

## ARTICLE / INVESTIGACIÓN

# Association of glutathione S-transferase 1 (GSTP1) polymorphisms with Breast Cancer susceptibility

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**Abstract.** Hereditary and environmental variables have a role in the development of breast cancer. This study aimed to examine the links between genetic Variations in the GSTP1 gene and Predisposition to breast cancer in an Iraqi population. The research included 40 Iraqi female breast cancer patients and 20 healthy volunteers. GSTP1-1695 A/G gene polymorphisms were investigated using polymerase chain reaction in Real-time (RT-PCR). The results showed the GSTP1 frequency of the wild GG genotypes was showed significantly ( $P<0.01$ ) higher in healthy women in comparison with Breast cancer women (GG, 80% vs. 32.5%, respectively; furthermore, heterozygous AG genotypes were significantly higher in Breast cancer women in comparison with healthy women 42.5% vs. 20%, respectively at ( $P<0.01$ ). While the mutant AA genotype (25%) in patient women appeared significantly ( $P<0.01$ ) higher compared to healthy women (0.0%). Finally, we discovered a connection between GSTP1 polymorphisms and a higher chance of developing breast cancer in an Iraqi female population sample.

**Keywords:** glutathione S-transferase1, breast cancer, polymorphism.

## Introduction

Most cancers are attributable to both hereditary and environmental causes and have been confirmed by molecular epidemiological research. The most frequent type of cancer is breast cancer—widespread breast cancer globally and the most common cause of cancer-related mortality. Breast cancer, which accounts for 18% of all female cancers in developed and developing countries, is a significant public health concern in developed and developing countries.<sup>1, 2</sup>. The risk of cancer development has increased with the finding of genetic enzyme polymorphisms that participate in carcinogen metabolism<sup>3, 4, 5</sup>. Carcinogen-metabolizing enzymes participate in numerous chemical agents, including xenobiotics and sex hormones, which are activated and deactivated in this way.<sup>6,7</sup>. Step II metabolic enzymes belong to a superfamily. Multiple carcinogens' detoxification catalyzes glutathione for chemotherapeutic medicines, environmental pollutants, and carcinogenic and diverse xenobiological substances. GST variants that GST-mediated detoxification of carcinogenic compounds that have been intensively explored as possible breast cancer susceptibility genes is impaired.<sup>8, 9, 10, 11</sup>. On chromosome 11 (11q13), GSTP1 genes are located<sup>12</sup>. GSTP1 is mainly present in the heart, spleen, and lungs, and In breast tissue, GST is the most prevalent.<sup>9,13,14</sup>. A single point mutation in the GSTP1 gene causes

the replacement of Ile for Val at codon105, resulting in a change in enzyme function.<sup>5,13</sup>. As a result, this research aimed to see how GSTP1 polymorphisms affected breast cancer susceptibility in an Iraqi population sample.

## Materials and methods

The current research included 40 patients diagnosed with breast cancer at the clinic between March 2020 and July 2020 from Oncology Teaching Hospital in Medical City. Before beginning care, each patient provided pertinent details such as age, menopause, number of children, history of lactation and breast cancer family history. Twenty healthy female volunteers served as the control group. Written informed consent was given to each study participant.

**Specimen collection.** Following an overnight, Every subject enrolled in the study had 5 mL of blood drawn and collected in EDTA tubes for DNA extraction to determine GSTP1 genetic polymorphisms. Genomic DNA was extracted immediately according to the manufacturer's instructions using a standard DNA Extraction Kit (Dsbio, China). Using a NanoDrop ND-1000, the purity of DNA was determined by

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calculating the absorbance ratio at 260 and 280 nm (A260/A280), DNA concentration, and 260/280 ratios.

### Detection of GSTP1 polymorphism.

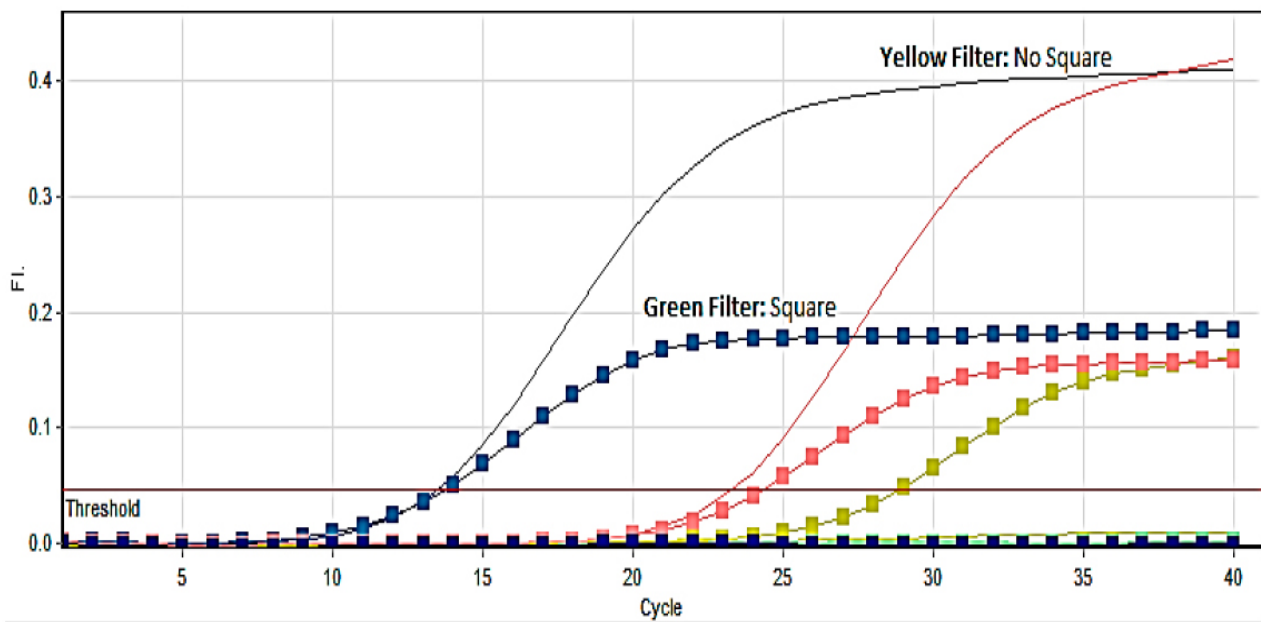
Genotypes of GSTP1-1695 A/G polymorphisms were determined by RT-PCR qPCR software and were used in conjunction with the QIAGEN Real-time PCR System ((Rotor-Gene Q, Germany). (allelic discrimination). The following primers for GSTP1-1695 A/G were used (designed using NCBI): forward primer: 5-CAGGGCTCTATGGGAAGGAC-3 and reverse primer: 5- CCCTTTCTTTGTTTCAGCCCC -3. Probe sequences for target GSTP1 gene. Dye Fam-BHQ: 5- ACATCTCCCTCATCTACACCAAC-3 and Dye Vic-BHQ: 5-ATACGTCTCCCTCATCTACACC-3 (Biosearch Technologies). Application program RT-PCR as follows: Hold 1 50 °C for 15min. Hold 2 94 °C for 15min. Denaturation 95 for 5 sec, annealing 60 °C for 20 sec, extension 72°C for 15 sec, (step one repeating for five cycles), then denaturation 95 °C for 5 sec., annealing 60 °C for 20 sec., finally extension 72 °C for 15 sec. (step two Repeating for 40 cycles). The component used in genotype RT-PCR for GSTP1 gene included forward primer 1µl, reverse primer 1µl, forward probe 1µl, reverse probe 1µl, probe master mix 10µl (WizPure™ qPCR Master (PROBE), South Korea, Dnase free water 3µl finally DNA 3µl. The total volume was 20 µl. The SPSS software was used to examine the impact of various variables on study parameters. To compare percentages, the Chi-square test was utilized in this study.

## Results

According to the results achieved by PCR, The GSTP1 frequency of the wild GG genotypes was shown significantly ( $P < 0.01$ ) higher in healthy women in comparison with Breast cancer women (GG, 80% vs. 32.5%, respectively; furthermore, heterozygous AG genotypes were significantly higher in Breast cancer women in comparison with healthy women 42.5% vs. 20%, respectively at ( $P < 0.01$ ). While the mutant AA genotype (25%) in patient women has appeared significantly ( $P < 0.01$ ) higher compared to healthy women (0.0%), however, there were no significant differences in wild GG, heterozygous AG and mutant AA genotypes in females patient. According to genetic models, the frequency of dominant AA+AG genotype was significantly ( $P < 0.01$ ) in female patients (67.5%) than in control (20%). However, the frequency of recessive GG+AG genotypes was significantly ( $P < 0.01$ ) higher in healthy women (100%) compared to breast cancer women (75%), while mutant AA genotype was more in patient women than control (25% and 0.0%) respectively, the frequency of additive 2AA+AG genotypes were significantly ( $P < 0.01$ ) increased in breast cancer female (92.5%) compared to healthy female (20%) (Figure 1 and Table 1).

Genotypes (GSTP1)	Patients (n=40)		Control (n=20)		Chi-Square value
	No.	%	No.	%	
GG	13	32.5%	16	80%	19.5**
AG	17	42.5%	4	20%	8.4**
AA	10	25%	0	0%	22.2**
Chi-Square value	---	4.9 NS		36**	---
<b>Genetic models</b>					
<b>Dominant</b>					
AA+AG	27	67.5%	4	20%	26.2**
<b>Recessive</b>					
GG+AG	30	75 %	20	100%	3.6**
AA	10	25%	0	0%	22.2**
Chi-Square value	---	26.2**		53.3	---
<b>Additive</b>					
2AA+AG	37	92.5%	4	20%	47.2**
<b>Allele</b>					
Minor allele frequency (MAF)	37	92.5%	4	10%	66.9**

**Table 1.** Distribution of genotype and allele frequencies of GSTP1 gene in patients and controls.



**Figure 1.** Determining GSTP1 gene polymorphism by RT-PCR using The Gentra Puregene Blood Kit ( Fam + Vic ) dyes.

## Discussion

Breast cancer (BC) is a condition affected by genetic and environmental factors. Twenty percent to twenty-five percent of all BC cases are caused by genes linked to susceptibility to the disease<sup>15</sup>. GSTs, which are involved in the cell's detoxification process, contain a common gene (GSTP1) related to the progression of BC<sup>16</sup>. In this research, we looked at the connection between GSTP1 genotypes with the risk of breast cancer in a group of Iraqi women. A substantial relationship between GSTP1 polymorphisms and the risk of breast cancer has been identified in the current investigation. Our findings agreed with Sergentanis and Economopoulos<sup>17</sup>, who discovered that GSTP1 polymorphisms were correlated with a higher incidence of breast cancer in Chinese people but disagreed with many authors<sup>8, 18, 19, 20, 21, 13</sup>, who observed that Genes were involved in the process of detoxification thought to be potential candidate genes for breast and another cancer susceptibility. GSTs are a group of enzymes involved in detoxifying a wide range of xenobiotics, including environmental carcinogens, hormones, and reactive oxygen species. Different liver functions and target tissue metabolizing enzymes may trigger individual differences in the metabolism of carcinogens. These differences could lead to varying vulnerabilities as breast cancer progresses. Increased oxidative stress and inflammation have long been linked to a high cancer risk<sup>22</sup>. By conjugating glutathione, GSTs help to detoxify a variety of toxic xenobiotics.<sup>23</sup> The majority of GST substrates are electrophiles. Products of oxidative stress (i.e., oxygen species that are reactive.) (24) Cells that lack GSTP1 activity are more vulnerable to oxidative DNA damage. Tumors are prone to relevant genetic alterations during breast carcinogenesis as a result of the formation of DNA base adducts<sup>14, 25</sup>. While some studies have looked into the connection between GST polymorphism and the risk of breast cancer, the link is still ambiguous and hotly debated.

## Conclusions

This may be the first research to look at the relationship between GSTP1 polymorphisms and breast cancer risk in an Iraqi population. More research with larger samples is required to confirm our findings. Finally, This study discovered a connection between GSTP1 polymorphisms and an increased risk of breast cancer in an Iraqi female population.

### Conflict of interest

No conflict of interest

### Acknowledgment

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