Association between *TNF-α* -308G>A and -238G>A gene polymorphisms and TNF-α serum levels in Mexican colorectal cancer patients

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ABSTRACT. The objective of this study was to examine the association between TNF-α serum levels and -308G>A and -238G>A

polymorphisms in the corresponding gene by comparing healthy subjects to colorectal cancer (CRC) patients from a Mexican population. Serum levels of TNF-α were found to significantly differ between CRC patients and controls (P = 0.001), but no relationship between the -308G>A and -238G>A polymorphisms and increased CRC risk was established (P > 0.05). However, an association between the -308G>A variant and disease became evident when the distribution of AA-GA genotypes was examined in patients with hematologic toxicity (neutropenia) and those without (odds ratio = 3.356, 95% confidence interval = 1.295-8.698, P = 0.013). The GG haplotype was more common in controls than CRC patients, with a frequency of 0.85 among the former, but this difference was not significant (P > 0.05). In conclusion, TNF-α serum levels and AA-AG genotypes of the *TNF-α* -308G>A polymorphism may significantly contribute to CRC susceptibility in the population examined in this investigation.

Key words: -308G>A; -238G>A; TNF-α; Haplotype; Colorectal cancer; Mexican population

INTRODUCTION

Colorectal cancer (CRC) is a disease characterized by the presence of uncontrolled cell growth in the colon and rectum (Alberts et al., 2014). The prognoses for stages I and II are favorable and have been associated with a 40% five-year survival rate. CRC is one of the most common diseases in developing countries and presents a significant public health problem due to its social implications, high healthcare costs, and increasing rates of diagnosis (Alberts et al., 2014; Binefa et al., 2014). This disease is estimated to be the third most common malignancy in men and the second such in women, representing 10% (746,000) and 9.2% (614,000) of all male and female cancer cases, respectively. Approximately 60% of CRC cases are diagnosed in developed countries, and its incidence varies between different ethnic groups (DeSantis et al., 2014; Parkin et al., 2014; Siegel et al., 2014; Ferlay et al., 2015). In Mexico, the cancer is considered to be the second leading cause of death, and particulary the CRC is responsible for 4% of all cancer-related mortalities (García-Osogobio et al., 2015). According to data generated by García-Osogobio et al. in 2015, the prevalence of adenomas detected by screening colonoscopy in the Mexican population is not different from that published in world literature. In 2008, Mexico reported 4031 CRC cases per 10,000 individuals (SINAIS, 2011). More than 80% of CRC patients are treated for stage III and IV tumors, and an increase in the frequency of CRC sufferers requiring treatment has been observed in recent years (Ferlay et al., 2015). The World Health Organization estimates that by 2030, there will be millions of symptomatic CRC cases and millions more currently asymptomatic individuals who will have developed cancer by that date. Previous research has implicated a variety of risk factors in sporadic CRC occurrence, including age, a diet rich in red meat and saturated fat and low in fiber, tobacco and alcohol consumption, a sedentary lifestyle, and overweight (Alberts et al., 2014; Binefa et al., 2014; DeSantis et al., 2014; Parkin et al., 2014; Siegel et al., 2014; Ferlay et al., 2015). CRC is regarded as a multifactorial disease that may result from interactions among genes, proteins, and environmental factors. It develops through a gradual accumulation

of genetic and epigenetic changes that transform normal colonic mucosa into invasive cancer tissue (Alberts et al., 2014). This process is promoted through chemical mediators, such as neuropeptides, cytokines, and growth factors, which participate in mucosal inflammation. Tumor necrosis factor-alpha (TNF-α) has been reported to be among the cytokines involved in this process (Bernstein, 2005).

The *TNF-α* gene, located on chromosome 6p21.3, is 3 kb long, contains four exons, and encodes a proinflammatory cytokine secreted primarily by macrophages. The soluble TNF-α molecule binds to its receptors, TNFR1 and TNFR2, to stimulate the expression of the *JUN* oncogene, which participates in apoptosis. TNF-α also plays a role in angiogenesis (Cereda et al., 2012; Gómez Flores-Ramos et al., 2013) by inducing the expression of adhesion molecules and facilitating the invasion of metastatic tumor cells (Gómez Flores-Ramos et al., 2013; Zelová and Hošek, 2013). *In vitro* and *in vivo* studies have shown that high levels of TNF-α are associated with poor prognoses in cancer patients due to its tumorigenic effects (Champ et al., 2012; Gómez Flores-Ramos et al., 2013). Several studies have demonstrated a significant association between cytokine levels and increased CRC risk (Fan et al., 2011) or severity (Stanilov et al., 2014). However, others have failed to establish a link between TNF-α and susceptibility to this disease (Landi et al., 2006; Crucitti et al., 2015). The -308 (rs1800629) and -238 (rs361525) polymorphisms in the promoter region of the *TNF-α* gene have been reported to correlate with increased TNF-α production (Cereda et al., 2012). Studies have also associated these polymorphisms with complex diseases, including breast cancer and CRC (Fan et al., 2011; Gómez Flores-Ramos et al., 2013; Stanilov et al., 2014). According to National Center for Biotechnology Information 2012 population data, the rs1800629 minor allele (A) frequency is 5-10, 0.8-14, 0-2, and 0-8% among European, African, Asian, and Mexican populations, respectively (Parra-Rojas et al., 2006; Gómez Flores-Ramos et al., 2013). The rs361525 minor allele is only present in the heterozygous genotype, and has previously been reported to be present at a frequency of 0.1-0.2%, based on different population data (Mohamed et al., 2010; Vázquez-Huerta et al., 2014). Although these polymorphisms have been connected to various diseases, including CRC (Fan et al., 2011; Shen et al., 2011; Cereda et al., 2012; Chu et al., 2012; Gómez Flores-Ramos et al., 2013; Stanilov et al., 2014; Vázquez-Huerta et al., 2014), study results have been inconsistent. The aim of this investigation was to measure TNF-α concentration in the plasma of Mexican CRC patients and determine its relationship with the -308G>A and -238G>A polymorphisms and haplotypes of the *TNF-α* gene.

MATERIAL AND METHODS

Plasma TNF-α quantification was performed using a human TNF-α Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA) following the manufacturer protocol. A standard curve was generated to determine plasma TNF-α concentrations using Excel (Microsoft, Redmond, WA, USA).

DNA was extracted from peripheral blood lymphocytes using standard protocols (Miller et al., 1988). Blood samples were collected from 209 healthy blood donors with an average age of 37.91 years. These volunteers were not age-matched with the patient group. Blood samples were also collected from 164 patients with clinically and histologically confirmed CRC. All patients were residents of the metropolitan area of Guadalajara and were recruited from June 2013 to September 2015. Samples were obtained after all patients

had provided written informed consent, as approved by the Ethics Committee 1305. No familial samples were included. Clinical and demographic data were obtained using written questionnaires. All patients were also interviewed to determine exposure to occupational risks and current drug regimens.

The *TNF-α* promoter region was amplified by polymerase chain reaction according to the details outlined in Table 1, and genotyped as in Figure 1A and B (Wilson et al., 1992; Sanguinetti et al., 1994; Gómez Flores-Ramos et al., 2013).

Table 1. Polymerase chain reaction conditions for amplification of *TNF-α* -308G>A and -238G>A sequences.

SNP	Primer sequence (5'-3')	Reaction mixture	Annealing temperature (°C)	Restriction enzyme	Genotype
-238G>A	AAACAGACCACAGACCTGGTC (Wilson et al., 1992)	0.2 mM dNTPs, 2.0 mM $MgCl_2$, 5 pmol primers	61	*Bam*HI	AA (155 bp) GA (155 + 130 + 25 bp) GG (130 + 25 bp)
	CTCACACTCCCCATCCTCCCGGATC				
-308G>A	AGGCAATAGGTTTTGAGGGCCAT (Gómez Flores-Ramos et al., 2013; Mohamed et al., 2010)	0.2 mM dNTPs, 2.5 mM $MgCl_2$, 5 pmol primers	60	*Nco*I	AA (107 bp) GA (109 + 87 + 20 bp) GG (87 + 20 bp)
	TCCTCCCTGCTCCCGGATTTCCG				

Allele discrimination was performed using 8% polyacrylamide gel (19:1) electrophoresis followed by silver staining (Sanguinetti et al., 1994). SNP = single nucleotide polymorphism, dNTPs = deoxynucleotide triphosphates

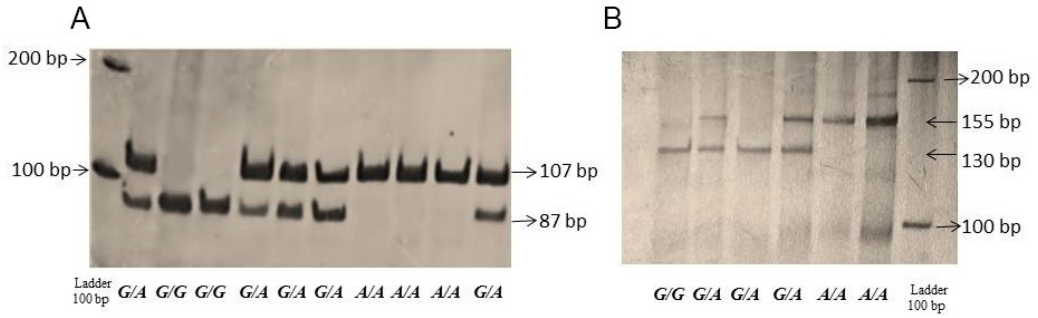

Figure 1. Electrophoresis on 8% polyacrylamide gels (19:1). **A.** Detection of *TNF-α* -308 GG (87 bp), GA (107 and 87 bp), and AA (107 bp) genotypes. **B.** Detection of *TNF-α* -238 GG (130 and 25 bp), GA (130, 155, and 25 bp), and AA (155 bp) genotypes.

Allele frequencies were obtained by direct counting. Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit chi-square test to compare observed and expected genotype frequencies among control subjects. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. A two-tailed P value <0.05 was considered to represent statistical significance. All statistical analyses were performed using the PASW Statistics Base 18 software (SPSS Inc., Chicago, IL, USA). Haplotype analysis was performed using the online program http://bioinfo.iconcologia.net/SNPstats.

RESULTS

Table 2 shows the comparative epidemiological data ascertained from CRC patients and control subjects. In the patient group, the average age was 59.51, ranging from 23 to 92 years. Fifty-four percent (88/164) of these patients were men. Tobacco (adjusted OR = 3.4,

95%CI = 1.6-7.3, P = 0.001) and alcohol consumption (OR = 5.1, 95%CI = 2.4-11.0, P < 0.0001), and family history of diabetes mellitus, arterial hypertension, and cancer (OR = 9.0, 95%CI = 1.4-60, P = 0.021) were found to be risk factors for CRC.

Table 2. Demographic characteristics of the study group.

	CRC patients (N = 164)		Controls (N = 209)		OR (95%CI)*	P
Age (years)						
Mean (SD)**	59.51	(12.12)	37.91	(11.60)		<0.0001
<50 years [(N) %]	(32)	19.5	171	82		
≥50 years [(N) %]	(132)	80.5	38	18	20.2 (10.6-38.8)	<0.0001
Gender						
Male [(N) %]	(88)	54	(83)	40		0.9
Female [(N) %]	(76)	46	(126)	60		
Tobacco consumption						
Yes [(N) %]	(80)	49	(29)	14	3.4 (1.6-7.3)	0.001
No [(N) %]	(84)	51	(180)	86		1111
Alcohol consumption						
Yes [(N) %]	(85)	52	(25)	12	5.1 (2.4-11.0)	<0.0001
No [(N) %]	(79)	48	(184)	88		
FH						
No [(N) %]	(116)	71	(153)	73		0.6
DM-AH [(N) %]	(33)	20	(54)	26		0.2
DM-AH-CA*** [(N) %]	(15)	9	(2)	1	9.0 (1.4-60.0)	0.021

CRC = colorectal cancer, SD = standard deviation, OR = odds ratio, CI = confidence interval, DM = type 2 diabetes, AH = arterial hypertension, CA = cancer, FH = family history. *ORs from adjusted regression analysis, **based on Student *t*-tests (P value), ***positive familial history of cancer, including leukemia, in first- and second-degree relatives of patients.

Table 3 shows the general clinical characteristics of the patient group. We observed that 63% had rectal cancer, approximately 10% had diverticulitis, 88% had stage III-IV tumors, 57% had metastasis, 89% had prognoses of 1-4 years, 70% were unresponsive to chemotherapy, and 63% had gastric toxicity. Some patients had higher than normal angiotensin-converting enzyme (73%), glutamate oxaloacetate transaminase (30%), alkaline phosphatase (53%), or glucose (20%) levels.

Serum levels of TNF-α among CRC patients (N = 45) and controls (N = 35) were 10.345 ± 0.664 and 9.942 ± 0.140 pg/mL (mean ± standard deviation), respectively, representing a statistically significant difference between these groups. A comparison of serum TNF-α levels between patients with stage I-II (N = 15) tumors and those with stage III-IV (N = 30) tumors also revealed a significant difference (10.017 ± 0.256 and 10.509 ± 0.745 pg/mL, respectively; P = 0.017; Figure 2A and B).

Genotype and allele frequencies of the *TNF-α* -308G>A and -238G>A polymorphisms did not significantly differ between patients and controls (Table 4). All genotype distributions demonstrated conformance with HWE. In addition, considering the general clinical characteristics of the study groups, significant associations were established between GA-AA genotypes of the -308G>A *TNF-α* polymorphism and neutropenia (adjusted OR = 3.35, 95%CI = 1.295-8.698, P = 0.013) and serum glutamic pyruvic transaminase (OR = 1.024, 95%CI = 1.008-1.041, P = 0.004) as risk factors for CRC (Table 5).

A comparative analysis failed to reveal a significant relationship between serum TNF-α levels and the -308G>A and -238G>A polymorphisms in the groups under investigation (data not shown). Haplotype frequencies, which showed linkage disequilibrium (D' = 0.6), are shown in Table 6.

Table 3. Clinical characteristics of colorectal cancer patients.

	N	%		N	%
Cancer type			Responsive to chemotherapy		
Colon	60	37	Yes	50	30
Rectal	104	63	No	114	70
Personal medical history			Chemotoxicity		
None	99	60	Gastric	103	63
DM-AH	48	30	Dermatologic	37	23
Diverticulitis	17	10	Hematologic	67	41
			Cardiologic	4	2
Body mass index*			Neurologic	25	15
18.5-19.9 (underweight)	27	17	Laboratory tests		
20-24.9 (normal weight)	68	42	CEA (µg/L)		
25-29.9 (overweight)	43	26	Normal	44	27
30-34.9 (obesity I)	22	13	Elevated	120	73
≥35 (obesity II-IV)	4	2	Hemoglobin (g/dL)		
Tumor stage			Normal	66	40
I-II	18	12	Low	98	60
III-IV	146	88	SGOT (IU/L)		
Adenocarcinoma histology			Normal	115	70
Well-differentiated	40	24	High	49	30
Poorly differentiated	121	74	SGPT (IU/L)		
Undifferentiated	3	2	Normal	138	84
Lymph node involvement			High	26	16
Yes	70	43	LDH (IU/L)		
No	94	57	Normal	85	52
Metastasis			High	79	48
Yes	94	57	ALP (IU/L)		
No	70	43	Normal	77	47
Prognosis			High	87	53
1-4 years	146	89	Glucose (g/dL)		
5-9 years	12	8	Normal	132	80
≥10 years	6	3	High	32	20

CEA = carcinoembryonic antigen, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic pyruvic transaminase, LDH = lactate dehydrogenase, ALP = alkaline phosphatase. *According to World Health Organization (2004) classifications.

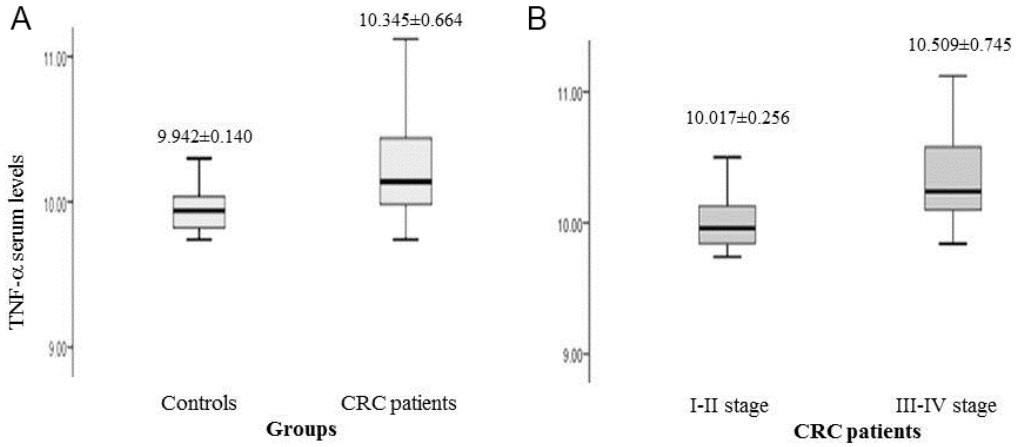

Figure 2. TNF-α serum levels in a) the two study groups, and b) colorectal cancer patients at different clinical stages. CRC = colorectal cancer.

Table 4. Genotype and allele distributions of *TNF-α* -308G>A and -238G>A polymorphisms in colorectal cancer patients and controls.

Polymorphism	CRC patients		Controls		OR	95%CI	P
-308G>A							
Genotypes	N = 164	%	N = 209	%			
GG	139	85	180	87	1.0*		
GA	21	13	27	12	0.98	0.53-1.8	0.97
AA	4	2	2	1	2.5	0.46-14	0.26
Alleles (2N)							
G	299	0.911	387	0.925	0.82	0.48-1.4	0.47
A	29	0.089	31	0.075	1.2	0.71-2.0	0.47
-238G>A							
Genotypes	N = 143	%	N = 49	%			
GG	127	89	42	86	1.0*		
GA	14	10	6	12	0.77	0.28-2.1	0.62
AA	2	1	1	2	0.68	0.06-7.6	1.0
Alleles (2N)							
G	268	0.937	90	0.918	1.3	0.55-3.1	0.52
A	18	0.063	8	0.082	0.75	0.31-1.8	0.52

CRC = colorectal cancer, OR = odds ratio, CI = confidence interval. *Reference genotype. Hardy-Weinberg equilibrium among controls: P = 0.9515 for -308G>A; and P = 0.199 for -238G>A

Table 5. Association between the *TNF-α* -308G>A polymorphism and more than one clinical variable in colorectal cancer patients.

							95%CI	
	B	SD	Wald	d.f.	P	OR	Lower	Upper
Neutropenia	1.211	0.486	6.206	1	0.013	3.356	1.295	8.698
SGPT	0.024	0.008	8.184	1	0.004	1.024	1.008	1.041
Constant	-1.622	0.168	93.111	1	0.000	0.197		

SGPT = serum glutamic pyruvic transaminase, OR = odds ratio, CI = confidence interval, SD = standard deviation, d.f. = degrees of freedom. Variables included in the analysis: colorectal cancer patients classified by GA-AA genotype (dependent); neutropenia and SGPT (independent).

Table 6. *TNF-α* -308G>A and -238G>A haplotype frequencies in the study groups.

Haplotype		Frequency			
-308	-238	Total	CRC patients	Controls	OR (95%CI)
G	G	0.8545	0.85	0.8525	0.22 (0.08-0.58)
A	G	0.078	0.0873	0.0648	0.10 (0.02-0.43)
G	A	0.0651	0.0616	0.0734	0.43 (0.07-2.56)
A	A	0.0024	0.0011	0.0094	

CRC = colorectal cancer, OR = odds ratio, CI = confidence interval. The polymorphisms were found to be in linkage disequilibrium (D' = 0.6).

DISCUSSION

CRC is a multifactorial disease with a complex etiology. In this sense, it is important to perform epidemiological studies that include risk factors to determine possible causes of this complicated disease in different populations. In Mexico, CRC is considered to be a major health issue, as its incidence has increased over the last 20 years, and it is currently one of the leading causes of death for both women and men (Gallegos-Arreola et al., 2009; Alberts et al., 2014; Binefa et al., 2014; Siegel et al., 2014). These facts are consistent with the observations made in our current study, in which the average patient age was 59.51 ± 12.12 years. Many studies have observed a high incidence of CRC in patients approximately 50 years old (Alberts

et al., 2014, Binefa et al., 2014; DeSantis et al., 2014; Parkin et al., 2014; Siegel et al., 2014; Ferlay et al., 2015). The increased frequency of this disease may be due to lifestyle changes in the Mexican population, such as diet (including food additives) and exposure to toxic substances. These lifestyle modifications combined with changes in longevity may contribute to the incidence of CRC in this country.

In this study, we found the consumption of tobacco and alcohol to be present as risk factors in 49 and 52% of CRC patients, respectively. CRC is not considered a strictly tobacco-related malignancy, but an association between smoking habit and this disease has been observed (Johnson et al., 2013). A connection between high alcohol intake and CRC risk has also been described (Chan and Giovannucci, 2010); however, this relationship remains controversial. The mechanisms by which alcohol promotes cancer are unknown, but some recent studies have suggested that it might reduce folate levels, contributing to abnormal DNA methylation and inducing cytochrome P450 enzymes to activate carcinogens (Gallegos-Arreola et al., 2009).

In the present study, we observed an association between CRC and first- and second-degree familial history of diabetes, arterial hypertension, and cancer. A number of articles have reported corresponding risk estimates (Peeters et al., 2015).

Rectal cancer was found to be present in 63% of our patient group, highlighting the clinical variability of CRC. New techniques and approaches that permit better detection of CRC are now being used in developing countries with a high incidence of this disease (Chen and Sheen-Chen, 2000; Gallegos-Arreola et al., 2009). Nine percent of the patients studied had a familial history of cancer (7% of which reported a family history of grade I CRC; data not shown). This frequency is consistent with the overall rates reported in the literature concerning cancer in individuals with or without family history of CRC (Gallegos-Arreola et al., 2009; Henrikson et al., 2015). The body mass index (BMI) of most patients in this study was categorized as normal or underweight. A possible explanation for this may be that many of these individuals presented advanced-stage CRC, a fact reflected in the percentage of patients diagnosed with stage III or IV tumors (88%). In addition, gastric complications secondary to chemotherapy (63%) may also be responsible for the BMI data in this study.

Advances in molecular and genetic epidemiology have increased our knowledge of the mechanisms behind colorectal carcinogenesis, and the relationship between disease susceptibility and exposure to carcinogens, diet, and individual genetic variations. TNF-α has been identified as an important proinflammatory regulator that participates in signal transduction pathways and activates cellular responses, apoptosis, proliferation, differentiation, migration, and angiogenesis signaling cascades (Cereda et al., 2012; Gómez Flores-Ramos et al., 2013). Chronic inflammation may promote tumor progression through stimulation of the vascular endothelium to recruit leukocytes to the tumor site and trigger angiogenesis, mitogenic stimulation, chemotaxis, and proteolysis. This in turn leads to recruitment of other inflammatory cells to stimulate angiogenesis, sustaining tumor growth and encouraging metastasis (Guadagni et al., 2007; Gómez Flores-Ramos et al., 2013). Some malignant CRC lesions originate from chronic inflammation; however, the exact mechanism responsible remains unknown. Several studies have shown that inflammatory cells can modulate colon carcinogenesis through mitogenic stimulation of stromal cells (Mueller and Fusenig, 2004), which may promote or inhibit tumor progression (Landi et al., 2006). The cytokines IL-6, IL-17A, IL-21, and TNF-α, among others, contribute to the creation of a favorable tumor environment by regulating cancer cell growth and survival. For this reason, inflammatory

infiltrate is considered to be the main trigger of colon carcinogenesis (Monteleone et al., 2012). It has been suggested that single nucleotide changes in the *TNF-α* gene promoter may modify the binding sites of specific transcription factors, thereby affecting transcriptional regulation and modulating the TNF-α secretory response (Aguillón et al., 2006; Gómez Flores-Ramos et al., 2013).

The A alleles of the *TNF-α* polymorphisms -308G>A and -238G>A are associated with high levels of TNF-α *in vitro* and have also been implicated in increased susceptibility to and severity of various diseases, including CRC (Berberoglu et al., 2004; Li et al., 2011; Cereda et al., 2012). However, the relationship between these polymorphisms and CRC remains controversial and depends on the population studied (Min et al., 2014). Moreover, little is known regarding this association in Mexican patients. In our study group, the frequency of -308G>A variant was 1% in controls and 2% in CRC patients, indicating that this polymorphism is not a risk factor for CRC. Recent data from our group revealed an association between the -308G>A polymorphism and breast cancer (Gómez Flores-Ramos et al., 2013); however, this relationship was not identified among CRC patients. These data are consistent with a recent meta-analysis demonstrating that this same polymorphism constitutes a risk factor in the development of breast cancer, but not CRC (Fan et al., 2011).

Serum TNF-α levels were significantly higher in CRC patients than in controls (10.345 ± 0.664 *vs* 9.942 ± 0.140 pg/mL; P = 0.001), and in advanced-stage compared to early-stage patients (10.50 ± 0.745 *vs* 10.017 ± 0.256 pg/mL; P = 0.017). Other studies have described similar results (Shimazaki et al., 2013; Stanilov et al., 2014). However, there was no association between TNF-α levels and -308G>A and -238G>A polymorphism haplotypes in Mexican CRC patients and controls.

Nevertheless, *TNF-α* -308G>A AA-GA genotypes were seen to be risk factors for neutropenia in CRC patients. Although the mechanisms by which TNF-α induces neutropenia are unknown, previous studies have observed that chemotherapy influences the number of neutrophils, which have a fundamental role in host defense. However, TNF-α upregulates the expression of proinflammatory cytokines involved in the differentiation and maturation of hematopoietic stem cells, and it is possible that TNF-α blockade mediates bone marrow failure by inhibiting stem cell differentiation. TNF-α has been shown to directly exert a complex and dual effect on neutrophils, accelerating apoptosis in some cells, yet prolonging the survival of others (Hastings et al., 2010).

In addition to these mechanisms, several factors may influence the development of CRC, including polymorphisms of the *TNF-α* gene capable of increasing enzyme activity and inducing changes in cell physiology resulting in neoplastic progression.

In conclusion, our results do not support an association between haplotypes of the *TNF-α* -308G>A and -238G>A polymorphisms and CRC. Differences in serum TNF-α levels were evident between a) CRC patients and controls, and b) advanced- and early-stage CRC patients. In addition, the prevalence of patients with -308G>A GA-AA genotypes and neutropenia confirmed that this factor may contribute significantly to CRC susceptibility, depending on clinical outcomes in this population. Further studies are required to confirm or reject these observations.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Aguillón JC, Cruzat A, Aravena O, Salazar L, et al. (2006). Could single-nucleotide polymorphisms (SNPs) affecting the tumour necrosis factor promoter be considered as part of rheumatoid arthritis evolution? *Immunobiology* 211: 75-84. http://dx.doi.org/10.1016/j.imbio.2005.09.005

Alberts SR, Citrin D and Rodriguez-Bigas M (2014). Colon, Rectal, and Anal Cancers. Cancer Management. http://www.cancernetwork.com/cancer-management/colon-rectal-and-anal-cancers. Accessed September 23, 2014.

Berberoglu U, Yildirim E and Celen O (2004). Serum levels of tumor necrosis factor alpha correlate with response to neoadjuvant chemotherapy in locally advanced breast cancer. *Int. J. Biol. Markers* 19: 130-134.

Bernstein JM (2005). Update on the molecular biology of nasal polyposis. *Otolaryngol. Clin. North Am.* 38: 1243-1255. http://dx.doi.org/10.1016/j.otc.2005.08.010

Binefa G, Rodríguez-Moranta F, Teule A and Medina-Hayas M (2014). Colorectal cancer: from prevention to personalized medicine. *World J. Gastroenterol.* 20: 6786-6808. http://dx.doi.org/10.3748/wjg.v20.i22.6786

Cereda C, Gagliardi S, Cova E, Diamanti L, et al. (2012). The role of TNF-Alpha in ALS: new hypotheses for future therapeutic approaches. In: Amyotrophic Lateral Sclerosis (Maurer MH, ed.). InTech, Rijeka, 413-436.

Champ CE, Volek JS, Siglin J, Jin L, et al. (2012). Weight gain, metabolic syndrome, and breast cancer recurrence: are dietary recommendations supported by the data? *Int. J. Breast Cancer* 2012: 506868. http://dx.doi.org/10.1155/2012/506868

Chan AT and Giovannucci EL (2010). Primary prevention of colorectal cancer. *Gastroenterology* 138: 2029-2043.e10. http://dx.doi.org/10.1053/j.gastro.2010.01.057

Chen HS and Sheen-Chen SM (2000). Obstruction and perforation in colorectal adenocarcinoma: an analysis of prognosis and current trends. *Surgery* 127: 370-376. http://dx.doi.org/10.1067/msy.2000.104674

Chu H, Yang J, Mi S, Bhuyan SS, et al. (2012). Tumor necrosis factor-alpha G-308 A polymorphism and risk of coronary heart disease and myocardial infarction: A case-control study and meta-analysis. *J. Cardiovasc. Dis. Res.* 3: 84-90. http://dx.doi.org/10.4103/0975-3583.95359

Crucitti A, Corbi M, Tomaiuolo PM, Fanali C, et al. (2015). Laparoscopic surgery for colorectal cancer is not associated with an increase in the circulating levels of several inflammation-related factors. *Cancer Biol. Ther.* 16: 671-677. http://dx.doi.org/10.1080/15384047.2015.1026476

DeSantis CE, Lin CC, Mariotto AB, Siegel RL, et al. (2014). Cancer treatment and survivorship statistics, 2014. *CA Cancer J. Clin.* 64: 252-271. http://dx.doi.org/10.3322/caac.21235

Fan W, Maoqing W, Wangyang C, Fulan H, et al. (2011). Relationship between the polymorphism of tumor necrosis factor-α-308 G>A and susceptibility to inflammatory bowel diseases and colorectal cancer: a meta-analysis. *Eur. J. Hum. Genet.* 19: 432-437. http://dx.doi.org/10.1038/ejhg.2010.159

Ferlay J, Soerjomataram I, Dikshit R, Eser S, et al. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* 136: E359-E386. http://dx.doi.org/10.1002/ijc.29210

Gallegos-Arreola MP, García-Ortiz JE, Figuera LE, Puebla-Pérez AM, et al. (2009). Association of the 677C→T polymorphism in the *MTHFR* gene with colorectal cancer in Mexican patients. *Cancer Genomics Proteomics* 6: 183-188.

García-Osogobio S, Téllez-Avila FI, Méndez N and Uribe-Esquivel M (2015). Results of the first program of colorectal cancer screening in Mexico. *Endoscopia* 27: 59-63.

Gómez Flores-Ramos L, Escoto-De Dios A, Puebla-Pérez AM, Figuera-Villanueva LE, et al. (2013). Association of the tumor necrosis factor-alpha -308G>A polymorphism with breast cancer in Mexican women. *Genet. Mol. Res.* 12: 5680-5693. http://dx.doi.org/10.4238/2013.November.18.17

Guadagni F, Ferroni P, Palmirotta R, Portarena I, et al. (2007). Review. TNF/VEGF cross-talk in chronic inflammation-related cancer initiation and progression: an early target in anticancer therapeutic strategy. *In Vivo* 21: 147-161.

Hastings R, Ding T, Butt S, Gadsby K, et al. (2010). Neutropenia in patients receiving anti-tumor necrosis factor therapy. *Arthritis Care Res.* 62: 764-769. http://dx.doi.org/10.1002/acr.20037

Henrikson NB, Webber EM, Goddard KA, Scrol A, et al. (2015). Family history and the natural history of colorectal cancer: systematic review. *Genet. Med.* 17: 702-712. http://dx.doi.org/10.1038/gim.2014.188

Johnson CM, Wei C, Ensor JE, Smolenski DJ, et al. (2013). Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* 24: 1207-1222. http://dx.doi.org/10.1007/s10552-013-0201-5

Landi S, Gemignani F, Bottari F, Gioia-Patricola L, et al. (2006). Polymorphisms within inflammatory genes and colorectal cancer. *J. Negat. Results Biomed.* 5: 15. http://dx.doi.org/10.1186/1477-5751-5-15

Li M, You Q and Wang X (2011). Association between polymorphism of the tumor necrosis factor alpha-308 gene promoter and colon cancer in the Chinese population. *Genet. Test. Mol. Biomarkers* 15: 743-747. http://dx.doi.org/10.1089/gtmb.2011.0068

Miller SA, Dykes DD and Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215. http://dx.doi.org/10.1093/nar/16.3.1215

Min L, Chen D, Qu L and Shou C (2014). Tumor necrosis factor-a polymorphisms and colorectal cancer risk: a meta-analysis. *PLoS One* 9: e85187. http://dx.doi.org/10.1371/journal.pone.0085187

Mohamed AA, Rashed LA, Shaker SM and Ammar RI (2010). Association of tumor necrosis factor-alpha polymorphisms with susceptibility and clinical outcomes of rheumatic heart disease. *Saudi Med. J.* 31: 644-649.

Monteleone G, Pallone F and Stolfi C (2012). The dual role of inflammation in colon carcinogenesis. *Int. J. Mol. Sci.* 13: 11071-11084. http://dx.doi.org/10.3390/ijms130911071

Mueller MM and Fusenig NE (2004). Friends or foes - bipolar effects of the tumour stroma in cancer. *Nat. Rev. Cancer* 4: 839-849. http://dx.doi.org/10.1038/nrc1477

Parkin DM, Bray F, Ferlay J and Jemal A (2014). Cancer in Africa 2012. *Cancer Epidemiol. Biomarkers Prev.* 23: 953-966. http://dx.doi.org/10.1158/1055-9965.EPI-14-0281

Parra-Rojas I, Ruíz-Madrigal B, Martínez-López E and Panduro A (2006). Influence of the -308 *TNF-α* and -174 *IL-6* polymorphisms on lipid profile in Mexican subjects. *Hereditas* 143: 167-172. http://dx.doi.org/10.1111/j.2006.0018-0661.01936.x

Peeters PJ, Bazelier MT, Leufkens HG, de Vries F, et al. (2015). The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. *Diabetes Care* 38: 495-502. http://dx.doi.org/10.2337/dc14-1175

Sanguinetti CJ, Dias Neto E and Simpson AJ (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 914-921.

Shen C, Sun H, Sun D, Xu L, et al. (2011). Polymorphisms of tumor necrosis factor-alpha and breast cancer risk: a meta-analysis. *Breast Cancer Res. Treat.* 126: 763-770. http://dx.doi.org/10.1007/s10549-010-1184-5

Shimazaki J, Goto Y, Nishida K, Tabuchi T, et al. (2013). In patients with colorectal cancer, preoperative serum interleukin-6 level and granulocyte/lymphocyte ratio are clinically relevant biomarkers of long-term cancer progression. *Oncology* 84: 356-361. http://dx.doi.org/10.1159/000350836

Siegel R, Desantis C and Jemal A (2014). Colorectal cancer statistics, 2014. *CA Cancer J. Clin.* 64: 104-117. http://dx.doi.org/10.3322/caac.21220

SINAIS/SINAVE/DGE/SALUD (2011). Perfil epidemiológico de los tumores malignos en México.

Stanilov N, Miteva L, Dobreva Z and Stanilova S (2014). Colorectal cancer severity and survival in correlation with tumour necrosis factor-alpha. *Biotechnol. Biotechnol. Equip.* 28: 911-917. http://dx.doi.org/10.1080/13102818.2014.965047

Vázquez-Huerta DI, Alvarez-Rodríguez BA, Topete-Reyes JF, Muñoz-Valle JF, et al. (2014). Tumor necrosis factor alpha -238 G/A and -308 G/A polymorphisms and soluble TNF-α levels in chronic kidney disease: correlation with clinical variables. *Int. J. Clin. Exp. Med.* 7: 2111-2119.

WHO Expert Consultation (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363: 157-163.

Wilson AG, di Giovine FS, Blakemore AI and Duff GW (1992). Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by *Nco*I restriction of PCR product. *Hum. Mol. Genet.* 1: 353. http://dx.doi.org/10.1093/hmg/1.5.353

Zelová H and Hošek J (2013). TNF-α signalling and inflammation: interactions between old acquaintances. *Inflamm. Res.* 62: 641-651. http://dx.doi.org/10.1007/s00011-013-0633-0