MIF* gene -173G/C polymorphism was genotyped in all study groups and the *MIF* gene was statistically correlated with the generated groups. Before the study was started, approval was obtained from Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee and all patients whose samples were taken signed informed consent forms.

In this study, DNA bank formed from individuals who participated in the study between 2015 and 2016 was used for the investigation of the association with *MIF* gene polymorphism. Blood samples obtained from atherosclerotic CAD cases and controls were retrieved from T.C. Ufuk University Faculty of Medicine Department of Cardiology. The polymorphism of the *MIF* gene in 259 individuals (mean age; 62.9±10.7, 69.8% male) analyzed in the CAD DNA bank was genotyped in the -173G/C region. Angiographic prevalence and severity were determined by calculating the Gensini and SYNTAX scores of the individuals in the significant CAD group.

The Gensini score has become the gold standard over time, in grading the severity and prevalence of CAD. In this system, the severity of stenosis in the epicardial coronary artery (1: 1–25% stenosis, 2: 26–50% stenosis, 4: 51–75% stenosis, 8: 76–90% stenosis, 16: 91–99% stenosis and 32: total occlusion of the coronary artery) and the appropriate coefficient determined according to the anatomical location of the lesion were multiplied, and all values were summed up to obtain the Gensini score[24] The higher the Gensini score, the more serious and so widespread is CAD.

In patients with significant stenosis of the coronary artery (≥50% stenosis) the SYNTAX score was also calculated to evaluate the degree of complexity of CAD. The SYNTAX scoring system takes into account factors such as the location and distribution of the lesions, the length of the lesion, the presence of tortuosity, bifurcation or trifurcation lesion (if any). SYNTAX score of each 50% stenotic epicardial coronary artery with a diameter of >1.5 mm, was calculated by entering the values into the online SYNTAX calculator (version 2.1 www.syntaxscore.com).

Low (≤22), intermediate (23–32) and high (≥33). SYNTAX scores were defined as indicated. Patients with a SYNTAX score of ≥23 were defined as patients with moderate to severely complex CAD.[25] In addition to the coronary artery stenosis score, presence of family history, smoking, diabetes, and biochemical parameters such as, HbA1c and fasting blood glucose were also taken into consideration in the selection of individuals included in the study.

In the selection of the control group, in addition to the coronary stenosis of ≤30%, lack of diabetes, family history of CAD, cerebrovascular/peripheral artery disease, and the advanced age (≥55 years) were taken into consideration.

Biochemical analyzes

Blood samples were collected before coronary angiographic examination and stored in a freezer at -75°C until analysis. Samples with high lipid content, hemolysis, and those obtained from patients with jaundice were excluded from the study.

Biochemical parameters were analyzed in two central laboratories. Total cholesterol, fasting triglyceride, high density lipoprotein (HDL) -cholesterol and low density lipoprotein (LDL) -cholesterol concentrations were measured with UniCell DxC 800 (Beckman Coulter, USA) device Blood glucose levels were measured by spectrophotometric method using the Hexokinase / G-6-PDH reaction principle in Abbott c8000 Architect autoanalyser (Abbott Lifesciences, USA). HbA1c levels were analyzed using the High Performance Liquid Chromatography (HPLC) system, which is considered as the gold standard. The results were given as the ratio of glycosylated hemogobin to total hemoglobin in percentages (Hb%).

Genotyping with RT-PCR

In this study, a DNA bank containing blood samples of individuals who were diagnosed as CAD or specified as controls based on coronary angiography findings recorded between 2015 and 2016 were used for the investigation of the correlation between CAD, T2DM and *MIF* gene polymorphism. DNA was isolated from 10 mL of peripheral blood samples obtained from individuals included in the study and DNA bank con-

Table 1. Primer-probe sequences used for genotyping

Gene	Primer sequence
MIF-Primer	5′-ggcttcatctctggaagggtaa-3′
	5′-cagcaaccgccgctaagc-3′
MIF-Prob	5′-LCRed-640-ggcggctagaaatcggcctgt-Pho-3’
	5′-gctccaagctgttctccac-Fluo-3′

MIF: Macrophage migration inhibitory factor.

taining samples of 259 individuals was established for the genotyping study. Genotyping was performed in DNA samples of total of 259 subjects in CAD-T2DM and control groups. Genotyping of DNA samples was performed on LightCycler® 480 (Roche, Germany) using labeled hybridization probes by Real-Time PCR method. The hybridization probe-primer sequences used in the study are shown in Table 1.

Statistical analysis

Continuous numerical (quantitative) values were expressed as mean ± standard deviation (SS) and categorical (nominal) values by percentage (%). Categorical variables in the study and control groups were compared with chi-square test and quantitative variables were compared with Student t-test and ANOVA. Univariate analysis or multiple regression analysis were used to determine the relationship between risk variables and cardiovascular diseases. The frequency of each allele was estimated, and the control of the studied population was determined by the Hardy-Weinberg equation for equilibrium and chi-square tests. Significance was evaluated at $p<0.05$. All statistical calculations were performed using the Windows SPSS 21.0 (IBM, USA) program.

RESULTS

The polymorphism of *MIF* genes (mean age; 62.9±10.7, 69.8% male) studied in the promoter region was genotyped using hybridization probes in Light Cycler 480 (Fig. 1).

In our study, clinical conditions and biochemical parameters are summarized in Table 2 according to T2DM in patients with and without significant CAD diagnosis.

MIF rs755622 genotype and allele frequencies

Genetic distributions of *MIF* rs755622 polymorphisms for GG, and GC + CC were found as 66.9% (n=93) and 33.1% (n=46), respectively. The frequency of C allele was found to be 18.7% in the significant CAD group. There was no statistically significant relationship between genotype and allele frequencies in significant CAD and control groups.

Similarly, the distributions of *MIF* rs755622 polymorphisms for GG, and GC+CC genotypes were found to be 65.8% (n=25) and 34.2% (n=13) in the CAD-DM group, respectively. The frequency of C allele was determined as 16.9% in the CAD-T2DM group. Any statistically significant relationship was not observed between genotype and allele frequencies in CAD-T2DM and control groups ($p>0.05$) (Fig. 2).

Table 2. Distribution of biochemical values in T2DM and non-T2DM in study group

	T2DM (n=112)	Non-T2DM (n=147)	*p**
Age (years)	62.8±10.8	59.6±11.7	0.002
Stenosis (%)	56.5±36.4	43.9±38.0	<0.001
Gensini score	41.7±49.1	26.9±42.2	<0.001
SYNTAX score	11.6±12.4	7.76±11.6	<0.001
T-cholesterol (mg/dL)	194.5±46.2	200.9±48.7	0.150
LDL-cholesterol (mg/dL)	111.5±35.1	118.5±37.6	0.040
HDL-cholesterol (mg/dL)	39.2±10.1	42.0±10.9	0.004
Glucose (mg/dL)	150±55.9	98.2±10.6	<0.001
Triglyceride (mg/dL)	154.9±1.73	134.9±1.66	0.004
HbA1c (%)	7.08±1.23	5.50±1.10	<0.001
BKI (kg/m^2)	29.8±4.37	28.1±3.73	<0.001

T2DM: Type 2 diabetes mellitus; apoA1: Apolipoprotein A1; ApoE: Apolipoprotein E; BKI: Body mass index; CRP: C-reactive protein; HbA1c: Hemoglobin a1c, HDL: High density lipoproteins; LDL: Low density lipoproteins; Lp(a): Lipoprotein (a); T-cholesterol: Total-cholesterol.

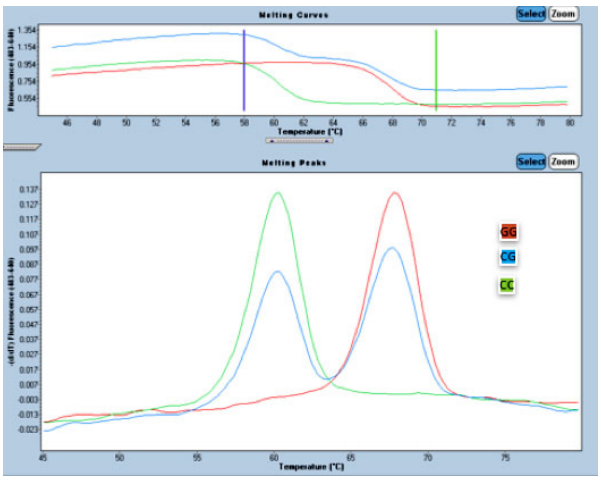

Figure 1. *MIF*-173G/C polymorphism genotypes. Melting curve graphs obtained by hybridization probes specific for the *MIF* rs755622 polymorphism in LC480 device.

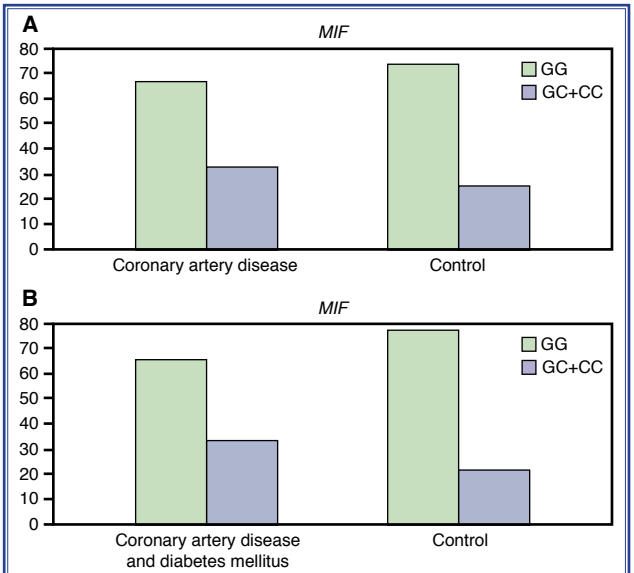

Figure 2. **(A)** Genotype frequencies of *MIF* rs755622 polymorphism in coronary artery disease and control groups. **(B)** Genotype frequencies of *MIF* rs755622 polymorphism in patients with coronary artery disease and also type 2 diabetes mellitus disease and in the control group.

On the other hand, in the same subgroups, the *MIF* common genotype showed a protection against T2DM disease development in the control group compared to those without C allele carriers ($p<0.05$).

The Relationship between *MIF* rs755622 polymorphism and Gensini and SYNTAX scores

There was no statistically significant relationship between angiographic severity and prevalence of CAD (Gensini and SYNTAX scores) and genotypes of *MIF* rs755622 polymorphism ($p>0.05$). There was no statistically significant relationship between angiographic severity and prevalenceof the disease and genotypes of *MIF* rs755622 polymorphism in the T2DM group (Table 3).

The relationship between *MIF* rs755622 polymorphism and cardiometabolic risk factors

In the CAD-T2DM group, C allele carriers of MIF rs755622 showed a statistically borderline relationship with HbA1c (GG: 0.83±0.093, GC+CC: 0.88±0.095, $p=0.07$). Regarding the biometric parameters and cardiovascular status of *MIF* genotypes in groups with and without T2DM independent of CAD group, no significant relationship was found with cardiovascular diseases.

In contrast, HbA1c ($p=0.038$) value was associated with C allele carriage in the T2DM group (Table 3). When the univariant analysis was adjusted for gender and obesity, C allele carriage posed a risk for high HbA1c (Table 4, $p=0.036$). No statistically significant correlation was found between these parameters and *MIF* rs755622 polymorphism in ANOVA and t-test analyzes which were specific for gender ($p>0.05$).

DISCUSSION

When groups with and without type 2 diabetes mellitus were compared C allele carriage was significantly associated with high HbA1c in the TD2M group. According to the results of our study, it is thought that *MIF* gene -173G/C polymorphism may increase the risk of CAD through T2DM in Turkish patients with significant CAD.

Recent studies have shown that MIF protein may play an important role in the development of T1DM and T2DM in glucose homeostasis.[26–28] MIF protein has a role in two different mechanisms of glucose metabolism; Firstly it exerts its effect on the response of insulin target cells and secondly, it directly induces insulin secretion from pancreatic beta cells.[19,29] The circulating level of MIF protein, which plays a pathogenic role in insulin resistance, increases in patients with impaired glucose tolerance (IGT) and in patients with T2DM.[14] In addition, it has been shown that the increase in systemic MIF protein concentrations occurs before the onset of T2DM. This may be related

Table 3. The relation of *MIF* rs755622 polymorphism with cardiometabolic risk factors in T2DM and non-T2DM groups

	Genotypes		*p**
	GG	GC+CC	
T2DM (n=112)			
Age (years)	62.3±11.1	63.4±11.2	0.605
Stenosis (%)	54.8±35.8	50.4±35.2	0.543
Gensini score	52.1±53.9	41.0±55.1	0.313
SYNTAX score	13.5±13.7	10.2±13.0	0.224
T-cholesterol (mg/dL)	194.7±51.0	193.7±40.8	0.917
LDL-cholesterol (mg/dL)	111.5±37.1	114.1±34.9	0.726
HDL-cholesterol (mg/dL)	38.5±11.2	40.6±8.79	0.330
Glucose (mg/dL)	142.2±54.7	147.2±40.6	0.627
Triglyceride (mg/dL)	158.5±1.66	151.3±1.78	0.543
HbA1c (%)	6.76±1.23	7.24±1.23	**0.039**
BKI (kg/m^2)	29.6±4.02	28.3±4.31	0.102
Non-T2DM (n=147)			
Age (years)	60.5±11.1	57.8±11.3	0.185
Stenosis (%)	37.2±33.9	48.3±39.1	0.091
Gensini score	26.5±44.2	40.8±58.0	0.111
Syntax score	6.64±11.8	9.63±13.0	0.182
T-cholesterol (mg/dL)	200.3±50.1	197.6±47.6	0.767
LDL-cholesterol (mg/dL)	118.6±38.8	116.8±35.7	0.802
HDL-cholesterol (mg/dL)	42.2±9.46	40.6±9.74	0.368
Glucose (mg/dL)	98.5±11.0	97.0±11.9	0.472
Triglyceride (mg/dL)	131.8±1.66	147.9±1.70	0.197
HbA1c (%)	5.50±1.10	5.50±1.10	0.492
BKI (kg/m^2)	27.8±3.63	28.5±3.74	0.291

T2DM: Type 2 diabetes mellitus; MIF: Macrophage migration inhibitory factor; GG: Common genotype; GC: Heterozygous genotype; CC: Rare genotype; ApoA1: Apolipoprotein A1; ApoE: Apolipoprotein E; BKI: Body mass index; CRP: C-reactive protein; HbA1c: Hemoglobin a1c; HDL: High density lipoproteins; LDL: Low density lipoproteins; Lp(a): Lipoprotein (a); T-cholesterol: Total cholesterol.

Table 4. The relation between *MIF* rs755622 polymorphism and HbA1c value when adjusted according to obesity, and gender in univariant analysis

	Genotypes		*p**
	GG	GC+CC	
T2DM			
HbA1c (%)	6.76±1.02	7.24±1.02	**0.036**
Non-T2DM			
HbA1c (%)	5.62±1.01	5.50±1.02	0.472

MIF: Macrophage migration inhibitory factor; T2DM: Type 2 diabetes mellitus; HbA1c: Hemoglobin a1c; GG: Common genotype; GC: Heterozygous genotype; CC: Rare genotype.

to the higher incidence of atherosclerotic vascular disease in diabetic patients relative to non-diabetic patients.[20]

Epidemiological and clinical trials have demonstrated the strong association between MIF protein and obesity, insulin resistance, and T2DM development.[30] In a previous study, it was found that serum MIF concentration increased from normoglycemia to impaired glucose intolerance and T2DM disease.[20] Herder et al.[20] reported an increase in circulating CRP, MIF and IL-6 levels in patients with IGT and T2DM compared to normoglycemic controls.

In another study, serum levels of MIF protein were measured in prediabetic patients (HbA1c: 6.0–6.5) and a significant increase was observed compared to normal controls.[31] Similarly, high serum MIF levels were found in American Pima Indians, who had a high incidence of T2DM.[32] In a study by Makino et al.,[14] MIF levels were measured in patients with stable CAD, including patients with IGT and T2DM. The results show that high MIF levels are independent risk factors for future coronary events in the CAD group with IGT/T2DM.

Gestational diabetes mellitus (GDM) is in many respects similar to T2DM disease, and in both cases the main pathophysiological mechanism leads to increased insulin resistance. The frequency of *MIF* rs755622 allele types was found to be statistically different between GDM and control groups. CC and CG genotypes were significantly associated with increased glucose levels and insulin resistance during OGTT in pregnant women with GDM. In conclusion, *MIF* gene rs755622 polymorphism was found to be associated with increased risk of GDM and insulin resistance in Han Chinese women.[33]

MIF protein plays a critical role in the pathogenesis of CAD caused by atherosclerosis. It activates hemorrhagic microvessels in atherosclerosis.[34] During formation and progression of lesions, increased *MIF* gene expression was observed in vascular endothelial cells compared to normal arteries.[35] In a study, it was shown that there was a close relationship between CAD and the polymorphism in the *MIF* gene -173G/C position and the risk of CAD was found to be associated with an increased plasma MIF concentration in MIF -173C allele carriers.[36] When plasma MIF levels were compared in CAD and control groups, it was found that individuals with *MIF* -173C alleles in the CAD group had produced significantly higher levels of MIF protein. It has been suggested that individuals with the *MIF* -173C allele produce higher amounts of MIF protein.[36]

In a population-based cohort study, the relationship between *MIF* genotypes, MIF serum levels and CAD risk was investigated. In women, the C-G-C-T haplotype was found to be significantly associated with increased CAD risk (HR 2.44, 95% CI 1.30–4.59). This haplotype contains rs755622C allele, which was previously associated with other proinflammatory conditions.[11] In another study, this polymorphism was associated with lower HDL-C and higher glucose, LDL-C, TG levels in the CAD group compared to the control group and a significant relationship was also found with the increased prevalence of hypertension and diabetes.[37]

The strong positive correlation between MIF protein concentrations in patients with IGT and T2DM is independent of other immune mediators. In contrast to CRP and IL-6, there was a significant increase in MIF concentrations in the T2DM group compared to the IGT group, suggesting an increase in systemic MIF concentrations before the onset of T2DM.[28] In this study, *MIF* C allele carriage was associated with higher HbA1c levels in T2DM CAD group.

We have previously reported the presence of a correlation between cardiovascular risk factors such as obesity and T2DM and the *MIF* gene polymorphism in the adult Turkish population.[38] The tendency of carriage of the -173C allele to independently predict new-onset diabetes was limited to men.[38] In addition, a significant correlation was found between MIF serum level and diabetes and metabolic syndrome in Turkish Adult Heart Disease and Risk Factors (TARF) study.[39] In our study, we found that Gensini and SYNTAX scores in the T2DM patient group were statistically significant when compared to the group without T2DM. The relationship between T2DM disease and the scores associated with angiographic prevalence and severity of CAD is important for the investigation of the genetic predisposition of diabetes in CAD groups.

One of the main limitations of the study is that the *MIF* gene polymorphism could not be investigated in a larger number of patients. It should be investigated in greater number of groups to allow statistical analyzes according to gender and gender specific findings should be demonstrated. Another limitation of our study was that other SNPs of the *MIF* gene could not be included in the study. In addition, the inability to measure the level of MIF protein is another limitation of the study.

These results suggest that *MIF* gene variant may contribute to CAD risk through T2DM in Turkish population. Considering that T2DM disease is closely related to predisposition to CAD and angiographic severity, the genetic predisposition of the diabetic condition in the CAD group should be studied in detail.

Further study is required with larger sample groups to better identify the role of *MIF* gene polymorphism in the T2DM-CAD group. As a result of these studies, testing of these markers in the *MIF* gene in the follow-up of CAD development will contribute to the early diagnosis and development of gene-specific treatment strategies.

Funding sources

This study was supported by Istanbul University Scientific Research Project Unit Project No: 47372.

Peer-review: Externally peer-reviewed.

Conflict-of-interest: None.

Authorship contributions: Concept: N.Ç.; Design: N.Ç.; Supervision: A.F.E., B.E., N.E.U.; Materials: A.F.E., B.E.; Data: N.Ç., A.F.E., B.E.; Analysis: N.Ç., M.K., E.V.; Literature search: N.Ç., M.K.; Writing: N.Ç.; Critical revision: N.E.U., A.F.E.

REFERENCES

1. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003;3:791–800.
2. Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, et al. A functional promoter polymorphism the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun 2002;3:170–6.
3. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med 1994;179:1895–902.
4. Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S, et al. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. Circulation 2002;105:1561–6.
5. Willis MS, Carlson DL, Dimaio JM, White MD, White DJ, Adams GA 4th, et al. Macrophage migration inhibitory factor mediates late cardiac dysfunction after burn injury. Am J Physiol Heart Circ Physiol 2005;288:H795–804.
6. Grieb G, Merk M, Bernhagen J, Bucala R. Macrophage migration inhibitory factor (MIF): a promising biomarker. Drug News Perspect 2010;23:257–64.
7. Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. Arteriosclerosis 1989;9:567–78.
8. Zernecke A, Bernhagen J, Weber C. Macrophage migration inhibitory factor in cardiovascular disease. Circulation 2008;117:1594–602.
9. Müller II, Müller KA, Schönleber H, Karathanos A, Schneider M, Jorbenadze R, et al. Macrophage migration inhibitory factor is enhanced in acute coronary syndromes and is associated with the inflammatory response. PLoS ONE 2012;7:e38376.
10. Donn R, Alourfi Z, De Benedetti F, Meazza C, Zeggini E, Lunt M, et al. Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum 2002;46:2402–9.
11. Herder C, Illig T, Baumert J, Müller M, Klopp N, Khuseyinova N, et al. Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. Atherosclerosis 2008;200:380–8.
12. Ji K, Wang X, Li J, Lu Q, Wang G, Xue Y, et al. Macrophage migration inhibitory factor polymorphism is associated with susceptibility to inflammatory coronary heart disease. Biomed Res Int 2015;2015:315174.
13. Yu XY, Chen HM, Liang JL, Lin QX, Tan HH, Fu YH, et al. Hyperglycemic myocardial damage is mediated by proinflammatory cytokine: macrophage migration inhibitory factor. PLoS ONE 2011;6:e16239.
14. Makino A, Nakamura T, Hirano M, Kitta Y, Sano K, Kobayashi T, et al. High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 2010;213:573–8.
15. Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Macrophage migration inhibitory factor levels in the vitreous of patients with proliferative diabetic retinopathy. Br J Ophthalmol 2000;84:636–9.
16. Kamchybekov U, Figulla HR, Gerdes N, Jung C. Macrophage migration inhibitory factor is elevated in obese adolescents. Arch Physiol Biochem 2012;118:204–9.
17. Kim H, Lee S, Kim HJ, Kong MH, Kim YR, Kang SH, et al. Elevated levels of macrophage migration inhibitory factor in women with metabolic syndrome. Horm Metab Res 2011;43:642–5.
18. Sanchez-Zamora YI, Rodriguez-Sosa M. The role of MIF in type 1 and type 2 diabetes mellitus. J Diabetes Res 2014;804519.
19. Toso C, Emamaullee JA, Merani S, Shapiro AM. The role of macrophage migration inhibitory factor on glucose metabolism and diabetes. Diabetologia 2008;51:1937–46.
20. Herder C, Kolb H, Koenig W, Haastert B, Muller-Scholze S, Rathmann W, et al. Association of systemic concentrations of macrophage migration inhibitory factor with impaired glucose tolerance and type 2 diabetes: results from the cooperative health research in the region of Augsburg, Survey 4 (KORA S4). Diabetes Care 2006;29:368–71.
21. Herder C, Klopp N, Baumert J, Müller M, Khuseyinova N, Meisinger C, et al. Effect of macrophage migration inhibitory factor (MIF) gene variants and MIF serum concentrations on

the risk of type 2 diabetes: results from the MONICA/KORA Augsburg Case-Cohort Study, 1984-2002. Diabetologia 2008;51:276–84.

22. Zhong X, Leng L, Beitin A, Chen R, McDonald C, Hsiao B, et al. Simultaneous detection of microsatellite repeats and SNPs in the macrophage migration inhibitory factor (MIF) gene by thin-film biosensor chips and application to rural field studies. Nucleic Acids Res 2005;33:e121.
23. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
24. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol 1983;51:606.
25. Serruys PW, Morice MC, Kappetein AP, Colombo A, Holmes DR, Mack MJ, et al; SYNTAX Investigators. Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. N Engl J Med 2009;360:961–72.
26. Finucane OM, Reynolds CM, McGillicuddy FC, Roche HM. Insights into the role of macrophage migration inhibitory factor in obesity and insulin resistance. Proc Nutr Soc 2012;71:622–33.
27. Stojanovic I, Saksida T, Stosic-Grujicic S. Beta cell function: the role of macrophage migration inhibitory factor. Immunol Res 2012;52:81–8.
28. Kleemann R, Bucala R. Macrophage migration inhibitory factor: critical role in obesity, insulin resistance, and associated comorbidities. Mediators Inflamm 2010;610479.
29. Sakaue S, Nishihira J, Hirokawa J, Yoshimura H, Honda T, Aoki K, et al. Regulation of macrophage migration inhibitory factor (MIF) expression by glucose and insulin in adipocytes in vitro. Mol Med 1999;5:361–71.
30. Sanchez-Niño MD, Sanz AB, Ihalmo P, Lassila M, Holthofer H, Mezzano S, et al. The MIF receptor CD74 in diabetic podocyte injury. J Am Soc Nephrol 2009;20:353–62.
31. Yabunaka N, Nishihira J, Mizue Y, Tsuji M, Kumagai M, Ohtsuka Y, et al. Elevated serum content of macrophage migration inhibitory factor in patients with type 2 diabetes. Diabetes Care 2000;23:256–8.
32. Nishihira J, Sakaue S. Overview of macrophage migration inhibitory factor (MIF) as a potential biomarker relevant to adiposity. J Tradit Complement Med 2012;2:186–91.
33. Li C, Qiao B, Qi W, Zhan Y, Ma C, Zhao L, et al. Association of macrophage migration inhibitory factor polymorphisms with gestational diabetes mellitus in Han Chinese women. Gynecol Obstet Invest 2016;81:84–9.
34. Benigni F, Atsumi T, Calandra T, Metz C, Echtenacher B, Peng T, et al. The proinflammatory mediator macrophage migration inhibitory factor induces glucose catabolism in muscle. J Clin Invest 2000;106:1291–300.
35. Sheu WH, Chang TM, Lee WJ, Ou HC, Wu CM, Tseng LN, et al. Effect of weight loss on proinflammatory state of mononuclear cells in obese women. Obesity (Silver Spring) 2008;16:1033–8.
36. Stosic-Grujicic S, Stojanovic I, Maksimovic-Ivanic D, Momcilovic M, Popadic D, Harhaji L, et al. Macrophage migration inhibitory factor (MIF) is necessary for progression of autoimmune diabetes mellitus. J Cell Physiol 2008;215:665–75.
37. Luo JY, Xu R, Li XM, Zhou Y, Zhao Q, Liu F, et al. MIF gene polymorphism rs755622 is associated with coronary artery disease and severity of coronary lesions in a Chinese Kazakh population. Medicine (Baltimore) 2016;95:e2617.
38. Coban N, Onat A, Yildirim O, Can G, Erginel-Unaltuna N. Oxidative stress-mediated (sex-specific) loss of protection against type-2 diabetes by macrophage migration inhibitory factor (MIF)-173G/C polymorphism. Clin Chim Acta 2015;438:1–6.
39. Onat A, Ademoğlu E, Can G, Çoban N, Kaya A, Yüksel H. Lower circulating migration inhibitory factor protein is associated with metabolic syndrome and diabetes. Biomark Med 2017;11:557–68.

Keywords: Coronary artery disease; macrophage migration inhibitory factor gene; polymorphism; type 2 diabetes mellitus.

Anahtar sözcükler: Koroner arter hastalığı; makrofaj migrasyon inhibitör faktör geni; polimorfizm; tip 2 diyabetes mellitus.