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ORIGINAL ARTICLE

Association of the *IL-10* gene rs1800872 (-592 C>A) polymorphism with breast cancer in a Mexican population

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Summary

Purpose: *Interleukin 10 (IL-10) gene polymorphisms are associated with different types of cancer, but these associations are inconsistent. The purpose of this study was to determine the frequency and association of the rs1800872 IL-10 gene polymorphism in Mexican women with breast cancer (BC).*

Methods: *The rs1800872 polymorphism was genotyped in 368 BC patients and 320 control women using the polymerase chain reaction (PCR).*

Results: *The rs1800872 polymorphism was a risk factor for BC compared to controls and BC patients with genotypes CA (p=0.004) and AA, and in the recessive model (p=0.0002), dominant model (CA+AA; p=0.0001), and allele A (p=0.0001). Additionally, differences were observed in BC patients with the CA and CAAA genotypes who had chemotherapy gastric and hematological toxicity (p=0.022) and tumor stage IV (p=0.013) as a risk factor. Genotypes were CA in breastfeeding (p=0.017), AA in gastric toxicity (p=0.048), and CAAA in tumor stage I-II (p=0.019) as protective risk factors. In BC carriers of: 1) CAAA genotype with tumor stage I-II and breast feeding (≥6 months), 2) CA genotype BC Luminal A with tumor stage I-II, 3) CA genotype BC Luminal B with breastfeeding (≥6 months), and 4) CAAA genotypes in BC HER2 with indices of cellular proliferation (Ki-67) that were elevated (≥20%), were considered to be protective factors in BC patients.*

Conclusion: *The IL-10 gene rs1800872 polymorphism was associated with BC susceptibility in this sample from the Mexican population.*

Key words: *IL-10, polymorphism, rs1800872, breast cancer, Mexican population*

Introduction

Breast cancer (BC) is the most frequent gynecological type of cancer [1], and its incidence varies between different types of ethnic groups [1]. In Mexico, BC is a major cause of mortality [1-3]. The gradual accumulation of genetic and epigenetic events might influence the development of BC [1,4]. Studies have shown an association between interleukin 10 (*IL-10*) and BC [5-7]. *IL-10* is an anti-inflammatory cytokine that regulates the immune response and inhibits the pro-inflammatory functions of antigen-presenting cells through expression of co-stimulated molecules. Its low expression is associated with a poor survival outcome through IL-6 expression and synthesis, which causes neoplastic cell proliferation and metastasis [6]. Thus, *IL-10* has dual biological functions because it acts as an inhibitor of pro-inflammatory cells and as a tumor promoter [6]. Additional functions, such as anti-angiogenic properties, inhibit tumorigenesis [6,7]. *IL-10* is activated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways through its receptor *IL-10* R1,

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which binds to STAT3. Then STAT3 is translocated into the nucleus, where it binds to STAT-binding elements in the promoters of various genes. One of these genes is *IL-10*, which is positively regulated by STAT3 [5-7]. *IL-10* mRNA expression was observed in over 50% of tumor samples and it promotes tumor cell proliferation and metastasis [6]. The human *IL-10* gene is located on chromosome 1q21-32 and contains five exons. There are several genes that are adjacent to *IL-10* that form the *IL-10* gene cluster and those encoding IL-20 and IL-19 are found upstream. However, genes encoding Mapkapk2 and Dyrk3 are localized downstream of *IL-10* [7]. Several polymorphism types including SNPs and VNTRs have been identified in *IL-10*. The *IL-10* promoter is highly polymorphic, and some of the most studied polymorphisms are rs1800871 (-819C>T), rs1800872 (-592C>A), and rs1800896 (-1082A>G).

The *IL-10* promoter region polymorphisms were reported to affect *IL-10* gene transcription and translation, resulting in abnormal cell proliferation and cancer development [5-7]. Studies have reported that genetic polymorphisms in the *IL-10* gene play an important role in the development of several diseases that include cancers such as BC and colorectal, lung, and gastric cancer [7]. Moreover, the *IL-10* gene rs1800872 polymorphism has been associated with an increased risk of BC [5-8]. However, other studies have shown inconsistent results [8]. The most frequently reported variety depends upon the population, and the *A* allele showed a frequency in controls of 22-32% among American and European populations, 30-65% among Iranian and Chinese populations, 73% in the Egyptian population, and 37-39% in the Mexican population [5, 9-10]. *IL-10* gene polymorphisms may determine BC susceptibility, but the association of studies that

Table 1. PCR conditions for the rs1800872 polymorphism in the IL-10 gene

	rs1800872C>A, promoter
*PCR Primers: sense	5′-GGTGAGCACTACCTGACTAGC-3′
antisense	5′-CCTAGGTCACAGTGACGTGG-3′
PCR product	412 bp
PCR conditions	Buffer enzyme 1X, 7.5 pmol of each primers, 0.2 mM of dNTPs, 2.0 mM of $MgCl_2$, 2.5 µL of Taq polymerase, 100 nM of genomic DNA
Alternating PCR temperature	59°C
Recognition enzyme restriction	RsaI, 37°C, (5′...GT↓AC...3′)
**Allele identified	
(wild type)	C: 412
(polymorphic type)	A: 236+176

*The primers were previously described in [16]. **The genotypes were identified in 6% polyacrylamide gels (29:1) followed by silver nitrate staining

Table 2. Demographic data for the study group

	BC patients (n=368)		*Controls (n=320)*		*p value***
	n (%)	*median±SD**	*n (%)*	*median±SD**	
20-29	10 (3)	26 ±2.6	19 (6)	25.31±2.98	0.548
30-39	49 (13)	34.73±3.05	53 (17)	35.01±3.06	0.638
40-49	113 (31)	44.80±2.96	85 (27)	45.17±2.71	0.361
50-59	101 (27)	54.08±2.56	103 (32)	54.01±2.74	0.852
60-69	67 (18)	64.37±3.14	58 (15)	64.24±3.16	0.817
≥70	28 (8)	74.78±3.46	2 (3)	76.0±1.49	0.394
Tobacco consumption					<0.0001
No	268 (73.0)		281 (91.0)		
Yes	100 (27.0)		39 (9.0)		
Alcohol consumption					<0.0001
No	270 (72.0)		288 (91.0)		
Yes	98 (27.0)		32 (9.0)		

SD: standard deviation, *Age, years, **Student's *t*-test

Table 3. Demographic and clinical data for the BC patients

Data	*(n=368) n (%)*	*Data*	*(n=368) n (%)*
FHC		Localization	
no	145 (40)	Unilateral	25 (7)
BC	108 (29)	Bilateral	343 (93)
Other type[1]	115 (31)		
PPA fibrosis		Type	
no	199 (54)	Ductal	338 (92)
uterine/breast	83 (23)	Lobular	29 (7.9)
DM-HAS	86 (23)	Mixed	1 (0.1)
Hormonal status (years)			
Menarche		Tumor stage	
7-10	27 (7)	I-II	151 (41)
11-13	246 (67)	III-IV	217 (59)
14-18	95 (26)	Metastasis*	111 (30)
Consumption			
no	153 (42)	Lymph node	
oral	168 (46)	Positive*	178 (48)
HRT	47 (13)		
Parity		Histological type	
Null-parity	29 (8)	Luminal A	108 (29.3)
Gestations (<5)	285 (77)	Luminal B	117 (31.8)
Gestations (≥5)	54 (15)	Her2	78 (21.2)
≥2 loss gestational		Triple-negative	65 (17.7)
Miscarriage *	98 (27)	Ki-67 (≥20%)*	281 (76.4)
Breastfeeding			
None	97 (26)	Chemotherapy***	
≤6 months	116 (32)	response	148 (40)
≥6 months	155 (42)	Partial response	96 (26)
premenopausal	143 (39)	Non-response	13 (4)
menopause	225 (61)	Non-response with recurrence	111 (30)
BMI (kg/m2)**			
Normal (18.5-24.9 kg/m^2)	125 (34)	Toxicity****	
Overweight (25-29.9 kg/m^2)	115 (31)	Gastric	84 (23)
Obesity I (30-34.9 kg/m^2)	89 (24)	hematological	46 (13)
Obesity II (35-39.9 kg/m^2)	30 (8)	Gastric and hematological	202 (55)
Obesity III (40-45.9 kg/m^2)	9 (3)	No toxicity	36 (9)

FHC: familial hereditary cancer, PPA: personal pathological antecedents, HRT: hormonal replacement therapy, DM: diabetes mellitus, HAS: systemic arterial hypertension.
[1]ovary, colorectal, lung, leukemia
*On base 368
**according to OMS classifications (appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Genève (Switzerland): World Health Organization, 2004).
***Chemotherapy (non-chemotherapy response, non-chemotherapy response by recurrence). Non-response to chemotherapy treatment with anthracyclines (e.g. doxorubicin, epirubicin, liposomal doxorubicin), taxanes (docetaxel, paclitaxel), and trastuzumab was evaluated according to the pathological Ryan's classification described as follows: 1) Moderate response (single cells or small groups of cancerous cells); 2) Minimum response (residual cancer surrounded by fibrosis); and 3) Poor response (minimal or no tumor destruction, extensive residual cancer) [27].
**** Gastric toxicity (nausea, diarrhea, vomiting, stomatitis, anorexia, abdominal pain, mucositis), hematologic toxicity (neutropenia, thrombocytopenia, anemia).

examined the *IL-10* rs1800872 polymorphism and BC risk have been inconclusive [5-8,11-13]. Some of them have shown an association [7,8,11] while others have shown no association [12,13]. In the Mexican population, the association of the *IL-10* rs1800872 polymorphism in BC remains unknown. Thus, the aim of this investigation was to determine the frequency and association of the *IL-10* rs1800872 polymorphism in Mexican women with BC.

Methods

Blood samples were collected from 320 healthy women who were donating blood and 368 patients with clinically and histologically confirmed BC. The patient and control groups were not age-matched, and no familial samples were included. All patients in the study group were recruited from June of 2014 to May of 2019, and samples were obtained after the patients and controls provided written informed consent, as approved by the ethical local committee (1305). All procedures performed in studies involving human participants were conducted in accordance with the Declaration of Helsinki. Clinical and demographic data were obtained using written questionnaires. All patients were interviewed to determine occupational exposure and current drug regimens. The BC patient database and patient DNA samples were examined for other polymorphisms [5,14]. DNA was extracted from peripheral blood lymphocytes using standard protocols [15].

The PCR amplification of the *IL-10* rs1800872 polymorphism was performed as reported in Table 1 [16].

Table 4. Genotype and allelic distribution of rs1800872 polymorphisms in IL-10 gene BC patients and controls

Polymorphism		*BC* (n=368)*	*Controls* (n=320)*	*OR*	*95% CI*	*p value*
rs1800872	*Genotype*	*n (%)*	*n (%)*			
	CC	172 (47)	209 (65)	1		
	CA	154 (42)	100 (32)	1.5	(1.15–2.16)	**0.0004**
	AA	42 (11)	11 (3)	3.6	(1.83–7.15)	**0.0002**
Dominant	CC	172 (47)	209 (65)	1		
	CA+AA	196 (53)	111 (35)	2.1	(1.57–2.91)	**0.0001**
Recessive	CC+CA	326 (87)	309 (97)	1		
	AA	42 (13)	11 (3)	3.6	(1.83–7.15)	**0.0002**
	Allele	*(2n=736)*	*(2n=640)*			
	C	498 (0.676)	518 (0.810)	0.5	(0.38–0.63)	**0.0001**
	A	238 (0.324)	122 (0.190)	2.0	(1.57–2.60)	**0.0001**

OR: odds ratio, CI: confidence intervals, p value, significant at< 0.05.
* Hardy-Weinberg equilibrium in controls of rs1800872 (chi-square test = 0.051, p = 0.8199) and BC patients (chi-square test=0.7029, p=0.4018) of the IL-10 gene polymorphism.
Bold numbers denote statistical significance

Table 5. Association of IL-10 rs1800872 polymorphism with clinical variables of BC patients

Polymorphism	*Histology*	*Genotype*	*Clinical variables**	*OR*	*95% CI*	*p value*
rs1800872		CA	Chemotherapy gastric and hematological toxicity	2.8	(1.16-6.9)	0.022
		CAAA	Metastasis presence	4.1	(1.35-12.6)	0.013
		CA	Breastfeeding (≥6 months)	0.5	(0.27-0.88)	0.017
		AA	Gastric toxicity	0.24	(0.065-0.937)	0.048
		CAAA	Tumor stage I-II	0.38	(0.173-0.853)	0.019
	Stage I-II	CAAA	Breastfeeding (≥6 months)	0.33	(0.15-0.77)	0.010
	Luminal A	CA	Tumor stage I-II	0.023	(0.01-0.78)	0.036
	Luminal B	CA	Breastfeeding (≥6 months)	0.22	(0.07-0.73)	0.014
	HER2	CAAA	Ki-67 (≥20%)	0.32	(0.12-0.73)	0.008

OR: odds ratio, CI: confidence intervals, p value, significant at< 0.05.
*Non-significant clinical variables included in the analysis were as follows: Age (<50, ≥50 years), tobacco and alcohol consumption, menopause; and cancer type (ductal, lobular), chemotherapy (non-chemotherapy response, non-chemotherapy response by recurrence). Non-response to chemotherapy with anthracyclines (e.g. doxorubicin, epirubicin, liposomal doxorubicin), taxanes (docetaxel, paclitaxel), and trastuzumab was evaluated according to the pathological Ryan's classification described as follows: 1) Moderate response (single cells or small groups of cancer cells); 2) Minimum response (residual cancer surrounded by fibrosis); and 3) Poor response (minimal or no tumor destruction, extensive residual cancer) [27]. Molecular classification (Luminal B, Triple-negative).

Allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit Chi-square test to compare the observed genotype frequencies to the expected frequencies among control subjects. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. A two-tailed p<0.05 was considered to be statistically significant. The association analysis was determined by the OR and binary logistic regression was performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA).

Results

Table 2 shows the comparative epidemiological data from the BC patients and control individuals. The age stratified by decade in BC patients was not different among the study groups, and both tobacco and alcohol consumption were statistically different in BC patients compared to controls (p<0.0001).

The clinical and demographic characteristics of BC patients are shown in Table 3. The rs1800872 polymorphism in the *IL-10* gene was significantly different between patients and controls. The genotypes *CA* (OR 1.5, 95%CI 1.15-2.16, p=0.004) and *AA* (OR 3.6, 95%CI 1.83-7.15, p=0.0002) and the allele *A* (OR 2.0, 95%CI 1.57-2.60, p=0.001) were observed as risk factors. The genotype distribution of the rs1800872 polymorphism in the *IL-10* gene was in Hardy-Weinberg equilibrium in the studied groups (Table 4).

There were no statistically significant differences in the association of the *IL-10* gene rs1800872 polymorphism in BC patients and controls that were stratified by demographic data (age years old, smoking, and alcohol consumption).

The association of clinical characteristics with the *IL-10* gene rs1800872 polymorphisms in the BC group is shown in Table 5. BC patients who showed gastric and hematological toxicity, those with genotypes *CA* (OR 2.8, 95% CI 1.16-6.90, p=0.022), and those who were carriers of *CA-AA* (OR 4.1, 95% CI 1.35-12.6, p=0.013) with stage IV of the rs1800872 polymorphism showed risk factor susceptibility. Additionally, in BC patients, breastfeeding (≥6 months), carriers of *CA* (OR 0.5, 95% CI 0.27-0.88, p=0.017), and gastric toxicity in patients who were carriers of *AA* (OR 0.24, 95% CI 0.06-0.93, p=0.048) genotypes were protective factors.

Additionally, rs1800872 polymorphism genotypes *CA* and *CAAA* were protective factors in BC patients stratified by stage I-II (*CAAA*, OR 0.38, 95% CI 0.15-0.77, p=0.010), Luminal B (*CA*, OR 0.22, 95% CI 0.07-0.73, p=0.014), breastfeeding (≥6 months), Luminal A with stage I-II (*CA*, OR 0.023, 95% CI 0.01-0.78, p=0.036), and HER2 with Ki-67 (≥20%) (*CA*, OR 0.32, 95% CI 0.12-0.73 p=0.008).

Discussion

The BC incidence in Mexico has increased in the last decade and it is currently one of principal causes of death in women [4,14,17]. BC was observed to occur at an average age of 50 years [4,14], which is consistent with data from this study, in which the major percentage occurred at the age of 50-59 years. Better knowledge about BC has contributed to improved treatment for BC patients, as well as to improving BC patients' quality of life in our country [4]. However, it is still necessary to implement new studies and strategies to detect BC in the early stages of the disease. In this study, we observed differences in tobacco and alcohol consumption between BC patients and controls. The relationship between these two factors and cancer development is well established. Alcohol and tobacco consumption cause more than 3 million of deaths per year [18]. The role of cytokines in carcinogenesis has been well established [7-8,11]. *IL-10* has dual biological functions in the tumor, as both a stimulator and an inhibitor, and both activities contribute to the regulation of angiogenesis in the tumor cell and allows the tumor to escape from immune surveillance [8,16]. Conversely, the immune-stimulator action of *IL-10* might inhibit tumor metastasis [16]. However, the exact molecular mechanism of how *IL-10* leads the proliferation of breast cancer cells remains unknown.

IL-10 was shown to participate in abnormal cell proliferation and stimulate the mitotic activity in ductal and lobular breast cells; this contributed to an increase in the cancer risk [5-7,16].

Relevant BC studies have been associated with different polymorphisms on the *IL-10* gene, including three in the promoter region (-1082 (A/G, -819 T/C, -592 A/C), which may influence the *IL-10* messenger RNA transcription and expression *in vitro* [5-8,12-13,16,18]. rs1800872 is one of the most extensively studied polymorphisms in the *IL-10* gene [5-8,12-13,16], but there have been contradictory results, some of which have been associated with susceptibility to BC development [5,11], whereas others showed no association [12,13].

Moreover, little is known about association of the *IL-10* gene rs1800872 polymorphism in Mexican BC patients. In our study group, the frequency of genotypes (*AA*), alleles (*A*), and dominant (*CA+AA* vs. *CC*) and recessive (*AA* vs. *CA+CC*) models of polymorphism were significantly different between BC patients and controls (p<0.05) and were associated with the susceptibility of the risk of developing BC. These data are in agreement with a meta-analysis study that included 21 case-control studies with 6054 cases and 6355 controls [5]. However, other

meta-analyses that included six studies with 16,785 cases and 19,713 controls for rs1800872 found a statistically significant association but a protective risk susceptibility for both dominant and recessive models [8,11]. This polymorphism has been studied in lung cancer [19], gastric cancer [20,21], head and neck cancer [22], and BC [5]. These results are in agreement with a study that demonstrated the association of the rs1800872 polymorphism with a susceptibility development risk for BC [5-8,11]. It is possible that this promoter polymorphism may affect DNA sites of regulation, which may influence *IL-10* gene expression or function and contribute to tumorigenicity in breast tissue [5-7].

We observed that the rs1800872 polymorphism was a risk factor for susceptibility to BC development in patients who were stratified by different clinicopathological parameters such as chemotherapy gastric and hematological toxicity (genotype *CA*), and tumor stage IV or metastasis presence (*CAAA* genotypes). However, the sample size was low and confidence intervals were high when the data were stratified. Thus, *IL-10* expression in BC has been analyzed in different tissues, but the regulatory mechanisms that are involved in the development of cancer remain unclear.

We observed that the *AC* and *CAAA* genotypes are associated with the risk susceptibility, and it is likely that the *C* allele is involved in increasing the *IL-10* levels. Studies have shown that increased serum *IL-10* levels could facilitate tumor development. Additionally, elevated serum *IL-10* levels were observed in patients with metastatic disease compared to those with undisseminated cancer [8].

Moreover, the precise mechanism to understand the therapeutic efficacy of *IL-10* is not well defined. It has been observed that the presence of metastasis is associated with adverse clinical outcomes and might alter the expression of different molecular factors including immunogenic mechanisms, which participate in the regulation of cellular processes. However, it has also been observed that the response to drugs is related to the monogenic inheritance of a protein variant, and it also depends on the interaction of several genes that are involved in multiple metabolic pathways and epigenetic events [23].

Additionally, genotypes *CA*, *AA*, and *CAAA* of the rs1800872 polymorphism in BC tumor stage I-II, breastfeeding (≥6 months), gastric toxicity, and BC tumor stage I-II with breastfeeding (≥6 months), Luminal A with tumor stage I-II , Luminal B with breastfeeding (≥6 months), and HER2 with Ki-67 (≥20%) showed protective effects against BC. Observations from other studies showed an inverse correlation for both tumor stage and breastfeeding with the *IL-10* -1082 (rs1800896) polymorphism [24]. However, no evidence exists that this documented association with BC showed a histological molecular classification, although these are a reflection of the common genotypes that were observed in this sample of the Mexican population. A plausible biological explanation is that, because of the important roles of *IL-10* in carcinogenesis, it is possible that *IL-10* polymorphism might modulate the risk of cancer. Thus, it has been observed that breastfeeding longer (≥6 months) and tumor stage I-II are associated with a decreasing risk of breast cancer. It has also been reported that the A variant from -1082 (rs1800896) and the -592 (rs1800872) polymorphisms of *IL-10* gene and their (ATA) haplotype were associated with lower *IL-10* expression. Thus, the -592 A variant can be regarded as a low-producer allele of the *IL-10* gene that might protect tumors by inhibiting cytotoxic T lymphocyte (CTL)-mediated tumor-specific cell lysis [8]. In a meta-analysis study, carriers of the *AA* genotype, which exhibit low production of *IL-10*, were associated with a lower cancer risk than participants with the *CC*. Moreover, the homozygous *IL-10*-592 *AA* genotype was revealed, indicating homozygosity for the haplotype (ATA) was protective against BC [8].

Investigations of *IL-10* expression showed different BC molecular subtypes including Luminal A, Luminal B, HER2, and triple-negative that are present in different *IL-10* expression profiles [5-8,25,26]. It has been suggested that IL-6 and *IL-10* may not have a prognostic significance in BC, but they have a role in modulating the tumor microenvironment and altering cancer cell motility, and, perhaps, metastatic ability. Thus, the complex role that *IL-10* plays in determining the immune response seems to be dependent upon the tissue microenvironment and *IL-10* receptor expression on different types of immune cells [25]. Additionally, these depend on the therapeutic treatment before surgery or after chemotherapy and radiotherapy, which could be a predictive factor for prognosis of patients with BC [6,19,21]. The allele A in *IL-10* gene polymorphisms might confer a significant genetic predisposition to a complex disease. Thus, a possible explanation for the protective role of polymorphisms in the *IL-10* gene could be that the function of the BC cell microenvironment shows different roles depending on the *IL-10* expression level. When they are lower, *IL-10* has an anti-proliferative function, and conversely when the *IL-10* levels expression is increased, this leads to proliferative effects. These variations and the different polymorphisms in the *IL-10* gene, as well as epigenetic factors, could contribute to

the variability of results in BC clinical studies [6,19,21,26].

In conclusion, our results showed that the rs1800872 polymorphism was an associated risk factor for BC compared with controls, and BC patients had genotypes *CA* and *AA*, the allele A, and recessive (*AA* genotype) and dominant (*CA+AA* genotype) models. Additionally, there were differences between patients with *CA* and *CAAA* genotypes with chemotherapy gastric and hematological toxicity, and tumor stage IV or metastasis presence. The presence of allele A was shown to be a protective factor for susceptibility in BC patients with the following: 1) gastric toxicity (*AA*) and tumor stage I-II (*CAAA*); 2) BC with tumor stage I-II and breastfeeding (*CAAA*); 3) Luminal A with stage I-II (*CA*); and 4) Luminal B with breastfeeding (*CA*) and HER2 with increased Ki-67 (*CAAA*). The presence of this evidence confirms that these factors significantly contribute to BC susceptibility in the analyzed sample from the Mexican population. Further studies are required to confirm these observations.

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Conflict of interests

The authors declare no conflict of interests.

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