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"Quantitative Profiling of GSTP1 Promoter Methylation in Breast Cancer Patients: Evidence from Central India"

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Abstract: Background: The Glutathione S-transferase Pi 1 (GSTP1) gene encodes an important detoxification enzyme involved in cellular defense against oxidative stress and carcinogenic compounds. Epigenetic silencing of GSTP1 through promoter hypermethylation has been widely reported in cancers such as prostate and liver cancer; however, its significance and role in breast cancer remain controversial and are still under investigation.**Objective:** To evaluate the role of GSTP1 gene promoter methylation in breast cancer patients. **Methods:** Using a cross-sectional case-control design, we analyzed tissue samples from 60 histologically confirmed breast cancer patients and 60 healthy controls. Genomic DNA was extracted, bisulfite-converted, and analyzed using Methylation-Specific PCR (MSP) and Pyrosequencing technology. **Results:** The mean GSTP1 methylation level was 45.24% (SD ± 25.09) in cancer cases and 51.93% (SD ± 29.39) in controls. The difference was not statistically significant (p = 0.248). **Conclusion:** GSTP1 methylation levels did not significantly differ between cancerous and benign tissues in this study. The high variability and lack of specific hypermethylation in cases suggest that GSTP1 may not serve as a universal standalone biomarker for breast cancer in this specific population.

Keywords: *Breast cancer, GSTP1, Glutathione S-transferase Pi 1, DNA promoter methylation, Epigenetics, Methylation-specific PCR (MSP), Pyrosequencing, Biomarker, Case-control study..*

1. Introduction

Breast cancer is a major public health concern and is currently the third most commonly diagnosed cancer worldwide, accounting for more than 20% of all cancers reported among women.[1]. In India, the disease trajectory is particularly concerning; it is rapidly becoming the most prevalent cancer among women, with incidence peaking between the ages of 50 and 64 [2, 3, 4]. While traditional models of carcinogenesis focused largely on genetic mutations, contemporary research has pivoted toward the "epigenetic landscape" to understand how tumors develop and progress [5,6,7].

Epigenetics refers to heritable changes in gene function that occur without altering the DNA sequence. Among these mechanisms, DNA methylation is the most stable and well-characterized. It typically involves the addition of a methyl group to the cytosine base within CpG dinucleotides. When this occurs in the promoter regions of tumor suppressor genes, it can act as a "switch," effectively silencing genes that are crucial for preventing uncontrolled cell growth [8,9,10].

One such critical guardian of the genome is the Glutathione S-transferase Pi 1 (GSTP1) gene, located on chromosome 11q13 [11]. The GSTP1 enzyme acts as a cellular housekeeper; it is part of the Phase II detoxification family, responsible for neutralizing electrophilic carcinogens and combating oxidative stress by conjugating glutathione to toxic compounds [12,13]. By detoxifying harmful substances before they can damage DNA, GSTP1 serves as a frontline defense against malignant transformation.

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In normal cells, the CpG-rich promoter region of GSTP1 remains unmethylated, ensuring the gene is active and the cell is protected. However, in several cancers—including liver, prostate, and breast cancer—this region becomes abnormally hypermethylated, leading to transcriptional silencing [14]. This phenomenon is so prevalent in prostate cancer that GSTP1 methylation is often considered a standard diagnostic hallmark. However, in the context of breast cancer, the picture is less clear. While some studies suggest GSTP1 silencing contributes to disease onset by allowing DNA damage to accumulate, findings have been inconsistent across different ethnic populations and tumor subtypes [15, 16, 17].

This study aims to bridge that knowledge gap by quantifying GSTP1 promoter methylation in a cohort of North Indian breast cancer patients. By comparing these levels with healthy controls, we seek to determine if GSTP1 epigenetic silencing is a driver of breast tumorigenesis in this specific population and whether it holds potential as a diagnostic biomarker.

2. Methodology

2.1 Study Design and Population A cross-sectional study was carried out in the Department of Biochemistry and the Department of Surgery at Index Medical College. The study comprised 120 participants, including 60 women with histopathologically confirmed breast cancer (cases) and 60 healthy women with benign breast conditions serving as controls. The age of the participants ranged from 25 to 65 years. Women with a history of other malignancies, chronic illnesses such as tuberculosis or AIDS, or recent steroid therapy were excluded from the study.

2.2 Sample Collection Tissue samples were collected from tumor specimens during mastectomy for cases and appropriate surgical procedures for controls. All fresh tissue samples were immediately stored in normal saline at -80°C to prevent degradation. Venous blood samples were also drawn under aseptic conditions for baseline comparisons .

2.3 Genomic DNA Extraction Genomic DNA (gDNA) was extracted from approximately 25 mg of tissue using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Tissue samples were lysed with Proteinase K at 56°C, followed by ethanol precipitation and spin-column purification. DNA concentration and purity were verified using a NanoDrop spectrophotometer, with an A260/A280 ratio of 1.8–2.0 considered acceptable .

2.4 Bisulfite Conversion To differentiate methylated from unmethylated cytosines, gDNA underwent bisulfite conversion using a Qiagen kit. This process deaminates unmethylated cytosine to uracil while leaving methylated cytosines unchanged. The reaction mixture and thermal cycling conditions are detailed in **Table 1** and **Table 2** .

Table 1: Bisulfite Conversion Reaction Mixture

Component	Volume for High Conc. DNA (1 ng - 2 µg)	Volume for Low Conc. DNA (1 - 500 ng)
DNA Solution	20 µl	40 µl
Bisulfite Mix	85 µl	85 µl
DNA Protect Buffer	35 µl	15 µl
Total Volume	140 µl	140 µl

Table 2: Thermal Cycler Program for Bisulfite Conversion

Step	Temperature	Time
Denaturation	95°C	5 min
Incubation	60°C	25 min
Denaturation	95°C	5 min
Incubation	60°C	85 min
Denaturation	95°C	5 min
Incubation	60°C	175 min
Hold	20°C	Indefinite

2.5 Methylation-Specific PCR (MSP) and Pyrosequencing Target regions of the GSTP1 gene were amplified using specific methylation primers. The PCR was performed in a total volume of 25 µl using GoTaq® Green Master Mix. The thermal cycling conditions included an annealing temperature of 58.7°C, as shown in **Table 3**. Quantitative methylation analysis was subsequently performed using the PyroMark Q48 Autoprep system (Qiagen) to determine the exact percentage of methylation at individual CpG sites.

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Table 3: PCR Thermal Cycling Conditions for GSTP1

Step	Temperature	Time
Initial Denaturation	95°C	3 min
Denaturation	95°C	30 sec
Annealing	**58.7°C**	30 sec
Extension	72°C	1 min
Final Elongation	72°C	3 min

2.6 Statistical Analysis Data were analyzed using the Mann–Whitney U test to compare methylation levels between the study groups. A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Demographic and Clinical Profile The mean age of breast cancer patients was 46.58 years, whereas the control group had a mean age of 43.95 years [Figure 1]. Among the cancer patients, 57% of tumors were left-sided and 43% were right-sided. Histopathological analysis revealed that 41.66% cases were infiltrating ductal carcinoma, 36.66% invasive ductal carcinoma, 13.33% cystosarcoma phyllodes, and 8.33% sarcoma of the breast. Based on histological grading, 26.67% of cases were classified as grade I, 55% as grade II, and 18.33% as grade III [Figures 2, 3, and 4].

3.2 GSTP1 Methylation Analysis Quantitative analysis of the GSTP1 promoter region yielded unexpected results. The breast cancer group exhibited a mean methylation level of **45.24%** (SD ± 25.09). In comparison, the control group showed a slightly higher mean methylation of **51.93%** (SD ± 29.39).

Statistical Significance A Mann–Whitney U test was performed to compare methylation levels between cases and controls. The analysis showed no statistically significant difference between the groups, with a p-value of 0.248. This finding suggests that GSTP1 promoter methylation levels were comparable between the two study groups and were not significantly associated with breast cancer in the present study.

Table 4: Comparative Mean Methylation of GSTP1 Gene

Group	Sample Size (n)	Mean Methylation (%)	Standard Deviation (SD)	p-value
Breast Cancer Cases	60	45.24	25.09	0.248
Healthy Controls	60	51.93	29.39	

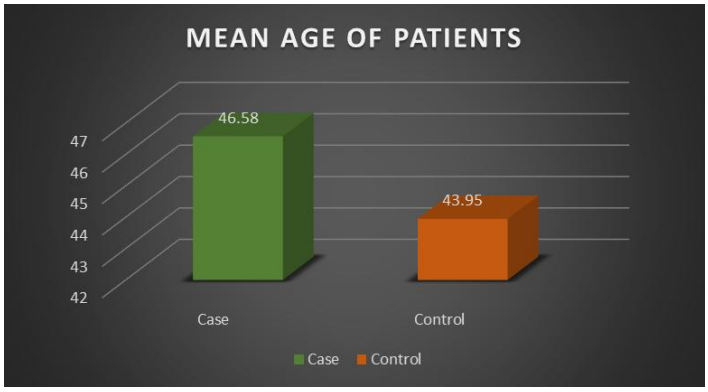

Figure 1: Mean Age of Study Population

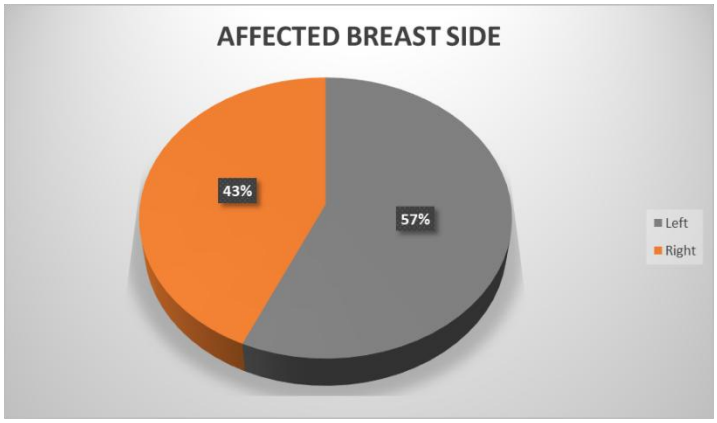

Figure 2: Affected Breast Side in Cancer Patients (Pie Chart)

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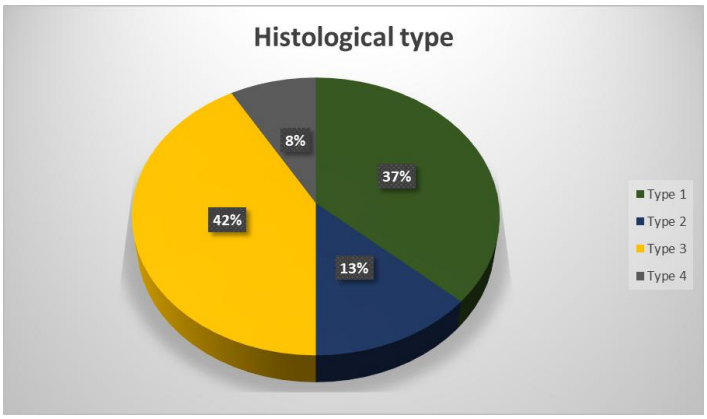

Figure 3: Histological Type 1= ductal invasive carcinoma, 2= cystosarcoma phyllodes, 3= infiltrating ductal carcinoma, 4= sarcoma of the breast

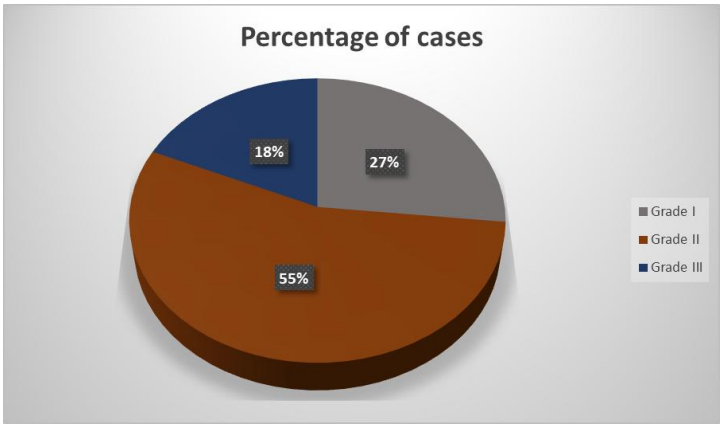

Figure 4: Histological Grading of Tumors

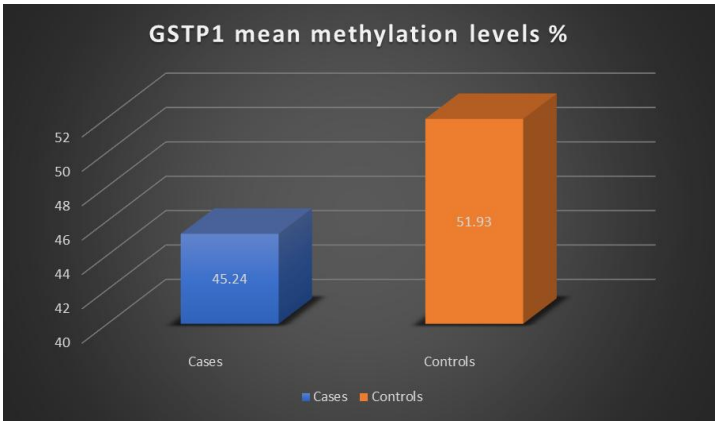

Figure 5: Mean GSTP1 Methylation level in each group

4. Discussion

The detoxification of carcinogens is a fundamental cellular defense mechanism, and the Glutathione S-transferase Pi 1 (GSTP1) gene plays a pivotal role in this process by neutralizing electrophilic compounds and combating oxidative stress. When this gene is silenced via promoter hypermethylation, the cellular defense is compromised, theoretically leaving the genome vulnerable to damage and subsequent malignant transformation. This study investigated the methylation status of the GSTP1 promoter in breast cancer patients from North India, yielding results that offer a distinct perspective compared to much of the existing global literature.

Contrary to the expectation that tumor suppressor genes are hypermethylated in cancer, our analysis did not reveal a statistically significant difference in GSTP1 methylation between breast cancer patients and healthy controls. In fact, the control group exhibited a slightly higher mean methylation level (51.93%) compared to the cancer group (45.24%). These findings diverge significantly from pivotal studies, such as those by Esteller et al., who identified GSTP1 promoter methylation in over 60% of breast cancer tissues and established it as a common mechanism for gene silencing [18]. Similarly, a meta-analysis involving 19 case-control studies by Fang et al. confirmed a strong association between GSTP1 hypermethylation and increased breast cancer risk [19]. The discrepancy between our findings and these established patterns highlights the complexity of epigenetic biomarkers and suggests that GSTP1 methylation may not be a universal driver of tumorigenesis across all populations.

Several factors may account for the lack of significant hypermethylation observed in our cohort. First, breast cancer is a highly heterogeneous disease comprising distinct molecular subtypes, such as Luminal A, Luminal B, HER2-enriched, and Triple-Negative Breast Cancer (TNBC). Research by Mokhtar et al. has demonstrated that GSTP1 methylation is significantly more frequent in TNBC cases compared to other subtypes and often correlates with higher tumor grades [20]. Since our study did not stratify patients by molecular subtype, it is plausible that the high-methylation

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signal from specific aggressive subsets was diluted by a larger proportion of tumors that do not harbor this specific epigenetic defect. Additionally, epigenetic patterns are known to be influenced by environmental factors and ancestry.

Furthermore, the observation of relatively high methylation levels in the control group is intriguing. A study by Alhayali et al. conducted in Iraq in 2025 reported that alterations in GSTP1 were associated with increased breast cancer susceptibility due to impaired detoxification capacity. These findings partially support the present study, although GSTP1 methylation was not statistically significant in the current investigation. [21]. Similarly, Danos et al. in Peru in 2023 reported significant GSTP1 promoter hypermethylation in plasma cell-free DNA of breast cancer patients and suggested its potential role as a minimally invasive biomarker for breast cancer detection, although their findings differed from the present study.[22]

5. Conclusion

In conclusion, while GSTP1 hypermethylation is a well-validated biomarker in cancers such as prostate cancer, our data suggests it may not serve as a robust, standalone diagnostic marker for breast cancer in the general North Indian population. The lack of statistical significance in our study cautions against the generalization of epigenetic biomarkers without local validation. Future research should focus on larger, well-stratified cohorts that account for molecular subtypes and environmental exposures to definitively determine the clinical utility of GSTP1 methylation in this region.

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Conflict of Interest

The author declares that there is **no conflict of interest** related to this study.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Ethics Committee of the participating institution before the commencement of the study. All study procedures were conducted in accordance with the approved ethical guidelines.

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