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The rs2234694 and 50 bp Insertion/Deletion polymorphisms of the *SOD1* gene are associated with breast cancer risk in a Mexican population

M.P. GALLEGOS-ARREOLA[1], M.A. RAMÍREZ-HERNÁNDEZ[1,2], L.E. FIGUERA[1,2], G.M. ZÚÑIGA-GONZÁLEZ[3], A.M. PUEBLA-PÉREZ[4]

[1]División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[2]Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México
[3]Laboratorio de Mutagénesis, División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[4]Laboratorio de Inmunofarmacología, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Guadalajara, Jalisco, México

Abstract. – OBJECTIVE: The rs2234694 and 50 bp insertion/deletion *(Ins/Del)* polymorphisms of the *SOD1* gene have been shown to be associated with many diseases, but in breast cancer (BC) their association has not been detected. The purpose of this study was to determine the frequency and association of *SOD1* gene polymorphisms (rs2234694 and 50 bp *Ins/Del*) in BC patients in the Mexican population.

MATERIALS AND METHODS: The *SOD1* polymorphisms were determined by Polymerase Chain Reaction (PCR) in Mexican healthy subjects and BC patients.

RESULTS: The rs2234694 polymorphism was associated with BC susceptibility when BC patients and the control group were compared for the AC genotype ($p<0.0001$), the AC/CC genotype (dominant model: $p<0.0001$), and the C allele ($p<0.0001$). The 50 bp *Ins/Del* polymorphism was associated with BC susceptibility for the Del allele (p=0.048), although the association between the dominant model AC/CC (rs2234694) and BC patients was evident for menopause [adjusted odds ratio (OR) 1.65 (95% CI 1.05-2.7); $p=0.048$], Ki-67 (≥15%) (OR1.9, 95% CI 1.14- 3.16, $p=0.016$), and the presence of DM2 (OR 2.4, 95% CI 1.35-4.31, $p=0.003$). A protective association for BC of the rs2234694 polymorphism was observed in patients younger than 50 years positive for estrogen receptor (ER) and progesterone receptor (PR), carrying the *AC* genotypes (OR 0.47, 95% CI 0.23-0.94, $p= 0.033$) and CC (OR 0.11, 95% CI 0.013-1.07, $p=0.047$). The association between the *InsDel/DelDel* (dominant model; 50 bp *Ins/Del*) genotype and BC with metastatic lymph nodes (OR 1.5, 95% CI 1.1-2.25, p=0.019), hematologic toxicity (OR 1.5, 95%CI 1.1-2.23, p=0.015), gastric toxicity (OR 1.5, 95%CI 1.1-2.07, p=0.030), and Ki-67 (≥15%) (OR1.6, 95%CI 1.2-2.26, p=0.002) was evident, indicating that these factors may contribute significantly to BC risk. The C/*Ins* haplotype was also associated with BC susceptibility (OR3.47, 95% CI 1.62-7.74, $p=0.001$).

CONCLUSIONS: rs2234694 and 50 bp Ins/Del polymorphisms in the *SOD1* gene were associated with BC susceptibility in a Mexican population. A protective association for BC of the rs2234694 polymorphism was observed in patients younger than 50 years positive for ER and PR, carrying the *AC* genotypes. The haplogenotypes *AA/InsIns* and *AC/InsDel* could contribute significantly to BC risk in gastric and hematologic toxicities, metastatic lymph nodes, and the presence of DM2 in the Mexican population analyzed.

Key Words:
Breast cancer, ROS, *SOD1*, Polymorphism, rs2234694, 50 bp *Ins/Del*.

Abbreviations

BC: Breast Cancer; BMI: Body Mass Index; CI: confidence intervals; Del: deletion; DM2: type 2 diabetes mellitus; ER: estrogen receptor; HRT: Hormonal Replacement Therapy; Ins: insertion; NF-kβ: nuclear factor kappa B; OR: Odds ratios; PPA: personal pathological antecedents; PR: progesterone receptor; RFLP: Restriction fragment length polymorphism; ROS: reactive oxygen species, SAH: Systemic Arterial Hypertension; SD: Standard Deviation; SNP: single nucleotide polymorphism; SOD: superoxide dismutase.

Corresponding Author: Martha Patricia Gallegos Arreola, Ph.D;
e-mail: marthapatriciagallegos08@gmail.com

Introduction

Breast cancer (BC) is the most frequent type of cancer in women and its incidence varies between different types of ethnic groups[1]. In Mexico, BC is a leading cause of mortality[1,2]. The gradual accumulation of genetic and epigenetic events might influence the development of BC[3]. Studies[4,5] have shown an association between superoxide dismutase (SOD), a key enzyme that plays a primary role in removing reactive oxygen species (ROS), and cancer. ROS are chemical compounds that contain one or more unpaired electrons and are derived from the oxygen molecule (O_2). Among the most important ROS are hydrogen peroxide, superoxide, and the hydroxyl radical[6]. They have a short half-life, are highly reactive and they are produced by ultraviolet rays, ionizing radiation, hyper-energetic diets, pharmaceutical drugs and the metabolism of oxygen in aerobic systems. Under physiological conditions, ROS activate inflammatory cells[6,7], which in the absence of efficient detoxification mechanisms to counteract these free radicals, can cause an imbalance and might produce health effects by the development of different disease manifestations, including cancer[6-8]. Regarding cancer, ROS are considered tumor promoters because of the DNA damage produced as they increase the range of mutations in the cell, and thus promote the transformation of oncogenes, so they are inducers by increasing cell proliferation, survival, and migration[6-8]. In cell proliferation, ROS can be identified as second messengers in the intracellular signaling cascade which induce and maintain the oncogenic phenotype of tumoral cells. It has been reported that ROS activate AP-1 and NF-kβ (nuclear factor kappa B) transcription factors in the signal transduction pathways, which carry the transcription of genes that participate in cell growth regulation pathways. This interaction determines the balance between proliferation and apoptosis of the cell[8].

The increase in the rate of cell proliferation and the suppression of apoptosis, constitutes the common platform in which all neoplastic changes occur. Essential alterations in the growth signals include several characteristics, such as self-sufficiency, lack of sensitivity to growth inhibitory signals, evasion of programmed cell death, unlimited replication potential, sustained angiogenesis, tissue invasion, and metastasis[9].

On the other hand, among the antioxidant defense systems activated by ROS, these include some classic antioxidant defense enzymes, such as SOD, catalase and glutathione peroxidase, distributed in the mitochondria, peroxisomes and cytoplasm, as well as non-classical enzymes [heme oxygenase-1; Phase II detoxifiers (glutathione reductase and transferase)] and non-enzymatic (vitamins E, C and catechins)[9].When the production of free radicals is excessive in the body and the antioxidant defense capacity is inefficient, then, oxidative stress is generated, which can become severe and even cause cell death[6-9].

SOD is the most important family of cellular defense enzymes in the body. It participates against agents that induce oxidative stress, particularly with superoxide anion. Different isoforms of SOD in mammals have been identified, SOD1 (copper zinc SOD), SOD2, and SOD3. SOD1 is located in the cytosol and contains copper and zinc in the catalytic center of the protein. Its function is to neutralize ROS in the mitochondrial respiratory chain[10]. Gene *SOD1* is located in chromosomal region 21q22 and it is structured by 5 exons and 4 introns. In its promoter region there are binding sites for different transcription factors, such as: NF1, Sp1, AP1, AP2, GRE, HSF, and NF-kB and more than 90 mutations, and some polymorphisms have been associated with different diseases[10]. One of the most studied is the SNP (single nucleotide polymorphism) rs2234694 (+35A/C) polymorphism, located at the junction site between the intron and exon 3 and it has been associated with the increase in SOD1 enzyme activity caused by the *AA* genotype and enzymatic activity reduction of the *CC* genotype[10]. The reported frequency of the rs2234694 polymorphism depends on the population studied, and the *C* allele showed a frequency in controls of 2-8% among Italian, Tunisian, Polish, and Indian populations[11-15]. Another polymorphism, characterized by an insertion or deletion of a 50 bp fragment, is located 1684 base pairs (bp) upstream of the ATG start codon in the promoter region of the *SOD1* gene, known as 50 bp Insertion/Deletion (*Ins/Del),* and it is associated with the reduction promoter activity of the gene, that is caused by the *Del* allele[16], and subsequently it may alter the level of ROS detoxification. Due to the high interaction of ROS with DNA, the *Ins/Del* genetic polymorphism may play an important role for inter-individual differences in maintaining the genome integrity[17]. The variation in the reported frequency also depends on the population analyzed, and the *Del* allele showed a frequency in controls of 12-14% among Iranian population[16-18].

The participation of SOD1 and ROS in the development of cancer are important in the activation of transcription factors, as well as initiation of multiple signal transduction and DNA

damage[19,20]. Moreover, it has been observed that the activity of SOD1 and SOD2 is regulated by the deacetylase SIRT3 and that the expression of SIRT3 is abolished or decreased in 87% of BC tissues. It has been suggested that SOD2 participates in post-translational regulation by means of acetylation and SIRT3 dependent deacetylation in response to oxidative stress[21]. It has also been observed that in absence of SOD2, SOD1 is required to maintain the integrity of the cell organelle[21]. Also, there is evidence[22] of the participation of SOD1 in regulating estrogen-responsive gene expression and its participation in the survival of BC cells and the progression of mammary tumors. The combined decrease of SOD2 and increase of SOD1 suggests that *SOD1* may have the main dismutase activity in BC[20]. Other studies[4-6,23] have described the participation of some polymorphisms of *SOD1* gene in the cancer development and the effect that might have in the cell, in the overall expression, function, or subcellular localization. So far, the participation of rs2234694 and 50 bp *Ins/Del* polymorphisms and their relationship with estrogen and progesterone receptor in the BC have not yet demonstrated. It only has been described[13-18,24,25] how these *SOD1* polymorphisms influence ROS generation and its participation in different pathologies.

SOD1 gene polymorphisms may determine BC susceptibility, but the association studies that examined the rs2234694 and 50 pb *Ins/Del* polymorphisms and BC risk have been inconclusive[26,27]. In the Mexican population, the association of the *SOD1* rs2234694 and 50 pb *Ins/Del* polymorphisms in BC remains unknown. Therefore, the aim of this investigation was to determine the frequency and association of *SOD1* gene polymorphisms in Mexican women with BC.

Patients and Methods

Blood samples were collected from 751 patients with clinically and histologically confirmed BC and 582 healthy women who donated blood. The patient and control groups were not age-matched and no familial samples were included. All patients were residents of the metropolitan area of Guadalajara (México) and were recruited from June 2014 to February 2020 and samples were obtained after patients and controls provided a written informed consent, as approved by the Local Ethics Committee (1305). This study was conducted in accordance with the 1964 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[3,28]. DNA was extracted from peripheral blood lymphocytes using standard protocols[29].

The PCR amplification of the rs2234694 and 50 pb *Ins/Del* polymorphisms of the *SOD1* gene was performed as reported in Table I. Allele frequencies were obtained by direct counting.

Table I. PCR conditions of the *SOD1* gene polymorphisms.

		rs2234694 (+35*A/C*)	50-bp *Ins/Del*
PCR Primers[13,17]	sense antisense	5'-CTATCCAGAAAACACGGTGGGCC-3' 5'-TCTATATTCAATCAAATGCTACAAAAC-3'	5'-AATTCCTTACCCCTGTTCTA-3' 5'-GGCAGATTTCAGTTCATTGT-3'
PCR product		278pb	297 bp and 247 bp
PCR conditions		Buffer enzyme 1X, 7.5 pmol of each primers, 0.2 mM of dNTPs, 3.0 mM of MgCl2, 2.5 u of Taq polymerase, DMSO 0.5%, 100 nM of genomic DNA	Buffer enzyme 1X, 7.5 pmol of each primers, 0.2 mM of dNTPs, 2.5 mM of MgCl2, 2.5 u of Taq polymerase, BSA 0.05%, 100 nM of genomic DNA
Alienating PCR temperature		55°C	58°C
Recognition enzyme restriction		*HhaI,* 37°C, (5'…GCG…C…3')(RFLP)	
***Allele identified:**	Wild type Polymorphic type	A = 278 bp C = 71+207 bp	Ins: 297 bp Del: 247bp

*The genotypes were identified in 6% polyacrylamide gels (29:1), followed by silver nitrate staining. Restriction fragment length polymorphism (RFLP). Ins=insertion, Del=deletion.

Table II. Demographic data of the study group.

BC patients(n=751)			Controls(n=582)		*p*-value
*Age media ± SD	53.55 +/- 11.65		47.84 +/- 13.33		0.0001
Tobacco consumption					
Yes (n), %	(194)	26.0	(69)	12.0	0.0001
No (n), %	(557)	74.0	(513)	88.0	
Alcohol consumption					
Yes (n), %	(151)	20.0	(56)	10.0	0.0001
No (n), %	(600)	80.0	(526)	90.0	

Standard deviation (SD), *Age, years, Mann-Whitney U test.

The 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 (OR) and 95% confidence intervals (CI) were also calculated. A two-tailed $p<0.05$ was considered statistically significant. The association analysis by the odds ratio and binary logistic regression

Table III. Demographic and clinical data of BC patients.

Characteristic		(n=751)	%*	Characteristics		(n=751)	%*
PPA	Breast fibrosis	(83)	11	**Localization**	Unilateral	(714)	95
	Uterine myomas	(105)	14		Bilateral	(37)	5
	DM2	(107)	14	**Type**	Ductal	(676)	90
	SAH	(112)	15		Lobular	(66)	9
Hormonal status	Oral/injection	(339)	45		Mixed	(9)	1
	HRT	(103)	14	**Lymph Nodes metastasis**	positive	(465)	62
Menarche	7-10 years	(52)	7	**Histological type**	Luminal A	(334)	44
	11-13 years	(482)	64		Luminal B	(173)	23
	14-18 years	(217)	29		Her2	(101)	13
Parity	Null-parity	(70)	9		Triple negative	(145)	19
≥ 2 loss gestational	Miscarriage	(226)	30		KI-67 (≥15%)(31)	(311)	41
	Pregnancies (< 5)	(546)	73	**Stage**	I	(46)	6
Breastfeeding	≤ 6 month	(178)	24		II	(211)	28
	≥ 6 month	(384)	51		III	(238)	32
	No	(189)	25		IV	(256)	34
	Premenopausal	(248)	33	**Chemotherapy****	Response	(221)	29.5
	Menopause	(503)	67		partial response	(93)	12
BMI (kg/m^2)*	Normal (18.5-24.9 kg/m^2)	(181)	24		Non-response	(199)	26.5
	Overweight (25-29.9kg/m^2)	(255)	34		Non-response by recurrence	(238)	32
	Obesity I (30-34.9 kg/m^2)	(199)	26	**Toxicity*****	Gastric	(186)	25
	Obesity II (35-39.9 kg/m^2)	(92)	12		Hematological	(170)	23
	Obesity III (40-45.9 kg/m^2)	(25)	3		Gastric and hematological	(192)	26
					Non-toxicity	(203)	27

Personal pathological antecedents (PPA), hormonal replacement therapy (HRT), Type 2 diabetes mellitus (DM2), Systemic Arterial Hypertension (SAH). *Body mass index (BMI), according to OMS classification (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). ***Gastric toxicity (nausea, diarrhea, vomiting, stomatitis, anorexia, pain abdominal, mucositis), hematological toxicity (neutropenia, thrombocytopenia, anemia).

Table IV. Genotype and allelic distributions of rs2234694 and 50 bp *Ins/Del* polymorphisms of the SOD1 gene in BC patients and controls.

Polymorphism		BC*	Controls*	OR	95% (CI)			*p*-value
rs2234694	***Genotype***	(n=751)	%	(n=582)	%			
	AA	(669)	89	(574)	99	0.11	(0.05-0.23)	**0.0001**
	AC	(78)	10	(8)	1	8.3	(3.98-17.3)	**0.0001**
	CC	(4)	1	(0)	0	3.89	(0.45-33.4)	0.24
	AC/CC	(82)	11	(8)	1	8.79	(4.21-18.3)	**0.0001**
	Allele$^{(2n1502)}$			$^{(2n=1164)}$				
	A	(1416)	0.9427	(1156)	0.9931	0.11	(0.05-0.23)	**0.0001**
	C	(86)	0.0573	(8)	0.0069	8.77	(4.23-18.1)	**0.0001**
50 pb Ins/del	***Genotype***	(n=749)	%	(n=443)	%			
	Ins/Ins	(492)	66	(314)	71	0.78	(0.60-1.01)	0.073
	Ins/Del	(230)	31	(119)	27	1.2	(0.92-1.56)	0.178
	Del/Del	(27)	3	(10)	2	1.6	(0.77-3.37)	0.261
	InsDel/InsIns	(257)	34	(129)	29	1.2	(0.98-1.64)	0.073
	Allele$^{(2n=886)}$							
	Ins	(1214)	0.8104	(747)	0.8431	0.79	(0.63-0.99)	**0.048**
	Del	(284)	0.1896	(139)	0.1569	1.25	(1.01-1.57)	**0.048**

Odds ratio (OR), confidence intervals (CI), significant p-value<0.05. *Hardy-Weinberg equilibrium in controls of rs2234694 (chi-square test=0.0278, p=0.8674 and BC patients (chi-square test=1.080, p=0.30) and 50 bp *Ins/Del* in controls (chi-square test=0.1053, p=0.7455) and BC patients (chi-square test=0.0003, p=0.9850) of the SOD1 gene polymorphisms.

analysis between the studied groups were performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA). Haplotype analysis was performed with https://www.snpstats.net/start.htm, program.

Results

Table II shows the comparative demographic data from BC patients and control individuals. The mean age, and tobacco and alcohol consumption were statistically different in BC patients compared with the control group (p<0.0001).

The clinical and demographic characteristics of BC patients are shown in Table III. The main characteristics of BC patients were overweight (34%), unilateral tumor localization (95%), ductal tumor type (90%), luminal A (44%), and stage IV tumor (34%).

The rs2234694 polymorphism in the *SOD1* gene was significantly different between BC patients and controls. The genotypes *AC* (OR 8.3, 95%CI 3.98-17.3, p=0.0001) and *AC/CC* (dominant model; OR 8.79, 95% CI 4.21-18.3, p=0.0001) and *C* allele (OR 8.77, 95%CI 4.23-18.1, p=0.0001) were observed as risk factors for developing BC (Table IV).

The genotype distribution of the 50 bp *Ins/Del* polymorphism in the *SOD1* gene did not show significant differences between BC and control groups (Table IV). However, the *Del* allele frequency showed a statistically significant difference (p<0.05). The rs2234694 and 50 bp *Ins/Del* polymorphisms in the *SOD1* gene were in Hardy-Weinberg equilibrium in the studied groups (Table IV).

The comparison of the rs2234694 and 50b p *Ins/Del* polymorphisms of the *SOD1* gene frequency in Mexican controls of this study with other control populations from different ethnic groups is shown in Table V.

No significant differences were observed when comparing the rs2234694 polymorphism stratified by age, tobacco and alcohol consumption (p>0.05). However, the 50 bp *Ins/Del* polymorphism showed significant statistical differences with age (OR 2.3, 95% CI 1.48-3.57, p=0.0001) and tobacco consumption (OR 1.9, 95% CI 1.01-3.56, p=0.045) between BC and control groups data not shown.

The association of clinical characteristics with the rs2234694 and 50 bp *Ins/Del SOD1* polymorphisms in the BC group is shown in Table VI. BC patients who had the dominant model *AC/CC* genotypes of the rs2234694 *SOD1* polymorphism and menopause (OR 1.65, 95% CI 1.05-2.7,

p=0.048), with DM2 (OR 2.4, 95% CI 1.35-4.31, *p*=0.003), and Ki-67 (≥15%) (OR 1.9, 95% CI 1.14-3.16, *p*=0.016), showed risk of developing BC. A protective association for BC of the rs2234694 polymorphism was observed in patients younger than 50 years positive for estrogen receptor (ER) and progesterone receptor (PR), carrying the *AC* genotypes (OR 0.47, 95% CI 0.23-0.94, *p*=0.033) and *CC* (OR 0.11, 95% CI 0.013-1.07, *p*=0.047). Nevertheless, the association of the *Ins/Del* polymorphism among patients younger than 50 years positive for estrogen and progesterone, there was no significant statistical differences.

In addition, in BC patients with the dominant model *InsDel/DelDel* genotypes of the 50 bp *Ins/Del SOD1* polymorphism showed BC susceptibility with metastatic lymph nodes (OR 1.5, 95% CI 1.1-2.25, *p*=0.019), Ki-67 (≥15%) (OR 1.6, 95% CI 1.2-2.26, *p*=0.002), gastric toxicity (OR 1.5, 95% CI 1.1-2.07, *p*=0.030), and hematologic toxicity (OR 1.5, 95% CI 1.1-2.23, *p*=0.015).

The haplotype frequency and association of *SOD1* polymorphisms are presented in the Table VII. The most frequent haplotype was *A/Ins,* however, no statistical differences were observed between the study groups. Nonetheless, evident differences were observed with the haplotype *C/Ins* (*p*<0.001).

Table VIII shows the haplogenotype association between BC patients and controls; the *AA/InsIns* was associated with protective susceptibility (OR 0.65, 95% CI 0.53-0.88, *p*=0.005) and on the contrary, the *AC/InsIns* (OR 2.89, 95% CI 1.34-6.27), *p*=0.007) was shown to be associated with BC risk.

The association of clinical variables with haplogenotypes in the group of patients with BC was analyzed (Table IX). The *AA/InsDel* was a risk factor for BC in patients with gastric (OR 1.55, 95% CI 1.04-2.30, *p*=0.036) and hematologic toxicities (OR 1.55, 95% CI 1.02-2.38, *p*=0.045). The *AC/InsDel* was a risk factor for BC in patients stratified by hormonal consumption (OR 1.91, 95% CI 1.09-3.35, *p*=0.024), the presence of metastatic lymph nodes (OR 2.37, 95% CI 1.08-5.19, *p*=0.031), and the presence of DM2 (OR 3.0, 95% CI 1.32-7.14, *p*=0.008)

Discussion

In Mexico BC is one of the leading causes of death in women[1,2]. BC was observed to occur at an average age of 50 years[2,3], which is consistent with data from this study since the mean age was 53.55 years. From this perspective, it still is necessary to implement new studies and strategies for BC detection in early stages of the disease[3]. In this study, we observed the differences in tobacco and alcohol consumption between BC patients and controls. The relationship between these two factors with cancer development is well established[3,30]. The

Table V. Frequency comparison of the rs2234694 and 50 bp *Ins/Del* genotypes of the *SOD1* gene polymorphisms in Mexican controls and other populations.

rs2234694	**Genotype**					
	AA	**AC**	**CC**	**AA**	**AC**	**CC**
Population				***p*-value**		
Mexican[(present study)]	574	8	0			
				vs.		
Italian[11]	222	19	1	0.0001	0.0001	0.44
Indian[12]	126	24	0	0.0001	0.0001	0.30
Polish[13]	107	16	2	0.0001	0.0001	**0.019**
Tunisian[14]	201	12	0	0.0001	0.0001	**0.007**
Bangladeshi[15]	140	4	0	0.0001	0.0001	1.0
50 bp *Ins/Del*	**Genotype**					
	InsIns	**InsDel**	**DelDel**	**InsIns**	**InsDel**	**DelDel**
Mexican[(present study)]	314	119	10			
				vs.		
Iranian[18]	590	188	2	0.290	0.2165	0.8224
Iranian[17]	151	44	5	0.2640	0.2246	1.0
Iranian[16]	268	86	7	0.3270	0.3669	0.9477

Table VI. Association of the rs2234694 and 50 bp *Ins/Del* genotypes of the *SOD1* gene polymorphisms with clinical variables of BC patients.

Polymorphism	Genotype*	Clinical variables	OR	95% (CI)	*p*-value
rs2234694	*AC/CC*	Menopause	1.65	(1.05-2.7)	0.048
		Ki-67 (≥15%)[31]**	1.9	(1.14-3.16)	0.016
		DM2	2.4	(1.35-4.31)	0.003
	AC	Age under 50 years old and ER and RP positive	0.47	(0.23-0.94)	0.033
	CC	Age under 50 years old and ER and RP positive	0.11	(0.013-1.07)	0.047
50 bp Ins/Del	*InsDel/DelDel*	Metastatic lymph nodes	1.5	(1.1-2.25)	0.019
		Hematological toxicity	1.5	(1.1-2.23)	0.015
		Gastric toxicity	1.5	(1.1-2.07)	0.030
		Ki-67 (≥15%)[31]**	1.6	(1.2-2.26)	0.002

Odds ratio (OR), Type 2 diabetes mellitus (DM2), confidence intervals (CI), p-value, significant < 0.05, ER (estrogen receptor), PR (progesterone receptor), * Dominant model, ** the cut-off point can be discriminated in these tumors with a low Ki-67 expression in both the primary lesion and the corresponding metastasis.

overexpression of *SOD1* has been observed frequently in the cytoplasm and nucleus of BC cells and it has been suggested that SOD1 in the cell nuclear fraction may play an important role in the survival of cancer cells. SOD1 probably binds to the estrogen receptor alpha for enhancing its transcriptional activity[31,32]. The *SOD1* gene has multiplex binding sites for different transcription factors that function as a ligand to activate the transcription and participate as a cell defense system against agents that induce oxidative stress[8,10]. Association studies of the rs2234694 polymorphism and BC remain unknown. However, only one study[33] has reported no association between 50 bp *Ins/Del* in *SOD1* gene with increased BC risk.

Moreover, little is known about the association of rs2234694 and 50 bp *Ins/Del* polymorphisms of the *SOD1* gene in Mexican BC patients. In our study, the frequency of *AC*, *AC/CC* (dominant model) genotypes and *C* allele showed statistically significant differences between BC patients and controls ($p<0.05$) and were associated with risk of developing BC.

Unfortunately, there are no association studies of rs2234694 polymorphisms with the development of BC. This is the first report where this association is analyzed. However, this polymorphism has been associated with the development of diabetic nephropathy among Bangladeshi population[14], with a protective association in chronic obstructive pulmonary disease[15] and macular degeneration[34] in a Tunisian and Poland population respectively.

In regard to the 50 pb *Ins/Del* polymorphism, our results show that only the *Del* allele was statistically significant different between BC patients and controls ($p<0.05$) and it was associated with risk susceptibility to BC. Mahjoub et al[33] from an Iranian population did not observe an association with susceptibility to BC. In this case, the expression of *SOD1* in BC has been analyzed in different studies[6,20,31], however, the regulatory mechanisms in the development of BC still need to be understood. The *C* and *Del* alleles have a deficient activity effect on the SOD1 enzyme and as a result, the cellular protective mechanisms as well as the antioxidant defense capacity are ineffi-

Table VII. Polymorphism rs2234694 and *Ins/Del* haplotype frequencies in the study groups.

Haplotypes		Frequency					
		BC		Controls		OR(95%CI)	*p*-value
rs2234694	Ins/Del	(n)	%	(n)	%		
A	Ins	(1134)	0.7980	(753)	0.8556	0.87(0.70-1.08)	0.2425
A	Del	(239)	0.1681	(159)	0.1352	0.96(0.77 - 1.20)	0.8140
C	Ins	(42)	0.029	(8)	0.0092	3.47(1.62-7.742)	0.001
C	Del	(6)	0.0049	(0)	0		

Breast Cancer (BC), Odds ratio (OR), Confidence Interval (CI). Linkage disequilibrium (D'=0.0)

Table VIII. Demographic data of the study group.

Haplogenotype				Patients(749)		Controls(440)		OR	95%(CI)	*p*-value
rs2234694		50 bp Ins/Del		(n)	%	(n)	%			
A	*A*	*Ins*	*Ins*	(453)	60.5	(303)	69	0.65	(0.53-0.88)	0.005
A	*A*	*Del*	*Del*	(24)	3	(10)	2	1.39	(0.66-2.9)	0.4840
A	*A*	*Ins*	*Del*	(190)	25.5	(119)	27.2	0.89	(0.68-1.16)	0.4221
A	*C*	*Ins*	*Ins*	(38)	5.1	(8)	1.8	2.89	(1.34-6.27)	0.007
A	*C*	*Del*	*Del*	(1)	0.1					
A	*C*	*In s*	*Del*	(38)	5.1					
C	*C*	*Ins*	*Ins*	(1)	0.1					
C	*C*	*Del*	*Del*	(2)	0.3					
C	*C*	*Ins*	*Del*	(2)	0.3					

cient. The oxidative stress is generated, therefore, gene regulatory mechanisms can anticipate and initiate the development of BC[6,20,31].

The comparative analysis of the rs2234694 polymorphism between our Mexican (control group) with control groups of other populations, the *AC* genotype showed statistically significant differences with the Italian, Indian, Polish and Tunisian populations[11-15]. The *DelDel* genotype frequency similarities of the 50 pb *Ins/Del* polymorphism were also observed in the Iranian population with respect to the Mexican population, which points to the genetic heterogeneity of these polymorphisms in other populations[16-18].

In our study, the association in the dominant model of the *InsDel/DelDel* genotype of the 50 bp *SOD1* polymorphism as risk factors in BC stratified by age (≥50 years old) and tobacco consumption, was also demonstrated.

Different researches have determined the association between ROS and many diseases. Previous papers[6-10,22,23] show that the decreased antioxidant enzyme activity of the SOD family produces DNA damage by oxidative stress, which could cause cancer. Additional data from the *C* and *Del* alleles of the rs2234694 and 50 bp *Ins/Del* polymorphisms of the *SOD1* gene, respectively, could reduce the promoter activity and the enzyme activity of SOD1, and therefore, participate in the development of cancer. Based on the above, we might suggest that in older ages and considering the consumption of tobacco, a greater production of free radicals could be generated in people carrying the *C* and/or *Del* alleles of these polymorphisms in the *SOD1* gene, and thus, a greater accumulation of free radicals in the breast tissue has an increased susceptibility to cancer.

Moreover, the analyzed association of rs2234694 and 50 bp *Ins/Del SOD1* gene polymorphisms in Mexican BC patients in our study demonstrated that in the dominant model of *AC/CC* genotype of rs2234694 polymorphism was a risk factor for BC susceptibility stratified by different clinical pathology parameters, such as menopause, Ki-67 (≥15) and the presence of DM2. In this case, the expression of *SOD1* in BC has been analyzed in different clinical samples (blood, serum, tissue, and cultures)[6,20,31,35-38], but the regulatory mechanisms in the development of cancer are still unclear. It is known that in women, menopause is a normal consequence of aging and it is characterized by the permanent cessation of ovarian follicular activity, which produces oxida-

Table IX. Association of the haplogenotypes of the SOD1 gene polymorphisms with clinical variables of BC patients.

Haplogenotype				Clinical variables	OR	95%(CI)	*p*-value
rs2234694		50 bp Ins/Del		(n)	%	(n)	%
A	*A*	*Ins*	*Del*	Gastric toxicity	1.55	(1.04-2.30)	0.036
				Hematology toxicity	1.55	(1.02-2.38)	0.045
A	*C*	*Ins*	*Del*	Hormonal	1.91	(1.09-3.35)	0.024
				Metastatic lymph nodes	2.37	(1.08-5.19)	0.031
				DM2	3.0	(1.32-7.14)	0.008

*Comparative data with *AA/InsIns* haplogenotype, Type 2 diabetes mellitus (DM2).

tive stress; this has been attributed to the deficiency of antioxidant enzymes that leads to production oxidative stress[35-38]. Ki-67 is a non-histone nuclear protein expressed only during the proliferative phases of the cell cycle and it is considered to be a proliferation marker of BC and others types of cancer[39]. Previous investigations[6-10,22,31-33,35-39] have revealed that antioxidant enzymes as SOD are closely linked to an increased cell proliferation in tumors. Therefore, there are many intrinsic factors in tumors that produce oxidative stress and damage DNA by producing mutations that give rise to carcinogenesis.

It is known that hyperglycemia induces the generation of ROS and at the mitochondrial level is the initial trigger of a vicious cycle of oxidative stress in DM2[14]. Akhy et al[14] reported the association of rs2234694 polymorphism in *SOD1* with the development of nephropathy in diabetic type 2 subjects in Bangladeshi population.

There are not studies that described the relationship of rs2234694 polymorphism and BC in women under 50 years with ER and PR positive, observed in this study. It should be also noted that the confidence intervals were high in the *CC* genotype due to the small sample size. Rao et al[22] demonstrated that SOD1 play an important role in the regulating estrogen-responsive gene expression and suggested that the increase of SOD1participated in the survival of BC cells and the progression of mammary tumors. According to the above, it is possible to think that the regulation of the activity of the enzyme SOD1 is age dependent and that in women older than 50 years the generation of oxidative stress may contributed to oxidative stress in the ductal and lobular cell of breast tissue, and contribute in the development of BC. However, in people under 50 years old may have the opposite effect. As observed by Rao et al[22] who demonstrated that the oxidative stress and the estrogen, increases *SOD1* expression in BC cells and it maybe protective from effects of oxidative stress.

Although the mechanism of the rs2234694 polymorphism of the *SOD1* gene is not well understood, we might suggest that in BC women under 50 years old, with ER and PR positive, carrying the *AC* genotype rs2234694 polymorphism of *SOD1*, a greater production of SOD1 enzyme, could be generated and an efficient elimination of free radicals has a protective effect in BC patients.

We indicated the dominant model of the *ins-Del/DelDel* genotype as a risk factor for BC development stratified by different clinical patholology parameters, the presence of metastatic lymph nodes, hematological and gastric toxicities, and Ki-67 (≥15%).

In addition, the haplotype and haplogenotype association of rs2234694 and 50 bp *Ins/Del SOD1* gene polymorphisms were determined between BC patients and control groups. The haplotypes showed no linkage disequilibrium with each other. We observed that the *C/Del* haplotype and haplogenotype *AC/InsIns* (OR 2.89; 95% CI 1.34-6.27, p=0.007) were associated with the susceptibility to BC; however, it should be noted that the confidence intervals were high due to the small sample size.

Moreover, we determined that the haplogenotype association was a risk factor for the development of BC stratified by different clinical pathology parameters, the *AA/InsDel* with hematologic and gastrointestinal toxicities and *AC/InsDel* with hormonal consumption, metastatic lymph nodes, and DM2 as risk factors for BC, were also demonstrated. To our knowledge, this is the first study to report this association, however, we could elucidate that the progression of cancer is associated with adverse clinical outcomes and it may modify the expression of different molecular factors including stress oxidative mechanisms, which could alter the regulation of cellular processes[6-8,35,38]. On the other hand, the progression of cancer is not only related to the monogenic inheritance of a protein variant, but it also depends on the interaction of several genes that are involved in multiple metabolic pathways and epigenetic events[3].

Conclusions

We showed that the rs2234694 and 50 bp *Ins/Del* polymorphisms were associated with BC risk when comparing controls and BC patients for the genotypes *AC, AC/CC* (dominant model) and *C* and *Del* alleles, respectively. In addition, there were evident differences in patients with the *AC/CC* genotype (dominant model) with menopause, Ki-67 (≥15%). A protective association for BC of the rs2234694 polymorphism was observed in patients younger than 50 years positive for ER and PR, carrying the *AC* genotypes. The presence of DM2, and the dominant model *InsDel/DelDel* genotype with metastatic lymph nodes, hematological and gastric toxicities, and Ki67(≥15%), respectively. The haplotype *C/Ins* was observed to be a risk factor for BC. The previous evidence confirms that these factors significantly contribute to BC susceptibility in the analyzed sample from a Mexican population, however, further studies are required to confirm these observations.

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Authors' Contribution

GAMP contributed in the design, analysis, experimentation, data collection and financing. RHAM and PPAM, contributed to data collection. FLE, ZGGM contributed to the design and analysis of the manuscript. All the authors read and approved the final manuscript.

Conflict of Interests

The authors declare no conflicts of interest.

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