Association of the −1031 T > C polymorphism and soluble TNF-α levels with Acute Coronary Syndrome

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ABSTRACT

Introduction: Inflammation has gained a pivotal role in the pathophysiology of Acute Coronary Syndrome (ACS). TNF-α is a pro-inflammatory cytokine that could be a potential biomarker in ACS due to its multiple functions. The rs1799964 *TNFA* polymorphism (−1031 T > C) has been associated with a decrease in gene transcription and cytokine levels.
Objective: To determine the association of rs1799964 *TNFA* polymorphism and TNF-α soluble levels in ACS.
Methods: A total of 251 patients diagnosed with ACS and 164 individuals without cardiovascular diseases classified as the reference group (RG), were included. The rs1799964 polymorphism was genotyped by PCR-RFLP. Soluble protein levels were determined by ELISA. Statistical analyses were performed using chi square and U-Mann Whitney tests.
Results: The genotype and allele frequencies were different between ACS and RG (OR = 0.317, p = 0.01; OR = 0.688, p = 0.03 respectively). ACS patients had higher soluble TNF-α levels compared with the RG (31.08 *vs* 23.00 pg/mL, p < 0.001); according genotype significant differences were observed (T/T: 24.06 *vs* T/C: 34.95 pg/mL, p = 0.0001) in patients. In the RG, T/T carriers showed discrete lower levels than C/C genotype (22.14 *vs* 27.83 pg/mL, p = 0.04).
Conclusions: The −1031 C allele of the *TNFA* polymorphism confers protection for the development of ACS. The T/C genotype carriers had higher TNF-α serum levels compared to the T/T genotype in ACS. In addition, the −1031 T > C *TNFA* polymorphism was associated with dyslipidemia in ACS in a Western Mexican population.

1. Introduction

Ischemic heart disease remains a major public health problem to date and is considered one of the leading causes of mortality worldwide including in emergency care services and is the third disease in terms of morbidity costs [1,2]. Since 1970 to date, the mortality rate associated with cardiovascular disease has increased consistently in Mexico, becoming the second cause of death [3,4].

Acute Coronary Syndrome (ACS) is one of the major cardiopathies [5,6]. The clinical manifestations that comprise ACS have the pathophysiology of ischemia or acute coronary insufficiency. In this scope,

unstable angina (UA), acute myocardial infarction with ST-segment elevation (STEMI) and acute myocardial infarction with non-ST segment elevation (NSTEMI) are included [7–9]. Acute ischemia is caused in most cases by the rupture of the atherosclerotic plaque in the coronary artery with rapid platelet accumulation and formation of an occlusive thrombus [7,10,11]. The average onset age is 40 years and is more prevalent in men than in women with a ratio of 3:1 [4,12–14].

The recruitment of circulating leukocytes to the vascular endothelium and increased migration to the sub endothelial spaces are the major steps in the development of atherosclerosis and this process is mediated by the participation of various molecules including pro-inflammatory cytokines and adhesion molecules [15].

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Tumor necrosis factor alpha (TNF-α) is a cytokine involved in lipid metabolism, coagulation, insulin resistance and endothelial function (OMIM *191160). Evidence suggests that pro-inflammatory cytokines play a key role, not only in atherogenesis, but also trigger ACS [16]. Similarly, it has been suggested that the enhanced release of TNF-α may contribute to the risk of myocardial infarction [17].

In relation to TNF-α levels in patients with ischemic stroke, these have been found significantly elevated compared with controls correlating positively with age, BMI and cholesterol levels [18–20]. Aside from promoting strong inflammation and thrombosis, it has been reported that elevated TNF-α levels also contribute to an increased activity of matrix metalloproteinase 2 (MMP2), collagen degradation, cardiomyocyte apoptosis and chronic left ventricular dysfunction after an Acute Myocardial Infarction (AMI) [21,22].

The identification of genetic variants has improved our understanding of pathogenic diseases. *TNFA* is a candidate gene for the development of ACS due to its involvement in the formation of the atherosclerotic plaque when the proinflammatory signaling cascade is initiated. Several *TNFA* polymorphisms have been related to atherosclerosis [23] and myocardial infarction [15,24–28]. Among the most studied are the −308 G > A and −238 G > A polymorphisms which have a lower variant frequency and thus, hamper genetic association interpretations. The −1031 T > C promoter polymorphism (rs1799964) has a reported minor allele frequency (−1031 C) of 22% in the 1000 Genomes Database. This polymorphism is located near the union sequence of transcriptional factors NF-kB and OCT-1 [29,30] and is considered a functional SNP influencing gene expression which is also related to decreased cytokine levels [31].

The lack of studies in Western Mexican population regarding the association of the −1031 T > C (rs1799964) polymorphism of the *TNFA* gene with ACS is what led us to study the genetic association of this polymorphism and the soluble levels of the protein in ACS patients and individuals without a history of cardiovascular disease matched by age from the region of Western Mexico.

2. Methods

2.1. Subjects. We studied 415 individuals genetically unrelated with the following characteristics

(1) One hundred and sixty-four Reference Group (RG) individuals were recruited (71 males and 93 females). They responded a questionnaire on their medical history and lifestyle characteristics. Inclusion criteria: individuals with a similar age distribution as cases, absence of cardiovascular diseases, not receiving medical treatment and recruited during the same period as the cases.
(2) Two hundred and fifty-one patients with ACS (187 males, 64 females) were diagnosed according to the American College of Cardiology (ACC) criteria [5]. All patients were enrolled between the second and the third day of the acute coronary event (AMI and UA).

Subjects were recruited from the "Hospital de Especialidades del Centro Medico Nacional de Occidente, Instituto Mexicano del Seguro Social (CMNO–IMSS)". Individuals with other diseases such as infectious, cancer and autoimmune pathologies were not considered.

The diagnosis of ACS was made by cardiologists. The diagnostic triad included clinical symptoms, electrocardiogram changes and alterations in cardiac biomarkers (CK: creatine kinase; CK-MB: creatine kinase muscle and brain and Troponine I). Routine biochemical test measures were registered. Classical risk factors, defined according to the ACC [5], were categorized as present or absent.

2.2. Ethical considerations

The study was performed in accordance to the Declaration of Helsinki. All patients and subjects accepted to participate in the study and an informed written consent was obtained. Ethical approval was obtained by the Centro Universitario de Ciencias de la Salud, CUCS, UdeG (CI/065/2014).

2.3. Ancestry

All participants were from the same ethnical origin region, and we considered as Mexican mestizos only those individuals who for three generations, including their own, had been born in Mexico having a Spanish-derived last name.

2.4. Genetic analyses

Genomic DNA (gDNA) was purified from total leukocytes in peripheral blood, by means of the Miller technique [32]. Then, gDNA concentration was determined spectrophotometrically at a wave length of 260 nm (absorbance of nucleic acids) and 280 nm (absorbance of proteins). Once gDNA concentration was obtained, the samples were stored at −20 °C until use.

The rs1799964 polymorphism was amplified by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) using the following primer sequences: forward, 5′- TAT GTG ATG GAC TCA CCA GG -3′; and reverse, 5′- CCT CTA CAT GGC CCT GTC TT -3′. PCR amplification was carried out in a total volume of 20 μL containing 10 ng/μL of gDNA, 0.08 U/μL of *Taq* DNA polymerase (Invitrogen™, Carlsbad, CA, USA), 1X of buffer, 0.6 pM of each primer, 1.5 mM of $MgCl_2$ and 0.1 mM dNTP. The thermocycling conditions had an initial denaturation step of 3 min at 94 °C followed by 30 cycles of 45 s each at 94 °C, 62 °C and 72 °C and the final extension step of 1 min at 72 °C. A 264 bp PCR fragment was digested using 5 U of *BbsI* enzyme (New England BioLabs®, Beverly, MA) in a final volume of 15 μL during 3 h at 37 °C. Digestion of the *TNFA* (−1031 T > C) polymorphism resulted in 251-bp and 13-bp (T allele) or 180-bp, 71-bp and 13-bp (C allele) fragments. The amplified PCR and digested products were analyzed by electrophoresis on 6% polyacrylamide gels (29:1; acrylamide:bis-acrylamide) stained with silver nitrate.

2.5. Analysis of soluble TNF-α levels

Soluble TNF-α levels (Invitrogen™, KHC3011) were measured in duplicate using all the serum samples from ACS patients and the RG by enzyme linked immunosorbent assay (ELISA) according to the specification of manufacturer. Once collected, blood was clotted in an upright position for at least 30 min before centrifugation. Serum samples were stored at −80 °C until use. Soluble TNF-α range of detection was 1.7–1000 pg/mL and the assays sensitivity was 1.7 pg/mL. Soluble TNF-α concentration was calculated using a four parameter logistic (4-PL) curve fit. Laboratory coefficients of covariance were in acceptable ranges (<10%).

2.6. Statistical analyses

The statistical analyses were carried out using SPSS statistical package version 21.0, Excel 2010, and Graph Pad Prism 6.04 (Graph Pad software, CA, USA). The X^2 or Fisher's exact test, when applicable, was used to compare discrete variables and to test the Hardy–Weinberg equilibrium. Soluble level comparisons were evaluated by Mann–Whitney U test representing median ± interquartile range 25–75.

The odds ratio (OR) was the measure of association. The significance level was <0.05. In order to rule out the age and gender bias

in the measure of soluble TNF-α levels, a linear regression was applied.

3. Results

3.1. Clinical and demographic characteristics

Baseline clinical and demographic characteristics of ACS patients and RG are shown in Table 1. The mean age of ACS patients was 65 years. By gender, 187 men and 64 women were included. Except for glucose, routine biochemical tests were found within reference values in both patients and the reference group. The average concentrations of cardiac markers were found to be elevated as expected since such markers are taken into consideration for the diagnosis of the disease (CPK: 386 IU/mL, CPK-MB: 51 IU/mL, Troponine I: 3.19 ng/mL). The main risk factors present in ACS patients were high blood pressure (62.9%), smoking (57.8%) and diabetes mellitus type 2 (49.4%).

3.2. Genetic contribution

In this study, the genotype distribution for the polymorphism −1031 T > C was in accordance with the Hardy-Weinberg equilibrium expectations ($p = 0.5$). Both genotype and allele frequencies showed significant differences (OR = 0.317, $p = 0.01$; OR = 0.688, $p = 0.03$, respectively). Regarding the heritage model, we found a significant difference in the recessive model (OR = 0.333, $p = 0.02$, Table 2).

Comparisons of clinical variables with the polymorphism according to heritage models in the ACS group are shown in Table 3. Conversely, we found a risk association of C allele carriers with dyslipidemia in the co-dominant and dominant models (OR = 2.29–2.31, $p < 0.05$). This association was confirmed and corrected by linear regression ($p < 0.003$).

3.3. Soluble levels

TNF-α soluble levels (Fig. 1a) were significantly higher in patients with ACS compared to the RG (median 31.08 *vs* 23.00 pg/mL, $p = 0.0001$).

When comparing serum TNF-α levels according to type of ACS, no statistically significant differences (Fig. 1b) were found. Neither age nor gender was different in the median of TNF-α serum levels (data not shown).

Table 2
Allele and genotype distribution of the −1031 T > C (rs1799964) *TNFA* polymorphism by groups.

	RG[a] *n* (%)	ACS *n* (%)	OR (CI)	*p*
Genotype				
T/T	102 (62.2)	173 (68.9)	–	–
T/C	49 (29.9)	71 (28.3)	0.854 (0.551–1.325)	0.48
C/C	13 (7.9)	7 (2.8)	0.317 (0.123–0.822)	0.01
Allele				
T	253 (77.1)	417 (83.1)	–	–
C	75 (22.9)	85 (16.9)	0.688 (0.486–0.973)	0.03
Dominant				
T/T	102 (62.2)	173 (68.9)		
T/C + C/C	62 (37.8)	78 (31.1)	0.742 (0.490–1.122)	0.15
Recessive				
T/T + T/C	151 (92.1)	244 (97.2)	–	–
C/C	13 (7.9)	7 (2.8)	0.333 (0.130–0.854)	0.02

RG: reference group; ACS: acute coronary syndrome; OR: odds ratio, *n*: sample size; CI: confidence interval; *p*: probability value using exact test.
[a] $p = 0.5$ for Hardy–Weinberg equilibrium.

Further, TNF-α levels were stratified by genotype (Fig. 1c). Marginal differences were observed in the RG between T/T and C/C genotype carriers (22.14 *vs* 27.83 pg/mL, $p = 0.042$). In the ACS group, significant differences were observed among individuals carrying the T/T and T/C genotype (24.06 *vs* 34.95 pg/mL, $p = 0.0001$). Furthermore, by genetic model testing, significant differences were found in the dominant model in the ACS group (T/T: 24.06 *vs* T/C + C/C: 34.88 pg/mL, $p = 0.0001$; data not shown).

Since TNF-α serum levels found in our study were slightly higher compared to other studies, we measured soluble levels of this cytokine in 38 females and 40 males (55–65 years) without cardiovascular diseases and without other associated risk factors with ACS, as an internal control group. The median value was 6.12 pg/mL (IQR$_{25-75}$: 2.13–16.67, data not shown).

4. Discussion

4.1. Clinical and demographic characteristics

Particularly in Mexico, cardiovascular diseases have been on the rise as well as associated comorbidities such as hypertension [33,34]. In this regard, high blood pressure was the most prevalent risk factor in both study groups (62.9% and 29.3%). The CARMELA study (assessment of cardiovascular risk in seven Latin American

Table 1
Demographic and clinical data of the RG and patients with ACS.

	RG median (IQR 25–75) *n* = 164	ACS median (IQR 25–75) *n* = 251	Reference value
Age (years)	58 ± 8.3	65 ± 11.3	–
Male/female	71/93	187/64	–
Glucose (mg/dL)	91.50 (77–108)	125 (95.5–180.5)	75–110
Cholesterol (mg/dL)	143.50 (119.5–164.5)	115 (91.25–134)	<200
Triglycerides (mg/dL)	83 (70–106)	88 (72–100)	<200
HDLc (mg/dL)	23 (18–27)	15 (11.5–20.5)	<60
LDLc (mg/dL)	51 (43–61)	40 (31–48.5)	<129
CK (IU/mL)	–	386 (142–1028.5)	24–195
CK-MB (IU/mL)	–	51 (23–126)	<130
Troponine I (ng/mL)	–	3.19 (0.6–7.9)	0.1–0.4
Risk factor	*n* (%)	*n* (%)	
Obesity	45 (27.44)	118 (47)	–
Diabetes mellitus type 2	25 (15.24)	124 (49.4)	–
Dyslipidemia	1 (0.61)	103 (41)	–
High blood pressure	48 (29.27)	158 (62.9)	–
Smoking	10 (6.09)	145 (57.8)	–

ACS: acute coronary syndrome; RG: reference group; IQR: interquartile range; HDLc: high density lipoprotein; LDLc: low density lipoprotein; CK: creatine kinase; CK-MB: creatine kinase muscle and brain. The upper limit of normal CK is defined by individual hospital laboratory standards.

Table 3
Association of clinical and demographic characteristics in the ACS group with the −1031 T > C polymorphism (rs1799964) in the *TNFA* gene by genetic models.

Phenotype	ACS n = 251	Co-dominant							Dominant				Recessive			
		T/T * n = 173	T/C n = 71	OR (CI)	p	C/C n = 7	OR (CI)	p	T/T n = 173	T/C + C/C n = 78	OR (CI)	p	T/T + T/C n = 244	C/C n = 7	OR (CI)	p
Diagnosis																
UA	20	14	6	1.042 (0.384–2.829)	0.936	0	0.729 (0.040–13.414)	0.432	14	6	0.940 (0.347–2.547)	0.904	20	0	1.376 (0.076–24.960)	0.430
STEMI	188	126	57	0.947 (0.346–2.591)	0.916	5	1.261 (0.066–23.985)	0.457	126	62	1.148 (0.421–3.132)	0.787	183	5	0.814 (0.043–15.252)	0.460
NSTEMI	42	32	8	0.583 (0.170–1.997)	0.388	2	2.231 (0.101–49.460)	0.354	32	10	0.729 (0.222–2.400)	0.603	40	2	0.395 (0.018–8.619)	0.321
Risk factors																
OBESITY	118	76	41	1.744 (0.998–3.049)	0.050	1	0.213 (0.025–1.805)	0.120	76	42	1.489 (0.870–2.547)	0.145	117	1	5.528 (0.656–46.600)	0.078
DM2	124	85	36	1.065 (0.613–1.850)	0.824	3	0.776 (0.169–3.573)	0.745	85	39	1.035 (0.607–1.767)	0.899	121	3	1.312 (0.287–5.984)	0.725
Dyslipidemia	103	60	39	2.295 (1.308–4.029)	0.003	4	2.511 (0.544–11.589)	0.224	60	43	2.314 (1.342–3.991)	0.002	99	4	0.512 (0.112–2.338)	0.380
HT	158	108	45	1.042 (0.588–1.847)	0.889	5	1.505 (0.284–7.980)	0.629	108	50	1.075 (0.617–1.873)	0.799	153	5	0.673 (0.128–3538)	0.637
COPD	2	1	1	2.457 (0.152–39.833)	0.513	0	7.667 (0.288–204.368)	0.840	1	1	2.234 (0.138–36.180)	0.562	2	0	0.155 (0.007–3.509)	0.810
Tobacco	145	98	42	1.108 (0.633–1.942)	0.719	5	1.913 (0.361–10.135)	0.438	98	47	1.160 (0.673–1.999)	0.592	140	5	0.538 (0.102–2.830)	0.458

ACS: acute coronary syndrome; UA: unstable angina; DM2: diabetes mellitus type 2; COPD, chronic obstructive pulmonary disease; HT: hypertension; CI: confidence interval; NSTEMI: I myocardial infarction with non-ST segment elevation; OR: odds ratio; STEMI: myocardial infarction with ST segment elevation.
* Variable of Reference. Significant *p* values were corrected by linear regression and are reported in this table.

cities) documented the most common risk factors: smoking (30%), obesity (23%), metabolic syndrome (20%), hypertension (18%), hypercholesterolemia (14%) and diabetes mellitus (7%) [35]. In this study, the importance of finding these comorbidities higher in patients with ACS, supports the knowledge that the sum of environmental and other factors such as genetic are involved in blood thrombogenicity, triggering an ACS [36].

Regarding baseline characteristics in the study groups, all biochemical parameters, except glucose in ACS, were within reference values. These findings could reflect treatment adherence and an adequate control and monitoring by the cardiologists in ACS patients; however, this does not apply to our RG due the selection criteria. Adherence allows the adequate evaluation of the clinical outcome, considering a therapeutic alliance between patient and physician is necessary for successful treatment [37]. In Mexico, there a few studies addressing this issue with a modest sample size. Zuart et al. reported treatment adherence among IMSS beneficiaries reaches 80% for diabetes mellitus 2 and 77.5 for hypertension [38]. Thus, indicating IMSS beneficiaries usually have a good adherence compared with other Mexican health institutions (25–60%, SISPA) [39]. In this regard, it is important to highlight that all individuals were recruited from the IMSS.

In our ACS group, glucose levels were upper limit of normal (125 mg/dL). This finding had been previously documented where the glucose level could experiment a transient rise in ACS on admission regardless of the diabetic status namely stress hyperglycemia [40]. This form of hyperglycemia is explained as the result of hormonal disorders characterized by an increase in insulin counter regulatory hormones (glucagon, cortisol, catecholamine and growth hormone) and systemic inflammatory response [41]. These changes are associated with increased hepatic gluconeogenesis, glycogenolysis and peripheral resistance to insulin action that characterize glucose metabolism during stress [42–46]. Also, hyperglycemia is a prognostic predictor which acts as a mortality marker during critical illness [47–54]. Meanwhile, among patients with AMI, hyperglycemia is associated with increased risk of congestive heart failure, cardiogenic shock and hospital mortality [55].

4.2. Genetic contribution

Different polymorphisms in the *TNFA* gene have been studied in the context of cardiovascular diseases. Several studies have shown the biological impact of SNPs located in the promoter region of *TNFA* affecting gene expression [56–58].

Regarding the genetic distribution of −1031 T > C (rs1799964) polymorphism, in our study the C allele had a frequency of almost 23% similar to the reported in the HapMap NCBI database in individuals with Mexican ancestry in Los Angeles, California and the European panel (p = 0.56, p = 0.59 respectively). In contrast with other populations, like Asiatic and Africans (CSHL-HapMap and HapMap-JPT), differences were evident (p < 0.002). These differences could be explained by the genetic structure of mestizos from Mexico, which highlight the genetic variability within different populations [59].

In this work, we found that C homozygous carriers had 3.15 times less susceptibility to present ACS (Table 2, p = 0.01). Scarce data exists regarding this polymorphism with cardiovascular disease. In this line, a meta-analysis study in a Chinese population found the −1031 T > C polymorphism associated with UA independently from gender [60]. In another study, Asifa et al., reported no association of this polymorphism with coronary heart disease in
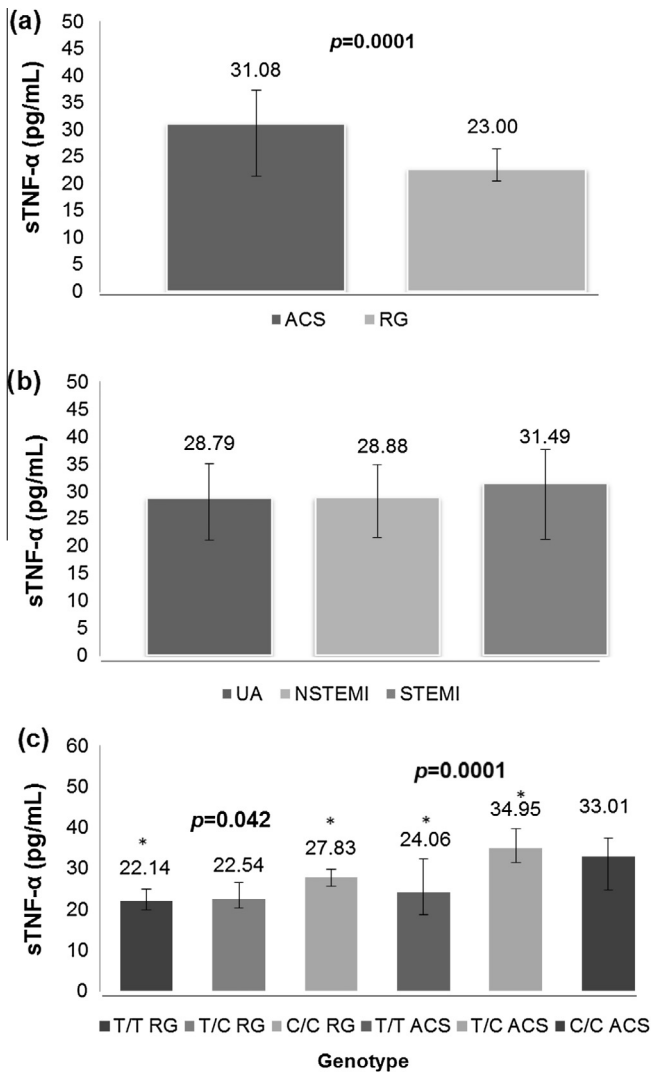
Fig. 1. TNF-α serum level comparisons. (a) Comparison of TNF-α levels in serum of **ACS** patients (IQR$_{25-75}$: 21.31–37.31), **RG** (IQR$_{25-75}$: 20.39–26.45). (b) Comparison of TNF-α levels in serum of ACS by type of diagnosis: **UA** (IQR$_{25-75}$: 21.14–35.02); **NSTEMI** (IQR$_{25-75}$: 21.51–34.92); **STEMI** (IQR$_{25-75}$: 21.25–37.63). (c) Comparison of TNF-α levels according to the -1031 T > C polymorphism (rs1799964): **ACS: T/T** (IQR$_{25-75}$: 18.66–32.40); **T/C** (IQR$_{25-75}$: 31.42–39.70); **C/C** (IQR$_{25-75}$: 24.77–37.33); **RG: T/T** (IQR$_{25-75}$: 19.78–24.89); **T/C** (IQR$_{25-75}$: 20.31–26.5); **C/C** (IQR$_{25-75}$: 25.67–29.82). Numbers above bars indicate median; Interquartile range (IQR 25–75) is shown in graphic representation. *Significant comparisons. ACS: Acute Coronary Syndrome; NSTEMI: myocardial infarction with non-ST segment elevation; RG: reference group; STEMI: myocardial infarction with ST segment elevation; UA: unstable angina, p: Mann–Whitney U-test.

Pakistanis [61]. On the other hand, Monraats et al., described a risk haplotype for coronary restenosis formed by GT alleles of the SNP -238 G > A and -1031 T > C [62]. Hence, further studies are required to determine the protective role of this polymorphism in ACS.

In addition, when the -1031 T > C polymorphism was evaluated according to risk factors associated with ACS, we found the T/C + C/C carriers had 2.31 times greater chance of developing dyslipidemia than the wild type genotype (p = 0.002, Table 3). In this sense, it is known that TNF-α exerts its effect on lipid metabolism in a complex manner depending on the type of tissue and organ involved, by increasing free fatty acid production, through the transcriptional regulation of enzymes involved in lipid metabolism or changing the expression and secretion of other adipokines such as leptin and adiponectin [63,64].

The association of the -1031 T > C polymorphism with dyslipidemia has not yet been evaluated to date. It has been reported in patients with rheumatoid arthritis (RA) with an atherogenic lipid profile. Vallvé et al., conducted a study in individuals with RA and found that the C allele was associated with a more atherogenic lipid profile and therefore was associated with an increased risk for atherosclerosis [65]. Although increased TNF-α serum levels in patients with dyslipidemias have been reported [66], subsequent studies have shown that long-term treatment with the use of TNF-α inhibitors has also been associated with an increased atherogenic index in patients with RA [67].

We would like point out that the association of the -1031 T > C polymorphism with dyslipidemia found in this study is contradictory considering the participation of TNF-α in lipid metabolism. This association could be masked by the presence of linkage disequilibrium (LD) with other promoter variants. Moreover, there are reports which correlate one of the most studied polymorphisms (-308 G > A, rs1800629) in the *TNFA* gene [68,69] with dyslipidemia. Roitenberg et al., reported that carriers of variant A showed 3 times more risk of elevated triglycerides [23]. This suggests that the role of TNF-α in regulating lipids is complex and requires a well-designed study where the effect of this and other polymorphisms in the promoter region be evaluated in patients with dyslipidemia and the measurement of plasma apolipoproteins A and B must be considered, as they have been proposed better risk predictors for AMI than the cholesterol ratio [70].

4.3. Soluble levels

As expected, TNF-α soluble serum levels were higher in patients with ACS than the RG (31.08 vs 23.00 pg/mL). These results were consistent with other reports in patients with ACS [71–73].

In our study, soluble TNF-α levels in the RG were higher than the internal control (23.00 vs 6.12 pg/mL), due to the presence of diabetes, obesity, and hypertension, conditions where TNF-α is increased [74–80]. Although a serum level reference value for TNF-α has not been established, the median value of TNF-α in a healthy median age population ranges between 6 and 46.42 pg/mL [76,77,81] and the establishment of specific cut-point values in each studied population and pathological entities has been suggested [74].

Regarding ACS type, TNF-α soluble levels were not different (Fig. 1b: 28.79, 28.88 and 31.49 pg/mL). Contrarily, Wang et al., demonstrated increased levels in patients with UA and AMI and these levels decreased during the 4 month follow-up, proposing TNF-α as a marker for these pathologies with diagnostic applicability [71]. Meanwhile, Gonzálvez et al., reported increased serum levels in patients with stable angina (n = 34, 7.51 ng/mL) and AMI (n = 15, 7.77 pg/mL). In this respect, the measurement of this cytokine in a larger UA sample size is desirable because ours was reduced (n = 20) [72].

In addition, TNF-α levels were stratified by age (<40, 41–50, 51–60 and >61 years, data not shown) with no significant differences. Several researchers have suggested the presence of unequal levels of TNF-α according to gender, where the females have a relative resistance to the depressive effects of myocardial function induced by TNF-α [82]. A female gender advantage in the cardiovascular system, mainly attributable to the beneficial effects of estrogen, has been reported [83–86]. In this respect, we did not find differences (data not shown). This is important because our findings rule out that the differences are due to age or gender in our study groups.

In regard to the TNF-α serum levels stratified by genotype, significant differences were observed in the RG, (T/T vs C/C, p = 0.042) and ACS (T/T vs T/C, p = 0.0001). In contrast to our findings, in patients with UA, Liu et al., reported statistically significant

differences between cases and controls ($p = 0.028$) but did not differ by genotype [60]. Cui et al., showed that T allele carriers with ischemic stroke have higher levels of TNF-α compared to T/C + C/C genotype carriers ($p < 0.01$) [31]. In this line, we found similar findings in the dominant model in ACS patients. In other diseases, such as malaria, Sohail et al., observed increased TNF-α levels in C/C genotype carriers (294.99 pg/mL, $p = 0.021$), supporting the suggestion of establishing population-based reference ranges or in pathological conditions [87].

5. Conclusions

The −1031 C allele of the *TNFA* polymorphism confers protection for the development of ACS. The T/C genotype carriers had higher TNF-α levels compared to the T/T genotype in ACS. The −1031 T > C *TNFA* polymorphism is associated with dyslipidemia in ACS in a Western Mexican population.

Acknowledgment

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