cardiogenetics

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Association of *GSTT1*, *GSTM1* and *GSTP1 (Ile105Val)* mRNA Expression with Cardiometabolic Risk Parameters in Women with Breast Cancer and Comorbidities

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Citation: Becerril Alarcón, Y.; Bastida González, F.; Camacho Beiza, I.R.; Dávila González, E.; Cruz Ramos, J.A.; Benítez Arciniega, A.D.; Valdés Ramos, R.; Soto Piña, A.E. Association of *GSTT1*, *GSTM1* and *GSTP1 (Ile105Val)* mRNA Expression with Cardiometabolic Risk Parameters in Women with Breast Cancer and Comorbidities. *Cardiogenetics* **2022**, *12*, 235–245. https://doi.org/10.3390/cardiogenetics12030022

Academic Editor: Giuseppe Limongelli

Received: 25 February 2022
Accepted: 12 July 2022
Published: 20 July 2022

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Abstract: Breast cancer (BC) and cardiometabolic diseases share a multifactorial and modifiable etiology, modulated by complex molecular pathways. *Glutathione S-transferase (GST)* plays a critical role, providing protection against xenobiotics and regulating levels of enzymes and proteins in the cell. *GST* variants have a significant impact on susceptibility to diseases whose pathogenesis involves oxidative stress, as is the case in many inflammatory diseases such as BC and cardiometabolic pathologies. However, the expression of these polymorphic variants has not been studied in BC. This study aimed to evaluate the presence of *GST* mRNA isoforms and their association with clinical and cardiometabolic parameters in women with BC. This was a case-control study, and a total of 57 participants were recruited. Concentrations of glucose and lipids in blood were measured in all the participants. *GST* variants (*GSTT1*, *GSTM1* and *GSTP1 Ile105Val* polymorphism) were evaluated in all the participants by real-time PCR analysis. There was a significant association ($p < 0.05$) between the frequency of *GSTP1* and LDL-c in the BC group. However, the control group showed significant associations between blood pressure with *GSTT1* and *GSTP1* variants with total cholesterol (TC), LDL-c, VLDL-c and triacylglycerols (TG). Therefore, *GSTT1* and *GSTP1* variants could be emerging biomarkers to discriminate between BC cases related or not to cardiometabolic disease factors.

Keywords: *glutathione S-transferase* genotypes; cardiometabolic risk; breast cancer; comorbidities

1. Introduction

Biological mechanisms, such as inflammation and molecular pathways in association with environmental and lifestyle risk factors, are linked to cancer development and some comorbidities [1]. Especially in breast cancer (BC) and cardiometabolic diseases, these interactions cause the incidence and mortality of BC in women worldwide [2,3]. According to the latest results of the National Survey of Nutrition and Health 2018–2019 (ENSANUT) of Mexican adult women, 11.4% develop diabetes, 20.9% hypertension and 21.0% present hypercholesterolemia and hypertriglyceridemia [4]. The Mexican Observatory of Non-Communicable Diseases (OMENT) in Epidemiological Panorama 2018 reports that, since 2010, the three main causes of death in Mexico are heart diseases, diabetes and malignant tumors. Since then, they have shown an increase in the mortality rate, sharing among them a multifactorial and modifiable etiology [5].

Cardiometabolic dysfunction, such as high blood pressure, hyperglycemia, obesity, high body fat and dyslipidemia, have been observed in postmenopausal women with

BC [6,7]. These parameters play an important role in prevention and prognosis of BC, and all of them are modulated by complex molecular pathways involved in stimulating or slowing the growth of malignant cells [8]. Moreover, cancer and cardiovascular diseases share the elevation of oxidative stress as a pathological mechanism [9,10].

An important molecular system is *Glutathione S-transferase* (*GST*), which comprises a family of enzymes involved in phase II detoxification. They play a critical role, providing protection against xenobiotics, oxidative stress and regulating levels of enzymes and proteins in the cell [6,10]. The *GST* superfamily includes eight classes of genes, and studies have suggested that genetic variants, such as *GSTT1*, *GSTM1* and *GSTP1*, are involved in susceptibility, progression and response to treatment in BC [7,11,12]. Specifically, in the *GSTP1* gene, the polymorphism that involves the change of adenine to guanine at codon 105 results in the substitution of isoleucine to valine (*Ile105Val*) [11,12]. Consequently, this change produces a *GSTP1* enzyme with reduced activity, increasing BC chemotherapy toxicity [11].

Furthermore, *GST* genotypes have shown to have protective effects against the development of metabolic syndrome, indicating that these enzymes are involved in the pathogenesis of cardiometabolic diseases [13]. *GST* enzymes with altered activity may have a significant impact on the susceptibility to diseases whose pathogenesis involves altered inflammatory processes, such as BC and cardiometabolic pathologies [14]. Aljboori et. al. studied the association of serum lipid profile with *GSTT1* and *GSTM1* polymorphisms in hypertension of post-menopausal women; they found that there is a strong association between *GSTT1* and hypertension and a weaker association between *GSTM1* and hypertension [15]. Furthermore, the risk of cardiovascular disease is associated with null genotypes of *GSTT1* and *GSTM1* [16,17]. Interestingly, the *GSTM1 null* genotype can also be related to increased oxidative stress and DNA damage in subjects with hypertension risk [6]. Nonetheless, a meta-analysis shows that there is no association between *GSTT1* and *GSTM1* polymorphisms and the risk of cardiovascular disease [18]. Therefore, the role of these polymorphisms and their expression in this type of disease remain ambiguous and should be further investigated.

Indeed, specific *GSTT1* cDNA is linked to a glutathione-dependent conjugation phenotype [19]. The expression of *GST* protein and enzyme activity can also be a biomarker of vascular and metabolic alterations [20,21]. *GST* isoforms have been studied in animals [22], but they are not well studied in humans and neither in BC. Moreover, it is not clear whether mRNA expression of *GST* variants is related to the presence of altered cardiometabolic variables in women with BC. Therefore, the aims of this investigation were to qualitatively characterize the mRNA expression of *GST* family enzymes and study their association with clinical, body composition and biochemical parameters in women with BC.

2. Materials and Methods

Study general description. A cross-sectional, case-control study was performed. The study included 57 women over 18 years old who gave their voluntary consent, recruited from the Specialized Medical Unit for the Detection of Breast Cancer (UNEME-DEDICAM, in Spanish) of Health Institute of the State of Mexico (ISEM, in Spanish). The cases group included patients with previous diagnosis by immunohistochemical analysis of BC (n = 23), and the control group included women without BC (n = 34), considering the inclusion and exclusion criteria for both groups as depicted in Figure 1.

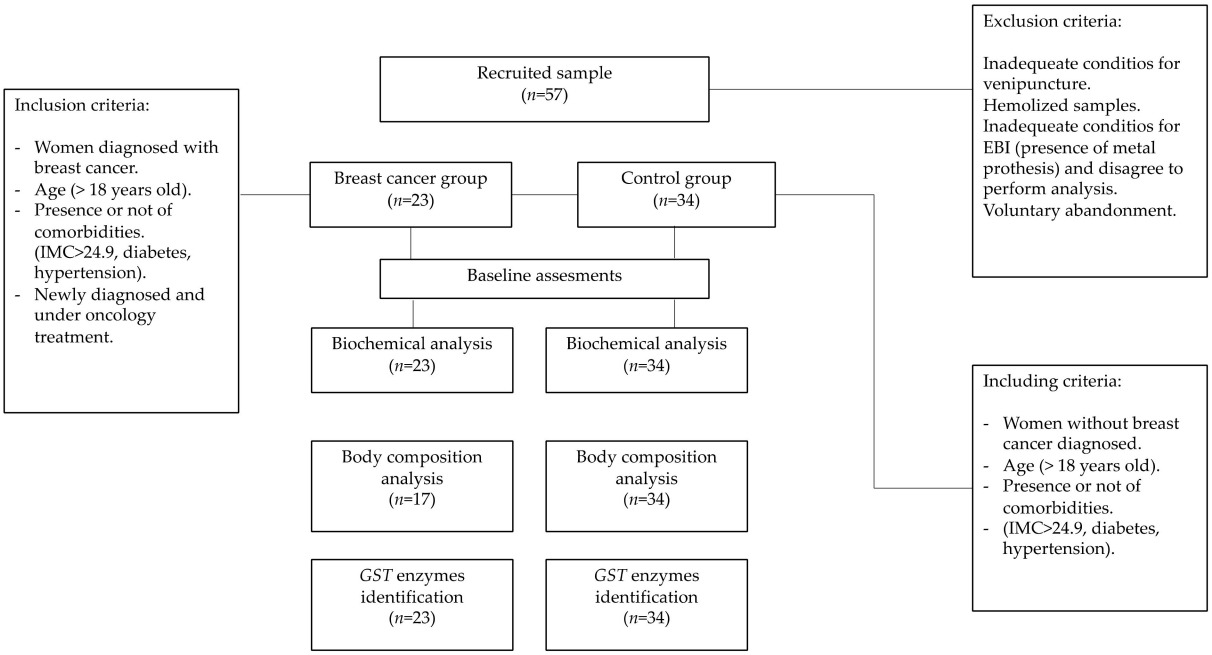

Figure 1. Study design and sample collection.

Sociodemographic, clinical and body composition information. We obtained sociodemographic, blood pressure, anthropometric and body composition information and blood samples for total RNA extraction and to measure glucose and lipids, such as total cholesterol (TC), triacylglycerols (TG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), very-low-density lipoprotein-cholesterol (VLDL-c) and atherogenic index (AI). All measurements were made by trained personnel, and blood samples were obtained by nursing staff from UNEME-DEDICAM Toluca, Mexico. Biochemical profile was performed by Quest Diagnostics® (Secaucus, NJ, USA) Clinical Analysis Laboratories, Toluca, Mexico using a used Beckman Coulter AU680® (Beckman Coulter, Brea, CA, USA) instrument.

Anthropometry and body composition analysis: all measurements were conducted using standardized procedures. Body weight and height were measured with a scale (TANITA® TBF 300 A, TANITA Corporation, Tokyo, Japan) and a stadiometer (SECA® S700, Hamburg, Germany), respectively. Furthermore, body fat percentage (BFP) was analyzed by electrical bioimpedance using a Biody Xpert^{ZM}® apparatus (Aminogram SAS, La Ciotat, France), and data were stored in the Biody Manager® software version 2.0.3. (Aminogram SAS, La Ciotat, France).

BMI classification was categorized in accordance with WHO criteria and the fat percentage according to Bray G. [18]. The classification of blood pressure (BP), glycemia and dyslipidemias were performed using the criteria from the guidelines of the American College of Cardiology (ACC) and American Heart Association (AHA).

***GSTT1, GSTM1, GSTP1 (Ile105Val)* detection by real-time PCR:** total RNA was isolated through a phenol isoamyl alcohol extraction protocol. After that, it was quantified by spectrometry using NanoPhotometer® P-Class (Implen, Inc., Westlake Village, CA, USA) equipment, and the concentration of each sample was normalized to 100 µg/1 µL. Subsequently, the detection of *GSTT1, GSTM1* and *GSTP (Ile105Val)* polymorphic variants was standardized by real-time PCR, using *GAPDH* as the reference gene. The primers used for the real-time PCR were published before by Arand [23] and Salimi [24] (Table 1).

Table 1. Characteristics of the genes under study.

Primer Designation	Sequence	Gene ID	SNP Number	Chromosome Position	Nucleotide	Amino Change
GSTM1 forward	5′-GAACTCCC TGAAAAGCTAAAGC-3′	2944	-	1:109690472 (GRCh38) 1:110233094 (GRCh37)	-	-
GSTM1 reverse	5′-GTTGGGCTC AAATATACGGTGG-3′					
GSTT1 forward	5′-TTCCTTACT GGTCCTCACATCTC-3′	2952	-	NT_187633.1:270497 (GRCh38) NT_187633.1:24376322 (GRCh37)	-	-
GSTT1 reverse	5′-TCACC GGATCATGGCCAGCA-3′					
GSTP1 forward outer primer	5′-AGGTTACGTAG TTTGCCCAAGGTC-3′	2950	rs1695	11:67585218 (GRCh38) 11:67352689 (GRCh37)	A313G	Ile105Val
GSTP1 reverse outer primer	5′-CGTTACTT GGCTGGTTGAT- GTCC-3′					
GSTP1 forward inner primer	5′-GAGGACCTC CGCTGCAAATTCG-3′					
GSTP1 reverse inner primer	5′-CATAGTTGG TGTAGATGAGGGAGCT-3′					

The real-time PCR reaction mix (25 µL) contained: DNA Polymerase AmpliTaq™ Fast Master Mix 6.25 µL (Applied biosystems, Thermo Fisher Scientific, Vilnius, Lithuania), EvaGreen™ Dye 1.25 µL of 20X (Biotium, Fremont, CA, USA) and 0.5 µL of each primer (Integrated DNA Technologies; San Diego, CA, USA). After that, the amplification was performed according to the real-time PCR protocol, as shown in Table 2, using a CFX 96 (Bio-Rad Inc., Mexico City, Mexico) thermal cycler. The results were reported as positive (+) or negative (−) according to high resolution melting (HRM) analysis.

Table 2. Real-time polymerase chain reaction protocol.

Step	Temperature	Duration	Cycles
RT	50 °C	10 min	×1
Primary denaturation	95 °C	3 min	×1
Denaturation	95 °C	15 s	×40
Annealing	60 °C	30 s	
Melt Curve	65–95 °C △ 0.05 °C	0.05 s	

Statistical analysis: Normal distribution analyses of biochemicals and body composition parameters were performed using the Kolmogorov–Smirnov test. Hardy–Weinberg equilibrium for *GSTP1 (Ile105Val)* was estimated ($p < 0.982$); for *GSTT1* and *GSTM1,* this analysis is not possible. Chi-square (X^2) and Student's *t*-test were used to find associations between control and breast cancer groups. Statistical analysis was carried out using the statistical software SPSS version 21.0 (IBM Corporation, Armonk, NY, USA) considering a significance level < 0.05.

3. Results

Demographic, clinical and biochemical characteristics of all the participants are summarized in Table 3 by group. SBP in the BC group was significantly lower than the control one ($p = 0.043$). DBP, BMI, BFP, glucose, TC, HDL-c, LDL-c, VLDL-c, TG and AI were similar in both cases and controls.

Table 3. Descriptive characteristics of the participants by study group (n = 57).

Indicators	BC Group (n = 23)	Control Group (n = 34)	p
Age (years)	50.48 (±8.25)	47.53 (±9.55)	0.233
	Menopause status		
Premenopause	12 (21.1)	23 (40.4)	0.239
Postmenopause	11 (19.3)	11 (19.3)	
	Presence of comorbidities [+]		
Yes	9 (15.8)	15 (26.3)	0.708
No	14 (24.6)	19 (33.3)	
SBP (mmHg)	100.00 (±11.67)	107.27 (±13.75)	0.043 *
DBP (mmHg)	66.96 (±7.64)	70.30 (±8.47)	0.136
BMI (kg/m^2)	27.96 (±4.72)	28.40 (±5.16)	0.746
BFP (%) (n = 17) ~	33.86 (±5.54)	34.54 (±5.32)	0.672
Glucose (mg/dL)	117.17 (±66.37)	102.79 (±30.20)	0.273
Total cholesterol (mg/dL)	183.91 (±33.66)	191.35 (±31.82)	0.401
HDL-cholesterol (mg/dL)	40.61 (±11.17)	43.41 (±8.05)	0.283
LDL-cholesterol (mg/dL)	108.61 (±21.94)	114.28 (±25.88)	0.392
VLDL-cholesterol (mg/dL)	34.69 (±27.38)	33.65 (±10.36)	0.841
Triacylglycerols (mg/dL)	214.96 (±192.19)	215.74 (±93.59)	0.984
Atherogenic Index	4.82 (±1.51)	4.53 (±1.02)	0.417

Data: mean (standard deviation), Student's t-test: * $p < 0.05$; n (%), X^2 test: * $p < 0.05$; ~ BC group n = 17. [+] Comorbidities include type II diabetes *mellitus* and hypertension; BC: breast cancer, SBP: systolic blood pressure, DPB: diastolic blood pressure, BMI: body mass index, BFP: body fat percentage.

3.1. Detection of GST Polymorphic Variants Expression

Table 4 shows the frequency of the presence (+) or absence (−) of *GSTT1, GSTM1* and *GSTP1 (Ile105Val)* polymorphism variants expression: isoleucine (*Ile*), valine (*Val*) and isoleucine/valine (*Ile/Val*) in BC and control groups. First, women with BC presented 60.9% *GSTT1*+ and 39.1% *GSTT1*−; *GSTM1*+ and *GSTM1*− showed similar frequencies to *GSTT1* (60.9% vs. 39.1%, respectively). The percentages of *GSTP1 (Ile105Val)* polymorphism variants were: absent (4.3%), *Ile/Ile* (17.4%), Val/Val (21.7%) and *Ile/Val* (56.5%). Compared to the BC group, the control group presented 58.8% *GSTT1*+ and 41.2% *GSTT1*−; 52.9% *GSTM1*+ and 47.1% *GSTM1*-; and *GSTP1 (Ile105Val)* polymorphism variants were: *Ile/Ile* (38.2%), *Val/Val* (14.7%) and the highest frequency was observed in *Ile/Val* presence (47.1%). The chi-squared analysis revealed that the frequencies of *GSTP1 (Ile105Val)* variants were significantly different between groups ($p < 0.001$); the *Ile/Ile* variant showed the lowest frequency in the BC group (17.4%) and the *Ile/Val* was the highest (56.5%).

Table 4. *GST* variants among participants according to study group.

GST Variants	BC Group (n = 23)	Control Group (n = 34)	p
GSTT1+	14 (60.9)	20 (58.8)	0.145
GSTT1−	9 (39.1)	14 (41.2)	
GSTM1+	14 (60.9)	18 (52.9)	0.354
GSTM1−	9 (39.1)	16 (47.1)	
	GSTP1 (Ile105Val)		
Negative	1 (4.3)	-	**0.001 ***
Ile/Ile	4 (17.4)	13 (38.8)	
Val/Val	5 (21.7)	5 (14.7)	
Ile/Val	13 (56.5)	16 (47.1)	

n (%), X^2 test: * $p < 0.05$; *GSTP1 (Ile105Val)* HWE $p > 0.05$; BC: breast cancer.

3.2. Cardiometabolic Risk Factors and GST Variants Expression

Analysis in Table 5 depicts clinical, body composition and biochemical variables according to GST variants. There were no significant differences in women of the BC

group regarding the *GSTT1* +/− and the categories of BMI, BFP, HDL-c and TG. Only the frequency of high LDL-c was significantly different among the *GSTP1 Ile105Val* variants ($p = 0.042$), presenting the highest frequency in normal LDL-c in heterozygous genotypes (Ile/Val, 52.2%).

Table 5. Categorized clinical, biochemical and body composition parameters according to GST expression among participants by study group.

	Breast Cancer Group (*n* = 23)										
	***GSTT1* Expression**		***p***	***GSTM1* Expression**		***p***	***GSTP1 (Ile105Val)***				***p***
Parameters	**Negative**	**Positive**		**Negative**	**Positive**		**Negative**	**Ile**	**Val**	**Ile/Val**	
BP (mmHg)											
Normal (No)	8 (34.8)	10 (43.5)	0.322	6 (26.1)	12 (52.2)	0.280	1 (4.3)	3 (13.0)	2 (8.7)	12 (52.2)	0.106
High (Yes)	1 (4.3)	4 (17.4)		3 (13.0)	2 (8.7)		-	1 (4.3)	3 (13.0)	1 (4.3)	
BMI (kg/m^2)											
Normal	1 (4.3)	4 (17.4)	0.322	2 (8.7)	3 (13.0)	0.964	-	1 (4.3)	-	4 (17.4)	0.510
>25	8 (34.8)	10 (43.5)		7 (30.4)	11 (47.8)		1 (4.3)	3 (13.0)	5 (21.7)	9 (39.1)	
BFP (%) [a]	n = 17										
Normal	-	3 (17.6)	0.110	1 (5.9)	2 (11.8)	0.761	-	-	-	3 (17.6)	0.466
High	7 (41.2)	7 (41.2)		6 (35.3)	8 (47.1)		1 (5.9)	1 (5.9)	5 (29.4)	7 (41.2)	
Glucose (mg/dL)											
Normal	7 (30.4)	8 (34.8)	0.311	6 (26.1)	9 (39.1)	0.907	1 (4.3)	2 (8.7)	2 (8.7)	10 (43.5)	0.372
High	2 (8.7)	6 (26.1)		3 (13.0)	5 (21.7)		-	2 (8.7)	3 (13.0)	3 (13.0)	
TC (mg/dL)											
Normal	8 (34.8)	10 (43.5)	0.322	6 (26.1)	12 (52.2)	0.280	1 (4.3)	3 (13.0)	3 (13.0)	11 (47.8)	0.661
High	1 (4.3)	4 (17.4)		3 (13.0)	2 (8.7)		-	1 (4.3)	2 (8.7)	2 (8.7)	
HDL-c (mg/dL)											
Low CVR	-	1 (4.3)	0.412	-	1 (4.3)	0.412	-	1 (4.3)	-	-	0.174
High CVR	9 (39.1)	13 (56.5)		9 (39.1)	13 (56.5)		1 (4.3)	3 (13.0)	5 (21.7)	13 (56.5)	
LDL-c (mg/dL)											
Normal	8 (34.8)	11 (47.8)	0.524	7 (30.4)	12 (52.2)	0.624	1 (4.3)	4 (17.4)	2 (8.7)	12 (52.2)	**0.042** *
High	1 (4.3)	3 (13.0)		2 (8.7)	2 (8.7)		-	-	3 (13.0)	1 (4.3)	
VLDL-c (mg/dL)											
Normal	9 (39.1)	10 (43.5)	0.078	6 (26.1)	13 (56.5)	0.106	1 (4.3)	3 (13.0)	5 (21.7)	10 (43.5)	0.633
High	-	4 (17.4)		3 (13.0)	1 (4.3)		-	1 (4.3)	-	3 (13.0)	
TG (mg/dL)											
Normal	5 (21.7)	4 (17.4)	0.196	4 (17.4)	5 (21.7)	0.675	-	1 (4.3)	3 (13.0)	5 (21.7)	0.595
High	4 (17.4)	10 (43.5)		5 (21.7)	9 (39.1)		1 (4.3)	3 (13.0)	2 (8.7)	8 (34.8)	
IA											
Normal	5 (21.7)	8 (34.8)	0.940	3 (13.0)	10 (43.5)	0.072	1 (4.3)	3 (13.0)	2 (8.7)	7 (30.4)	0.590
High	4 (17.4)	6 (26.1)		6 (26.1)	4 (17.4)		-	1 (4.3)	3 (13.0)	6 (26.1)	
	Control Group (*n* = 34)										
	***GSTT1* Expression**		***p***	***GSTM1* Expression**		***p***	***GSTP1* (Ile105Val)**				***p***
Parameters	**Negative**	**Positive**		**Negative**	**Positive**		**Negative**	**Ile**	**Val**	**Ile/Val**	
BP (mmHg)											
Normal (No)	4 (11.8)	15 (44.1)	**0.007** *	8 (23.5)	11 (32.4)	0.515	-	6 (17.6)	2 (5.9)	11 (32.4)	0.353
High (Yes)	10 (29.4)	5 (14.7)		8 (23.5)	7 (20.6)		-	7 (20.6)	3 (8.8)	5 (14.7)	
BMI (kg/m^2)											
Normal	3 (8.8)	6 (17.6)	0.577	3 (8.8)	6 (17.6)	0.336	-	4 (11.8)	2 (5.9)	3 (8.8)	0.582
>25	11 (32.4)	14 (41.2)		13 (38.2)	12 (35.3)		-	9 (26.5)	3 (8.8)	13 (38.2)	
BFP (%)											
Normal	3 (8.8)	3 (8.8)	0.628	1 (2.9)	5 (14.7)	0.100	-	3 (8.8)	2 (5.9)	1 (2.9)	0.182
High	11 (32.4)	17 (50.0)		15 (44.1)	13 (38.2)		-	10 (29.4)	3 (8.8)	15 (44.1)	
Glucose (mg/dL)											
Normal	11 (32.4)	11 (32.4)	0.157	9 (26.5)	13 (38.2)	0.331	-	9 (26.5)	3 (8.8)	10 (29.4)	0.905
High	3 (8.8)	9 (26.5)		7 (20.6)	5 (14.7)		-	4 (11.8)	2 (5.9)	6 (17.6)	
TC (mg/dL)											
Normal	11 (32.4)	11 (32.4)	0.157	8 (23.5)	14 (41.2)	0.091	-	12 (35.3)	3 (8.8)	7 (20.6)	**0.024** *
High	9 (25.7)	9 (26.5)		8 (23.5)	4 (11.8)		-	1 (8.3)	2 (5.9)	9 (26.5)	
HDL-c (mg/dL)											
Low CVR	-	1 (2.9)	0.396	-	1 (2.9)	0.339	-	-	-	1 (2.9)	0.560
High CVR	14 (41.2)	19 (55.9)		16 (47.1)	17 (50.0)		-	13 (38.2)	5 (14.7)	15 (44.1)	
LDL-c (mg/dL)											
Normal	13 (38.2)	13 (38.2)	0.059	12 (35.3)	14 (41.2)	0.849	-	13 (38.2)	3 (8.8)	10 (29.4)	**0.039** *
High	1 (2.9)	7 (20.6)		4 (11.8)	4 (11.8)		-	-	2 (5.9)	6 (17.6)	
VLDL-c (mg/dL)											
Normal	12 (35.3)	14 (41.2)	0.288	10 (29.4)	16 (41.7)	0.070	-	13 (38.2)	3 (8.8)	10 (29.4)	**0.039** *
High	2 (5.9)	6 (17.6)		6 (17.6)	2 (5.9)		-	-	2 (5.9)	6 (17.6)	
TG (mg/dL)											
Normal	6 (17.6)	4 (11.8)	0.150	3 (8.8)	7 (20.6)	0.198	-	7 (20.6)	-	3 (8.8)	**0.035** *
High	8 (23.5)	16 (47.1)		13 (38.2)	11 (32.4)		-	6 (17.6)	5 (14.7)	13 (38.2)	
IA											
Normal	9 (26.5)	9 (26.5)	0.268	6 (17.6)	12 (35.3)	0.089	-	10 (24.9)	2 (5.9)	6 (17.6)	0.088
High	5 (14.7)	11 (32.4)		10 (29.4)	6 (17.6)		-	3 (8.8)	3 (8.8)	10 (29.4)	

[a] n = 17; n (%), X^2 test: * $p < 0.05$; BP: blood pressure, BMI: body mass index, BFP: body fat percentage, TC: total cholesterol, CVR: cardiovascular risk, TG: triacylglycerols, IA: atherogenic index. Cut points: BP: high > 110/70; glucose: normal ≤ 99 mg/dL, high ≥ 100 mg/dL; TC: normal ≤ 199 mg/dL, high ≥ 200 mg/dL; HDL-c risk: high ≤ 59 mg/dL, low ≥ 60 mg/dL; LDL-c: normal ≤ 129 mg/dL, high ≥ 130 mg/dL; VLDL-c: normal ≤ 39 mg/dL, high ≥ 40 mg/dL; TG: normal ≤ 149 mg/dL, high ≥ 150 mg/dL; IA: normal ≤ 4.49, high ≥ 4.5.

In contrast to the BC group, in the control group, the frequency of women with *GSTT1+* and *GSTT1−* variants as well as normal and high BP was significantly different ($p < 0.05$). The frequency of women with high BP and *GSTT1+* (14.7%) was lower than women with *GSTT1−* (29.4%). We did not find significant differences in BMI, BFP, HDL-c, LDL-c or VLDL-c in control women with *GSTT1*. Interestingly, *GSTP1* expression in association with TC (20.6%), LDL-c (29.6%) and VLDL-c (29.4%) presented statistically significant differences ($p < 0.05$), with higher percentages in normal levels of homozygous variant (Ile/Ile). The frequency of women with high TC was the highest in the Ile/Val variant (26.5%).

4. Discussion

The aim of this study was to identify the association between *GSTT1, GSTM1* and *GSTP1 (Ile105Val)* polymorphism mRNA expression with BP, BMI, BF and blood lipids in Mexican women with and without BC. In fact, this is the first qualitative study that evaluates the mRNA expression of GST superfamily variants in BC. Our results suggest that the expression of *GSTP1* variants and *GSTT1* can have an association with cardiometabolic disease factors in patients with BC.

The function of *GST* in cancer is controversial; the deletion of their genotypes is considered as a biomarker in cancer development and in treatment success [25]. There are studies that have investigated the association of *GST* genotypes in BC development [26]. Specifically, deletions of *GSTT1, GSTM1* and *GSTP1* are related to a higher susceptibility, resulting in impaired enzymatic functions, causing a lower response to exogenous carcinogenic agents in different populations and ethnic groups [27,28].

We did not observe significant differences regarding the expression of *GSTT1* and *GSTM1* between the BC and control group (Table 4), but we found a significant difference within the *GSTP1* variants. Interestingly, the *GSTP1 (Ile105Val)* variant is the most frequent in the BC (56.5%) and control (47.1%) groups, suggesting this could be the most abundant in this Mexican population, but it may not necessarily be related to a higher incidence of BC. Further investigations with a larger population are required to clarify that.

In this study, according to *GST* variants expression in the BC group, the frequencies of *GSTT1* and *GSTM1* present similar percentages (60.9% and 39.1%, respectively), which suggests that 39.1% of the women in this group might have a better response to chemotherapy treatment [29]. Other studies in Mexican women have reported a similar distribution in *GSTT1* and *GSTM1*; they show a similar percentage in the absence of *GSTT1* (31.3%) and *GSTM1* (38% and 43.3%) but in larger study samples (n = 342–558) [12,29]. On the other hand, the frequency of the *GSTP1* variants is similar to that in northeastern Mexican women (*Ile/Val*: 43.4%; *Val/Val*: 32.6%; *Ile/Ile*: 24%); in women from Mexico City, the higher frequency is reported in the homozygous type (*Val/Val*:38%; *Ile/Val*: 34.6%; *Ile/Ile*: 27.3%) [30]. In this case, the sample size could be a factor in the distribution differences.

Regarding the ethnic background of this sample in relation to *GST* family, it is important to indicate that they are part of three generations of Mexican Mestizo parents. We did not investigate the Indigenous or Latin background in this sample. However, it is worth mentioning that the study sample meets the equilibrium conditions of Hardy–Weinberg. In addition, the combined *GSTT1+* and *GSTM1−* genotypes was not found in a previous study performed in a Mexican Mestizo population in the State of Mexico, which included women with BC [31]. Moreover, *GSTP1 A313G* is linked to the risk of preeclampsia in Mayan Mestizo women [32]. This could suggest that there is a genetic susceptibility derived from environmental intervening variables, such as diet and carcinogenic agents as well as the presence of comorbidities [33].

The association between *GST* genotypes and metabolic markers has been raised as an important factor in the development of BC. Results reported by Aljboori, M of serum lipids and *GST* genotypes show that the deletion of *GSTM1* is related to the reduction in TG, HDL-c and VLDL-c but not in TC and LDL-c concentrations; *GSTT1* deletion may be associated with a reduction in HDL-c and an increase in TC, TG, LDL-c and VLDL-c; TG and HDL-c are positively affected by *GSTT1* and *GSTM1* deletion [15]. Our findings show that, in the

BC group, the expression of *GSTP1 Ile/Val* is linked to normal levels of LDL-c. Therefore, the reduction in *GSTP1* enzyme activity may not affect LDL-c concentrations. This was similar for LDL-c and VLDL-c in the control group. However, high TG concentrations were observed in the *GSTP1 Ile/Val* carriers from this group as well. These findings may indicate that, in women from the central region of Mexico, mRNA expression of *GSTP1* variants may have a closer association with LDL-c blood concentrations. A study in a young adult population that investigated the association between *GSTP1 Ile/Val* polymorphism with obesity and markers of cardiometabolic risk shows that subjects carrying the (*Val/Val*) genotype present a higher percentage of weight, BMI, neck, waist and hip circumference, body fat mass, SBP and higher levels of glycated hemoglobin (HbA1C) [34]. In contrast, we did not find such associations with *GSTP1 Val/Val*.

The prevalence of dyslipidemia is common in the Latino population [35]. It is clear that overweight, obesity and dyslipidemia represent risk factors for BC and its mortality [36]. An observational study in Italy for four years in patients with early BC shows that weight, waist circumference, BP, fasting glucose, TC, HDL-c and TG have fundamental functions in BC biology and increase, up to 16 times, the risk of BC mortality in women with more than three metabolic components [37]. However, we do not observe significant differences in our study groups in the levels of LDL-c, VLDL-c, HDL-c and TC. These results agree with another study we performed in women survivors of BC [38]. This suggests that, in this particular Mexican population, comorbidities, including BC and dyslipidemia, may not be completely derived from *GST* variants traits, as shown before [39]. Instead, they may involve other xenobiotic metabolizing genes or non-genetic factors, such as exposure to pollutants and unhealthy lifestyles [40–42]. This association may also include the immune system and inflammation. For instance, *GSTP1* is present in dendritic cells (DCs) that contain the estrogen receptor (ER), which is highly involved in the development of BC; indeed, it is a tumor marker [43]. The absence of *GSTP1* can increase the rate of proliferation of DCs and the binding affinity of estradiol to the ER [44]. Furthermore, DCs modulate the induction of pro-inflammatory cytokines and chemokines, such as IL6, IL8 and monocyte chemoattractant protein 1 (MCP-1) [45]. The role of *GSTP1* variants and their link to this type of immune response should be further investigated to identify potential biomarkers of these comorbidities.

The elevation of LDL-c is a common feature in dyslipidemias observed in the urban children population of central Mexico (including the states of Puebla, Estado de Mexico and Mexico City) [46,47]. In the northern Indigenous, dyslipidemia is characterized by decreased HDL-c [47]. Furthermore, dyslipidemias involving high LDL-c and TG as well as altered HDL-c have a strong link to Native American ancestry in Mexicans [48]. Genes with SNPs associated with dyslipidemias are implicated in lipid metabolism and homeostasis, lipoprotein particle composition and organic hydroxy compound transport; in the Mexican population, some of them include *LDLR*, *FADS 1-2-3*, *APOB*, *APOC3*, *GCKR* and *HMGCR* [48].

Dyslipidemias are linked to cardiovascular risk due to the development of atherosclerosis and vascular damage, including alterations in angiogenesis, vasculogenesis and vessels repair [49]. Similarly, BC also shows alterations in angiogenic factors, such as IL-8 and VEGF [50]. Therefore, vascular alterations may be a common feature in the pathology of comorbidities between BC and cardiovascular diseases. Interestingly, in our study, there is a significant association between BP and *GSTT1* ($p = 0.007$) in the control group, and the frequency of women with normal BP was higher in the *GSTT1+* variant, indicating that women with *GSTT1−* may be more susceptible to present higher BP. Nonetheless, this result is not seen in women with BC, indicating that the *GSTT1−* and BP association may not be relevant in BC.

Finally, the liver is a common reservoir for *GST* activity and lipids metabolism [51]. *GST* expression might influence lipids' hepatic metabolism. For example, the effect of dietary fat intake on antioxidant enzymes could depend on phase I and II metabolizing enzymes [52], but the mechanisms behind that are not clearly known. Therefore, future

investigations could be performed to comprehend their signaling pathways of interaction. These should involve novel non-lipid biomarkers of cardiovascular function, such as uric acid [53] and the enzymes related to its synthesis. Uric acid is tightly correlated to high blood pressure and certainly could be a more precise predictor of cardiovascular alterations in women with BC.

5. Conclusions

Our results suggest that mainly *GSTP1* variants mRNA expression is associated with BC and cardiometabolic parameters in Mexican women. Firstly, the mRNA expression of the *GSTP1 Ile105Val* variant has an association with normal LDL-c levels in women with or without BC. Secondly, women without *GSTT1* expression may be more prone to develop high blood pressure, but this is not related to BC. It is likely that the limitations of the study, such as sample size and study design, could have an effect on our results. This is the reason why it is necessary to perform future investigations with larger samples and longitudinal follow-ups. Those should include the evaluation of inflammatory and angiogenic factors as well as other genes involved in the signaling pathways of vascular damage and xenobiotic metabolism.

Author Contributions: Y.B.A., A.E.S.P., F.B.G., E.D.G., A.D.B.A. and I.R.C.B. contributed to the conception, study design, data collection and analysis as well as writing the manuscript. Y.B.A., A.E.S.P., F.B.G., E.D.G. and J.A.C.R. performed molecular and statistical analysis of GST genotypes. R.V.R. and J.A.C.R. participated in writing and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Autonomous Mexico State University (Project number 4755/2019/CI).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. This project was approved by the Research Ethics Committee of the Medical Sciences Research Center of Autonomous Mexico State University (Project number: 2018/10) as well as the Institute of Health of the State of Mexico Academic Coordination (217B50025/888/19).

Informed Consent Statement: All women were informed about the aim and procedures of the study, and their authorization to participate was requested by signing informed consent.

Acknowledgments: We would like to acknowledge the support provided by Health Institute of the State of Mexico (ISEM), especially Specialized Medical Unit for the Detection of Breast Cancer (UNEME-DEDICAM) and State Public Health Laboratory (LESP).

Conflicts of Interest: The authors declare that there is no conflict of interest related to the content of this study.

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