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Association of the tumor necrosis factor-alpha -308G>A polymorphism with breast cancer in Mexican women

L. Gómez Flores-Ramos[1,2], A. Escoto-De Dios[1], A.M. Puebla-Pérez[3], L.E. Figuera-Villanueva[4], A. Ramos-Silva[1,5], R. Ramírez-Patiño[1,2], J.I. Delgado-Saucedo[3], E. Salas-González[6], G.M. Zúñiga-González[7], A. Alonzo-Rojo[1,2], I. Gutiérrez-Hurtado[1,2] and M.P. Gallegos-Arreola[1]

[1]Laboratorio de Genética Molecular, División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[2]Programa de Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México
[3]Laboratorio de Inmunofarmacología, Centro Universitario de Ciencias Exactas e Ingenierias, Universidad de Guadalajara, Guadalajara, Jalisco, México
[4]División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[5]Programa de Doctorado en Farmacología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México
[6]Unidad Medica de Alta Especialidad, Hospital de Gineco-Obstetricia, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[7]Laboratorio de Mutagénesis, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México

Corresponding author: M.P. Gallegos-Arreola
E-mail: marthapatriciagallegos08@gmail.com

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ABSTRACT. The tumor necrosis factor-alpha (TNF-α) gene plays an important role in cell proliferation, differentiation, apoptosis, lipid metabolism, coagulation, insulin resistance, and endothelial function. Polymorphisms of *TNF*-α have been associated with cancer. We examined the role of the -308G>A polymorphism in this gene by comparing the genotypes of 294 healthy Mexican women with those of 465 Mexican women with breast cancer. The observed genotype frequencies for controls and breast cancer patients were 1 and 14% for AA, 13 and 21% for GA, and 86 and 65% for GG, respectively. We found that the odds ratio (OR) for AA genotype was 2.4, with a 95% confidence interval (95%CI) of 5.9-101.1 (P = 0.0001). The association was also evident when comparing the distribution of the AA-GA genotype in patients in the following categories: 1) premenopause and obesity I (OR = 3.5, 95%CI = 1.3-9.3, P = 0.008), 2) Her-2 neu and tumor stage I-II (OR = 2.5, 95%CI = 1.31-4.8, P = 0.004), 3) premenopause and tumor stage III-IV (OR = 1.7, 95%CI = 1.0-2.9, P = 0.034), 4) chemotherapy non-response and abnormal hematocrit (OR = 2.4, 95%CI = 1.2-4.8, P = 0.015), 5) body mass index and Her-2 neu and III-IV tumor stage (OR = 2.8, 95%CI = 1.2-6.6, P = 0.016), and 6) nodule metastasis and K-I67 (OR = 4.0, 95%CI = 1.01-15.7, P = 0.038). We concluded that the genotypes AA-GA of the -308G>A polymorphism in *TNF-α* significantly contribute to breast cancer susceptibility in the analyzed sample from the Mexican population.

Key words: -308G>A; Tumor necrosis factor alpha; Risk factor; Obesity; Breast cancer; Mexican population

INTRODUCTION

Breast cancer (BC) is a disease characterized by the presence of uncontrolled cell growth in the ducts (ductal carcinoma) or lobules (lobular carcinoma) of the mammary gland. These tumors can invade the surrounding tissues of the breast in later stages. Most breast tumors are invasive or infiltrating, and they are classified by the American Joint Committee on Cancer with several defined stages. BC is one of the most common diseases in developing countries as well as a major health problem owing to its social implications, high health costs for states, and increasing incidence rates of the diagnosis each year (Bandi et al., 2010). An estimated millions of symptomatic women are affected by BC, and millions more are currently asymptomatic and they will develop cancer. Incidence rates are variable among ethnic groups, with 122.6 per 100,000 cases in white women, 118 per 100,000 cases among women of African ethnicity, 92.8 per 100,000 cases in Hispanics, 87.9 per 100,000 cases in Asian/Pacific Islanders, and 65.6 per 100,000 in American Indian/Alaskan Natives (Miller et al., 2012). In many countries, and particularly in Mexico, the incidence of BC has increased within the last 6 years and is now one of the main causes of death in women

of reproductive age. The mortality rate for the disease has increased since 2006 and has surpassed that of cervical cancer. The mortality rate in Mexico was 4,854 deaths in 2009 and it has been projected to increase to over 16,500 deaths per year by 2020. Only 10% of BC cases are detected at stage I. The mortality rate in the State of Jalisco is among the highest for BC (Knaul et al., 2009).

BC is considered a multifactorial disease and it might result from a combination of abnormal protein levels and their interaction with environmental factors. Previous research has implicated a variety of risk factors for BC, including age, early menarche, menopause, oral contraceptive use, cigarette smoking, alcohol consumption, and family history of BC, breast fibrosis, nulliparity, breastfeeding, ethnicity, nutrition, and genetics (Abdulrahman and Rahman, 2012). Elucidating the genetic variants among ethnic groups may help to explain the progression of cancer as well as differences in chemotherapy response.

The tumor necrosis factor-alpha (TNF-α) gene, which is located at chromosome 6p21.3, encodes a pro-inflammatory cytokine secreted primarily by macrophages that is involved in cell proliferation, differentiation, apoptosis, lipid metabolism, coagulation, insulin resistance, and endothelial function. This cytokine consists of a chain of 233 amino acid synthesized as a protein of membrane-binding of 26 kDa, which is cleaved by processing enzymes and released as a soluble protein of 17 kDa. The soluble molecule can bind to its receptors, TNFR1 and TNFR2. TNF-α has a receptor on tumor cells that stimulates activation and prolonged expression of the JUN oncogene; participates in apoptosis; stimulates the hydrolysis of sphingomyelin, ceramide, and phosphorylcholine; and induces the production of reactive oxygen species (Cereda et al., 2012). *In vitro* and *in vivo* experiments have shown the tumorigenic effects of TNF-α, and it has been associated with elevated plasma levels of TNF-α in cancer patients with a poor prognosis (Champ et al., 2012).

TNF-α is a key molecule during angiogenesis, promoting it directly by stimulating endothelial cell proliferation and indirectly by enhancing the expression of pro-angiogenic factors (Cereda et al., 2012). Furthermore, TNF-α induces the expression of adhesion molecules, facilitating the invasion of metastatic tumor cells (Champ et al., 2012). Multiple studies have found a significant association between this cytokine and increased risk for BC (Fang et al., 2010; Shen et al., 2011), and an increase in the severity of the disease (Azmy et al., 2004).

TNF-α has a length of 3 kilobases and contains 4 exons that code for more than 80% of the secreted protein (Cereda et al., 2012). Several polymorphisms and mutations have been described for this gene. The TNF-α-308G>A polymorphism is located in the promoter region of the gene and involves the substitution of a guanine (G) by an adenine (A). This change effects gene expression, increasing production of TNF-α (Cereda et al., 2012) and has been extensively associated with complex diseases, including BC (Azmy et al., 2004; Fang et al., 2010; Shen et al., 2011).

According to the National Center for Biotechnology Information population diversity in 2012 (rs361525), in the population of European origin, the allele frequency of the mutant (A) allele corresponds to 5-10%, to 0.8-14% in the African population, and to 0-2% in the Asian population. In Hispanic populations, the frequency of the mutant allele has been reported at approximately 11%. In the Mexican population, studies have reported a frequency of 0-8% of the mutant allele (Parra et al., 2006; Sánchez et al., 2008). Although this polymor-

phism has been associated with diseases such as rheumatism (Cereda et al., 2012), cardiovascular disease (Chu et al., 2012), diabetes (Feng et al., 2011), and cancer (Yang et al., 2011), the results have been inconsistent. The aim of this study was to determine the association between the -308G>A polymorphism in the TNF-α gene and BC in Mexican women.

MATERIAL AND METHODS

DNA was extracted from peripheral blood lymphocytes using standard protocols (Miller et al., 1988). Blood samples were collected from 294 healthy women recruited as volunteer blood donors (average age, 34 years). These volunteers were not age matched with the patient group. Blood samples were also collected from 465 patients with clinical and histological confirmation of BC. All of the patients were residents of the metropolitan area of Guadalajara. The patients were recruited from June 2010 to April 2012. All the samples were obtained after a written informed consent previously approved by the ethics committee. No familial samples were included. Clinical and demographical data were obtained using written questionnaires. All the patients were also interviewed to determine occupational exposure and the use of therapeutics.

The amplification of the *TNF-α* promoter region was performed via PCR using the following primers: 5'-AGGCAATAGGTTTTGAGGGCCAT-3' (sense) and 5'-TCCTCCCTG CTCCCGGATTTCCG-3' (antisense) (Wilson et al., 1992). The PCRs were performed in a total volume of 15 µL containing 0.2 mM deoxyribonucleotide triphosphates (Invitrogen, Carlsbad, CA, USA), 5 pmol primers, 2.5 mM $MgCl_2$, 2.5 U Taq polymerase (Invitrogen), and 0.8% bovine serum albumin (Promega, Madison, WI, USA). The PCR thermocycler conditions were 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min. The amplified products were digested with *Nco*I restriction enzyme (Promega) at 37°C for 12 h. Allele discrimination was performed using 8% polyacrylamide gel (19:1) electrophoresis followed by silver staining (Sanguinetti et al., 1994). A 107-bp fragment was found to indicate a polymorphic type genotype (-308AA). Two fragments of 87 and 20 bp indicated the wild-type genotype (-308GG).

Allele frequencies were obtained through direct counting. Hardy-Weinberg equilibrium was tested by a goodness-of-fit chi-square test to compare the observed genotype frequencies with the expected frequencies among control subjects. Odds ratios (ORs) and 95% confidence to be intervals (95%CIs) were also calculated. A 2-tailed P of <0.05 was considered to be statistically significant. All statistical analyses were performed using PASW Statistic Base 18 software (2009, Chicago, IL, USA).

RESULTS

Table 1 shows the comparative epidemiological data from the BC patients and control subjects. In the patient group, the average age was 54.11 years, ranging from 28 to 88 years. Menarche occurred at a mean age of 12.43 years in patients and 12.14 years in controls. Body mass index (BMI) of ≥30-34.9 (crude OR = 16.6, 95%CI = 7.2-38.4, P < 0.0001) and ≥35 to >40 (OR = 24.5, 95%CI = 7.6-77.3, P < 0.0001), oral contraceptive use (crude OR = 1.8, 95%CI = 1.3-2.4, P < 0.0001), pregnancies (OR = 5.7, 95%CI = 4.0-2.2, P < 0.0001), abortions (OR = 6.8, 95%CI = 4.1-11.4, P < 0.0001), breast-feeding (OR = 1.8, 95%CI = 1.3-2.5, P < 0.0001), postmenopause (OR = 11.6, 95%CI = 8-16.8, P < 0.0001), family history (OR = 3.2,

95%CI = 2.4-4.4, P < 0.0001) maternal (OR = 2.2, 95%CI = 1.6-3.1, P < 0.0001) and paternal (OR = 2.1, 95%CI = 1.3-3.3, P < 0.001) were identified as risk factors.

Table 2 shows the general clinical characteristics of the patient group. We observed that 15% of the patients had hysterectomies, approximately 50% were positive for hormonal receptors, 87% had ductal histology, and 57% had stage III-IV tumors. Some patients had levels over the rates of normality of platelets (22%), glutamate oxaloacetate transaminase (23%), alkaline phosphatase (26%), gamma glutamyl transferase (31%), and glucose (30%).

Table 1. Demographic data from the study groups.

	Breast cancer patients		Controls		OR (95%CI)	P
	(N = 465)	%	(N = 294)	%		
Age (years, Means ± SD)[1]	54 ± 11.6		34 ± 11.4			
Menarche (years, Means ± SD)[1]	12.43 ± 1.6		12.14 ± 1.1			
7-10	(42)	9	(8)	3	3.5 (1.6-7.6)	<0.0001[2]
11-14	(372)	80	(272)	93	0.33 (0.20-0.54)	<0.0001[2]
15-18	(51)	11	(14)	4	2.3 (1.2-4.3)	0.004[2]
Body Mass Index (BMI)*						
18.5- 24.9 (normal)	(97)	21	(131)	45	0.39 (0.23-0.45)	<0.0001[2]
≥25- 29.9 (overweight)	(156)	34	(154)	52	0.47 (0.34-0.63)	<0.0001[2]
≥30- 34.9 (obesity I)	(119)	25	(6)	2	16.6 (7.2-38.4)	<0.0001[2]
≥35- >40 (obesity II-III)	(93)	20	(3)	1	24.5 (7.6-77.3)	<0.0001[2]
Oral contraceptive use						
Yes	(180)	39	(75)	26	1.8 (1.3-2.4)	<0.0001[2]
No	(285)	61	(219)	74		
Pregnancies						
Yes	(406)	87	(160)	54	5.7 (4.0-2.2)	<0.0001[2]
No	(59)	13	(134)	46		
Abortion						
Yes	(150)	32	(19)	6	6.8 (4.1-11.4)	<0.0001[2]
No	(315)	68	(275)	94		
Breastfeeding						
Yes	(321)	69	(160)	54	1.8 (1.3-2.5)	<0.0001[2]
No	(144)	31	(134)	46		
Breastfeeding (1-3 month)	(63)	20	(45)	28		0.523[2]
Breastfeeding (≥ 6 month)	(258)	80	(115)	72	1.9 (1.4-2.6)	<0.0001[2]
Menopause						
Postmenopausal	(319)	69	(46)	16	11.6 (8.0-16.8)	<0.0001[2]
Premenopausal	(146)	31	(248)	84		
Tobacco consumption						
Yes	(106)	23	(44)	15	1.6 (1.1-2.4)	0.009[2]
No	(359)	77	(250)	85		
Alcohol consumption						
Yes	(131)	28	(77)	26		0.55[2]
No	(334)	72	(217)	74		
Familial history (FH)**						
Yes	(303)	65	(107)	36	3.2 (2.4-4.4)	<0.0001[2]
No	(162)	35	(187)	64		
Cancer type of FH						
No	(162)	35	(187)	64	0.30 (0.22-0.40)	<0.0001[2]
Breast cancer	(57)	12	(10)	3	3.9 (1.9-7.9)	<0.0001[2]
Cancer	(46)	10	(28)	9		0.901[2]
Diabetes mellitus (DM)- Arterial hypertension (AH)	(103)	22	(54)	18		0.141[2]
DM-AH-Cancer	(97)	21	(15)	6	4.9 (2.7-8.6)	<0.0001[2]
Inheritance						
No	(162)	35	(187)			
Maternal	(173)	37	(62)	21	2.2 (1.6-3.1)	<0.0001[2]
Paternal	(85)	18	(29)	10	2.1 (1.3-3.3)	0.001[2]
Both	(45)	10	(16)	5	1.8 (1.0-3.3)	0.040[2]

[1]Fisher's exact test, [2]Student's *t*-test. SD = standard deviation. *According to OMS classifications (Who Expert Consultation, 2004). **Positive familial history of cancer, leukemia, DM, and HAS in first and second degree relatives of patients.

Table 2. Clinical data from patients with breast cancer.

	Breast cancer	
	(N = 465)	%
Personal medical history		
No	(220)	47
DM-AH	(136)	29
Breast fibrosis	(36)	8
Hysterectomy	(68)	15
Depression, pregnancy, preeclampsia	(5)	1
Tumor localization		
Right	(209)	45
Left	(234)	50
Bilateral	(22)	5
BirradI-III grade	(33)	7
IV grade	(326)	70
V grade	(106)	23
Diagnostic time		
1 - 4 years	(359)	77
5 - 9 years	(87)	19
10 - 15 years	(10)	4
Tumor markers		
Estrogen receptor	(263)	57
Progesterone receptor	(242)	52
Her 2-neu	(141)	30
P53	(41)	8
E-caderine	(14)	3
K167	(63)	14
Histology		
Ductal	(406)	87
Lobular	(41)	9
Mixed	(18)	4
Tumor stage		
I-II	(201)	43
III-IV	(264)	57
Lymph node status (presence)	(332)	71
Metastasis (presence)	(156)	34
Metastasis site (N =156)		
Bone	(73)	48
Bone-lung	(21)	13
Bone-liver	(24)	15
Lung-liver	(14)	9
Lung-central nervous system-liver	(24)	15
Chemotherapy response		
Yes	(158)	34
No	(307)	66
Chemotherapy type		
FEC	(334)	72
Other	(119)	26
No chemotherapy	(12)	2
Chemotherapy toxicity		
Yes	(367)	80
No	(98)	20
Laboratory test		
Hemoglobin (g/dL)		
<11	(32)	7
11-16.4	(433)	93
Hematocrit (%)		
<37	(148)	32
37-47	(317)	68
Platelets (mm^3)		
<150,000	(21)	4
150,000-450,000	(343)	74
>450,000	(101)	22

Continued on next page

Table 2. Continued.

	Breast cancer	
	(N = 465)	%
Leukocytes (mm^3)		
<150,000	(57)	12
150,000 -500,000	(357)	77
>500,000	(51)	11
Urea (mg/dL)		
>20	(52)	11
6-20	(413)	89
Creatine (mg/dL)		
>1.1	(21)	5
3-1.1	(444)	95
Glutamate-oxaloacetate transaminase (SGOT) (mI/L)		
>35	(107)	23
0-35	(358)	77
Glutamic pyruvic transaminase (SGPT) (mI/L)		
>45	(58)	12
5-45	(407)	88
Lactate dehydrogenase (LDH) (mI/L)		
>333	(84)	18
105-333	(381)	82
Alkaline phosphatase (ALP) (mI/L)		
>45	(120)	26
5-45	(345)	74
Gamma glutamyl transferase (GGT) (mI/L)		
>45	(146)	31
5-45	(319)	69
Glucose (µI/L)		
>106	(326)	70
74-106	(139)	30

FEC = 5-Fluorouracil, Epirrubicine, Cyclophosphamide; others = paclitaxel, docetaxel, herceptin.

Table 3 summarizes the multivariate logistic regression analysis in which the BC group was classified with menopause as the dependent variable. The presence of obesity I and II (adjusted OR = 2.6, 95%CI = 1.5-4.4, P = 0.0001), abortion (OR = 1.7, 95%CI = 1.1-2.7, P = 0.021), and high levels of glucose (OR = 1.6, 95%CI = 1.03-2.7, P = 0.036) were found to be risk factors. By contrast, the presence of Breast Imaging Reporting and Data System score I-III (OR = 0.33, 95%CI = 0.15-0.71, P = 0.004) was a protective factor.

The genotypes and allele frequencies of TNF-α -308G>A were different between patients and controls (Table 4). The GG genotype was observed in 65% (302/465) of patients compared with 86% (254/294) of the controls (OR = 0.29, 95%CI = 0.19-0.42, P = 0.0001). The heterozygous genotype (GA) was observed in 21% of patients (96/465) and 13% (38/294) of controls (OR = 1.7, 95%CI = 1.1-2.6, P = 0.006). The polymorphic genotype (AA) was observed in 14% (67/465) of patients and in 1% (2/294) of controls (OR = 2.4, 95%CI = 5.9-101.1, P = 0.0001). Control genotype distributions were in Hardy-Weinberg equilibrium. In addition, significant differences were found with regard to the general characteristics of the study groups and genotype GA-AA of the -308G>A TNF-α polymorphism with Her 2-neu (adjusted OR = 1.6, 95%CI = 1.05-2.4, P = 0.027) as a risk factor. However, leukocyte level (OR = 0.59, 95%CI = 0.37-0.93, P = 0.025) and menopause (OR = 0.61, 95%CI = 0.40-0.93, P = 0.023) were protective factors (Table 5).

Table 6 shows that the heterozygous and homozygous (GA, AA, respectively) genotypes were associated with more than 1 variable listed in Tables 1 and 2. The GA-AA genotypes were associated with BMI 30-34.9 (crude OR = 3.5, 95%CI = 1.3-9.3, P = 0.008) as risk factors in premenopausal patients. GA-AA carriers with stage I-II were associated with Her-2 neu positivity

(OR = 2.5, 95%CI = 1.31-4.8, P = 0.004) and patients with stage III-IV with post-menopause (OR = 1.7, 95%CI = 1.03-2.9, P = 0.034). In GA-AA carrier patients non-responders to chemotherapy showed association with abnormal hematocrit (OR = 2.4, 95%CI = 1.2-4.8, P = 0.015), BMIs of >30 to >40 were associated with Her 2 (OR = 2.8, 95%CI = 1.2-6.6, P = 0.016), and K I67 positivity was associated with metastatic nodules (OR = 4.0, 95%CI = 1.01-15.7, P = 0.038).

Table 3. Regression binary logistic of the patient group.

						95%CI		
	B	SD	Wald	d.f.	P	OR	Low	Upper
Obesity I and II	0.960	0.272	12.49	1	0.000	2.612	1.534	4.449
Abortion	0.545	0.237	5.303	1	0.021	1.725	1.085	2.742
Birrad I-III	-1.091	0.383	8.113	1	0.004	0.336	0.159	0.712
Glucose (high levels)	0.515	0.245	4.416	1	0.036	1.673	1.035	2.705
Constant	0.366	0.144	6.426	1	0.011	1.442		

Variables included in the analysis: dependent: breast cancer patients classified by menopause. Independent: personal medical history, menarche ranges 7-10, 11-13, 14-18 years; pregnancies, abortion, breastfeeding, oral contraceptive use, tobacco, and alcohol consumption, HF, HF type: breast cancer, DM, AH, DM-AH-cancer, parental inheritance: mother, father, both, and BMI: 18.5-24.9, ≥25-29.9, ≥30-34.9, ≥35, years of diagnosis; birrad, histology, tumor markers, tumor stage, lymph node status, metastasis, MTS tissue, response to chemotherapy, type of chemotherapy, toxicity in chemotherapy, laboratory tests (HB, HTO, platelets, leukocytes, urea, creatinine, SGOT, SGPT, LDH, ALP, GGT, and glucose levels).

Table 4. Genotype and allelic distribution of the -308G>A TNF-a polymorphism in healthy controls and breast cancer patients.

	Genotypes				Alleles	
	GG	GA	AA	GA-AA	G	A
Patients (N = 465)* (N, %)	302 (65)	96 (21)	67 (14)	163 (35)	700 (0.75)	230 (0.25)
Controls (N = 294) (N, %)	254 (86)	38 (13)	2 (1)	40 (14)	546 (0.93)	42 (0.07)
Patients *vs* Controls						
OR	0.29	1.7	2.4	3.4	0.43	2.2
95%CI	(0.19-0.42)	(1.1-2.6)	(5.9-101.1)	(2.3-5.0)	(0.16-0.33)	(3.0-6.0)
P value	0.0001	0.006	0.0001	0.0001	0.0001	0.0001
Marker informativity**		0.87				

*Hardy-Weinberg equilibrium in the controls (X^2 = 0.015; P = 0.90); **marker informativity was assessed within a range of 0-1: markers with a score greater than 0.7 were considered to be highly informative, whereas markers with a value of 0.44 were considered to be moderately informative (Gallegos et al., 2012).

Table 5. Regression binary logistic of the patient group.

						95%CI		
	B	SD	Wald	d.f.	P	OR	Low	Upper
Her2-neu	0.473	0.214	4.894	1	0.027	1.604	1.055	2.439
Leucocytes (normal levels)	-0.518	0.231	5.050	1	0.025	0.595	0.379	0.936
Menopause	-0.481	0.212	5.154	1	0.023	0.618	0.408	0.936
Constant	-0.361	0.285	1.605	1	0.205	0.697		

Variables included in the analysis: dependent: AA-AG -308G>A *TNF*-α polymorphism. Independent: personal medical history, menarche ranges 7-10, 11-13, 14-18 years; menopause, pregnancies, breastfeeding, oral contraceptive use, tobacco and alcohol consumption, HF, HF type: breast cancer, DM, AH, DM-AH-cancer, parental inheritance: mother, father, both, and BMI: 18.5-24.9, ≥25-29.9, ≥30-34.9, ≥35, years of diagnosis; birrad, histology, tumor markers, lymph node status, metastasis, MTS tissue, response to chemotherapy, laboratory tests (HB, HTO, platelets, leukocytes, urea, creatinine, SGOT, SGPT, LDH, ALP, GGT, and glucose).

Table 6. Association of the -308G>A *TNF*-α polymorphism with more than one variable of the general characteristics of the breast cancer patients.

		Variable				
Variable	Genotype	N	N	OR	95%CI	P
BMI (30-34.9)		Pre-menopause				
		Yes	No			
	AG-AA	23	11	3.5	(1.3-9.3)	0.008
	GG	74	10			
		Her2-neu				
Tumor stage		Yes	No			
I-II	AG-AA	26	41	2.5	(1.31-4.8)	0.004
	GG	27	107			
		Pre-menopause				
		Yes	No			
III-IV	AG-AA	39	57	1.7	(1.03-2.9)	0.034
	GG	47	121			
		Hematocrit				
Non chemotherapy response		Abnormal	Normal			
	AG-AA	28	33	2.4	(1.2-4.8)	0.015
	GG	25	72			
		Her-2 neu				
BMI		Yes	No			
>30->40	AG-AA	16	17	2.8	(1.2-6.6)	0.016
	GG	21	64			
K-I67		Nodule metastasis				
		Yes	No			
	AG-AA	20	3	4.0	(1.01-15.7)	0.038
	GG	25	15			

DISCUSSION

BC is a multifactorial disease with a complex etiology. Therefore, epidemiological studies that include risk factors are important for providing information about possible causes in various populations. In Mexico, BC is considered a major health issue because its incidence has increased over the last 6 years, and it is currently among the leading causes of death in women of productive age (Knaul et al., 2009). These facts are consistent with our observations in the current study, in which the average age was 54 ± 11.6 years. Many studies have observed a high frequency of BC in women aged approximately 50 years (Abdulrahman and Rahman, 2012).

In this study, we found early menarche to be a risk factor because it was present in 9% of the patients and observed to occur at early ages (7-10 years). The risk of BC reportedly increases when menarche occurs early and menopause occurs late, which can be influenced by nutritional and socioeconomic status and is likely due to prolonged exposure to estrogen (Parkin, 2011).

When we adjusted the study groups according to BMI, we observed that obesity II-III (≥30 to >40) was a risk factor for patients with BC. In fact, several theories have attempted to explain this association and involve the roles of leptin, insulin, and other molecules that mediate inflammatory processes independently of estrogen. In addition, peripheral circulating estrogens (from aromatization of androgens) are elevated in obese postmenopausal women. Another current hypothesis is that obesity is associated with metabolic syndrome, which activates molecular processes that are mitogenic in breast epithelial cells and stimulate neoplasia.

Yet another hypothesis suggests that adipocytes and their autocrine mechanisms are important for BC development (Macciò and Madeddu, 2011).

In the present study, the majority of BCs (69%) occurred in postmenopausal women. Approximately 75% of BCs are diagnosed at this stage (Parkin, 2011). Therefore, our data are consistent with previously reported rates. Additionally, the use of contraceptives was a risk factor observed in 39% of the patients in this study. Exogenous hormone use has also been hypothesized to increases the risk of BC, but this risk may depend on many other factors, including nutritional status, physical activity, BMI, and tobacco-alcohol addiction (Parkin, 2011; Abdulrahman and Rahman, 2012). Another risk factor for BC is gestation, which is thought to reduce the risk of BC, and a higher number of pregnancies carried to term is considered to be a protective factor. The risk of BC is reduced by 7% with each term pregnancy, and generally, women who have had children have a 30% lower risk than that of nulliparous women (Parkin, 2011). However, in this study, the presence of pregnancies was not a risk factor, probably because we did not take into account the number of term deliveries and age at first birth. In this study, breast-feeding was recorded in 69% of patients in contrast with 54% of controls, which made it a risk factor. However, these results should be interpreted with caution because the control group was not age matched, and changes in the lifestyles of younger generations of women has put breast-feeding in decline. Prolonged breast-feeding has been hypothesized to reduce the risk of BC 4% for each 12 months of breastfeeding (Parkin, 2011). Studies about abortion as a risk factor for BC have been considered inconsistent. In the present study, abortion was observed to be a risk factor. Also we found the association between BC and tobacco consumption; this association of tobacco use with BC is still controversial; studies have observed that smokers have a higher BC risk than that in nonsmokers (Abdulrahman and Rahman, 2012; Parkin, 2011). As well this association depends on the number of cigarettes smoked per day and exposure time. However, in most studies, these parameters are not considered.

Conversely, a study has estimated that approximately 3% of BCs in UK women are associated with breast-feeding for less than 6 months (Parkin, 2011). A familial history of cancer also increases the risk for BC. In this study, 12% of patients had a familial history of BC compared with 3% of controls. Moreover, a familial history of diabetes mellitus, hypertension, and other cancer types also accompanies increased risk (Lazcano et al., 1996).

When the group was stratified by menopause as either premenopausal or postmenopausal and compared with clinical and biochemical characteristics of BC, obesity I and II (discussed above), abortion, and high levels of glucose emerged as risk factors. In this regard, our results showing glucose as a risk factor are consistent with previously reported findings (Emerging Risk Factors Collaboration et al., 2011). Some studies have suggested that glucose metabolism may contribute to BC development. Because insulin can stimulate cell proliferation through its receptor, it regulates insulin-like growth factor binding proteins and sex hormones, increasing bioavailable mitogens (Duggan et al., 2011).

Advances in molecular and genetic epidemiology have increased our knowledge of the mechanisms underlying breast carcinogenesis and the relationship between susceptibility and exposure to carcinogens, diet, and individual genetic variations. TNF-α has been identified as a pro-inflammatory molecule central to the regulation of inflammatory response. The function of TNF-α is complex; it interacts with 2 receptors, TNFR1 and TNFR2, which participate in signal transduction pathways and the signaling cascade of cellular responses such as apoptosis, proliferation, differentiation, migration, and angiogenesis (Cereda et al., 2012). Chronic

inflammation may promote tumor progression through stimulation of the vascular endothelium via recruitment of leukocytes to the tumor, triggering angiogenic, mitogenic, and chemotactic factors and proteolytic enzymes that recruit other inflammatory cells to stimulate angiogenesis, which in turn sustains tumor growth and facilitates metastasis (Guadagni et al., 2007).

Changes in single nucleotides in coding regions of the *TNF*-α promoter have been suggested to modify the binding site of specific transcription factors, and therefore affect transcriptional regulation and modulate their secretory responses. Allele A of polymorphism -308G>A of *TNF*-α is associated with a high level of *in vitro* TNF expression and has also been associated with increased susceptibility and severity of various diseases, such as diabetes, immunological diseases, malaria, infection, and cancer (Berberoglu et al., 2004; Duggan et al., 2011; Cereda et al., 2012; Chu et al., 2012). However, the associations between -308G>A *TNF-α* polymorphisms and BC remain controversial, and their association depends on the population studied. Moreover, little is known about the association of this polymorphism with BC in Mexican women. In our population, the frequency of this polymorphism was 14% in BC patients and 1% in controls and was identified as a risk factor. Other studies have observed the association between the -308G>A polymorphism and BC (Li et al., 2008; Pooja et al., 2011; Shen et al., 2011).

In our study, the presence of the Her-2 neu immunohistochemical marker in patients with genotype AA- was a risk factor. Although the mechanism by which Her-2 neu oncogene overexpression induces resistance to TNF is unknown, a study has observed that overexpression is associated with poor prognosis in breast and ovarian cancer patients because it induces metastatic potential and resistance in cancer cells (Zhou et al., 2000). Zhou et al. (2000) have demonstrated the molecular mechanisms of TNF resistance in cancer cells because of Her-2 neu overexpression through the activation of Akt, nuclear factor-kappaB, the anti-apoptotic cascade, and reduced host defenses against neoplasia. Another proposed mechanism by Lyu et al. (2005) is that the overexpression of Her-2 neu is mediated by signaling pathway cytotoxicity, suggesting that the overexpression of TNFR-1 is important in TNF sensitivity in Her-2 neu-overexpressing cancer cells.

In this study, we also observed an association between the GA-AA genotypes and risk in premenopausal patients who had obesity grade I. A study by Lorincz and Sukumar (2006) supports this association. In this sense, the association between obesity and BC risk is a complex biological mechanism that has not been clearly established; generally, obesity is accepted as a risk factor in postmenopausal BC (Braun et al., 2011). However, studies of mortality and survival between BC and obesity have shown that adiposity is associated with poorer survival and increased likelihood of recurrence among those with the disease, regardless of menopause status and after adjustment for stage and treatment (Lorincz and Sukumar, 2006). The exact mechanism through which TNF-α cytokine is secreted by adipocytes is unknown but it has been proposed to participate in several processes: 1) the development of insulin resistance, 2) autocrine and paracrine processes influence apoptosis and the synthesis of cytokines and adipokines, and 3) regulation of the synthesis of interleukin-6 and the biosynthesis of estrogen by stimulating aromatase expression in adipose tissue (Lorincz and Sukumar, 2006). Therefore, BC patients with obesity grade II who are carriers of allele A of *TNF*-α polymorphism -308G>A may have an increase in circulating TNF-α that contributes to breast tumorigenesis through insulin resistance and interleukin-6 synthesis (Alokail et al., 2009). We also observed an association of the GA-AA genotype in Her-2 neu patients with tumor stage (I-II) and premenopause with tumor stage (III-IV) as a risk factor (explained above).

Non-response to chemotherapy in BC patients with abnormal hematocrit was influenced by the GA-AA *TNF*-α genotype in this study. Response to chemotherapy depends on several factors, including the presence of metastatic nodes, tumor markers, menopause, time of diagnosis, and tumor stage, and adjuvant chemotherapy can induce persistent resistance to therapeutic drugs with longer exposure (Mihlon et al., 2010). Henry et al. (1995) have observed that the lowest response rates occur in patients who lack a substantial increase in hemoglobin in the early weeks of therapy; a lack of oxygen transport to tissues likely assists the development of cancer resistance. Conversely, several studies have linked high levels of TNF-α with poor chemotherapy response in BC (Berberoglu et al., 2004).

The GA-AA genotypes are a risk factor in BC patients with BMI ≥30 to > 40 who were immunohistochemical Her-2 neu positive. Gilbert and Slingerland (2013) have observed that high cytokine levels in primary BCs and in the circulation of affected patients have been associated with poor outcome, likely owing to the interaction of adipokines and cytokines in the fat tissue. Many of the cytokines associated with a proinflammatory state are not only up-regulated in obese adipose tissue but also may stimulate the self-renewal of cancer stem cells. Thus, enhanced cytokine production in obese adipose tissue may serve both as a chemoattractant for invading cancers and for augmenting their malignant potential.

The presence of Her-2 neu in BC patients has been correlated with 1) metastases, to detect the early appearance of recurrent BC and to predict response to hormonal therapy or chemotherapy, 2) insulin resistance and overexpression of fatty acid synthase, and 3) regulation of expression by metformin in *in vitro* models (Perks and Holly, 2011). Fernández et al., (2010) have observed that serum Her-2 neu concentrations in patients with diabetes type 2 are associated with serum TNFR1 because TNF-α might underlie concomitant insulin resistance and epidermal growth factor resistance, leading to increased circulating Her-2 levels.

The GA-AA genotype is a risk factor in BC patients with nodule metastasis who were immunohistochemically K I67 positive. A variety of mechanisms for TNF activity has been proposed that could contribute to the development of metastasis. In fact, significant correlation has been observed between the number of K I67 positive cells and tumor cell proliferation (Orosz et al., 1993).

In addition to these mechanisms, several factors could influence at the development of BC. Polymorphisms in *TNF*-α can increase enzyme activity, produce changes in the cell mechanisms, and subsequently participate in neoplastic progression. Our results showed that the frequencies of homozygous and heterozygous genotypes of the -308G>A polymorphism in *TNF*-α were significantly different in controls compared with those in BC patients. The differences were most evident in patients with 1) premenopause and obesity, 2) Her-2 neu and tumor stage I-II, 3) premenopause and tumor stage III-IV, 4) non-response to chemotherapy and abnormal hematocrit, 5) Body mass index (BMI), Her-2 neu, and III-IV tumor stage, and 6) nodule metastasis and K I67, confirming that these factors contribute significantly to BC susceptibility in the analyzed sample from the Mexican population. Further studies are required to confirm these observations.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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