Polymorphism of cytokine genes: Cardiovascular risk factors in the Venezuelan population?

Polimorfismo de genes de citocinas: ¿factores de riesgo cardiovascular en la población venezolana?

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Abstract

Objective: To examine whether the polymorphisms of the IL6, TNFA and IL10 genes represent a risk marker for acute myocardial infarction (AMI) and to analyze their correlation with risk factors, age of occurrence and type of AMI. **Method:** Association study that included 310 unrelated Venezuelan individuals, grouped in 190 patients with AMI and 120 controls with or without cardiovascular risk factors. The IL6-174 G/C (rs1800795), TNFA -308 G/A (rs1800629), and IL10-1082 A/G (rs1800896), -819 C/T (rs1800871) and -592 C/A (rs1800872) polymorphisms were determined using the polymerase chain reaction technique with sequence-specific primers. **Results:** Comparison of genotypic and allelic frequencies, using adjusted logistic regression analysis for risk factors, showed a significantly increased frequency of the genotype combination G/G-A/A of TNFA-308 G/A (odds ratio [OR]: 3.8; $p = 0.00007$), GG/-C/C of IL6-174 G/C (OR: 2.3; $p = 0.009$), A/G-G/G of IL10-1082 A/G (OR: 3.8; $p = 0.00001$) and the GCC haplotype of IL10 (OR: 3.71; $p = 0.0053$) in infarcted patients compared to controls. Interactions between the IL10-1082 A/G and TNFA-308 G/A polymorphisms and hypertension were observed. **Conclusions:** The association of the variants of the TNFA, IL6 and IL10 genes with AMI suggest that the imbalance in the production of cytokines promotes an exacerbated inflammatory process, supporting the fundamental role of inflammation in all stages of the atherosclerotic process.

Key words: Polymorphism. Cytokines. Acute myocardial infarction. Acute coronary syndrome.

Resumen

Objetivo: Examinar si los polimorfismos de los genes IL6, TNFA e IL10 representan un marcador de riesgo de infarto agudo de miocardio (IAM) y analizar su correlación con los factores de riesgo, la edad de ocurrencia y el tipo de IAM. **Método:** Estudio de asociación que incluyó 310 individuos venezolanos, no relacionados, agrupados en 190 pacientes con IAM y 120 controles con o sin factores de riesgo cardiovascular. Los polimorfismos IL6-174 G/C (rs1800795), TNFA-308 G/A (rs1800629) e IL10-1082 A/G (rs1800896), -819 C/T (rs1800871) y -592 C/A (rs1800872) se determinaron utilizando la técnica reacción en cadena de la polimerasa con iniciadores de secuencias específicas. **Resultados:** La comparación de frecuencias genotípicas y alélicas, mediante el análisis de regresión logística ajustado para los factores de riesgo, mostró una frecuencia significativamente incrementada de la combinación de genotipos G/G-A/A de la variante TNFA-308 G/A (odds ratio [OR]: 3.8; $p = 0.00007$),

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GG/-C/C de la variante IL6-174 G/C (OR: 2.3; $p = 0.009$), A/G-G/G de la variante IL10-1082 A/G (OR: 3.8; $p = 0.00001$) y del haplotipo GCC de IL10 (OR: 3.71; $p = 0.0053$) en los pacientes con IAM con respecto a los controles. Se observaron interacciones entre los polimorfismos IL10-1082 A/G y TNFA-308 G/A e hipertensión. **Conclusiones:** La asociación de las variantes de los genes TNFA, IL6 e IL10 con IAM sugiere que el desbalance en la producción de citocinas promueve un proceso inflamatorio exacerbado, apoyando el papel fundamental de la inflamación en todas las etapas del proceso aterosclerótico.

Palabras clave: Polimorfismo. Citocinas. Infarto agudo de miocardio. Síndrome coronario agudo.

Introduction

Acute myocardial infarction (AMI) is caused by an interaction of environmental and genetic factors¹. In fact, the incidence of AMI additively increases depending on the number of conventional cardiovascular risk factors (CVRF), such as hypertension, diabetes mellitus and hypercholesterolemia. Although each CVRF is by itself partially under genetic control, several studies suggest the existence of additional susceptibility genes in the development of infarction². Many epidemiological studies have suggested that various genetic variants increase the risk of AMI. Based on this, the main purpose of genetic research is to provide comprehensive risk assessment, added to traditional clinical and biochemical risk factors. There is growing evidence that inflammation plays a central role in the genesis of atherosclerosis, the main etiopathogenic mechanism of AMI³. Inflammation appears to be involved at all stages of atherosclerotic development, including oxidative damage, cell proliferation, and evolution and atheroma plaque destabilization. However, despite evidence implying that a group of markers are related to inflammation and AMI, interactions with environmental factors and involved mechanisms are poorly understood^{4,5}. While vascular wall inflammation can be a consequence of some environmental factors (bacterial or viral infections, tissue damage, eating habits, etc.), it is possible that genetic factors may contribute to an altered or exacerbated inflammatory response. Studies in older individuals have demonstrated that some inflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF- α), predict cardiovascular outcome². Moreover, variability of these inflammatory genes can influence on human beings longevity by affecting inflammatory responses associated with age-related diseases, such as coronary artery disease⁶. It is important pointing out that genetic variants, unlike biochemical markers, are less influenced by environmental factors, which is why they constitute better individual markers of susceptibility to AMI, in particular when there is a family history thereof⁷. Another

important cytokine in coronary artery disease that has anti-inflammatory activity is interleukin 10 (IL-10), which has the capability to inhibit low-density lipoproteins adhesion to the endothelium and negatively regulate fibrinogen biosynthesis. IL-10 serum concentration has been described to decrease in patients with acute coronary syndrome (ACS) and to be inversely related to future events in patients with AMI, which suggests an important anti-inflammatory role that compensates pro-inflammatory responses². Based on the fact that polymorphisms located at cytokine genes' promoter region determine their concentrations and affect the inflammatory process involved in the genesis of atherosclerosis, in this work we examine whether polymorphisms of the IL6, TNFA and IL10 genes represent a marker of AMI risk in the Venezuelan mestizo population. Furthermore, the possible correlation of these polymorphisms with traditional risk factors, the age of occurrence and the type of AMI are analyzed.

Method

A retrospective, case-control association study was carried out, which included 310 unrelated Venezuelan individuals, classified in two groups:

- Patients ($n = 190$): individuals diagnosed with AMI, determined by clinical, paraclinical and electrocardiographic findings, who attended the coronary care unit and the special studies unit of the cardiology department at Hospital General del Este Dr. Domingo Luciani, in Caracas, Venezuela. Based on AMI patients admission electrocardiogram and clinical characteristics, ACS was classified as non-ST-segment elevation ACS (NSTEMI-ACS) and ST-segment elevation ACS (STEMI-ACS).
- Controls ($n = 120$): apparently healthy individuals with or without CVRF.

The samples of both groups were collected during the period from March 2010 to November 2013. Once all samples were obtained, cytokine gene polymorphisms genotyping was carried out.

In both groups, individuals with a history of liver, autoimmune or neoplastic diseases or AMI secondary to other causes were excluded. All participants signed an informed consent document approved by the Bioethics Committee at Hospital General del Este Dr. Domingo Luciani, Caracas, Venezuela (document No. 00211).

For the determination of polymorphisms, genomic DNA was extracted from peripheral blood leukocytes and lymphocytes using the Bunce protocol⁸. Polymorphisms of *IL6*, *TNFA* and *IL10* genes were determined by the polymerase chain reaction (PCR) technique with specific sequence primers. The *IL6*-174 G/C (rs1800795), *TNFA*-308 G/A (rs1800629) and *IL10*-1082 A/G (rs1800896), -819 C/T (rs1800871) and -592 C/A (rs1800872) variants were determined using the primers described by Ambrozova et al.⁹, Verjans et al.¹⁰ and Kingkeow et al.¹¹, respectively. For each polymorphism, two reaction mixtures were prepared, one for the ancestral or wild allele (-308 G, -174 G, -1082G, -819 C and -592 C, respectively) and another for the mutated or infrequent allele (-308 A, -174C, -1082 A, -819T and -592 A, respectively). As PCR internal control, the ABO blood group primers described by Olsson et al.¹² were included in all reactions.

Allelic and genotypic frequencies were calculated by direct count. The strength of association was determined by the odds ratio (OR), with the corresponding 95% confidence interval (CI). Statistical significance of frequency differences (genotypes, haplotypes) between groups was estimated by the chi-square test, using 2 x 2 contingency tables. P-values were considered significant when they were < 0.05. *IL10* gene promoter region polymorphism haplotypes (-1082, -819, -592) Hardy-Weinberg equilibrium, inheritance models and interaction with CVRFs were determined using the SNPStats tool¹³. Correlations between the analyzed polymorphisms, traditional CVRFs and AMI occurrence and type were made using the SPSS Statistic statistical package, version 20¹⁴.

Results

Of the 190 included patients, 33.7% were females and 66.3% were males, with an age range between 34 and 90 years (average of 54 ± 12.26 years). Regarding the 120 controls, 45.8% were females and 54.2% were males, with an age range between 33 and 83 years (average of 47.5 ± 2.12 years). As for infarction clinical presentation, 37.9% of patients were clinically classified as NSTEMI-ACS and 62.1% as STEMI-ACS. When between-group comparisons were established, in the

Table 1. Demographic and clinical characteristics of patients with AMI and of apparently healthy individuals (controls)

	Patients with AMI (n = 190)	Controls (n = 120)	p
Gender			
Females	33.7 (64)	45.8 (55)	0.016
Males	66.3 (126)	54.2 (65)	
Age range (years)	34-90	33-83	< 0.00001
Average age $\pm$ SD (years)	61.05 $\pm$ 12.25	52.14 $\pm$ 10.34	
CVRFs present			
Hypertension	70.5 (134)	27.5 (33)	< 0.00001
Diabetes mellitus	20.5 (39)	15.0 (18)	NS
Smoking	41.1 (78)	10.8 (13)	< 0.00001
Obesity	70.5 (134)	28.3 (34)	< 0.00001
Sedentary lifestyle	23.7 (45)	24.2 (29)	ns
Clinical presentation			
NSTEMI-ACS	37.9 (72)	-	-
STEMI-ACS	62.1 (118)	-	-

SD: standard deviation; CVRF: cardiovascular risk factors; AMI: acute myocardial infarction; NS: non-significant ($p \geq 0.05$); STEMI-ACS: ST-segment elevation acute coronary syndrome; NSTEMI-ACS: non-ST-segment elevation acute coronary syndrome. Frequencies are expressed as percentages, and the number of individuals is indicated in brackets.

group of patients, we observed a significantly increased frequency of males (OR: 1.66; 95% CI: 1.04-2.66; $p = 0.016$), hypertension (OR: 6.3; 95% CI: 3.79-10.48; $p < 0.00001$), obesity (OR: 6.1; 95% CI: 3.65-10.03; $p < 0.00001$) and smoking (OR: 5.7; 95% CI: 3.01-10.91; $p < 0.00001$), as well as a higher average age (95% CI: 6.26-11.57; $p < 0.00001$), with regard to controls (Table 1).

Using the SNPStats program, the existence of Hardy-Weinberg equilibrium was confirmed for the studied polymorphisms genotypic distribution. In addition, the inheritance model of each polymorphism was determined, with that with the lowest probability value (p) being established as the most adjusted inheritance model, with the over-dominant inheritance model being the most adjusted for the *TNFA*-308 G/A and *IL10*-592 C/A variants, codominant for the *IL6*-174 G/C variant and dominant for the *IL10*-1082 A/G and *IL10*-819 C/T variants. When genotypic frequencies were compared between patients and controls, considering each variant inheritance model and using the logistic regression analysis adjusted for gender, age, hypertension, smoking, obesity, dyslipidemia, diabetes and sedentary lifestyle, in patients with AMI we observed a significantly increased frequency of the combination of G/G-A/A

Table 2. Association of *TNF*, *IL-6* and *IL10* gene polymorphisms with the development of acute myocardial infarction, age of occurrence and type of acute coronary syndrome

Polymorphisms	Genotype	AMI patients (n = 174)	Controls (n = 68)	OR (95% CI)	p
<i>TNF-308</i> G/A over-dominant	G/G-A/A vs. G/A	90.2 (157) vs. 9.8 (17)	70.6 (48) vs. 29.4 (20)	3.8 (1.87-7.93)	0.00007
<i>IL6-174</i> G/C co-dominant	C/C+GG vs. G/C	86.2 (150) vs. 13.8 (24)	73.5 (50) vs. 26.5 (18)	2.3 (1.13-4.48)	0.009
<i>IL10-1082</i> A/G dominant	A/G-G/G vs. AA	54.0 (94) vs. 46.0 (80)	23.5 (16) vs. 76.5 (52)	3.8 (2.02-7.20)	0.00001
<i>IL10-819</i> C/T dominant	C/T-T/T vs. C/C	47.1 (82) vs. 52.9 (92)	48.5 (33) vs. 51.5 (35)	0.9 (0.54-1.66)	0.9
<i>IL10-592</i> C/A over-dominant	C/C-A/A vs. A/C	62.6 (109) vs. 37.4 (65)	57.4 (39) vs. 42.6 (29)	1.2 (0.70-2.21)	0.22
Polymorphisms	Genotype	AMI at ≤ 55 years of age	AMI at ≥ 56 years of age	OR (95% CI)	p
<i>TNF-308</i> G/A over-dominant	G/G-A/A vs. G/A	89.6 (60) vs. 10.4 (7)	90.7 (97) vs. 9.3 (10)	0.9 (0.32-2.45)	0.4
<i>IL6-174</i> G/C co-dominant	C/C+GG vs. G/C	86.6 (58) vs. 13.4 (9)	86.0 (92) vs. 14.0 (15)	1 (0.43-2.56)	0.45
<i>IL10-1082</i> A/G dominant	A/G-G/G vs. AA	53.7 (36) vs. 46.3 (31)	54.2 (58) vs. 45.8 (49)	0.9 (0.53-1.81)	0.47
<i>IL10-819</i> C/T dominant	C/T-T/T vs. C/C	47.8 (32) vs. 52.2 (35)	46.7 (50) vs. 53.3 (57)	1 (0.56-1.92)	0.44
<i>IL10-592</i> C/A over-dominant	C/C-A/A vs. A/C	61.2 (41) vs. 38.8 (26)	63.5 (68) vs. 36.5 (39)	0.9 (0.48-1.69)	0.37
Polymorphisms	Genotype	STE-ACS (n = 110)	NSTE-ACS (n = 64)	OR (95% CI)	p
<i>TNF-308</i> G/A over-dominant	G/G-A/A vs. G/A	89.1 (98) vs. 10.9 (12)	92.2 (59) vs. 7.8 (5)	0.7 (0.22-2.06)	0.25
<i>IL6-174</i> G/C co-dominant	C/C+GG vs. G/C	85.5 (94) vs. 14.5 (16)	87.5 (56) vs. 12.5 (8)	0.8 (0.34-2.09)	0.35
<i>IL10-1082</i> A/G dominant	A/G-G/G vs. AA	53.6 (59) vs. 46.4 (51)	54.7 (35) vs. 45.3 (29)	0.9 (0.52-1.78)	0.45
<i>IL10-819</i> C/T dominant	C/T-T/T vs. C/C	43.6 (48) vs. 56.4 (62)	53.1 (34) vs. 46.9 (30)	0.7 (0.37-1.27)	0.11
<i>IL10-592</i> C/A over-dominant	C/C-A/A vs. A/C	63.6 (70) vs. 36.4 (40)	60.9 (39) vs. 39.1 (25)	1 (0.59-2.12)	0.36

AMI, acute myocardial infarction; CI: confidence interval; OR: odds ratio; STE-ACS, STsegment elevation acute coronary syndrome; NSTE-ACS: non-ST-segment elevation acute coronary syndrome. Frequencies are expressed as percentages, and the number of individuals is indicated in brackets.

genotypes of the *TNFA-308* G/A variant (90.2 vs. 70.6%; OR: 3.8; 95% CI: 1.87-7.93; $p = 0.00007$), of the G/G/-C/C combination of the *IL6-174* G/C variant (86.2 vs. 73.5%; OR: 2.3; 95% CI: 1.13-4.48; $p = 0.009$) and of the A/G-G/G combination of the *IL10-1082* A/G variant (54 vs. 23.5%; OR: 3.8; 95% CI: 2.02-7.20; $p = 0.00001$) with regard to controls. On the other hand, when the frequencies of each variant genotypes were compared between patients who suffered AMI at an age ≥ 56 years and patients who suffered it at an age ≤ 55 years, and between patients with STE-ACS and with NSTE-ACS, no significant differences were observed (Table 2).

When the haplotypes formed by the *IL10-1082*, *-819* C/T and *-592* C/A variants were analyzed, eight haplotypes were observed in patients and seven in controls, with the haplotype formed by wild-type alleles (ACC) showing the highest frequency in both groups. In contrast, the haplotype formed by mutated

alleles (GTC) was only present in patients. The analysis of the association between the *IL10* haplotypes and AMI by means of logistic regression adjusting for CVRFs showed a significantly increased frequency of the GCC haplotype in patients with regard to controls (27.5 vs. 16.0%; OR: 3.71; 95% CI: 1.49 - 9.26; $p = 0.0053$). However, when haplotype frequencies were compared between patients who suffered AMIs at ≥ 56 years of age and those who suffered them at ≤ 55 years of age, and between patients with STE-ACS and NSTE-ACS, no significant differences were observed. Finally, the interaction analysis, between patients and controls, of cytokine gene polymorphisms with CVRFs showed that there was interaction of polymorphism *IL10-1082* A/G with hypertension (interaction $p = 0.015$). In addition, the analysis of the interaction between patients who suffered AMI at ≤ 55 years of age and patients who suffered them at ≥ 56 years of age showed that there was interaction of the *TNFA-308*

G/A polymorphism and hypertension (interaction $p = 0.019$).

Discussion

AMI is a multifactorial health condition, and often it is caused by epicardial coronary arteries unstable plaque, where inflammatory processes play a central role. In this sense, several circulating pro-inflammatory molecules have been associated with cardiovascular thrombotic events, including acute phase proteins, cell adhesion molecules and cytokines¹⁵. Studies have shown that pro-inflammatory cytokines, such as TNF- α , IL-1 and IL-6, have an important function in the development of atherosclerotic plaque and, consequently, in the development of coronary artery disease. Furthermore, different studies have shown that IL-10 is crucial in inflammation regulation. This cytokine is an important inhibitor of pro-inflammatory cytokines synthesis and has been detected within human atherosclerotic plaques, with high concentrations of it being associated with a significant decrease in cell death and inducible nitric oxide synthase enzyme expression¹⁶. Given the importance of cytokines in the pathogenesis of the disease, studying whether *TNFA*, *IL6* and *IL10* gene polymorphisms represent a marker of AMI risk in the Venezuelan mestizo population was proposed, as well as to analyze their correlation with risk factors, age of occurrence and AMI type.

In the present study, a significantly increased frequency of the combination of *TNFA*-308 G/G-A/A variant and *IL6*-174 GG/-C/C variant genotypes was observed in patients with regard to controls. Functional studies have shown that *TNFA*-308 variant wild-type G allele and *IL6*-174 variant wild-type G allele are correlated with low production of TNF- α and high production of IL-6, respectively. In contrast, *TNFA*-308 variant mutated allele A and *IL6*-174 variant mutated allele C are correlated with a high production of TNF- α and low production of IL-6, respectively. These pro-inflammatory cytokines play an important role in the regulation of the synthesis of acute phase proteins, such as fibrinogen and factor VIII, which are risk factors for atherosclerosis¹⁵. Therefore, a deregulation in the production of these cytokines could be conferring risk for developing AMI. On the other hand, the results suggest an advantage for the heterozygous genotype, with an increased frequency of polymorphisms *TNFA*-308 and *IL6*-174 G/A and G/C heterozygous genotypes, respectively, being observed in control individuals. Both genotypes would be associated with moderate production of these cytokines, thus

conferring protection against the development of AMI. Different studies have described that patients with ACS have increased concentrations of IL-6¹⁷⁻²¹ and TNF²²⁻²⁷. Increased IL-6 production may be promoting atherogenesis, contributing to AMI risk, or may be the result of atherosclerosis, associated with strong inflammatory reactions²⁸. Simultaneously, IL-6 regulates acute phase response, and thus constitutes an important activator in the production of C-reactive protein, which is an acute phase protein whose concentrations predict the risk for developing AMI. In fact, an increase in IL-6 and C-reactive protein concentrations has been described in patients with cardiovascular disease, and a relationship between IL-6, C-reactive protein and myocardial necrosis or infarction size has been even established, thus confirming the connection between infarction and inflammation²⁹⁻³⁰. Together, studies in patients with ACS suggest that IL-6 is associated with ischemia-reperfusion-associated myocardial injury and mortality³⁰. On the other hand, TNF- α , produced by endothelial cells, smooth muscle cells and coronary atheroma-associated macrophages, has been involved in several cardiovascular processes, and is overexpressed in the myocardium in response to transient myocardial ischemia and reperfusion. Furthermore, it has been hypothesized that TNF- α persistent overexpression, after ischemia, could lead to coronary adverse outcomes, and it has been suggested that TNF- α concentrations significantly increase with coronary ischemia²⁴. Furthermore, this cytokine induces vasoactive substances secretion in endothelial cells, which produce vasorelaxation or vasoconstriction and, consequently, influence the regulation of blood pressure, with high concentrations of TNF- α being observed in the serum of hypertensive patients with regard to normotensive individuals³¹, which would explain the interaction of polymorphism *TNF*-308 and hypertension in patients with AMI. Regarding *IL10* gene polymorphisms, a significantly increased frequency of the combination of variant *IL10*-1082 A/G-G/G genotypes and *IL10* GCC haplotype was observed in patients with regard to controls. Three of *IL10* gene most important polymorphisms (-1082, -819 and -592) generate three common haplotypes, GCC, ACC and ATA, which determine high, intermediate and low expression of the gene, thus affecting the concentrations of this cytokine³². Specifically, the G allele at position -1082 and the haplotypes that contain this allele have been associated with IL-10 high production, while allele A and haplotypes that contain this allele have been associated with low production of this cytokine³³. In this research, a significantly increased frequency of the combination

of genotypes A/G-G/G and haplotype GCC, which have been associated with high IL-10 expression, was observed in patients. These results agree with what is described in the literature, as shown by a meta-analysis of 12 eligible studies, in which an association between high concentrations of IL-10 and adverse events in patients with ACS was described³⁴. Moreover, Målarstig et al.³⁵ demonstrated that patients with ACS had higher IL-10 plasma concentrations than healthy individuals, and that said elevation at admission was associated with the number of CVRFs, to conclude that IL-10 reflects a pro-inflammatory state in patients with ACS and suggest that IL-10 is a biomarker that is as efficacious for predicting the risk of future cardiovascular events as other systemic inflammation markers. This would be in agreement with the interaction observed between polymorphism *IL10-1082* and hypertension. Despite the biological plausibility of IL-10 as a cardioprotective cytokine, high plasma concentrations could be a strong and independent predictor of long-term adverse cardiovascular outcomes in patients with ACS^{36,37}. One possible explanation is that numerous cytokines have both pro-inflammatory and anti-inflammatory properties, depending on target cells properties, on the number of cytokines and the activated signaling pathway³⁴. Another explanation is that IL-10 concentration increase in patients with cardiovascular disease could be a compensatory response against pro-inflammatory mediators elevation and vascular injury³⁶. In fact, a study carried out in a small cohort of patients with AMI showed that IL-10 spontaneous production by cultured leukocytes obtained from patients with AMI, but not IL-10 increased plasma concentrations, predicts a more favorable cardiac remodeling after ST-segment elevation AMI, which suggests that the association between IL-10 high plasma concentrations and recurrent cardiac events, adverse remodeling or heart failure could be spurious³⁸. Furthermore, studies in animal models have demonstrated the importance of IL-10 in cardiac remodeling after infarction. In the treatment of murine cardiac ischemia with stem cells, IL-10 deficiency in the ischemic myocardium affects survival and the function of exosomes derived from endothelial progenitor cells originating in bone marrow, whereas treatment with IL-10 improves cardiac remodeling and function, and reduces fibrosis³⁹. On the other hand, mice pericardial adipose tissue is enriched with IL-10-producing B cells, which facilitate the termination of infarction-induced inflammation, impact on the balance between pro-inflammatory and anti-inflammatory monocytes after infarction and reduce myocardial damage in order to preserve cardiac

function after infarction⁴⁰. Consequently, these results suggest that the use of recombinant IL-10 could be appropriate for the prevention of adverse cardiac remodeling in patients with ST-segment elevation AMI³⁹, and IL-10-producing B cells in pericardial adipose tissue might constitute a therapeutic target for improving AMI outcome⁴⁰.

Therefore, *IL10* gene polymorphisms may have an important role in the pathogenesis of AMI, thus supporting that IL-10 would be a prognostic biomarker of cardiac remodeling after AMI.

Conclusions

The results of the present study, based on the *TNFA*, *IL6* and *IL10* genes variants associated with risk for developing AMI, suggest the existence of an imbalance in the production of cytokines that promotes an exacerbated inflammatory process, which supports the essential role of inflammation at all stages of the atherosclerotic process, from its initiation to the progression of its complications.

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Conflicts of interest

None.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures that were followed adhered to the ethical standards of the responsible committee for experimentation on human beings and were in agreement with the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

References

- Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med*. 1994;330:1041-6.
- Olivieri F, Antonicelli R, Cardelli M, Marchegiani F, Cavallone L, Mocchegiani E, et al. Genetic polymorphisms of inflammatory cytokines and myocardial infarction in the elderly. *Mech Ageing Dev*. 2006;127:552-9.
- Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res*. 2002;53:31-47.
- Woods A, Brull DJ, Humphries SE, Montgomery HE. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. *Eur Heart J*. 2000;21:1574-83.
- Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur Heart J*. 2005;17:1765-73.
- Bonafe M, Olivieri F, Cavallone L, Giovagnetti S, Mayegiani F, Cardelli M, et al. A gender-dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur J Immunol*. 2001;31:2357-61.
- Ianni M, Callegari S, Rizzo A, Pastori P, Moruzzi P, Corradi D, et al. Pro-inflammatory genetic profile and familiarity of acute myocardial infarction. *Immun Ageing*. 2012;9:14.
- Bunce M. PCR-SSP typing in histocompatibility testing. Bidwell and Navarette C. Imperial College Press, London; 2000. p. 149-86.
- Ambruzova Z, Mrazek F, Raida L. Association of IL6 and CCL2 gene polymorphisms with the outcome of allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2009;44:227-35.
- Verjans GM, Brinkman BMN, van Doornik CE, Kijlstra A, Verweij CL. Polymorphism of tumor necrosis factor-alpha (TNF- α) at position -308 in relation to ankylosing spondylitis. *Clin Exp Immunol*. 1994;97:45-7.
- Kingkeow D, McNicoll JM, Maneeekam N, Wongtrakul J, Taecareonkul S, Suriyanon V, et al. Frequencies of IL10 SNP genotypes by multiplex PCR-SSP and their association with viral load and CD4 counts in HIV-1 infected Thais. *Asian Pac J Allergy Immunol*. 2011;29: 94-101.
- Olsson ML, Hansson C, Arent ND, Akesson IE, Green CA, Daniels GL. A clinically applicable method for determining the three major alleles at the Duffy (FY) blood group locus using polymerase chain reaction with allele-specific primers. *Transfusion*. 1998;38:168-73.
- Sole X, Guino E, Valls J, Iñiesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006;22:1928-29.
- Nie N, Hull C, Bent D. IBM Statistical Package for the Social Sciences (SPSS Version 20). Computer Software. Chicago; 2011.
- Biswas S, Ghoshal PK, Mandal SC, Mandal N. Relation of anti- to pro-inflammatory cytokine ratios with acute myocardial infarction. *Korean J Intern Med*. 2010;25:44-50.
- Fragoso JM, Vallejo M, Álvarez-León E, Delgadillo H, Peña-Duque MA, Cardoso-Saldaña G, et al. Alleles and haplotypes of the interleukin 10 gene polymorphisms are associated with risk of developing acute coronary syndrome in Mexican patients. *Cytokine*. 2011;55:29-33.
- Ito T, Ikeda U. Inflammatory cytokines and cardiovascular disease. *Curr Drug Targets Inflamm Allergy*. 2003;2:257-65.
- Wang YN, Che SM, Ma AQ. Clinical significance of serum cytokines IL-1 beta, siL-2R, IL-6, TNF-alpha and IFN-v in acute coronary syndrome. *Chin Med Sci J*. 2004;19:120-4.
- Aggarwal A, Schneider DJ, Terrien EF, Glibert KE, Dauerman HL. Increase in interleukin-6 in the first hour after coronary stenting: an early marker of the inflammatory response. *J Thromb Thrombolysis*. 2003;15:25-31.
- Funayama H, Ishikawa SE, Kubo N, Katayama T, Yasu T, Saito M. Increases in interleukin-6 and matrix metalloproteinase-9 in the infarct-related coronary artery of acute myocardial infarction. *Circ J*. 2004;68:451-4.
- Lino DOC, Freitas IA, Meneses GC, Martins AMC, Daher EF, Rocha JHC, et al. Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. *Braz J Med Biol Res*. 2019;52:e8658.
- González M, Ruiz-Ríos JA, Pérez-Paredes M, Lozano ML, García-Almagro FJ, Martínez-Corbalán F, et al. Prognostic value of tumor necrosis factor-alpha in patients with ST-segment elevation acute myocardial infarction. *Rev Esp Cardiol*. 2007;60:1233-41.
- Valgimigli M, Ceconi C, Malagutti P, Merli E, Soukhomovskaia O, Francolini G, et al. Tumor necrosis factor- α receptor-1 is a major predictor of mortality and new-onset heart failure in patients with acute myocardial infarction: The Cytokine-Activation and Long-Term Prognosis in Myocardial infarction (C-ALPHA) study. *Circulation*. 2005;111:863-70.
- Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*. 2000;101: 2149-53.
- Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, et al. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. *Curr Vasc Pharmacol*. 2015;13:26-36.
- Aydin S, Ugur K, Aydin S, Sahin I, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. *Vasc Health Risk Manag*. 2019;15:1-10.
- Mahmoud AH, Taha NM, Zakhary M, Tadros MS. PTEN gene and TNF-alpha in acute myocardial infarction. *Int J Cardiol Heart Vasc*. 2019;23:100366.
- Bennet AM, Prince JA, Fei GZ, Lyrenäs L, Huang Y, Wiman B, et al. Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction. *Atherosclerosis*. 2003;171:359-67.
- Moriya J. Critical roles of inflammation in atherosclerosis. *J Cardiol*. 2019;73:22-7.
- Groot HE, Al Ali L, van der Horst ICC, Schurer RAJ, van der Werf HW, Lipsic E, et al. Plasma interleukin 6 levels are associated with cardiac function after ST-elevation myocardial infarction. *Clin Res Cardiol*. 2019;108:612-21.
- Li Y-Y. Tumor necrosis factor-alpha G308A gene polymorphism and essential hypertension: a meta-analysis involving 2244 participants. *PLoS One*. 2012;7:e35408.
- Iyer SS, Cheng G. Role of Interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol*. 2012;32:23-63.
- Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, et al. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet*. 1997;349:170-3.
- Liu J, Li X, Xu R, Zhu C, Guo Y, Wu N, Li J. Serum interleukin 10 levels and adverse events in patients with acute coronary syndrome: a systematic review and meta-analysis. *Chin Med J*. 2014;127:150-6.
- Målarstig A, Eriksson P, Hamsten A, Lindahl B, Wallentin L, Siegbahn A. Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary. *Heart*. 2008; 94:724-9.
- Tabrez S, Ali M, Jabir NR, Firoz CK, Ashraf GM, Hindawi S, et al. A putative association of interleukin-10 promoter polymorphisms with cardiovascular disease. *IUBMB Life*. 2017;69:522-7.
- Cavusoglu E, Marmur JD, Hojati MR, Chopra V, Butala M, Subnani R, et al. Plasma interleukin-10 levels and adverse outcomes in acute coronary syndrome. *Am J Med*. 2011;124:724-30.
- Falcão RA, Christopher S, Oddi C, Reznikov L, Grizzard JD, Abouzaki NA, et al. Interleukin-10 in patients with ST-segment elevation myocardial infarction. *Int J Cardiol*. 2014;172:e6-8.
- Yue Y, Wang C, Benedict C, Huang G, Truongcao M, Roy R, et al. Interleukin-10 deficiency alters endothelial progenitor cell-derived exosome reparative effect on myocardial repair via integrin-linked kinase enrichment. *Circ Res*. 2020;126:315-29.
- Wu L, Dalal R, Cao CD, Postojak JL, Yang G, Zhang Q, et al. IL-10-producing B cells are enriched in murine pericardial adipose tissues and ameliorate the outcome of acute myocardial infarction. *PNAS*. 2019;116:21673-84.