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Glutathione -S-transferase gene polymorphism (GSTT1 and GSTM1) and Cytochrome P450 (CYP2D6) polymorphism and breast cancer susceptibility in Egyptian women

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Abstract

Background: The enzymes GSTM1, GSTT1, GSP1, CYP1A1, and CYP2E1 promote the bio-activation and detoxification of many xenobiotics that can be found in food, organic solvents, tobacco smoke, medications, pesticides, environmental pollutants, and alcoholic beverages. The genes that encode these enzymes have variations that have been associated with certain indicators of sickness vulnerability.

Aim: This study seeks to understand whether there is a correlation between breast cancer risk in Egyptian women and variations in Glutathione-S-transferase and CYP2D6.

Methodology: Out of 202 participants in the case-control study, 46.0% had the GSTT1 (0) /GSTM1 (0) genotype. A tumor-node-metastasis staging approach, adapted from the International Union Against Cancer and the American Joint Committee on Cancer, was used to compare breast cancer patients with and without the disease. Using immune histochemistry approaches, prognostic indicators such as HER2/neu and ER/PR were found. Comprehensive blood counts (CBCs), cancer antigen 15-3 and carcinoembryonic antigen (CEA), albumin, bilirubin, albumin-lowering plasma protein (ALP), creatinine, and uric acid were assessed.

Results: The study indicated that the frequencies of GSTT0/GSTM0 were considerably higher in breast cancer cases (43% vs. 49.5% in healthy controls). Cases (54.5%) had a higher frequency of the '*2' allele compared to controls (9.5%). Case and control groups also differed significantly in genetic distribution. Cases had a larger proportion of the Wt/Wt genotype, whereas controls had higher proportions of the Wt/*2 and *2/*2 genotypes. A crude odds ratio of 4.1 indicates that the prevalence of GSTT1/0 was higher in cases (75.2%) compared to controls (42.6%). There was a little increase in GSTM1 in cases (55.4% vs. 49.5% in controls). When comparing controls and cases, the former had more GSTT1 with or without GSTM1 and the latter had more GSTT1 without or with GSTM1.

Conclusion: The results showed that among Egyptian women, GSTM1/0 mutations had a 4.1-fold increased risk of breast cancer. Breast cancer risk is elevated in people with CYP2D6 *2 alleles. When these genotypes are coupled with characteristics related to lifestyle or the environment, the risk can be amplified.

Key words: Cytochrome P450, polymorphism, glutathione-S-transferase, and breast cancer.

Introduction

More than one in ten new cancer diagnoses are attributed to breast cancer each year; it is also the second leading cause of cancer-related mortality among females globally. Reducing the incidence of breast cancer relies heavily on addressing the well-established risk factors. In Western countries, routine screenings often detect breast cancer, which often develops undetected. Breast cancer is frequently discovered as a noticeable lump in the breast when screening is not done. Breast cancer treatment varies by tumor type and stage but often involves a mix of radiation, chemotherapy, immunotherapy, surgery, and/or surgery. There has been a considerable improvement in overall survival and patient-reported outcomes due to advancements in various therapy approaches. Healthcare providers can improve their breast cancer management competence through this activity, which aims to update their knowledge, skills, and strategies for early detection, effective interventions, and better care coordination, all of which contribute to better patient outcomes and reduced morbidity.¹The exact process by which breast cancer develops is unknown; however, environmental variables and genes that cause decreased diffusion may play a role.²For postmenopausal women with estrogen receptor-positive breast cancer, tamoxifen is one of the top treatments. Treatment with tamoxifen alleviates symptoms for around 30% of women whose breast cancer has spread. Patients with estrogen receptor-positive breast cancer who take adjuvant tamoxifen had a lower risk of death and recurrence of the disease compared to those who do not.³The liver increases the potency of tamoxifen's active metabolites, which include endoxifen and 4-hydroxy-tamoxifen. Doxifen and 4-hydroxy-tamoxifen have forty-five times higher estrogen receptor affinities than tamoxifen. The reason endoxifen is more effective than 4-hydroxy-tamoxifen is that it reaches plasma concentrations that are 5-10 times higher.⁴One important participant in the conversion of tamoxifen to endoxifen is Cytochrome P450 2D6 (CYP2D6). Genetic polymorphisms mainly control CYP2D6 activity and not expression induction or inhibition; as a result, there is a 1,000-fold difference in CYP2D6 metabolic capacity. If a person's enzyme activity is normal and they have two functional CYP2D6 alleles, they are said to be an extensive metabolizer (EM); on the other hand, if they have two non-functional CYP2D6 alleles, they are said to be poor metabolizers (PM). Carriers of both a functioning and a non-functional allele are commonly referred to as intermediate metabolizers (IMs). A CYP2D6*4 variation allele is among the most prevalent among Caucasians and is responsible for the PM phenotype.⁵

The CYP2D6 alleles can be categorized as either inactive, reduced, normal, or enhanced, depending on their functional state. Genotype and diplotype, the combination of alleles, allow one to anticipate metabolizer phenotypes, ranging from ultrarapid metabolizers (UMs, boosted enzyme activity) to poor metabolizers (PMs, no enzymatic activity). Ultrametabolizers (UMs) exhibit high enzyme activity, intermediate metabolizers (IMs) exhibit lowered enzyme activity, and poor metabolizers (PMs) display no enzyme activity when contrasted with the normal metabolizer (NM) phenotype. The most prevalent reason for elevated enzyme activity is a change in the number of functional gene copies, often known as gene copy number variation.^{6,7} When it comes to chromosomal polymorphism, the frequency of CYP2D6 alleles differs from one group to another. Clinicians can find out who might gain the most from proactive genetic testing for actionable pharmacogenetic variation by looking at data on the frequency of certain alleles and metabolizer phenotypes across sub-populations of different ethnic or geographical groups, or both. There is a plethora of material regarding population-specific allele and phenotypic frequencies related to the CYP2D6 gene because of how variable it is. Possible BC genes include GST and other low-developed genes that belong to five families. One of the key components in the process of microbe detoxification is GSTT1, which is found on the long arm of chromosome 22 (22q11.23). Particularly intriguing among the GST isoforms is GSTM1, which includes an empty polymorph known as GSTM1^{-/-} (GSTM1 - null) that renders the GSTM1 enzyme inactive overall. Depending on race, 27-65% of the population possesses the GSTM1 ^{-/-} gene.⁸ Reduced enzyme function, elevated cancer risk, and cellular damage have all been associated with the prevalent GSTT1 gene homozygous deletion. The genes family which code for hydrophilic molecules that can be extracted from carcinogenic substances. In large portions of the human population, null alleles result in the loss of protein-coding regions. People are at a higher risk of developing cancer if they are homozygous mutant for the GSTM1 and GSTT1 enzyme genes.⁹ Reactive metabolites can cause chemical harm to normal breast tissue, but GST mechanisms protect it. Along with providing a synopsis of recent studies on phenotypic and allele frequencies.¹⁰ In order to find out if polymorphisms in the GSTM1 and GSTT1 genes were associated with cancer, this study compared Egyptian breast cancer patients with matched healthy controls. The research indicates that CYP2D6 is involved in the metabolism of over 25% of the medicines available today. Because of differences in heredity, the CYP2D6 enzyme's activity varies substantially across individuals. The CYP2D6 gene duplication 16947 is one of the factors that affects genotype interpretation; other

factors include biomarker panels that use CYP2D6*41 and CYP2D6*2A as surrogate markers and studies that examine coexistence or structural abnormalities.¹¹

Subjects and method

Study Design: A case-control study.

Study Setting: The study was conducted at Mansoura University.

Subjects Eligibility Criteria:

Inclusion Criteria: Two hundred women participated in this hospital-based case-control study; 101 were breast cancer patients (mean age: 53) and 101 were healthy volunteers.

Exclusion Criteria: Patients who had a history of cancer during pregnancy or while nursing were not included in the study.

Data Collection Tools: Information was gathered by analyzing test results and patient registry records.

Outcome Measurements: The following tests were assessed: genotyping, allelic frequencies, and biochemical, tumor marker, and hemostatic parameters.

Sample handling and study participants

Ethical approval

After obtaining ethical authorization from the institutional review board and ethical committee at Mansoura University, each lady who participated in this study was given allocated consent. From November 2019 to December 2020, patients were recruited from the Oncology Center's Outpatient Clinics at Mansoura University in Egypt for this hospital-based case-control research. The Faculty of Medicine at Mansoura University granted ethical clearance (ID: R.23.03.2111). Prior to participating in this study, all ladies were given the necessary permissions after the institution's review board and ethics committee at Mansoura University granted their approval. Participants for this case-control study were enrolled between November 2019 and December 2020 at the Outpatients' Clinics of Oncology Center at Mansoura University in Egypt. There was a total of 202 female participants in this study; 101 were cancer patients with breast cancer and 101 were healthy volunteers who served as age- and socioeconomic-matched controls. Using a hollow core needle biopsy, the histological diagnosis of breast cancer was confirmed. Hormonal, radiological, chemical, or immunological treatments were not administered to any of the breast cancer patients. The pathologist evaluated the tumor's stage and grade by validating the breast samples. Three criteria formed the basis of the Scarf-Bloom-Richardson (SBR) grading system

for breast tumors: (1) tubule development, (2) nuclear polymorphism, and (3) mitotic count, which may be scored as 1, 2, or 3. There were three main groups based on the final SBR grade: grades I through III, with scores ranging from 3 to 5 (highly differentiated), and grades II through VII, with scores ranging from 6 to 7 (moderately differentiated). The tumor-node-metastasis (TNM) staging approach, which incorporates revisions and updates to the International Union against Cancer staging Criteria (UICC) and the American Joint Committee on Cancer (AJCC) criteria, was also used for tumor staging. Using immune histochemistry techniques, predictive indicators of breast cancer, such as human epidermal growth factor receptor 2 (HER2/neu) and estrogen and progesterone receptors (ER/PR), were found. Inclusion criteria for breast cancer patients and cancer-free controls were absence of the disease, as well as a history of hepatic, renal, cardiac, autoimmune, or any other type of cancer in the body. From the medical records, we were able to extract the demographic and clinical details of the breast cancer patients and the controls that did not have the disease.

Evaluation of Biochemical, Tumor Marker, and Hematological Measurements

Albumin, total bilirubin, alkaline phosphatase (ALP), albumin, uric acid, creatinine, and serum transaminase enzymes (ALT and AST) were estimated using a fully automated biochemical analyzer (Cobas c501, Roche Diagnostics, Mannheim, Germany). Each woman's complete blood count (CBC) also included measurements of her white blood cells, red blood cells, hemoglobin level, platelets, and lymphocytes (CELL-DYN 3700 SL, Abbott Diagnostics, USA). In addition, enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3).

DNA extraction and amplification

All subjects had their blood drawn before the interventions began, and the DNA was extracted using a QIAmp DNA Blood Mini Kit (Qiagen, Germany) in accordance with the instructions provided by the manufacturer. We dissolved the extracted DNA in enzyme-free water and stored it at -20°C until we required it. A NanoDrop™ spectrophotometer from Thermo Scientific, USA was used to evaluate DNA's integrity and quality. Genomic DNA was isolated from whole blood samples by standard phenol-chloroform extraction of the leukocyte pellet. Until it was needed, the produced DNA was kept at a temperature of -20°C. The presence of GSTM1, GSTT1, and CYP2D6 polymorphism was assessed in a multiplex PCR experiment. As an internal control, the CYP1A1 gene was used with the following primers: 5'-

GAACTGCCACTTCAGCTGTCT-3' for the forward primer and 5'-AGCTGCATTTGGAAGTGCTC-3' for the reverse primer. There were positive and negative controls in every reaction series.

Performing genotyping and amplification of GST (M, T) and CYP2D6.

A total of 20 µl was used for the amplification experiment, with 1 µl of DNA (100 ng), 10 µl of Taq polymerase master mix (amplicon, Biomol, Germany), 1 µl of each primer (10 µmol l-1), and 7 µl of DEPC water coming together. We used an Eppendorf gradient thermal cycler, made in the US by Life Science, to do the polymerase chain reaction (PCR). The amplifying condition was as previously mentioned: The process begins with a 5-minute heating step at 94°C, then 30 cycles of 1-minute heating at 94°C, 30-second cooling at 60°C, and 30-second heating at 72°C. Lastly, a final heating step of 5 minutes at 72°C is required. Using DNA-safe dye and a UV transilluminator (cleaver, UK), the amplicons were resolved by electrophoresis on a 2% agarose gel.

The primer sequences of **GST** are forward 5'- GAACTCCCTGAAAAGCTAAAGC-3' and reverse 5'GTTGGGCTCAAATATACGGTGG-3',

GSTT1, forward:5'-TTCCTTACTGGTCCTCACATCTC-3'andreverse 5'-AAGACTTGGACCAGCACC-3'.

GSTM1, forward: 5'- GAACTCCCTGAAAAGCTAAAGC-3' and reverse 5'-GTTGGGCTCAAATATACGGTGG-3' CYP2D6 are tetra primers as following:

Primers 3 5- GCC CAC CCA GAA CCC ACC A-3

4 new 5- GGT CCG GCC CTG AGA CTC CTT CT-3

U2 wt 5-AAT GAT GAG AAC CTG G-3

L2 mut 5- GGC AGG ACT ATG CA-3

Gel electrophoresis.

To separate DNA fragments according to their molecular weights, agarose gel electrophoresis is used, according to Rasmussen. Smaller fragments move more rapidly than larger ones. As the molecular weight logarithmically increases, the migration distance on the gel decreases. By

comparing the distance of the unknown fragment to known size standards and calibrating the gel, the size of the fragment can be determined. shown in figure 4 and5.

Genotyping

Every single person who took part in the Rotterdam Study had their CYP2D6*4 polymorphism genotyped as previously stated (1846G>A). One nanogram of genomic DNA was amplified using 40 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute in Taqman assays (Applied Biosystems, Foster City, USA). The absence of the 1846G>A SNP led to the classification of individuals as homozygous *4/*4 (PM), heterozygous *1/*4 (IM), or *1/*1 (EM).¹²

Statistical analysis

The data was entered and analyzed using IBM-SPSS, a program that was launched in 2017 by IBM Corporation. Version 25.0 of IBM SPSS Statistics for Windows is the program that was utilized. Based in Armonk, New York, IBM Corp. develops it. Frequency and percentage were used to represent the qualitative data. The Chi-Square or Fisher Exact (WI) test is what the user is referring to. The original Bonferroni method was used to adjust p-values when comparing column proportions. The researchers in this study used letter comparisons to show differences in column proportions; comparable characters denoted a slight difference and dissimilar characters a significant one. When it was thought to be appropriate, the Monte Carlo approach was used. The Shapiro-Wilk test revealed that the data did not adhere to a normal distribution (P-value> 0.05). Outliers were tested using boxplots. When comparing two sets of data, the Mann-Whitney U test was employed.

Results

The Baseline Characteristics of the Study Population

Table 1 displays the demographic features of the patients who were evaluated. The average age of the patients is 50.9 years, and 39.6% of the cases had early tumor stages, while 60.4% had late stages. Out of all the instances, 35.6% have a low G1 grade and 64.4% have a high G2-3 rating. Lymph node involvement was found in 67.3% of cases, while 32.7% of cases were classified as negative. In contrast to 29.4%, 70.3% show that the cancer has spread to other organs. In this study, estrogen receptors were found to be positive in 61 patients and negative in 30. Progesterone receptors are positive in 68 cases and negative in 33 others. A total of 62 patients

tested positive for progesterone receptors, while 39 patients tested negative, according to the HER-2/neu test.

Table 1; Demographic characteristic of studied patient

	Breast cancer patients (n=101)
Age (Mean±SD, years)	50.9±10.6
T stage, no. (%)	
Early T≤2	40 (39.6)
Late T>2	61 (60.4)
Histological grade, no. (%)	
Low G1	35 (35.6)
High G2-3	65 (64.4)
Lymph node involvement, no. (%)	
Negative (N0)	33 (32.7)
Positive (N1)	68 (67.3)
Distant organs metastasis, no. (%)	
Negative (M0)	71 (70.3)
Positive (M1)	30 (29.7)
Tumor size (small<3cm/large>3cm)	45/56
Estrogen receptor ER (negative/positive)	30/71
Progesterone receptor PR (negative/positive)	33/68
HER-2/neu (negative/positive)	39/62
Healthy females (n=101)	
Age (mean±SD, years)	49.6±10.3

Evaluation of Biochemical, Tumor Marker, and Hematological Measurements

Table 2 showed the tested subjects' biochemical parameters. All the liver function tests that we did came back normal, with no notable variations, including SGPT, SGOT, T. bilirubin, albumin, and alkaline phosphatase. There was no statistically significant difference between the normal

and abnormal ranges for creatinine and uric acid in our renal function tests. Nevertheless, when it comes to CEA and CA 15-3, two markers of tumors, there is a no difference between the two groups. Serum creatinine, CEA, and CA-15-3 levels were considerably normal in the two groups, as shown in the table.

Table2; Evaluation of Biochemical, Tumor Marker, and Hematological Measurements

Characteristic	Control	Case	P value
N	101	101	
SGPT	19 (16-32)	18 (14-29.7)	0.364
SGOT	23 (20-33)	23 (17-33)	0.792
T. bilirubin	0.5 (0.4-0.7)	0.48 (0.4-0.5)	0.150
Albumin	4.1 (3.8-4.3)	4 (3.8-4.2)	0.425
Alkaline phosphatase	132 (110-212)	138 (121-195)	0.480
Creatinine	0.8 (0.7-1.0)	0.7 (0.6-0.8)	0.012
Uric acid	4.2 (3.6-5.4)	4 (3.3-5)	0.111
CEA	2 (1-3)	1 (1-2.9)	0.049
CA-15-3	25 (17.7-35)	19 (15.7-24.3)	0.035
WBCs	6.6 (5.3-8.7)	7.1 (5-8.9)	0.735
Hemoglobin	11.5 (10.6-12.5)	11.7 (10.7-12.6)	0.718
Platelet count	256 (222-336)	260 (196-332)	0.894

Data expressed as Median (IQR) and compared by Mann-Whitney U-test.

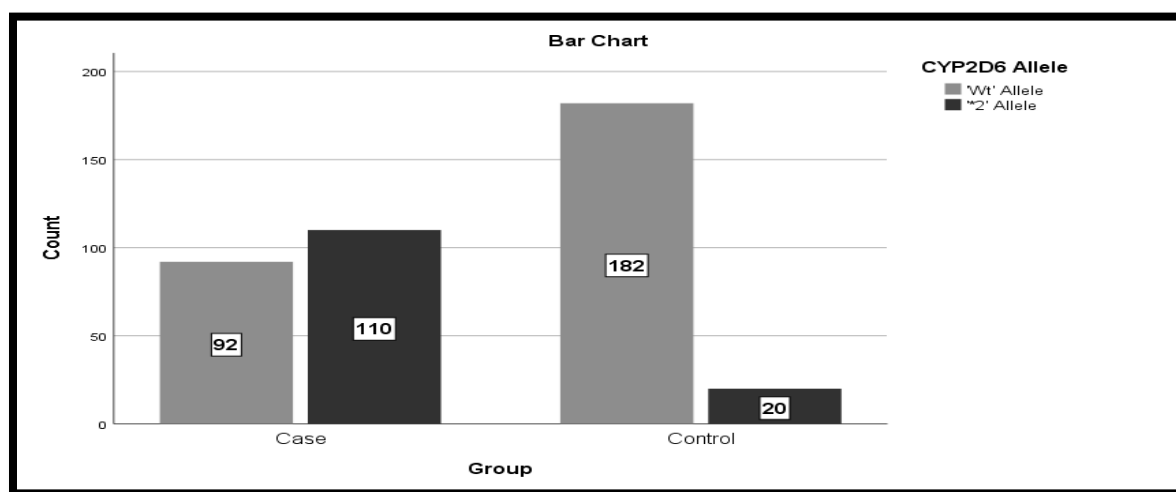
Genotyping and allelic frequencies of CYP2D6

Table 3 shows that the '*2' allele was much more common in cases (54.5%) than in controls (9.5%). A basic binary logistic regression analysis revealed a crude odds ratio of 10.9 for those with the '*2' allele, meaning they have a 10.9 times higher chance of developing breast cancer. A overview of this can be found in Figure 1.

Table 3: Genotyping and allelic frequencies of CYP2D6

Allele	Case	Control	χ^2	P1	COR	95% CI	P2
N of participants	101	101					
N of alleles	202	202					
‘Wt’ allele	92 (45.5%)	182 (90.1%)	91.870	<0.001	R	R	<0.001
‘*2’ allele	110 (54.5%)	20 (9.5%)			10.88	6.35-18.64	

P1: Chi-Square test. P2: Simple binary logistic regression analysis. R=reference category. COR = Crude Odds Ratio. 95% CI = 95% Confidence Interval.

**Fig. 1 Genotyping and allelic frequencies of CYP2D6**

Association of CYP2D6 variants with the risk of breast cancer patients

Table 4 displays the significantly different distributions of the three genotypes between the case and control groups. The proportion of Wt/Wt genotype was substantially greater in the case group compared to the control group, and the proportions of Wt/*2 and *2/*2 genotypes were also substantially higher in the control group compared to the case.

Table 4 showed a statistically significant difference in the distribution of the three genotypes between case and control groups.

Genotype		Case	Control	χ^2	P1	COR (95% CI) P2
N of participants		101	101			
Wt/Wt (EM)	19 (18.8%) a	85 (84.16%) b	86.612	<0.001	R	
Wt/*2 (IM)	54 (53.5%) a	12 (11.88%) b			20.1 (9.1-44.7)	<0.001
*2/*2 (PM)	28 (27.7%) a	4 (3.96%) b			31.3 (9.8-99.9)	<0.001

Data expression: N (%). P1: Chi-Square test.P2: Binary logistic regression. Comparisons of column proportions: Z-test with Bonferroni adjustment (different letters = significant difference). EM=Extensive metabolizer. IM=Intermediate metabolizer. PM=Poor metabolizer.

Genetic analysis and GSTT1 allelic frequency in women with breast cancer

For specifics, see Table 5, however in the case group the proportion of GSTT1/0 was 75.2%, while in the control group it was 42.6%. A basic binary logistic regression analysis revealed a

Allele	Case	Control	χ^2	P1	COR	95% CI	P2
N of	101	101					

crude odds ratio of 4.1, which means that those with GSTT1/0 have a 4.1 times higher likelihood of showing cancer. Figure 2 summarizes this.

Table 5 Genotyping and allelic frequency of GSTT1 in breast cancer patients

participants							
GSTT1/+	25 (24.8%)	58 (57.4%)	22.272	<0.001	R	R	<0.001
GSTT1/0	76 (75.2%)	43 (42.6%)			4.10	2.25-7.47	

P1: Chi-Square test. P2: Simple binary logistic regression analysis. R=reference category. COR = Crude Odds Ratio. 95% CI = 95% Confidence Interval.

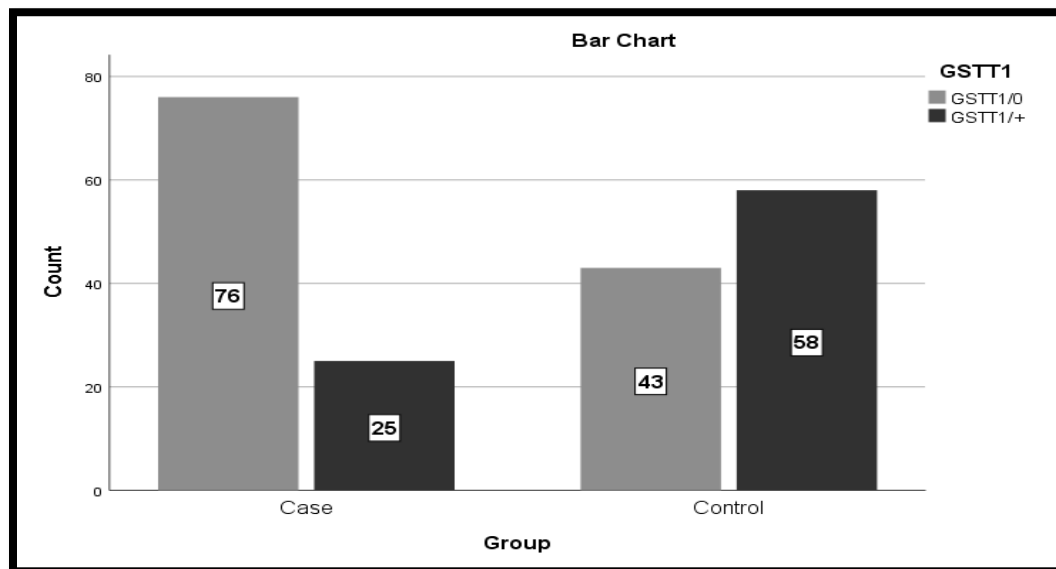


Fig.2 Genotyping and allelic frequency of GSTT1

Genotyping and allelic frequency of GSTM1

The distribution of GSTM1 was not significantly different between cases and controls, according to Table 6. However, the percentage of cases with GSTM1/0 was slightly higher (55.4%) than the percentage of controls (49.5%). The lack of a significant correlation between GSTM1/0 and breast cancer was shown using a basic binary logistic regression study. The genotyping and allelic frequency of GSTM1 were shown in Figure 3.

Table 6: Genotyping and allelic frequency of GSTM1

Allele	Case	Control	χ^2	P1	COR	95% CI	P2
N of participants	101	101					
GSTM1/+	45 (44.6%)	51 (50.5%)	0.715	0.398	R	R	0.398

GSTM1/0	56 (55.4%)	50 (49.5%)			1.27	0.73-2.21	
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P1: Chi-Square test. P2: Simple binary logistic regression analysis. R=reference category. COR = Crude Odds Ratio. 95% CI = 95% Confidence Interval

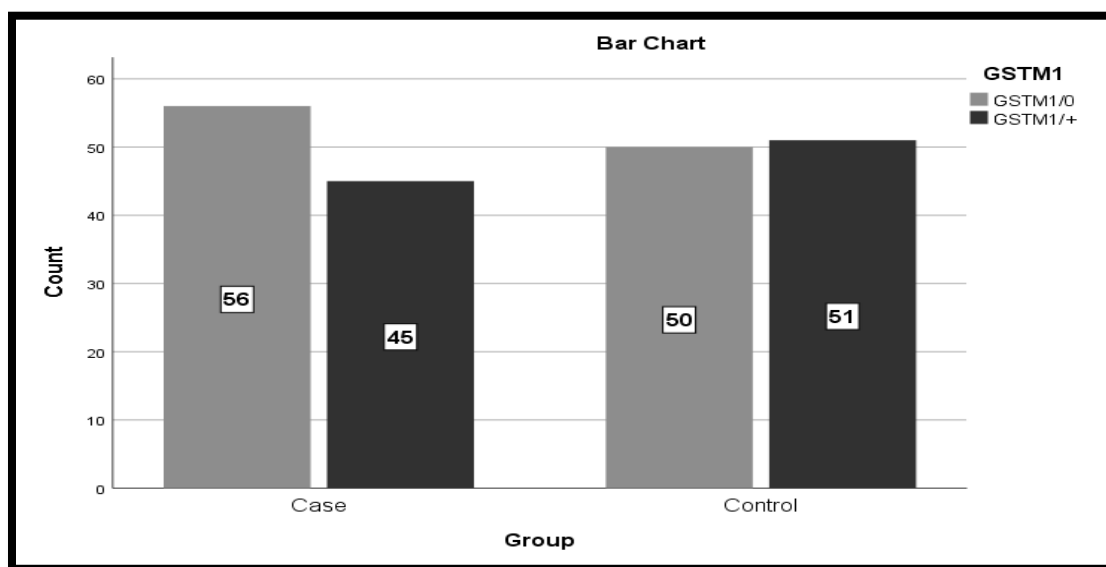


Fig 3. Genotyping and allelic frequency of GSTM1

Genotyping and allelic frequency of GSTT1/GSTM1 and risk of breast cancer.

According to Table 7, there was a notable disparity in the distribution of combined GSTM1 and GSTT1 between the control group and the cases. Statistical analysis revealed that controls had a higher prevalence of GSTT1 with or without GSTM1 compared to cases, while cases had a higher prevalence of GSTT1 without GSTM1 compared to controls. Results from a basic binary logistic regression study showed that the risk of breast cancer was 3.1 times higher for GSTT1/0-GSTM1/+ and 4.1 times higher for GSTT1/0-GSTM1/0. Figure 4 summarizes this.

Table 7 Genotyping and allelic frequency of GSTT1/GSTM1 and risk of breast cancer.

Allele	Case	Control	χ^2	P1	COR	95% CI	P2
N of participants	101	101					
GSTT1/+ - GSTM1/+	15 (14.9%)	31 (30.7%)	23.043	<0.001	R	R	
GSTT1/+ - GSTM1/0	10 (9.9%)	27 (26.7%)			0.77	0.3-1.98	0.582

GSTT1/0-GSTM1/+	30 (29.7%)	20 (19.8%)			3.1	1.34-7.16	0.008
GSTT1/0-GSTM1/0	46 (45.5%)	23 (22.8%)			4.1	1.87-9.15	<0.001

P1: Chi-Square test. P2: Simple binary logistic regression analysis. R=reference category. COR = Crude Odds Ratio. 95% CI = 95% Confidence Interval.

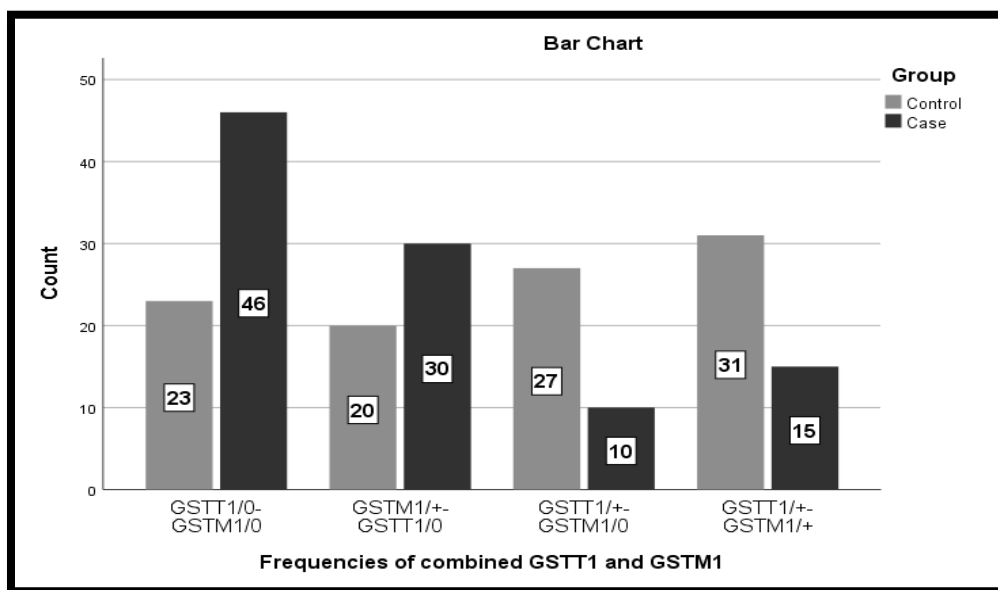


Fig 4. Genotyping and allelic frequency of GSTT1/GSTM1

The gel electrophoresis pattern of CYP2D6 was shown in Figure 4. DNA ladder lane 1 indicates low expression, heterozygous lane 2 indicates low expression (150 bp) and highly expressed lane 6 indicates highly expressed (50 bp); lanes 3 and 6 indicate low expression and homozygous pattern of mutant type, respectively.

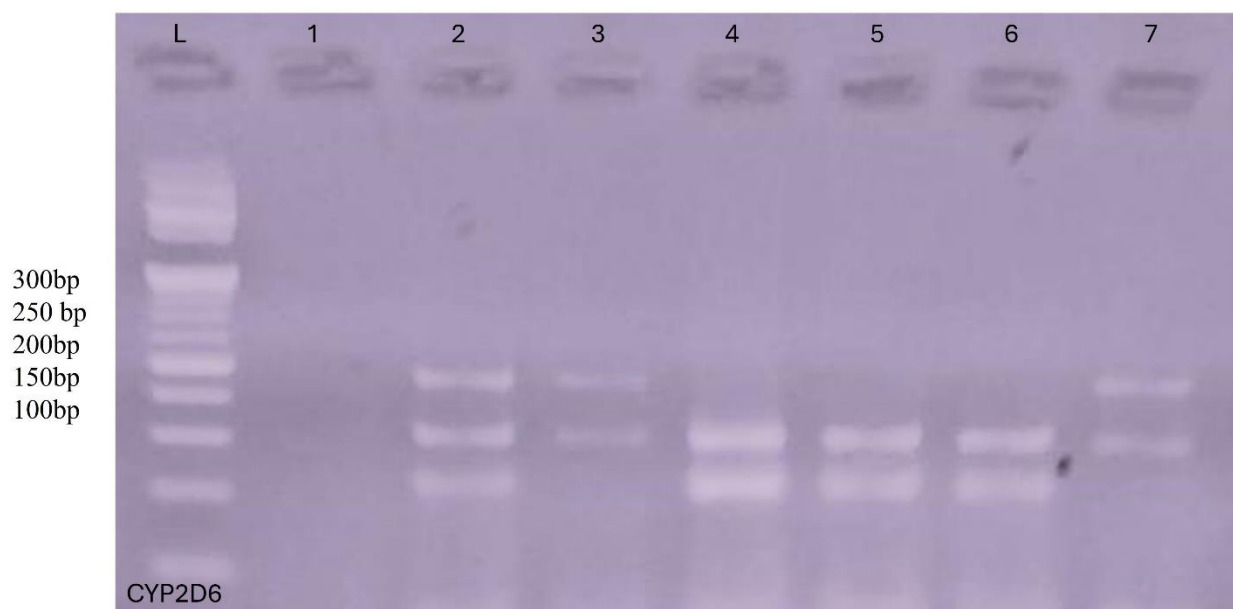


Fig 4. Pattern of gel electrophoresis of CYP2D6

Fig 5. Demonstrated a pattern of gel electrophoresis of GSTT/GSTM (as illustrated in Lane 1) measuring 309 bp as highly expressed pattern, 201 bp and 108 bp respectively with moderate expressed pattern).

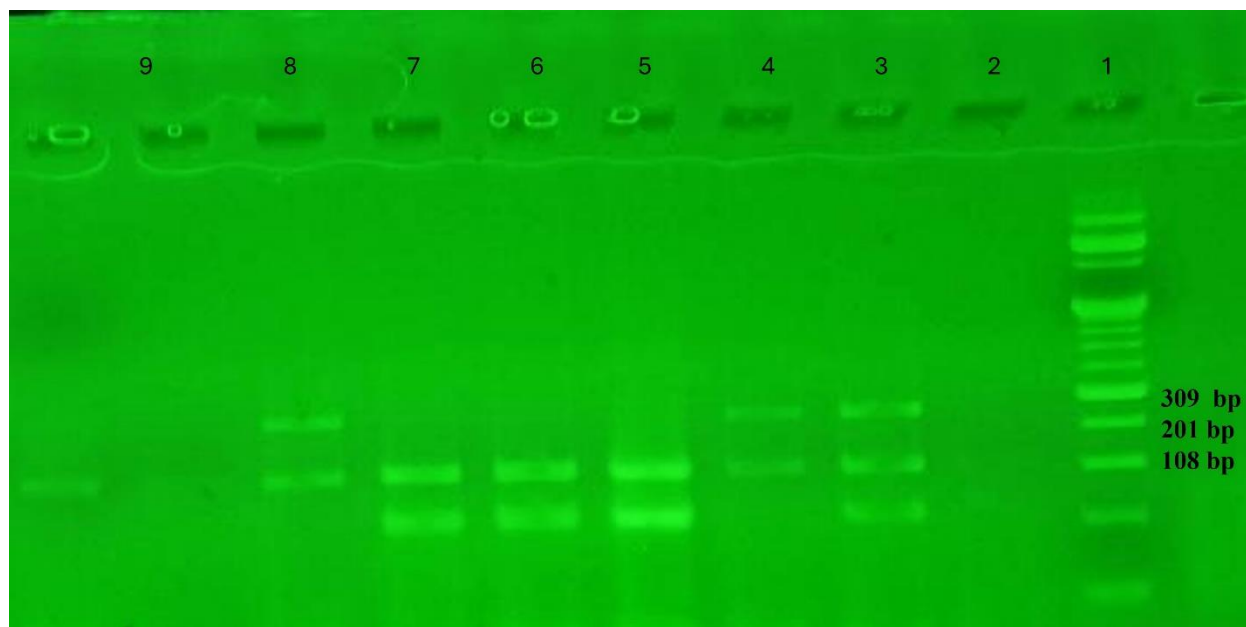


Fig 5. Pattern of gel electrophoresis of GSTT/GSTM

Discussion

A large amount of functional polymorphism variety is observed in many isoenzymes belonging to the glutathione S-transferases (GSTs) superfamily. GSTs detoxify DNA-reactive electrophiles that could be toxic or mutagenic. This contains byproducts of many chemotherapeutic medications, some of which are suspected carcinogenic for humans. Varieties in the phase II metabolizer enzymes GST T1, GST M1, and GST P1 affect the body's ability to detoxify specific environmental leukinogens.¹³ A growing amount of evidence from epidemiological and experimental studies implicates gene-environment interactions in a broad variety of human diseases. Genetic variations have been linked to cancer and other long-term illnesses in humans in multiple investigations. But remember that many SNPs are somatic mutations caused by environmental stressors. More and more research are showing that many chronic diseases have a complex etiology that includes both genetic predisposition and environmental variables. In addition, research has demonstrated that environmental factors interact with allelic differences to substantially alter disease risk. Genetic and environmental variables interact to influence the prevalence and severity of human diseases, as has been demonstrated by a plethora of research in gene-environment interactions.¹⁴ The cytosol detoxifying enzymes known as GSTs have been associated with many cancers, according to the available evidence. A preliminary odds ratio of 4.1 indicates that 75.2% of the Egyptian women in the study had GSTT1/0, whereas 42.6% of the control group did. People who have the GSTM1/0 genotype are more likely to develop breast cancer.^{15,16} In Iran,¹⁷ Lebanon,¹⁸ and Iceland,¹⁹ no GSTM1-null genotype was found. However, in northeastern India,²⁰ southern Taiwan,²¹ and China,²² an association was found. Some environmental and lifestyle factors, as well as a personal or familial history of breast cancer, increase a person's risk of developing the illness. Oral contraceptives, a woman's family history, and the age she reaches menopause, the frequency and severity of her periods, and other comparable factors all increase her risk. Lifestyle variables that increase the likelihood of acquiring the condition include smoking, drinking excessively, not getting enough exercise, and eating a fatty diet.²³ Enzymes such as GSTM1, GSTT1, GSTP1, CYP1A1, and CYP2E1 aid in the bio-activation and detoxification of xenobiotics, which can be found in food, organic solvents, tobacco smoke, medicines, pesticides, environmental pollutants, and alcoholic

beverages. Studies have linked polymorphisms in these enzyme-encoding genes to several disease susceptibility markers.²⁴ Finding out if polymorphisms in Glutathione-S-transferase and CYP2D6 are associated with breast cancer risk in Egyptian women is the main goal of this research.

In a case-control study with 202 individuals, the highest frequency of the GSTT1 (0) /GSTM1 (0) genotype was 42.0%. To compare breast cancer patients with and without the disease, a tumor-node-metastasis staging system was utilized, which was derived from the International Union Against Cancer and the American Joint Committee on Cancer. Prognostic markers including HER2/neu and ER/PR were identified using immune histochemistry techniques. Albumin, bilirubin, albumin-lowering plasma protein (ALP), creatinine, uric acid, cancer antigen 15-3, and carcinoembryonic antigen (CEA) were all measured. According to the study, the frequency of GSTT0 and GSTMO was significantly higher in breast cancer patients compared to healthy controls (43% and 49.5%, respectively). When comparing cases (54.5%) to controls (9.5%), the '*2' allele was more common in the former group. The genetic distribution of the case and control groups was likewise substantially different. The Wt/Wt genotype was more common in cases, but the Wt/*2 and *2/*2 genotypes were more common in controls. A crude odds ratio of 4.1 suggests that GSTT1/0 was more common in cases (75.2%) than in controls (42.6%). A little higher percentage of patients has GSTM1 than controls (55.4% vs. 49.5%). The control group had a greater GSTT1 without GSTM1 prevalence than the patients, while the cases had a higher GSTT1 with GSTM1 prevalence than the controls.

In terms of the distribution of the three genotypes, the case and control groups differed significantly. A crude odds ratio of 10.9 was shown by the binary logistic regression analysis, implying that the risk of developing breast cancer was 10.9 times higher for those who possessed the '*2' allele. Regarding the proportions of Wt/Wt, Wt/*2, and *2/*2 genotypes, there was a statistically significant difference between the case and control groups. A binary logistic regression analysis found that the risk of breast cancer was 31 times higher for individuals with the PM genotype and 20 times higher for those with the IM genotype.

Researchers found that there is a strong correlation between GSTT1 genotype and an elevated risk of breast cancer (95% CI: 1.08-4.29). When looking at the case-series data, researchers found no association between GSTT1 genotypes and menopause ($p=0.86$), tumor stage ($p=0.12$),

grade ($p=0.32$), or size ($p=0.07$). Results from the survival research showed that overall survival (OS) was much lower in GSTT1-negative cases compared to GSTT1-positive ones (mean OS: 23 months vs. 33 months). When comparing patients with and without GSTT1, the former had a better overall health hazard ratio (OS HR) of 8.13.²⁵ The risk of death from breast cancer was significantly increased in patients with the *4/*4 genotype compared to the wild type. With each additional variation allele, the risk of breast cancer death increased by 2.0. There was no increased risk of cancer or death from any cause in tamoxifen users with the CYP2D6*4 allele. Users of tamoxifen who have reduced CYP2D6 activity are at a higher risk of breast cancer mortality, according to the model where tamoxifen production is dependent on CYP2D6 activity.²⁶ Out of 157 patients, forty-four (28%) had CYP2D6 polymorphisms; fourteen (31.8%) shown improvement, while thirty (68.0%) were resistant ($P=0.027$). The responders' frequency of *10/*3 and *10/*10 variants was greater (12/14, 85.71%, $P=0.027$), whereas the refractory group's frequency of *3,*4 variants was much higher (26/30, 86.6%). The overall survival rates of both refractory and responder patients were lower ($P<0.001$), and there was a substantial relationship between CYP2D6 polymorphism and Her-2 amplification ($P=0.001$).²⁷

The average duration of follow-up was 6.3 years. After 9 years of follow-up, the recurrence rates for those who metabolized well were 14.9%, for those who were heterozygous for both good and bad metabolizers it was 20.9%, and for those who metabolized poorly it was 29.0%. Different percentages of deaths occurred due to different causes: 16.7%, 18.0%, and 22.8%. The time to recurrence adjusted hazard ratio [HR] for heterozygous extensive/intermediate metabolizers was 1.40 while the time to recurrence HR for poor metabolizers was 1.90 (95% CI, 1.10-3.28). Extensive metabolizers fared worse in event-free survival and disease-free survival compared to individuals with reduced CYP2D6 activity (heterozygous extensive/intermediate and poor metabolism), regardless of the difference in overall survival.²⁸ The CYP2D6*10 polymorphism was discovered in 356 Chinese women who were considered healthy and 286 Chinese women who had breast cancer. The 188C/C genotype was associated with a modestly increased risk of breast cancer as compared to the 188T/T genotype in women. Although the association did not achieve statistical significance, the 188T/T genotype was more strongly linked to an increased risk of breast cancer among postmenopausal women. In addition, the risk of having a positive lymph node status was greater in individuals with the 188T/T or T/C genotype compared to those with the 188C/C genotype. Based on our research, the 188T/T genotype is related to a slightly

increased risk of breast cancer (not statistically significant). Also, it's important to remember that 188C/C genotype patients tend to be less aggressive than 188T/T or T/C genotype patients. We need further studies to confirm our current findings, but it's possible that the link we saw between genotype and clinical outcome was just coincidental.²⁹

Preoperative tamoxifen treatment significantly reduced the Ki-67 labeling index in breast cancer tissues ($P = 0.00000000000000013$). Allred scores ($P = 0.0023$) and the fraction of estrogen receptor-positive cells in breast cancer tissues ($P = 0.0076$) were also significantly associated with Ki-67 response. When comparing two groups, one with one wild-type allele and the other without, CYP2D6 variants substantially correlated with Ki-67 response following preoperative tamoxifen treatment ($P = 0.018$), but they had no effect on pathologic response or hot flashes.³⁰ Genetic variations in GSTM1 and GSTT1 have been associated with an increased risk of breast cancer in Asians, according to meta-analyses. Research indicates that deletions of GSTM1 and GSTT1 could raise the risk of cancer in Caucasus, whereas in African Americans, Swedes, and Indians it could increase the risk of lung cancer. Southerners.³¹ Leukemogenesis likely has multiple initiating factors, although the exact mechanisms are largely unclear. Hereditary differences in GST expression and activity have been observed, and changes in GST enzymatic activity have been associated with several cancers.³² The impact of GST polymorphisms on B-ALL susceptibility and outcome is evaluated in this unique case-control study. Specifically, GSTO1 (rs4925: C>A) and GSTO2 (rs156697: A>G) are examined. Research takes place in northern India.³³ Heredity has a significant role in determining how an individual's metabolism and body respond to specific medications. The metabolism of various pharmacological drugs is facilitated by enzymes that belong to the cytochrome P450 family. Metabolized by CYP2D6 are a wide variety of medications, including those for pain, cancer, mental illness, certain cardiovascular symptoms, chorea, and Gaucher disease.³⁴ The CYP2D6 gene, which encodes this enzyme, is very variable. At last, our research showed that Egyptian women carrying GSTM1/null mutations had a 4.1-fold increased risk of breast cancer. Having the Cyp2d6 *2 allele increases a person's chance of getting breast cancer. Multiple environmental and behavioral factors, in addition to these hereditary ones, may increase the risk.

Conclusion

The results demonstrated that the risk of breast cancer was 4.1 times higher in Egyptian women with GSTM1/null mutations. Women who carry the Cyp2d6 *2 gene variant are more likely to develop breast cancer. A higher risk may result from a combination of these genetic factors plus environmental or lifestyle factors.

Declarations

Consent for publication

Not applicable.

Declaration of Generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technologies were used by the author(s) throughout the writing process of this work.

Availability of data and materials

All information gathered or processed throughout the course of this research is detailed in this final report.

Competing interests

The authors affirm that they are not involved in any conflicts of interest.

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