**MBL2 and MIF gene polymorphisms in cardiovascular patients with atherosclerotic lesions undergoing heart valve replacement**

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**ABSTRACT**

The basic underlying factor for cardiovascular diseases is atherosclerosis, which is a multifactorial disease driven by environmental and genetic factors. We aimed to study the genetic polymorphism in mannose binding lectin-2 (MBL2) and macrophage migration inhibitory factor (MIF) in the arteries of patients with atherosclerotic lesions who underwent cardiac valve replacement for cardiac valve stenosis. Thirty-five patients (38.9%) operated with coronary bypass surgery (coronary group, CG), 55 (61.1%) patients operated with aortic or mitral valve replacement (valve group, VG) and 100 healthy controls were analyzed for codon 54 A/B polymorphism in the MBL2 gene and –173 G/C polymorphism in the MIF gene by using the method of polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The comparison of the healthy control group with CG and VG in terms of the MBL2 genotypes revealed significantly lower AA genotype and A allele ratios. The comparison of the healthy control group with CG and VG in terms of the MIF genotypes showed significantly lower GG genotype and G allele ratios. We suggest that the lower frequency of the GG genotype/G allele of the MIF gene and of the AA genotype/A allele of the MBL2 gene may be associated with the ethiopathogenesis of CG and VG patients.

**Introduction**

Atherosclerosis is classified as an inflammatory disease resulting from atherosclerotic plaques formed by cholesterol accumulation in arterial intima [1]. Vascular intimal injury leads to cholesterol accumulation and foam cell formation which result in plaque formation due to smooth muscle cell increase. As a result, the vessel wall thickens, the arterial lumen narrows and the vessel is occluded [2]. Valvular heart disease (VHD) is a common cause of cardiac dysfunction and death [3]. Mannose binding lectin-2 (MBL2) and macrophage migration inhibitory factor (MIF) play important roles in the pathogenesis of several inflammatory and autoimmune disorders. Significant evidence has been found regarding the migration of circulating immune cells into vessel walls and mural inflammation in atherosclerotic lesion development [4].

Rheumatic heart diseases occur in children as a complication of rheumatic fever developing in relation to group A streptococcal (GAS) pharyngitis [5]. It has been shown that GAS strongly bind to the MBL2 protein [6]. MBL2 plays an important role in natural immunity through opsonizing pathogens, improving phagocytosis and activating the complement chain through the lectin pathway [7,8]. MBL2 can also bind directly to macrophages. Each of these activities results in pro-inflammatory immune system activation [9]. Based on the above-described findings, we investigated the potential association of genetic polymorphisms of MBL2 and MIF in atherosclerotic lesions of patients with coronary artery disease (CAD) and in stenotic aortic or mitral valves of patients undergoing heart valve replacement.

**Subjects and methods**

**Patients and controls**

This study enrolled 90 patients admitted to the Department of Cardiovascular Surgery at Sanko Hospital in Gaziantep, Turkey, in the period of March–October 2007. Fifty-eight (64.4%) of the patients were male and 32...
(35.6 %) were female. Fifty-six subjects (56 %) in the control group were male and 34 (34 %) were females. The mean age in the group of patients was 50 (17–73) years and in the control group, 39 (27–56) years. Thirty-five patients (38.9 %) underwent coronary bypass surgery (coronary group, CG) and 55 patients (61.1 %) were referred to aortic and/or mitral valve replacement (valve group, VG). Atherosclerotic samples from coronary arteries were obtained via endarterectomy during bypass surgeries of the coronary artery patients. Decisions for thrombo-endarterectomy were always made intraoperatively on the basis of coronary morphology. Coronary artery segments of 10–12 millimeters with atherosclerotic lesions were obtained from the patients with CAD. Twenty-four (43.6 %) of 55 valve patients had aortic valve replacement (AVR) and 31 (56.4 %) had mitral valve replacement (MVR). All patients that had AVR underwent surgery due to adult-type calcific aortic valve stenosis and all patients that had MRV underwent surgery due to mitral valve stenosis that developed secondary to rheumatic heart disease. The coronary and valve samples were stored in sterile bottles and were frozen at −20 °C. Peripheral blood samples were taken from 100 healthy persons included as the control group. A written informed consent was obtained from each patient before surgery and from the subjects in the control group. The Local Board of Ethics Committee at Sanko Hospital in Gaziantep approved the study (BH/IY/2-24.01.2007).

**DNA isolation and genotyping of MBL2 and MIF genes**

Coronary vascular segments approximately 10 mm in length and 25–30 mg of valvular tissues were used for DNA isolation using the QIAamp Tissue kit (Qiagen GmbH, Hilden, Germany). For the controls, genomic DNA was extracted from peripheral blood samples using the QIAamp blood kit (Qiagen GmbH, Hilden, Germany). For the controls, genomic DNA isolation using the QIAamp Tissue kit (Qiagen GmbH, Hilden, Germany). For the controls, genomic DNA isolation and genotyping of MIF gene polymorphisms between the CG, VG and the healthy control group.

| MBL2 gene Genotypes | CG patients n (%) | VG patients n (%) | Healthy controls N (%) | OR* | %95 CI* | p* |
|---------------------|-------------------|-------------------|------------------------|-----|--------|----|
| AA                  | 14 (40)           | 31 (56.4)         | 64 (64)                | 0.375 a | 0.170–0.826 a | 0.012 a |
| AB                  | 17 (49.6)         | 18 (32.7)         | 35 (35)                | 0.192 b | 0.092–0.399 b | 0.001 b |
| BB                  | 4 (11.4)          | 6 (10.9)          | 1 (1)                  | 1.754 a | 0.804–3.826 a | 0.112 a |
| Alleles             |                   |                   |                        |      |         |    |
| A                   | 45 (64.3)         | 80 (72.7)         | 163 (81.5)             | 2.692 a | 1.471–4.926 a | 0.001 a |
| B                   | 25 (35.7)         | 30 (27.3)         | 37 (18.5)              | 1.652 b | 0.952–2.866 b | 0.050 b |

\[1 n = 35, \bar{n} = 55, 1 n = 100, ^a\text{Fisher’s Exact Test}, ^a\text{comparison of genotype frequencies between coronary group (CG) and healthy control group}, ^b\text{comparison of genotype frequencies between valve group (VG) and healthy control group.} \]
In the VG patients, the distributions of GG, GC and CC genotypes for MIF were 49.1%, 40% and 10.9%, respectively, compared with 58%, 40% and 2%, respectively, for the controls (Table 2). The allelic frequencies of G/C were 69.1%; 30.9% and 10.9%, respectively, for the VG patients compared with 78%, 22% and 10.9%, respectively, for the controls. The frequencies of the GG genotype and the G allele were significantly lower among VG patients (p = 0.024, p = 0.001, respectively).

We identified three common polymorphic areas in MBL2. The Gly3Asp variant at MBL2 codon 54 (G54D) in exon 1 is designated as B allele. This variant is associated with recurrent infections. MBL G54D also destabilizes the 6th collagen repeat of MBL2, and the B-type MBL chains expressed in vitro fail in activating the complement [12–14]. In addition, subjects who are heterozygotes for the wild type MBL A allele (g54) and the poorly functioning MBL B allele (D54) have a 20-fold reduction in MBL concentrations compared with G54 homozygotes, because the MBL B allele product apparently acts as a dominant negative [12–14]. The MBL2 mutant allele is named ‘O’ and the wild-type allele as ‘A’ for these three mutations [15]. In this study, when the healthy control group and CG patients were compared in terms of MBL2 genotypes, a statistically significant reduction was found in the AA genotype and A allele ratios. The presence of this genotype and allele may play a role in the protection from the disease (2.7-fold reduction inhibits the protection from the disease). A 12.7-fold increase in the BB genotype frequency in CG patients suggests that this genotype may have an important role in disease susceptibility, being a risk-associated genotype. When the healthy control group and VG patients were compared in terms of MBL2 genotypes, a statistically significant reduction was identified in the frequency of the AA genotype. Thus, it may be speculated that the presence of this genotype may play a role in the protection from the disease (5.2-fold reduction inhibits the protection from the disease) (Table 1). These results are in accordance with previous reports that some variations in the MBL2 genotype distributions may be associated with cardiovascular disease. For example, Ramasawmy et al. [16] found a significantly higher D allele frequency in patients with severe rheumatic aortic regurgitation than in the healthy controls (11% versus 4%). The frequencies of B and C alleles were similar in both groups. The frequencies of the O/O homozygotes were significantly different between patients and controls, whereas the frequencies of the A/O heterozygotes were similar in both groups. Schafranski et al. [17] concluded that the MBL2 genotype associated with high MBL2 production may be connected with the pathogenesis of rheumatic carditis and the progress of chronic rheumatic heart diseases. Garred et al. [18] suggested that the variant alleles in rheumatoid arthritis are connected with the early development of the disease, whereas the MBL2-A/A genotype is associated with late development of the disease. In addition, Zhang et al. [19] indicated that the polymorphism of codon 54 in MBL2 may predispose to rheumatoid arthritis (RA), especially seropositive or erosive RA.

In another study, patients homozygously carrying the normal MBL2 genotype and patients with a genotype determining a low serum MBL2 level were compared, and the former were found to have the highest ratio of early restenosis development after carotid endarterectomy [20].

These observations have indicated complement activation with the lectin pathway and an important side effect of MBL2 in the pathogenesis of cardiovascular diseases. The last complement complexes are associated with endothelial cell damage in rheumatoid nodules; the complement activation can influence the vascular damage in RA. In this study, endothelial cell damage causing endothelial dysfunction was described for RA, which predicts the development of coronary artery disease [21–23]. In previous studies, similar alleles have been found in patients with a similar echocardiographic picture of the disease: mitral stenosis has been associated with the A allele, which codes for a higher production of MBL2; conversely, aortic regurgitation appeared to be associated with the O allele, which codes for a lower production of MBL2 [24]. Orsatti et al. [25] found the

**Table 2. Comparison of MIF gene polymorphism between the CG, VG and the healthy control group.**

| Genotype | CG patients | VG patients | Healthy controls | OR* | %95 CI* | p* |
|----------|-------------|-------------|------------------|-----|---------|----|
| GG       | 13 (37.1)   | 27 (49.1)   | 58 (58)          | 0.428* | 0.194–0.945* | 0.027* |
| GC       | 16 (45.7)   | 22 (40)     | 40 (40)          | 0.698* | 0.360–1.353* | 0.185* |
| CC       | 6 (17.2)    | 6 (10.9)    | 2 (2)            | 1.263* | 0.581–2.745* | 0.346* |
| Allele G | 42 (60)     | 76 (69.1)   | 156 (78)         | 2.582* | 1.442–5.627* | 0.001* |
| Allele C | 28 (40)     | 34 (30.9)   | 44 (22)          | 1.586* | 0.938–2.681* | 0.057* |

* p = 0.024, *p = 0.001, *comparison of genotype frequencies between coronary group (CG) and healthy control group. *comparison of genotype frequencies between valve group (VG) and healthy control group.
polymorphic allele for codon 54 in 25.8% of postmenopausal women (A/B = 22.6%, B/B = 3.2%) and for codon 57, in 12.2% (A/C = 10.8%, C/C = 1.4%). The polymorphism at codon 54 was significantly associated with the presence of hypertension.

MIF is an important proinflammatory cytokine regulator that modulates the activation of monocyte/macrophages and T lymphocytes, and in animal studies, it has been determined that it plays a critical role in the inflammatory pathologies responsible for arthritis, glomerulonephritis and acute lung injury, as well as the host response in septic shock [26]. Fragile plaques are characterized by high inflammatory activity coupled with high contents of inflammatory cells such as monocyte/macrophage and chemokines playing an important role in plaque rupture [27].

Radstake et al. [28] claimed that the increase (variants) in the serum MIF level in RA patients carrying the C allele play a role in the development of cardiovascular diseases. Palomino-Morales et al. [29] supported the idea that MIF-173 biallelic polymorphism is responsible for an increased sensitivity to atherosclerosis in patients with RA. Lehmann et al. [30] determined that patients carrying the C-allele showed significantly increased levels of the proinflammatory cytokine MIF compared to G/G homozygous subjects when revascularization was carried out using cardiopulmonary bypass (CPB). Herder et al. [31] found that while the MIF serum level showed no correlation with coronary heart disease (CHD), the haplotypes including −173C alleles were associated with increased risk.

In our study, when the healthy control group and CG patients were compared in terms of MIF genotypes, a statistically significant reduction (a 2.5-fold decrease) was found in the GG genotype and G allele ratios. Thus, it could be hypothesized that the presence of this genotype and allele may play a role for the protection from the disease. A 10.1-fold increase in the CC genotype in CG patients suggested that this genotype may pose a risk for the development of this disease. When the healthy control group and VG patients were compared in terms of MIF genotypes, a statistically significant increase was observed in the CC genotype ratio in the patient group, suggesting that this genotype may play a role in the susceptibility to the disease (Table 2).

Coronary arterial segment samples were obtained from the patients who underwent coronary bypass surgery and valve samples from the patients who underwent aortic and/or mitral valve replacement. Because obtaining these samples is quite a difficult task, the number of the samples was limited, which is a limitation of our study. Despite this limitation, the results obtained in our study may constitute a background for further studies on this subject.

Conclusions
This study identified lower AA genotype and A allele frequency of the MBL2 gene, and lower GG genotype and G allele frequency of the MIF gene in the CG and VG patient groups than in the control subjects. We conclude that functional polymorphisms of MIF (173) and MBL2 (codon 54) may be associated with the ethiopathogenesis of CG and VG.

Declaration of interest
The authors have no conflict of interest.

Acknowledgements
The authors thank the patients of the Şahinbey Research and Application Hospital of Gaziantep University and SANKO Hospital for their participation in this study.

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