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Investigation of the Possible Role of Macrophage Migration Inhibitory Factor Gene -173G/C Polymorphism ın Patients with Atherosclerosis

Aterosklerozlu Hastalarda Makrofaj Migrasyon İnhibitör Faktör Geni -173G/C Polimorfizminin Olası Rolünün Araştırılması

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ÖZ

Amaç: Ateroskleroz, arteriyel intimada kolesterol birikiminin neden olduğu aterosklerotik plak oluşumu ile sonuçlanan enflamatuar bir hastalık olarak tanımlanmaktadır. Damarın intima tabakasındaki hasarlanma sonucunda kolesterol birikmesini takiben köpük hücre oluşumu ve düz kas hücre artışına bağlı olarak plak gelişimi görülmektedir. Aterosklerozun farklı aşamalarında, lökositlerin damar duvarına göçü fonksiyonel türdeki kemokinler ile sağlanmaktadır; kemokinlerle aynı fonksiyonel özelliklere sahip olduğu için Migrasyon İnhibitör Faktör (MIF) -173G/C polimorfizminin bu fonksiyona bağlı olarak ateroskleroz hastalığındaki olası rolünün araştırılması amaçlanmıştır.
Araçlar ve Yöntem: Çalışmaya, anjiyografi ile %70 tıkanıklık tespit edilen 30 hasta ve 30 sağlıklı birey dahil edildi. Çalışmaya katılan bireylerden EDTA'lı tüplere alınan kan örneklerinden DNA izolasyon kiti ile DNA izolasyonu yapıldı. MIF -173G/C polimorfizminin analizi Real Time PCR (LC480, Roche) cihazında gerçekleştirildi. İstatistiksel analizler STATISTICA version 13.5.0.17 (TIBCO Software Inc. (2017)) programı ile yapıldı. Tüm karşılaştırmalarda istatistik önem seviyesi $p \leq 0.05$ alınmıştır.
Bulgular: MIF -173G/C polimorfizminde, hasta grubunun GG, GC ve CC genotipi sıklığı sırasıyla %55.26 %41.18 ve %66.66 kontrol grubunda ise %44.74 %58.82 ve %33.33 olarak saptandı. GG genotipiyle karşılaştırıldığında, GC genotipine sahip olanlar 0.567 kat (p=0.3367), CC genotipine sahip olanlar 1.6190 kat (p=0.7038) hastalık geliştirme riskine sahip olduğu belirlendi.
Sonuç: MIF -173 G/C polimorfizminde C aleline sahip olanların ateroskleroz hastalığı için risk oluşturduğu saptandı.

Anahtar Kelimeler: ateroskleroz; migrasyon inhibitör faktör; PCR

ABSTRACT

Purpose: Atherosclerosis is defined as an inflammatory disease that results in the formation of atherosclerotic plaques caused by the deposition of cholesterol in the arterial intima. As a result of damage to the intima layer of the vessel, foam cell formation following cholesterol accumulation and plaque development due to smooth muscle cell increase are observed. In different stages of atherosclerosis, migration of leukocytes to the vessel wall is provided by functional type chemokines; ıt was aimed to investigate the possible role of Migration Inhibitory Factor (MIF) -173G/C polymorphism in atherosclerosis disease depending on this function, since it has the same functional properties as chemokines.
Materials and Methods: Thirty patients with 70% occlusion detected by angiography and 30 healthy individuals were included in the study. DNA isolation was performed with DNA isolation kit from blood samples taken into EDTA tubes from individuals participating in the study. Analysis of MIF -173G/C polymorphism was performed on Real Time PCR (LC480, Roche). Statistical analyzes were performed with the program STATISTICA version 13.5.0.17 (TIBCO Software Inc. (2017)). Statistical significance level $p \leq 0.05$ was taken in all comparisons.
Results: The frequency of GG, GC and CC genotypes in the MIF -173G/C polymorphism was 55.26%, 41.18% and 66.66% in the patient group, respectively, and 44.74%, 58.82% and 33.33% in the control group. When compared with the GG genotype, it was determined that those with the GC genotype had a 0.567-fold (p=0.3367) risk of developing the disease and those with the CC genotype had a 1.6190-fold (p=0.7038) risk of developing the disease.
Conclusion: It was determined that those with the C allele in the MIF-173 G/C polymorphism pose a risk for atherosclerosis disease.

Keywords: atherosclerosis; migration ınhibitory factor; PCR

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INTRODUCTION

Atherosclerosis is known as a disease that affects the intima layers of medium and large arteries, the typical lesion of which is thermoma plaques. The event that plays an important role in the onset of atherosclerosis is endothelial dysfunction. At the same time, endothelial dysfunction is the common point of atherosclerosis risk factors. Inflammatory cells begin to accumulate in the intima under the influence of a number of cytokines and chemotactants released as they pass under the low-density lipoprotein (LDL) endothelium. While macrophages phagocytize oxidized LDL in the intima, foam cell formation is observed as yellow lines under the intima. These lesions, which constitute the first lesion of atherosclerosis and do not cause narrowing of the lumen, are called fat streaks. Extracellular connective tissue (capsule) synthesis begins around the lipid core of smooth muscle cells that migrate from the media to the intima.[1]

This is due to the proliferative property of muscle cells. Macrophage migration inhibitory factor(MIF) is a protein that has proinflammatory, hormonal and enzymatic activities, and has important roles in inflammation with the activity of macrophages. The macrophage migration inhibitory factor (MIF) protein superfamily consists of the cytokine MIF (more recently also termed MIF-1) and its homolog D-dopachrome tautomerase (D-DT, also called MIF-2). Both members are abundantly expressed in adipose tissue.[2,3] Macrophage inhibitory factor was first discovered in 1966 and was defined as a cytokine with immune activity that was released from T lymphocytes and prevented random migration of macrophages during this period.[4] In 1989, human MIF cDNA was isolated and today MIF has been cloned and its molecular structure has been fully demonstrated by developing methods. MIF is a mediator protein with a molecular weight of 12.5 kDa and 115 amino acids with cytokine, hormone, and enzyme properties.[5,6] The MIF gene is located on the q arm(22q11.2) of chromosome 22 in humans and is separated by two introns of base pairs 94 and 188; it is a gene of less than 1 kilobase with 3 exons of base pairs 66, 107 and 172.[6,7]

In different stages of atherosclerosis, migration of leukocytes to the vessel wall is provided by chemokines. Since it has the same functional properties as chemokines, it was aimed to investigate the possible role of MIF -173G/C polymorphism in atherosclerosis disease due to this function.

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MATERIALS and METHODS

Aged and gender matched 60 people, including 30 patients with 70% occlusion detected by angiography (patient group) and 30 healthy individuals, between 15.10.2021 and 15.03.2022 and met the inclusion and exclusion criteria were included in the study. Our study was approved by the Ethics Commitee of the School of Medicine of Mersin University with E-78017789-050.01.04-1715214 reference number date on 28.07.2021. The examination of the patients admitted to the outpatient clinic was performed. The blood taken into EDTA tubes from the patients who accepted to participate in the study was used. Blood samples were stored at +4°C until the study day. DNA isolation from blood samples will be done using DNA isolation kit (Roche Diagnostics, Mannheim, German) and then analysis of MIF -173G/C (rs755622) polymorphism (ID number C-2213785-10; [VIC/FAM] TTTCTAGCCGCCAAGTGGAGA-ACAG$_{[C/G]}$TTGGAGCGGTGCGCCGGCTTAGCG) will be performed in RT-PCR device. When sufficient sample size was reached, MIF -173G/C polymorphism analysis was performed using a DNA isolation PCR instrument (Roche LightCycler 480). Statistical analyzes were performed with the program STATISTICA version 13.5.0.17 (TIBCO Software Inc. (2017)). Categorical variables are summarized by number (n) and percentage (%). Relationships between categorical variables were investigated with Chi-square (chi-square test) analysis and the appropriate one of Chi-Square, likelihood ratio or Fisher's exact test statistics was used. Hardy Weinberg balance was checked in groups for each genotype and allele distributions were given. Risk factors that may be effective in disease formation were evaluated with binary and multip-

le logistic regression analysis. The p values obtained from the univariate analyzes were examined and the risk factors thought to have a statistically or clinically significant effect according to the p<0.25 rule were added to the binary and multiple logistic regression model. The december obtained from the models and the confidence intervals of the ratio are given. Odds ratio with a confidence interval not including "1" was considered statistically significant. Statistical significance level p≤ 0.05 was taken in all comparisons.

RESULTS

The frequency of GG, GC and CC genotypes in MIF -173G/C polymorphism was 55.26%, 41.18% and 66.66% in the patient group; in the control group, ıt was determined as 44.74%, 58.82% and 33.33%, respectively. Compared to the GG genotype, it was determined that those with the GC genotype had a 0.567-fold (p=0.3367) risk of developing the disease, and those with the CC genotype had a 1.6190-fold (p=0.7038) (Table 1).

Table 1. Relationship between patıent and control groups in terms of allele frequencies and genotypes.

Genotype	Control n(%)	Patient group n(%)	OR	Confidence interval(95%)	P
TT	28 (100.00)	30 (100.00)			
GG	17 (60.7)	21 (70.0)			0.55
GC	10 (35.5)	7 (23.3)	0.567	.178-1.805	0.337
CC	1 (3.6)	2 (6.7)	1.619	.135-19.414	0.704
Allelic frequencies					
G	44 (0.78)	49 (0.81)			0.72
C	12 (0.21)	11 (0.18)			0.85

n: number of individuals, p: significance, OR: odds ratio

According to the Chi-square analysis, genotypes were not found to be a statistically significant risk factor for the disease (p>0.25). However, since genotypes are thought to be clinically important risk factors, a risk study was performed with binary logistic regression analysis. Those with the CC genotype have a 1.619 times higher risk of disease than those with GG, but the calculated odds ratio was not statistically significant (p=0.55) (G.A 95% 0.135-49.414). Those with the GC genotype have a 0.567 times less risk of disease than those with GG, but the calculated odds ratio is not statistically significant (p=0.337) (G.A 95% 0.178-1.805). Gender in the comparison made without considering genotypes, there is a statistically significant relationship between patient-control groups and gender (p<0.05). Accordingly, the rate of sick men (64.9%) is higher than the rate of sick women (28.6%). There was no relationship between allele genes and men and women in the control group (p=0.76). There was no relationship between allele genes and men and women in the patient group (p=0.86). There was no statistically significant difference in allele frequencies between the patient and control groups. According to the chi-square analysis, it was determined that genotypes were not a statistically significant risk factor for the disease (p>0.25). There was no relationship between allele genes of men and women in the control group and men and women in the patient group (Table 2).

Table 2. Distribution of genotypes according to gender of patients and controls.

Group	Genotype	Women	Men	p
Control	TT	15(100.00)	6(100.00)	0.84
	GG	9(60)	4(66.7)	
	GC	6(40)	2(33.3)	
	CC	0(0.0)	0(0.0)	
Patient	TT	13(100.00)	24(100.00)	0.76
	GG	8(61.5)	17(70.8)	
	GC	4(30.8)	5(20.8)	
	CC	1(7.7)	2(8.3)	
Allelic frequencies				
Control	G	24(0.8)	10(0.83)	0.8
	C	6(0.2)	2(0.17)	
Patient	G	20(0.67)	39(0.81)	0.65
	C	60(0,23)	9(0.19)	

p: significance

According to the chi-square analysis, genotypes are not a statistically significant risk factor for the disease (p>0.25). Gender was determined as a statistically significant risk factor for the disease (p<0.05). However, since genotypes are thought to be clinically significant risk factors on the disease, the effect of gender was investigated with multiple logistic regression analysis. According to the result, patients with CC genotype have a 0.966 times lower risk of disease than those with GG genotype, but the calculated odds ratio is not statistically significant (p=0.979) (G.A 95% 0.077-12.098)). Patients with GC genotype have a 0.645 times lower risk of disease than those with GG genotype, but the calculated odds ratio is not statistically significant (p=0.486) (G.A 95% 0.188-2.217). It was determined that men were 4.421 times

more likely to develop the disease than women(p=0.01) (95% CI 1.35-14.477)(Table 3).

Table 3. Multiple logistic regression analysis of the effect of genotype distribution with gender.

Genotype		P	Odds ratio (OR)	95% C.I.for OR	
				Lower	Upper
Gender	GG	0.783			
	GC	0.486	0.645	0.188	2.217
	CC	0.979	0.966	0.077	12.098
	Women				
	Men	0.014	4.421	1.35	14.477
	Constant	0.151	0.468		

p: significance, Lower: lower limit, Upper: Upper limit

DISCUSSION

In previous studies, it had been suggested that macrophage migration ınhibitory factor(MIF) has a proinflammatory role in atherosclerosis.[8,9] MIF is the founding member of the MIF protein family that also comprises D-DT/MIF-2 and MIF-like orthologs in numerous species. MIF is an upstream regulator of the host innate and adaptive immune response, but—if dysregulated—it is a driver of inflammatory diseases as well as cardiovascular diseases including atherosclerosis. Contrary to its eponymous name, MIF has been classified as an ACK that, similar to arrest chemokines such as CXCL1/8, enhances atherogenic leukocyte chemotaxis and arrest. It has been suggested that inhibition of random macrophage migration as observed in the historic experiments is likely to represent a desensitization effect as well-known for chemokines.[10,11] Serving as an inflammatory, chemokine-like cytokine and upstream regulator of innate immunity, it is not unexpected that MIF has a key role in numerous inflammatory and autoimmune conditions, including septic shock, rheumatoid arthritis (RA), systemic lupus erythematosus, Crohn's disease, obesity, glomerulonephritis and inflammatory and allergic lung conditions.[12,13,14,15,16] Owing to the close mechanistic links between chronic inflammation and cancer, MIF also has been identified as a pro-tumorigenic factor in several tumour entities, enhancing cancer cell proliferation, promoting tumour angiogenesis and modulating anti-tumour immunity.[17,18,19,20] Its chemokine-like and inflammatory properties render MIF a potent regulator of the atherogenic process. MIF expression is up-regulated in human and murine atherosclerotic lesions with peak levels observed in advanced plaques.[21,22,23] It is not only up-regulated in the atherogenic endothelium and infiltrating leukocytes, but also in VSMCs and platelets following inflammatory stimulation.[24,25] Antibody-mediated neutralization in Apoe / mice resulted in reduced lesional immune cell content and lowered levels of inflammatory mediators associated with atherosclerosis.[26] Similarly, Mif-deficient Ldlr / mice showed reduced atherosclerotic plaque areas compared with controls.[27] Targeting MIF with neutralizing antibodies resulted in significant plaque regression.[10]

MIF is abundantly expressed at all stages of plaque development in humans.[28] However, MIF appears to play a more important role in sensitive lesions as it induces MMP-1 expression and activity in SMCs and leads to fibrous cap thinning.[29] Atherosclerosis is defined as a chronic inflammatory disease and is characterized by the accumulation of macrophages and T lymphocytes in the arterial Wall.[30] oxidized-LDL(OxLDL) formation in the vessel wall affecting ECs, leading to changes in adhesion molecule expression and promoting the migration of macrophages and other inflammatory cells is seen in early stage atherosclerosis.[31,32] Foam cells formation is seen as a result of OxLDL uptake by macrophages and vascular smooth muscle cell(VSMCs).[31]

In particular, foam cells derived from macrophages produce and secrete proinflammatory cytokines. It also strengthens the inflammatory cascade in the early stages of plaque development. The increased levels of MIF have been shown to result from its release from macrophages upon stimulation with oxLDL in vitro and in vivo. It also serves to protect inflammation in advanced plaques. Furthermore, MIF expression is more prevalent in chronically inflamed areas containing VSMC and macrophage-derived foam cells. This indicates that OxLDL also plays an important role in macrophage MIF induction in vivo. It is interesting to note that MIF-stimulated mac-

rophages secrete tumor necrosis factor, IL1-β, -3, -5, -8, -12 proinflammatory cytokines, which are abundant in atherosclerotic lesions.[33,34] Key events in lesion development are that MIF regulates nitric oxide production in macrophages and matrix metalloproteinases in fibroblasts.[35]

Emmanuel Valdés-Alvarado et al.[36] demonstrated that MIF may be associated with its potential to trigger the expression of inflammatory mediators and mediate leukocyte recruitment and arrest, either directly or through the induction of adhesion molecules and chemokines in ECs and monocytes, which influences atherosprogression. Benigni F et al.[37] showed that MIF plays a critical role in the pathogenesis of coronary artery disease (CAD) and causes atherosclerosis. It activates hemorrhagic microvessels in atherosclerosis. Sheu WH et al.[38] shown that During lesion formation and progression, increased MIF gene expression was seen in vascular endothelial cells compared to normal arteries. In a study by Stosic-Grujicic S et al.,[39] it was shown that there was a close relationship between the polymorphism in the -173G/C position of the MIF gene and CAD, and it was found that the risk of CAD in carriers of the MIF -173C allele was associated with an increased plasma MIF concentration. When plasma MIF levels were compared in the CAD and control groups, individuals with the MIF -173C allele in the CAD group were found to have significantly higher MIF levels. It has been suggested that individuals carrying the MIF -173C allele produce higher amounts of MIF protein. Calandra T et al. It has been shown that MIF is secreted in high amounts as a result of activation of macrophages with lipopolysaccharide (LPS), TNF-α, and interferon gamma (IFNγ). This may explain the role of macrophage-derived MIF in atherogenesis.

In our study, MIF -173G/C polymorphism (rs755622), the frequency of GG, GC and CC genotypes in the patient group was 55.26%, 41.18% and 66.66%, and 44.74%, 58.82% and 33.33% in the control group, respectively. we determined that those with the GC genotype have a 0.567-fold (p=0.3367) risk of developing the disease, and those with the CC genotype have a 1.6190-fold (p= 0.7038) compared with the GG genotype.In the light of these data, further studies can be carried out in which the number of samples is increased and grouped according to the number of occluded vessels. Advanced age in women was more strongly associated with lower carotid elasticity than men aged 45-84 years. One standard deviation lower carotid artery resilience is associated with a 13-19% higher risk of stroke. Thus, gender differences in arterial stiffness, and thus stiffness-related cardiovascular disease, may explain the observation in US and British national-level cohort analyzes that the age-related increase in heart disease death rates atrophy in men after age 45, not like that for women. In conclusion, although genotype distribution was not found to be a statistically significant risk factor for atherosclerosis, it is thought that genotypes may be clinically significant risk factors for the disease according to our data. In addition, further studies can be performed by grouping them according to the number of occluded vessels and increasing the number of samples.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

Ethics Committee Permission

Approval for this study was obtained from the Mersin Üniversitesi Tıp Fakültesi Etik Committee (28.07.2021dated and E-78017789-050.01.04-1715214 numbered).

Authors' Contributions

Concept/Design: RT, SB, MTŞ, AÇ, LT. Data Collection and/or Processing: RT, SB, MTŞ, AÇ, LT. Data analysis and interpretation: RT, SB, MTŞ, AÇ, LT. Literature Search: RT. Drafting manuscript: RT, SB, MTŞ, AÇ, LT. Critical revision of the manuscript: RT, SB, MTŞ, AÇ, LT.

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