ORIGINAL ARTICLE

Relationship of Interleukin-6 G-174C and G-572C and Interleukin-1 C-511T and C+3953T Polymorphisms with Ischemic Heart Disease in Quito, Ecuador

Relación de los polimorfismos G-174C y G-572C de la interleucina-6 y C-511T y C+3953T de la interluecina-1 con la cardiopatía isquémica en Quito, Ecuador

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ABSTRACT

Background: It has been recently found that inflammatory processes are directly related to the development of atherosclerotic plaque, causing ischemic heart disease. For this reason, every molecule related to these processes is critically important. Interleukins (IL) are proinflammatory cytokines, and their polymorphisms seem to increase the inflammatory progress. IL-1 and IL-6 polymorphisms are the ones most significantly associated with ischemic heart disease.
Objectives: The aim of this study was to establish the relationship of IL-6 G-174C and G-572 C and IL-1 C-511T and C+3953T polymorphisms with ischemic heart disease.
Methods: A retrospective study of 76 cases and 76 controls was carried out in patients attending the hemodynamics service of Carlos Andrade Marín Hospital (HCAM) of Quito, Ecuador. Genotyping was done on the basis of polymerase chain reaction with restriction enzymes (PCR-RFLP).
Results: Among the four polymorphisms studied, only IL-6 -174 GG was significantly associated with ischemic heart disease. The logistic regression analysis used to determine the most important predictors of ischemic heart disease showed that the IL-6 -174 GG genotype was associated with an increased risk of independently presenting ischemic heart disease (OR 4.065, $p \leq 0.001$).
Conclusions: The GG genotype of IL-6 G-174C polymorphism confers a fourfold higher risk of developing ischemic heart disease, while the remaining three polymorphisms do not pose risks in this human population.

Key words: Coronary Artery Disease - Atherosclerosis - Polymorphism - Interleukin-1 - Interleukin-6.

RESUMEN

Introducción: En los últimos años se ha evidenciado que los procesos inflamatorios están directamente relacionados con la formación de la placa ateroesclerótica, causante de la cardiopatía isquémica (CI). Por esta razón, toda molécula relacionada con aquellos procesos es de vital importancia. Las interleucinas (IL) son citoquinas proinflamatorias y sus polimorfismos aparentemente incrementan el proceso inflamatorio. Los más asociados con la cardiopatía isquémica son algunos polimorfismos de las interleucinas 1 (IL-1) y 6 (IL-6).
Objetivos: Establecer la relación de los polimorfismos G-174C y G-572C de la interleucina-6 y C-511T y C+3953T de la interleucina-1 con la cardiopatía isquémica.
Material y métodos: Se desarrolló un estudio de tipo analítico retrospectivo, de 76 casos y 76 controles, de pacientes atendidos en el servicio de hemodinámica del Hospital Carlos Andrade Marín (HCAM), de Quito. La genotipificación se hizo mediante la reacción en cadena de la polimerasa con enzimas de restricción (PCR-RFLP).
Resultados: De los cuatro polimorfismos estudiados, únicamente el IL-6174 GG tuvo una asociación estadísticamente significativa con la cardiopatía isquémica. La regresión logística usada para determinar los predictores más importantes de cardiopatía isquémica mostró que el genotipo IL-6 174 GG (OR 4,065, p=<0,001) se asoció con un incremento del riesgo de presentar cardiopatía isquémica de forma independiente.
Conclusiones: El genotipo GG del polimorfismo IL-6 G-174C confiere un riesgo 4 veces superior de desarrollar cardiopatía isquémica, mientras que los tres polimorfismos restantes no confieren riesgos.

Palabras claves: Enfermedad arterial coronaria - Aterosclerosis - Polimorfismos interleucina-1 e- interleucina-6

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INTRODUCTION

Cardiovascular diseases are the main cause of morbidity and mortality in the Western hemisphere, and are associated with diabetes, dyslipidemia and hypertension, as well as toxic habits such as smoking and alcohol consumption, among others. (1) In the last years, however, new risk factors unrelated with patient lifestyle but with inflammatory processes have been investigated, suggesting that their mechanism is essential for atherosclerotic plaque development. (2) This is the case of mutations found in interleukins (IL), molecules that participate in inflammatory activity. (3) For example, IL-1 and IL-6 are signaling peptides expressed as a consequence of tissue aggression, activating an inflammatory response.

Several studies have demonstrated that some IL-1 and IL-6 simple nucleotide polymorphisms are associated with ischemic heart disease (IHD), the best studied being IL-1β C-511T (Rs16944), IL-1β C+3953T (Rs1143634), IL-6 G-174C (Rs1800795) and IL-6 G-572C (Rs1800796). The aim of this study was to associate these four IL-6 and IL-1 polymorphisms with the risk of presenting IHD in an Ecuadorian population sample.

METHODS

Study population

A case-control study was designed. The case group consisted of 76 patients fulfilling the IHD diagnostic criteria of ACCC/AHA guidelines. These criteria were: two angina episodes at rest or an angina episode lasting more than 20 minutes in the last 48 hours, electrocardiographic (ECG) ST-segment elevation during angina, with or without cardiac ischemia enzyme release (CK-MB/troponin T), or more than 50% angiographic stenosis.

The control group comprised 76 individuals without history of cardiovascular disease, who attended the hospital for reasons unrelated to IHD. In both cases, individuals were Ecuadorian, selected in the hemodynamics service of Hospital Carlos Andrade Marín in Quito, from August 2014 to December 2015.

DNA procurement and genotyping

Venous blood samples were collected in EDTA tubes to obtain genomic DNA. This was isolated from leukocytes using a commercial Wizard Genomic DNA Purification Kit. DNA was quantified in a Thermo Scientific™ NanoDrop 2000 spectrophotometer at 260 nm and 280 nm and diluted to a concentration of 20 ng/μL.

The DNA region containing the polymorphic site was amplified by polymerase chain reaction (PCR). A final volume of 25 μl: 12.5 μL of GoTaq® Green Master Mix (Promega), 0.5 μL of FW primer and 0.5 μL of RV primer (Invitrogen), 3 μL of DNA and 8,5 μL of UltraPure™ nuclease-free water (Invitrogen) was obtained for each sample (IL-1 and Il-6 polymorphisms). Cycling parameters for Il-1 were 3 minutes at 94°C and then 35 cycles at 95°C for 2 minutes, at 53°C for 1 minute and at 74°C for 1 minute, followed by a final extension at 72°C for 5 minutes. Cycling parameters for Il-6 were 10 minutes at 94°C and then 35 cycles at 95°C for 1 minute, at 55°C for 1 minute 35 seconds and at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. The amplification products were digested with specific restriction enzymes and then separated by horizontal electrophoresis in agarose gels (2.5% w/v) and stained with Gel Stain (Lonza). Primers and enzymes used in the study are detailed in Table 1.

Statistical analysis

Sample size was calculated based on a 95% confidence level and 80% study power. The expected exposure frequency was 32% for controls and 60% for cases, adding an extra 20% for loss and error.

A retrospective case-control analytical study was developed. SPSS 23 was used for statistical analysis. Frequency differences of alleles and other categorical variables were compared between cases and controls using Pearson's chi-squaretest or Fisher's exact test. Odds ratio and logistic regression were calculated considering $p < 0.05$ as significant.

A chi-square test was used to analyze whether the study population was at Hardy-Weinberg equilibrium for the polymorphisms studied.

Ethical considerations

The study was approved by the Research Ethics Committee of Pontificia Universidad Católica del Ecuador and by the HCAM Health Research Committee, and fulfilled the declaration of Hesinki guidelines. A written informed consent was obtained from all participants.

RESULTS

The case-control population consisted of subjects with average age between 40 and 70 years; however, the case population was older than the control population. Male gender was predominant in both cases and controls. Diabetes, hypertension, dyslipidemia and alcohol and tobacco consumption were more prevalent in the case population (Table 2). A chi-square analysis showed that the population was at Hardy-Weinberg equilibrium for the polymorphisms studied, except for IL-6 G-174C (chi-square = 1,551.45) polymorphism.

The CC genotype of IL1β -511 polymorphism was more frequent in cases of IHD (17.11%) compared with controls (11.84%) with an OR of 1.53 (95% CI 0.61-3.84; $p=0.05$). A significant difference was also found in the GG genotype of IL-6 -174, which was more frequent in cases of IHD (73.68%) than in controls (40.79%) with an OR of 4 (95% CI 2.04-8.06; $p<0.001$) (Table 3).

A logistic regression analysis was performed (Table 4) to establish the most important predictors of IHD. The GG genotype of IL-6 -174 polymorphism was associated with an increased risk of presenting IHD in this Ecuadorian population sample. This genotypic association was independent of other usual risk factors, as alcoholism and dyslipidemia, which also presented a significant association. No significant risk association was found with the other polymorphisms.

DISCUSSION

In our population, comorbidities most frequently associated with IHD were hypertension (HTN), dyslipidemia and type 2 diabetes mellitus (DM-2), consist-

Table 1. Primer and restriction enzyme sequence used to detect IL-1 and IL-6 polymorphisms

Polymorphism	SNP Identification	Primer sequence	Fragment	Enzyme	Size of the digested fragment (bp)			Reference
					N	H	M	
IL-1β C-511T	rs16944	FW: TGG CAT TGA TCT GGT TCA TC RV: GTT TAG GAA TCT TCC CAC TT	305 bp	Ava I	305	305, 190, 115	190,115	5
IL-1β C+3953T	rs1143634	FW: GTT GTC ATC AGA CTT TGA CC RV: TTC AGT TCA TAT GGA CCA GA	249 bp	Taq I	249	249,135,114	135, 114	20
IL-6 G-174C	rs1800795	FW: TGA CTT CAG CTT AC TCT TGT RV: CTG ATT GGA AAC CTT ATT AAG	198 bp	SfaNI	198	198, 140, 58	140,58	19
IL-6 G-572C	rs1800796	FW: ACGCCTTGAA GTAACTGC RV: TTTCCTCTGACT CCATCGCAG	163 bp	MbiI	163	163, 88, 75	88,75	21

FW: Forward primer, RV: reverse primer, N: Normal homozygous, H: Heterozygous, M: Mutant homozygous. bp: Base pair

Table 2. Clinical characteristics of ischemic heart disease cases and controls

Variables	Cases n= 76	Controls n=76	p value
Age	64.7 ± 12	57.5 ± 12	b
Male	66 (86.84%)	52 (68.42%)	a
Female	10 (13.16%)	24 (31.58%)	a
HTN	44 (57.89%)	28 (36.84%)	b
DM 2	25 (32.89%)	12 (15.79%)	a
Dyslipidemia	46 (60.53%)	18 (23.6%)	b
Alcohol	40 (52.63%)	18 (23.7%)	b
Tobacco	36 (47.37%)	23 (30.26%)	a

HTN: Hypertension; DM 2: Type 2 Diabetes mellitus 2; n: Number of patients, a: p <0.05; b: p <0.01

ent with Italian, Brazilian and other Latin American countries' findings. (4-6)

Regarding IL-1β C-511T (Rs16944), IL-1β C+3953T (Rs1143634), IL-6 G-174C (Rs1800795) and IL-6 G-572C (Rs1800796) polymorphisms, results showed that only the GG genotype of IL-6 G-174C polymorphism (Rs1800795) was significantly associated with the presence of coronary events (OR=4.06; 95% CI 2.04-8.06; p<0.001).

The CC genotype of IL-1β C-511T (Rs16944) polymorphism presented p=0.05 for association with IHD, with arguable significance. (7)

A Brazilian study showed that the GG genotype is associated with increased risk of coronary heart disease (OR=2.037; p=0.028). (8) In that study, the genotypic distribution was present in 69.6% of cases versus 60% in the control population. These results are consistent with the observations of the present study, where the genotypic frequency was 73.68% in cases and 40.79% in controls.

The explanation for this finding is that IL-6 is a proinflammatory cytokine, and hence, polymorphism carriers in its encoding sequence have increased susceptibility for the development if IHD. (9). It is considered that IL-6 promotes the atherosclerotic signaling pathway.

In a sample of 106 Turkish patients with acute coronary syndrome (ACS), Ozdemir et al. found a frequency of 82% for the homozygous GG genotype. Moreover, 31% of patients presenting Il-6 G-174C polymorphism developed multivessel lesion vs. 8% evidencing single lesion at coronary angiography (p<0.05). This investigation showed a relationship between polymorphism and coronary involvement severity. Patients with the GG genotype presented longer hospital stay and more severe complications due to higher IL-6 plasma levels. (10)

Different investigations have sought to correlate IL-6 values with coronary artery severity, under the assumption that IL-6 levels become higher as vascular involvement increases. Deliargyris et al. showed that IL-6 is generated in the coronary circulation of patients with unstable angina, but not in those with stable angina. (11) A Turkish study describes higher

Genotypes	Cases n= 76	Controls n=76	p value	OR (95% CI)
IL-1B C-511T				
TT	33 (43.42%)	48 (63.16%)	NS	0.47 (0.24-0.90)
TC	30 (39.40%)	19 (25%)	NS	1.95 (9.97-3.91)
CC	13 (17.11%)	9 (11.84%)	NS	1.53 (0.61-3.84)
TT+TC vs CC			NS	0.65 (0.26-1.62)
TT vs TC+CC			a	0.44 (0.23-0.85)
IL-1B C-3953T				
TT	0 (0%)	4 (5.26%)	a	1.05 (1.00-1.11)
TC	14 (18.42%)	11 (14.47%)	NS	1.33 (0.56-3.16)
CC	62 (81.58%)	61 (80.26%)	NS	1 (0.44-2.27)
TT+TC vs CC			NS	0.92 (0.48-2.06)
TT vs TC+CC			a	0.00 (0.00-1.09)
IL-6 G-174C				
CC	5 (6.58%)	32 (42.11%)	b	0.09 (0.03-0.26)
GC	15 (19.74%)	13 (17.11%)	NS	1.19 (0.52-2.71)
GG	56 (73.68%)	31 (40.79%)	b	4.06 (2.04-8.06)
CC+GC vs GG			b	0.24 (0.12-0.48)
CC vs GC+GG			b	0.09 (0.03-0.26)
IL-6 G-572C				
CC	12 (15.79%)	18 (23.68%)	NS	0.6 (0.26-1.36)
GC	31 (40.79%)	31 (40.79%)	NS	1 (0.52-1.91)
GG	33 (43.42%)	27 (35.53%)	NS	1.39 (0.72-2.67)
CC+GC vs GG			NS	0.71 (0.37-1.37)
CC vs GC+GG			NS	0.60 (0.26-1.36)

n= Number of patients, a: p <0.05; b: p.<0.01; NS: Not significant

Table 3. Genotypic frequencies of IL-1β C-511T, IL-1βC+3953T, IL-6 G-174C and IL-6 G-572C polymorphisms in cases with ischemic heart disease and controls

Variables	B	Standard error	Wald	OR	95% CI	p value
Alcohol	1.098	0.495	4.931	3.58	1.78-7.17	a
Tobacco	0.750	0.506	2.200	2.07	1.06-4.03	NS
HTN	0.475	0.441	1.159	2.35	1.22/4.55	NS
DM 2	0.624	0.499	1.569	2.61	1.19-5.70	NS
Dyslipidemia	1.423	0.443	10.299	4.94	2.45-9.95	b
IL6 -174 GG	0.380	0.529	0.517	4.065	2.04-8.06	b

IL6-174GG vs. GC+CC; HTN: Hypertension; DM 2: Diabetes mellitus 2; a: p <0.05; b: p <0.01; NS: Not significant.

Table 4. Logistic regression for risk of presenting ischemic heart disease

IL-6 and C-reactive protein levels in patients who developed an acute myocardial infarction compared with those presenting stable IHD. (10) Different studies have shown that IL-6 plasma levels are higher in patients carrying the GG genotype of IL-6 G-174C polymorphism. (12-14)

The results of the present study are in agreement with reported findings on IL-6 G-174C polymorphism (6, 8, 12, 13, 15). However, it is noteworthy that other studies have associated the CC, instead of the GG genotype, with the risk of cardiovascular disease. (16-18) As stated by M. Libra, "these conflicting results suggest a complex relationship of IL-6 G-174C polymorphism with the development of cardiovascular diseases". (19)

CONCLUSIONS

In this group of patients, the presence of the GG genotype of IL-6 G-174C polymorphism confers a fourfold higher risk of developing IHD. We consider it is necessary to perform further studies with a larger and more diverse sample in order to generalize the results to the whole population of Ecuador.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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