# Significance of GSTP1 Protein Expression in Invasive Ductal Carcinoma of Breast

[a]Mandalia Toral[1], [b]Gajjar Kinjal[1], Ghosh Nandita[2]
Research Assistant[1], Associate Professor[2]
Tumor Biology Lab, Cancer Biology Department
The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, Gujarat, India
Corresponding Author: nandita.ghosh@gcriindia.org,
[a]https://orcid.org/0000-0002-3495-1600
[b]https://orcid.org/0000-0002-1126-0217
https://orcid.org/0000-0002-0210-7317

**Summary**

Glutathione S-transferases (GSTs) are important isoenzymes that play an essential role in detoxification of carcinogens and acts as endogenous inhibitor of MAP kinase pathway. GST Pi 1 (GSTP1) isoform has been documented to contribute to drug resistance in breast cancer patients. Hence, present study aimed to investigate the prevalence of GSTP1 protein expression in breast cancer patients by immunohistochemistry method and further to examine its correlation with various clinicopathological parameters. Total 70 untreated patients with invasive ductal carcinoma of breast cancer (70 tumor tissues and 30 adjacent normal tissues) were included in the study. Statistical analysis was carried out using SPSS software. The results indicated that- cytoplasmic and/or nuclear GSTP1 immunoreactivity was observed in 76% tumors and 97% adjacent normal tissues of the breast cancer patients. Significant higher GSTP1 protein expression was observed in high BR score tumors (78%; P=0.007), ER-ve patients (68%; P=0.008), TNBC patients (78%; P=0.004) and patients having absence of perinodal extension (56%; P=0.050) as compared to their respective counter parts. Hence, there is loss of GSTP1 protective function during the transition of malignant transformation. Higher GSTP1 expression is associated with aggressive prognosticators of breast cancer. However, confirmation in larger set of patients and longer follow up details is needed to evaluate the potential of GSTP1 as a prognostic marker.

**Keywords:** GSTP1, breast cancer, immunohistochemistry, TNBC, Glutathione S-transferases

## Introduction

Epidemiological studies suggest that breast cancer is the most common type of cancer among women with continuous prevalence throughout the world.[1] Although its incidence is not the same in different countries and ethnic groups, breast cancer has become a significant public health challenge among women worldwide.[2,3] It is a multifactorial and polygenic disease which may be influenced by both environmental and genetic factors.[4,5] Although there are several comprehensive treatment options, such as surgery, chemotherapy, and endocrine therapy, many patients still have high rates of metastasis and recurrence, which remain the primary cause of death in patients with breast cancer.[6] Patients with triple negative breast cancer (TNBC) account for about 15–20% of total breast cancer cases, which have higher rates of metastasis and recurrence, and lower survival rates compared to other subtypes because these patients do not receive anti-receptor therapy. Therefore, other potential prognostic markers and new therapeutic targets for BC should be explored.[7] In recent years, some genes have been confirmed as potential cancer susceptible genes. Glutathione S-transferases (GSTs) are overwhelmingly important genes, which play key role in the detoxification of toxic, potentially carcinogenic compounds, and a host of basic physiological processes of the human body.[8–11] They are a super family of dimeric phase-II metabolic enzymes that have an irreplaceable role in the cellular defense system.[12,13] In human, classes of GST enzymes include alpha-α, mu-μ, pi-π, sigma-σ, omega- Ω and theta-θ.[14] Louie S M. found that GST Pi 1 (GSTP1) was a new breast cancer oncogene that governed the pathogenicity of cancer by regulating glycolysis, and energy and fat metabolism.[15] Although some reports had shown the association between GSTs and overall survival in breast cancer patients, the results were not consistent.[16-19] Therefore, the aim of the present study was to investigate the relationship between the GSTP1protein expression and the clinicopathological characteristics of breast cancer patients.

## Materials and Methods

### Patients

Seventy untreated and histopathologically confirmed invasive ductal breast carcinoma female patients diagnosed at Gujarat Cancer & Research Institute (GCRI) were included in this retrospective study. The study was approved by Institutional Scientific and Ethical Committees and informed consent was obtained from all subjects prior to treatment administration. Detailed clinical and pathological history i.e. age, menopause status, tumor size, diseases stage, histological grade, treatment given, disease status, were obtained from the case files maintained at the Medical Record Department of the institute.

**Immunohistochemistry (IHC)**

Three to five micron thick sections were cut from the formalin fixed paraffin embedded tissue blocks of IDC patients using Leica microtome and mounted on APES coated glass slides. The protein expression of GSTP1 was studied by immunohistochemistry technique using HRP/DAB (ABC) detection IHC kit (Abcam). The instructions in the kit insert were followed for carrying out the procedure. Mouse monoclonal GSTP1 primary antibody (Cat#sc-66000, Santa Cruz) was used at 1:100 dilution. Antigenicity was retrieved by heating the sections in 10 mM sodium citrate buffer (pH, 6.0) for 15-20 minutes in a pressure cooker. The specific immune reaction was identified using 3,3′-Diaminobenzidine (DAB) chromogen and the sections were counterstained with haematoxylin. Finally, the stained sections were mounted with DPX and observed under a light microscope (Nikon, Japan).

**Scoring by Modified H- Score method**

Scoring of the immunohistochemically stained sections was done by independently by two individual observers in a blinded manner. Semi quantitative H-score method based on staining positivity and staining intensity was used. The staining intensity was graded on a four-point scale from 0-3 (0- No staining, 1- weak staining intensity, 2-moderate staining intensity and 3- strong staining intensity). The percentage positivity of stained tumor cells (0-100%) was counted by 10% intervals. Final histoscore was calculated by multiplying the staining intensity and the staining positivity resulting in a range from 0 to 300.

**Statistical analysis**

The data was analysed using Statistical package for Social Sciences-SPSS software (SPSS Inc. version 20). Two-tailed chi square test and Spearman's correlation was used to determine the correlation between the GSTP1 protein expression and various clinicopathological parameters of breast cancer patients. P values ≤0.05 were considered to be significant.

**Results**

The detailed clinicopathological characteristics of total 70 histologically confirmed breast cancer patients with invasive ductal carcinoma are shown in Table 1.

**Incidence of GSTP1 protein expression in primary tumors and adjacent normal tissue of patients with breast cancer:**

Immunostaining pattern of GSTP1 expression in primary breast tumor cells was found to

**Table 1:** Patient and Tumor characteristics of Invasive Ductal Breast Carcinoma patients

| Variables | | N | Percentage (%) |
|---|---|---|---|
| Age (Range:33-85 years) (Medianage:50 years) | <50 | 38 | 54 |
| | >50 | 32 | 46 |
| Family History | Absent | 60 | 86 |
| | Present | 10 | 14 |
| Site | Left | 34 | 49 |
| | Right | 35 | 50 |
| | Bilateral | 1 | 1 |
| Menopausal Status | Pre-Menopausal | 18 | 26 |
| | Post-Menopausal | 52 | 74 |
| Histological Type | Invasive Ductal Carcinoma | 70 | 100 |
| | Invasive Lobular Carcinoma | 0 | 0 |
| | Paget's Diseases | 0 | 0 |
| BR Score | Score-3-5 | 9 | 13 |
| | Score-6-7 | 43 | 61 |
| | Score-8-9 | 18 | 26 |
| | Unknown | 0 | 0 |
| | Grade2 | 43 | 61 |
| | Grade3 | 18 | 26 |
| | Unknown | 0 | 0 |
| Tumor Size | T1 | 13 | 18 |
| | T2 | 55 | 79 |
| | T3 | 2 | 3 |
| | T4 | 0 | 0 |
| Lymphnode Involvement | N0 | 28 | 40 |
| | N1 | 21 | 30 |
| | N2 | 14 | 20 |
| | N3 | 7 | 10 |
| Metastasis | M0 | 70 | 100 |
| | M1 | 0 | 0 |
| Stage | I | 7 | 10 |
| | II | 40 | 57 |
| | III | 23 | 33 |
| | IV | 0 | 0 |
| Stromal Response | Positive | 28 | 40 |
| | Negative | 42 | 60 |
| ER Status | Positive | 42 | 60 |
| | Negative | 28 | 40 |
| PR Status | Positive | 25 | 36 |
| | Negative | 45 | 64 |
| Her2 neu Status | Positive | 21 | 30 |
| | Negative | 49 | 70 |

| | | | |
|---|---|---|---|
| Molecular Subtype | LuminalA | 31 | 44 |
| | LuminalB | 11 | 16 |
| | Her2amplification | 10 | 14 |
| | TNBC | 18 | 26 |
| Lymphatic Permeation | Positive | 30 | 43 |
| | Negative | 40 | 57 |
| Vascular Permeation | Positive | 11 | 16 |
| | Negative | 59 | 84 |
| Perineural Invasion | Positive | 7 | 10 |
| | Negative | 63 | 90 |
| Perinodal Extension | Positive | 20 | 29 |
| | Negative | 50 | 71 |
| Necrosis | Positive | 16 | 23 |
| | Negative | 54 | 77 |
| Elastosis | Positive | 4 | 6 |
| | Negative | 66 | 94 |
| Treatment | Surgery | 4 | 6 |
| | S+CT | 16 | 22 |
| | S+RT | 2 | 3 |
| | S+HT | 4 | 6 |
| | S+CT+HT | 10 | 14 |
| | S+RT+HT | 2 | 3 |
| | S+CT+RT | 9 | 13 |
| | S+CT+RT+HT | 23 | 33 |
| Recurrence | Presence | 1 | 1 |
| | Absence | 69 | 99 |
| Survival | Died | 1 | 1 |
| | Alive | 69 | 99 |

be heterogeneous and cytoplasmic and/or nuclear. GSTP1 immunoreactivity was detected in 76% (53/70) patients, while only 24% (17/70) of patient were negative for GSTP1 expression. The staining intensity was observed to be 28% (19/70) of +1, 24% (17/70) of +2 and 24% (17/70) of +3. The median H-score for GSTP1 immunoreactivity was 40 (Range 0 to 300) and this was used as a cut-off value to subgroup the patients into low (<40) and high (≥40) expression groups. Accordingly, 51% (36/70) patients displayed low (<40) and 49% (34/70) displayed high (>40) GSTP1 protein expression. (Table 2)

In adjacent normal tissues the staining pattern of GSTP1 expression was intensely nuclear or/and cytoplasmic distributed throughout the epithelium. No membranous staining of GSTP1 was seen. Further, positive GSTP1 immunoreactivity in adjacent normal tissue was observed in 97% (29/30), with staining intensity of +1 in 27% (8/30), +2 in 30% (9/30) and +3 in 40% (12/30) in breast cancer patients (Table 2). The median H-score for immunoreactivity in adjacent normal adjacent tissue was 100 (Range 40 to 300).

**Table 2:** Incidence of GSTP1 immunoreactivity in primary tumors and adjacent normal tissues of breast cancer patients

| GSTP1protein expression | Primary tumors (N=70) | | Adjacent normal tissues (N=30) | |
|---|---|---|---|---|
| | N | % | N | % |
| Negative | 17 | 24 | 1 | 3 |
| Positive | 53 | 76 | 29 | 97 |
| +1 | 19 | 28 | 8 | 27 |
| +2 | 17 | 24 | 9 | 30 |
| +3 | 17 | 24 | 12 | 40 |
| Median H-score (Range) | 40 (0 to 300) | | 100 (40 -300) | |
| <Medianscore | 36 | 51 | 16 | 53 |
| >Medianscore | 34 | 49 | 14 | 47 |

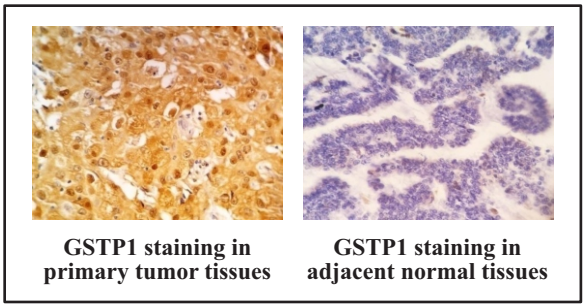


**Figure 1:** Representative photomicrographs of GSTP1 staining in primary tumors and adjacent normal tissue of breast cancer

This was used as a cut-off value to stratify the patients into low (<100) and high (≥100) expression group. Accordingly, 53% (16/30) patients displayed low (<100) and 47% (14/30) displayed high (>100) GSTP1 protein expression. (Table 2). Figure 1 shows the representative photomicrographs of GSTP1 immunoreactivity in primary tumor tissue and adjacent normal tissues.

**Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with clinical factors:**

A trend of decreased GSTP1 expression in both, the tumor ($\chi 2=2.890$, r=-0.203, P=0.091) and adjacent normal tissues ($\chi 2=3.210$, r=-0.320, P=0.070) was observed with increase in age of breast cancer patients. Similarly, a trend towards low GSTP1 protein expression was observed in primary tumor ($\chi 2 =3.170$, r=-0.210, P=0.070) and adjacent normal tissues ($\chi 2=3.51$, r=-0.342, P=0.064) in patients with post menopausal as compared to pre menopausal breast cancer patients. On the other hand, no significant difference was observed in the GSTP1 expression between the left and right sided breast tumor or adjacent normal tissues. (Table 3)

**Table 3:** Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with clinical factors of patients with breast cancer

| | Primary Tumor N=70 | | Adjacent Normal Tissue N=30 | |
|---|---|---|---|---|
| | GSTP1 Protein | | GSTP1 Protein | |
| | Low-expression N(%) | High-expression N(%) | Low-expression N(%) | High-expression N(%) |
| Age(years) | | | | |
| ≤50 | 16(42) | 22(58) | 4(33) | 8(67) |
| >50 | 20(62) | 12(38) | 12(67) | 6(33) |
| | $\chi2$=2.890,r=-0.203, P=0.091 | | $\chi2$=3.210,r=-0.320, P=0.070 | |
| Menopausal status | | | | |
| Pre | 6(33) | 12(67) | 2(25) | 6(75) |
| Post | 30(58) | 22(42) | 14(64) | 8(36) |
| | $\chi2$=3.170,r=-0.210, P=0.070 | | $\chi2$=3.510,r=-0.342, P=0.064 | |
| Site | | | | |
| Left | 17(50) | 17(50) | 9(56) | 7(44) |
| Right | 19(51) | 17(49) | 7(50) | 7(50) |
| | $\chi2$=0.972,r=-0.053, P=0.734 | | $\chi2$=0.110,r=+0.063, P=0.743 | |

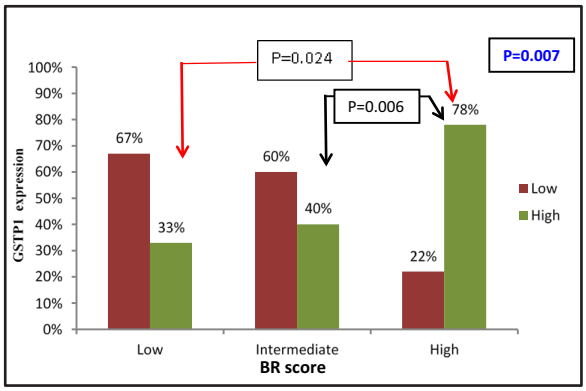


**Figure 2:** Correlation of GSTP1 expression in primary tumor with BR score

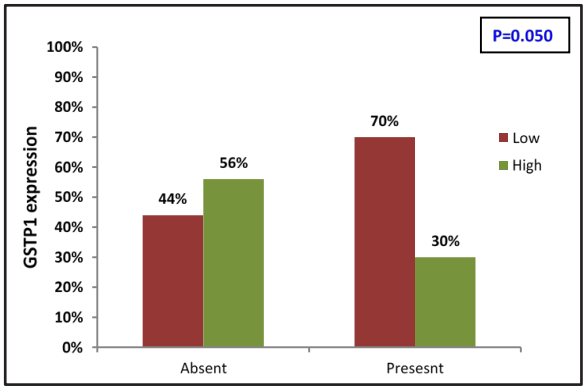


**Figure 3:** Correlation of GSTP1 expression in primary tumor with perinodal extension

**Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with pathological characteristics**

When correlated with the pathological parameters, in primary tumors GSTP1 expression showed a significant positive correlation with increasing BR score. Furthermore, it was observed that GSTP1 expression was significantly higher in patients with high BR score (78%) as compared to low BR score (33%; $\chi2$=5.082, r=+0.434, P=0.024) and intermediate BR score (40%; $\chi2$=7.425, r=+0.349, P=0.006). (Table 4; Figure 2). Moreover, its expression significantly decreased in patients with perinodal extension ($\chi2$=3.866, r=-0.235, P=0.050) indicating an inverse correlation of GSTP1 with perinodal extension of tumor. (Table 4; Figure 3). Apart from this, GSTP1 expression did not show any significant correlation with any of the pathological parameters in primary tumors or the adjacent normal tissues.

**Table 4:** Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with pathological characteristics of patients with breast cancer

| | **Primary Tumor N = 70** | | **Adjacent Normal Tissue N = 30** | |
|---|---|---|---|---|
| | **GSTP1 Protein** | | **GSTP1 Protein** | |
| | **Low-expression N (%)** | **High-expression N (%)** | **Low-expression N (%)** | **High-expression N (%)** |
| **Tumor Size** | | | | |
| T1 | 6(46) | 7(54) | 3(60) | 2(40) |
| T2 | 30(56) | 24(44) | 12(52) | 11(48) |
| T3 | 0(0) | 3(100) | 1(50) | 1(50) |
| | $\chi2$=3.68, r=+0.054, P=0.738 | | $\chi2$=0.111, r=+0.057, P=0.763 | |
| **Nodal Status** | | | | |
| N0 | 14(50) | 14(50) | 6(37) | 10(63) |
| N1 | 8(40) | 12(60) | 4(68) | 2(32) |
| N2 | 10(67) | 5(33) | 4(80) | 1(20) |
| N3 | 4(57) | 3(43) | 2(67) | 1(33) |
| | $\chi2$=2.55, r=-0.090, P=0.450 | | $\chi2$=3.683, r=-0.300, P=0.760 | |
| **Stage** | | | | |
| I | 4(57) | 3(43) | 2(50) | 2(50) |
| II | 18(45) | 22(55) | 8(44) | 10(56) |
| III | 14(61) | 9(39) | 6(75) | 2(25) |
| | $\chi2$=1.574, r=-0.095, P=0.436 | | $\chi2$=2.098, r=-0.212, P=0.261 | |
| Early | 22(47) | 25(53) | 10(45) | 12(55) |
| Advanced | 14(61) | 9(39) | 6(75) | 2(25) |
| | $\chi2$=1.22, r=-0.130, P=0.276 | | $\chi2$=2.058, r=-0.262, P=0.162 | |

| **BR Score** | | | | |
|---|---|---|---|---|
| Low (BR3-BR5) | 6(67) | 3(33) | 1(50) | 1(50) |
| Intermediate (BR6 - BR7) | 26(60) | 17(40) | 10(59) | 7(41) |
| High (BR8-BR9) | 4(22) | 14(78) | 5(45) | 6(55) |
| Overall | $\chi2$=8.38, r=+0.310, P=0.007 | | $\chi2$=0.480, r=+0.090, P=0.595 | |
| Low vs High | $\chi2$=5.082, r=+0.434, P=0.024 | | | |
| Intermediate vs High | $\chi2$=7.425, r=+0.349, P=0.006 | | | |
| **Lymphatic Permeation** | | | | |
| Absent | 20(50) | 20(50) | 9(50) | 9(50) |
| Present | 16(53) | 14(47) | 7(58) | 5(42) |
| | $\chi2$=0.076, r=-0.033, P=0.786 | | $\chi2$=0.201, r=-0.082, P=0.667 | |
| **Vascular Permeation** | | | | |
| Absent | 31(53) | 28(47) | 14(50) | 14(50) |
| Present | 5(46) | 6(54) | 2(100) | 0(0) |
| | $\chi2$ =0.186, r=+0.052, P=0.671 | | $\chi2$=1.87, r=-0.250, P=0.183 | |
| **Perineural Invasion** | | | | |
| Absent | 31(49) | 32(51) | 14(54) | 12(46) |
| Present | 5(71) | 2(29) | 2(50) | 2(50) |
| | $\chi2$=1.245, r=-0.133, P=0.271 | | $\chi2$=0.021, r=+0.026, P=0.891 | |
| **Perinodal Extension** | | | | |
| Absent | 22(44) | 28(56) | 11(50) | 11(50) |
| Present | 14(70) | 6(30) | 5(63) | 3(37) |
| | $\chi2$=3.866, r=-0.235, P=0.050 | | $\chi2$=0.368, r=-0.111, P=0.560 | |
| Elastosis | | | | |
| Absent | 33(50) | 33(50) | 16(55) | 13(45) |
| Present | 3(75) | 1(25) | 0(0) | 1(100) |
| | $\chi2$=0.944, r=-0.116, P=0.338 | | $\chi2$=1.180, r=+0.199, P=0.293 | |
| **Necrosis** | | | | |
| Absent | 28(52) | 26(48) | 12(55) | 10(45) |
| Present | 8(50) | 8(50) | 4(50) | 4(50) |
| | $\chi2$=0.017, r=+0.016, P=0.898 | | $\chi2$=0.049, r=+0.040, P=0.833 | |
| **Stromal Response** | | | | |
| Absent | 20(48) | 22(52) | 12(48) | 13(52) |
| Present | 16(57) | 12(43) | 4(80) | 1(20) |
| | $\chi2$=6.100, r=-0.093, P=0.442 | | $\chi2$=1.714, r=-0.239, P=0.203 | |

### Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with surface receptors and molecular subtypes

The ER–ve patients and TNBC positive patients showed significantly higher GSTP1 expression in the primary tumors than ER+ve patients ($\chi2$=6.940, r=-0.315, P=0.008) (Figure 4) and TNBC

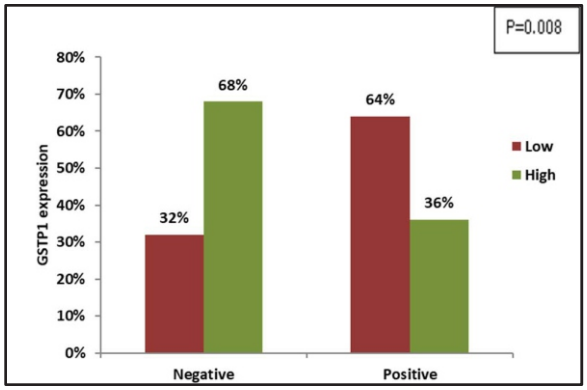


**Figure 4:** Correlation of tumoral GSTP1 expression with ER status

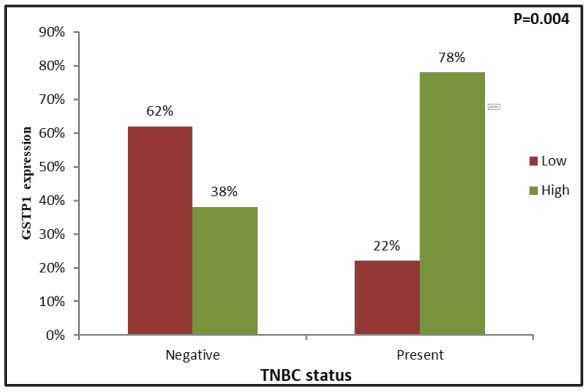


**Figure 5:** Correlation of tumoral GSTP1 expression with TNBC status

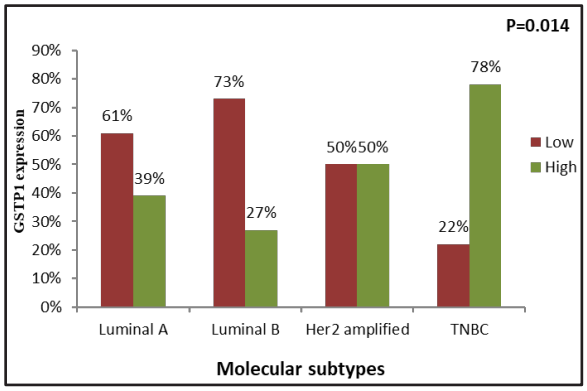


**Figure 6:** Correlation of tumoral GSTP1 expression with molecular subtype

negative patients ($\chi2$=8.274, r=+0.344, P=0.004) (Figure 5), respectively. According to molecular subtypes, GSTP1 protein expression was significantly higher in breast cancer patients with TNBC (78%), followed by Her-2 (50%), Luminal A (39%) and Luminal B (27%) ($\chi2$=9.359, r=+0.292, P=0.014) (Figure 6) (Table 5).

### Comparison of GSTP1 protein expression according to ERPR status in breast cancer patients

As depicted in Table 6, patients when sub grouped according to surface receptor i.e. ERPR

**Table 5:** Correlation of GSTP1 protein expression in tumor and adjacent normal tissues with surface receptors and molecular subtypes in patients with breast cancer

| | Primary Tumor N = 70 | | Adjacent Normal Tissue N = 30 | |
|---|---|---|---|---|
| | GSTP1 Protein | | GSTP1 Protein | |
| | Low-expression N (%) | High-expression N (%) | Low-expression N (%) | High-expression N (%) |
| **ER** | | | | |
| Negative | 9(32) | 19(68) | 7(54) | 6(46) |
| Positive | 27(64) | 15(36) | 9(53) | 8(47) |
| | $\chi2$=6.940, r=-0.315 , P=0.008 | | $\chi2$=0.002, r=+0.009 , P=0.962 | |
| **PR** | | | | |
| Negative | 21(47) | 24(53) | 12(60) | 8(40) |
| Positive | 15(60) | 10(40) | 4(40) | 6(60) |
| | $\chi2$=1.144, r=-0.128 , P=0.292 | | $\chi2$=1.071, r=+0.189, P=0.317 | |
| **Her2** | | | | |
| Negative | 23(47) | 26(53) | 13(57) | 10(43) |
| Positive | 13(62) | 8(38) | 3(43) | 4(57) |
| | $\chi2$=1.31, r=-0.137, P=0.257 | | $\chi2$=0.403, r=+0.116, P=0.542 | |
| **TNBC** | | | | |
| Negative | 32(62) | 20(38) | 10(53) | 9(47) |
| Positive | 4(22) | 14(78) | 6(55) | 5(45) |
| | $\chi2$=8.274, r=+0.344 , P=0.004 | | $\chi2$=0.018, r=-0.018, P=0.923 | |
| **Molecular subtype** | | | | |
| Luminal A | 19(61) | 12(39) | 7(58) | 5(42) |
| Luminal B | 8(73) | 3(27) | 2(40) | 3(60) |
| Her2 | 5(50) | 5(50) | 1(50) | 1(50) |
| TNBC | 4(22) | 14(78) | 6(55) | 5(45) |
| | $\chi2$=9.359, r=+0.292, P=0.014 | | $\chi2$=0.493, r=-0.033, P=0.863 | |

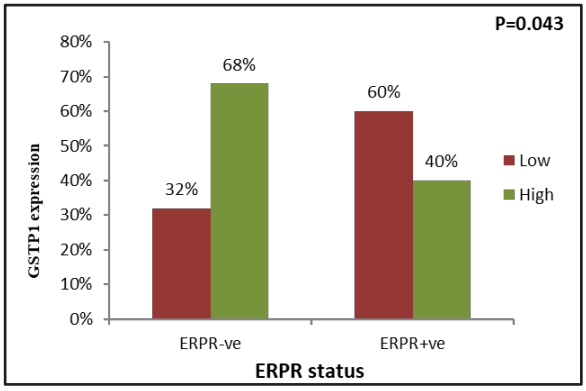


**Figure 7:** Correlation of tumoral GSTP1 expression with ERPR status

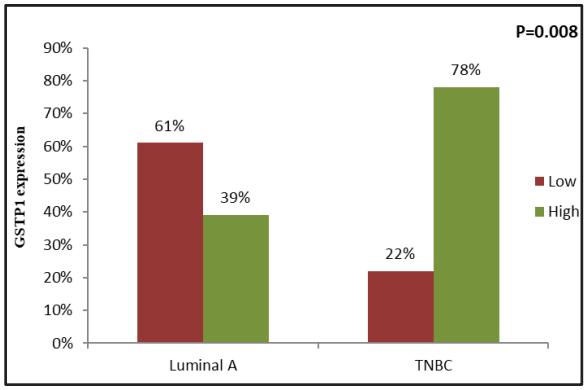


**Figure 8:** Correlation of tumoral GSTP1 expression between Luminal A and TNBC subtypes

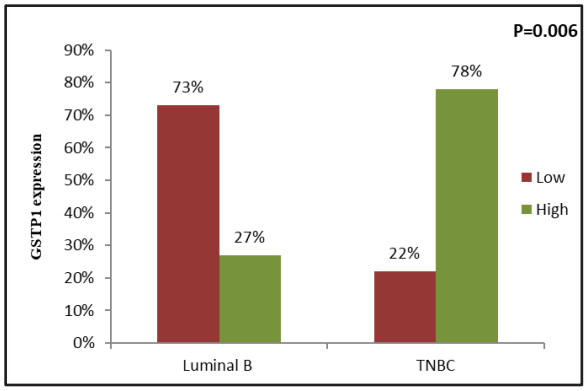


**Figure 9:** Correlation of tumoral GSTP1 expression between Luminal B and TNBC subtypes

status, in the primary tumors the incidence of GSTP1 expression was significantly higher in patients with ERPR–ve tumors as compared to patients having ERPR+ve tumors ($\chi2$= 4.137, r=-0.277, P=0.043) (Figure 7). Patients with TNBC molecular subtype had significantly high tumoral GSTP1 protein expression as compared to patients with luminal A ($\chi2$=6.979, r=+0.377, P=0.008) (Figure 8) and luminal B ($\chi2$=7.180, r=+0.498, P=0.006) (Figure 9) molecular subtype, respectively.

## Discussion

Breast cancer is the most common malignant tumor in women worldwide accounting for approximately one third of all female cancers. It is clinically a heterogeneous disease with multi-factorial etiology. Factors influencing prognosis and treatment outcome are solely based on clinicopathological factors and molecular surface based markers such as tumor size, grade, histological type, lymph node involvement, ER, PR, Her2 and TNBC status. Although these parameters guide therapeutic decision making, a great variability in disease outcome and ultimately prognosis have been observed amongst individual patients and within same stage. Due to variability in clinical progression of disease, identification of markers, that could predict tumor behavior is necessary. Identification of novel

**Table 6:** Comparison of GSTP1 protein expression with ERPR status, Luminal A versus TNBC, Luminal B versus TNBC and Luminal A versus Luminal B in patients with breast cancer

| | Primary Tumor | | Adjacent Normal Tissue | |
|---|---|---|---|---|
| | GSTP1 Protein | | GSTP1 Protein | |
| | Low-expression N (%) | High-expression N (%) | Low-expression N (%) | High-expression N (%) |
| **Estrogen receptor and Progesterone receptor status** | | | | |
| | (N=53) | | (N=23) | |
| ERPR-ve | 9(32) | 19(68) | 7(54) | 6(46) |
| ERPR+ve | 15(60) | 10(40) | 4(40) | 6(60) |
| | $\chi2$=4.137, r=-0.277, P=0.043 | | $\chi2$=0.434, r=+0.137, P=0.532 | |
| **Luminal A versus TNBC** | | | | |
| | (N=49) | | (N=23) | |
| Luminal A | 19(61) | 12(39) | 7(58) | 5(42) |
| TNBC | 4(22) | 14(78) | 6(55) | 5(45) |
| | $\chi2$= 6.979, r=+0.377, P=0.008 | | $\chi2$=0.034 , r=+0.038 , P=0.863 | |
| **Luminal B versus TNBC** | | | | |
| | (N=29) | | (N=16) | |
| Luminal B | 8(73) | 3(27) | 2(40) | 3(60) |
| TNBC | 4(22) | 14(78) | 6(55) | 5(45) |
| | $\chi2$=7.180, r=+0.498, P=0.006 | | $\chi2$=0.291, r=-0.135, P=0.169 | |
| **Luminal A versus Luminal B** | | | | |
| | (N=42) | | (N=17) | |
| Luminal A | 19(61) | 12(39) | 7(58) | 5(42) |
| Luminal B | 8(73) | 3(27) | 2(40) | 3(60) |
| | $\chi2$=0.463, r=-0.105, P=0.508 | | $\chi2$=0.476, r=+0.167, P=0.521 | |

biomarkers and an understanding of their clinical significance would benefit both current therapies and prognosis.[7]

GSTP1s are multifunctional enzymes that play a critical role in cellular detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione and may influence mutagenesis and carcinogenesis. It is known to protect normal cells from the influence of carcinogenic materials. Goto et al (2009) found GSTP1 is present in mitochondria and cytosol and nucleus in mammalian cell line and these enzymes play an important role in maintaining physiological function in these structures.[20] In the present study, in histological confirmed adjacent normal tissues, the staining pattern of GSTP1 expression was intensely nuclear and/ or cytoplasmic and distributed throughout the epithelium. Ninety-seven percent of the tissues had positive GSTP1 immuno-reactivity. Similar to the present study, Vecanova et al (2011) in breast cancer also observed cytoplasmic and/or nuclear GSTP1 positive expression in 100% normal tissues. The presence of GSTP1 in normal tissue indicates a probable protective function of the enzyme.[21]

Although present study observed GSTP1 expression in histologically confirmed adjacent normal tissues, studies have shown loss of GSTP1 expression in approximately 2/3rd of the carcinoma in situ cases.[22] Ramos-Gomez et al (2001) observed that breast epithelial cells with lack of expression of GSTP1 suffer from DNA damage more easily upon exposure to carcinogens.[23] Thus, GSTP1 probably acts to protect cells from cancer initiation. The present study observed reduced tumoral GSTP1 protein expression (76%) when compared to GSTP1 expression in histologically confirmed adjacent normal tissues (97%). Similarly, Haas et al (2006), also observed GSTP1 expression was consistently weaker in invasive carcinomas than in non-neoplastic mammary glands.[24] Thus, probably indicating that with the decrease of GSTP1 protein there might be a loss of protective function during the transition from normal to malignant transformation. However, no consensus has been achieved yet regarding the association between GSTP1 expression and malignant transformation.

In addition, the present study observed cytoplasmic and/or nuclear immuno expression in primary tumors (76%). Similar to the present study, Vecanova et al (2011) observed that cytoplasmic and/or nuclear GSTP1 positive expression in 63% of invasive carcinoma showed positive GSTP1 immunoreactivity.[21] Moreover, several reports are available in invasive breast cancer, showing cytoplasmic or nuclear GSTP1 immunoreactivity in nearly 77%-50% of patients.[7,25-27] Beside breast cancer, in accordance to present study, positive GST π nuclei or cytoplasmic immunoreactivity was observed in 71.4% of cases in advanced CRC,28 nasopharyngeal cancer,[29] NSCLC,[30,31] and in patients with advanced gastric cancer.[32] Contradictory to above, Ali-Osman et al (1997) observed in patients with gliomas, 38% high, 33% moderate and 29% low staining intensity with cytoplasmic and/or nuclear GST-π expression in tumor cells.[33]

Further in the present study, when relationship of GSTP1 and clinical parameters such as age, menopausal status, tumor site was evaluated, no significant association was noted, however a decreasing trend of GSTP1 protein expression was observed in elderly patient group and in post menopausal patients when compared to respective counterparts. Muftin et al (2015) observed significantly higher GSTP1 positivity in elderly age group patients but the authors had not correlated with menopausal status.[27] Huang et al (2003),[26] Haas et al (2006)[24] and Chen et al (2017)[7] failed to find any significant difference of GSTP1 according to patients

age. Miyake et al (2012)[34] and Chen et al (2017)[7] could not find any significant difference of GSTP1 protein expression and menopausal status. To best of our knowledge, there exist very rare reports on association of GSTP1 protein expression and age, menopausal status, site in patients with invasive breast cancer.

When relationship between GSTP1 and pathological variables were evaluated, it was observed that high tumoral GSTP1 protein expression was associated with breast cancer patients having N0 and N1 nodal status, T1 and T2 tumor size and in early disease stage when compared to their respective counterparts. Although, the difference was found to be statistically non significant but it confers a probable role of GSTP1 as an early event in breast carcinogenesis. Likewise, Buser et al (1997) showed that lower GSTs levels are associated with more advanced breast cancer.[35] Haas et al (2006) linked smaller tumor sizes with high GSTP1 expression.[24] Recently, Chen et al (2017) reported significantly higher GSTP in smaller tumors (P=0.023), early clinical stage of the tumor, but no significant association with the remaining clinicopathological characteristics, axillary lymph node status (P=0.071), pathological type (P=0.607), histological grade (P=0.750).[7] Contrary to the present study, Muftin et al (2015) found high GSTP1 expression was significantly associated with stage III and large tumor size (>2cm), ($p< 0.05$).[27] On the other hand, higher GSTP1 protein expression was significantly associated with aggressive prognostic factor such as high BR (8-9) score and presence of perinodal invasion. In accordance to the present results, Jardim et al (2012)[36] and Li et al (2014),[37] associated the highest GSTP1 expression with high histological levels of invasive ductal carcinomas. Nevertheless, other authors have demonstrated contrary results. Cairns et al (1992) associated an absence of GSTP1 in tumor tissue with the highest histological grade.[38] According to Miyake et al (2012), GSTP1 positivity significantly varied according to histological grade (HG) that is, HG2 tumors showed a lower positivity (32/81, 39.5%) than HG1 tumors (9/19, 47.4%) and HG3 tumors (16/22, 72.7%).[34] Muftin et al (2015) found high GSTP1 expression was significantly associated with grade III histology,[27] whereas Haas et al (2006) linked GSTP1 with well differentiated tumors.[24] Additionally, Huang et al observed GST-pi immunoreactivity was not significantly correlated with any of the traditional histological factors known to influence prognosis.[23] The plausible reason for this difference between our results and those conflicting results may be due to the diversity of GSTP1 assessment methods and the difference in sample size.

Since, GSTs isoenzyme facilitate clearance of endogenous hydrophobic compounds such as hormones, steroids, etc. GSTP1 binds non-covalently to steroids and hormones, allowing it to act as an intracellular buffer to minimize short-term changes in steroid levels. The breast being an important organ of the body which is continuously exposed to these steroids and it is therefore estrogens act as endogenous tumor initiators in the breast tissue when GSTP1 is inactivated by promoter methylation. Therefore, expression of GSTP1 protein and surface receptor was evaluated, higher GSTP1 protein expression was observed in tumors with ER-ve patients (68%), PR-ve (53%) and TNBC patients (78%) as compared to their respective counter parts. Similar high GSTP1 protein expression was noted in patients with ERPR–ve tumors. Consistent with present study, Miyake et al (2012),[34] Peters et al (1993)[39] and Gilbert L et al (1993)[40] found that GSTP1 expression was significantly associated with ER negativity and PR negativity in patients with breast cancer. On the other hand, Huang et al (2003),[23] and Haas et al (2006)[24] failed to observe any significant correlation between GSTP1 and ER, PR status.

Additionally, when sub grouped according to molecular subtypes, GSTP1 protein expression was significantly higher in breast cancer patients with TNBC (78%), followed by Her-2 (50%), luminal A (39%) and luminal B (27%) ($\chi 2 = 9.359$, $r = 0.292$, P=0.014). A recent study by Pakdeethai et al (2012), speculated a significant correlation of estrogen receptor negativity with high GSTP1 expression (p 0.001).[25] The other parameters - tumor size, tumor grade, lymph node status, HER2- IHC score, Ki67 index did not correlate with high or low GSTP1 protein expression. It is evident that TNBC subtypes are considered more aggressive than the luminal A or B subtypes, or even those overexpressing HER-2/neu. Louie et al (2016) found that GSTP1 was a new TNBC oncogene that governed the pathogenicity of cancer by regulating glycolysis, and energy and fat metabolism.[15] They believed that GSTP1, a new TNBC target, was a risk factor for breast cancer and promoted breast cancer. Chen et al (2017), found approximately 77% positive rate of GSTP1 protein expression in TNBC patients.[7] Interestingly, the current study demonstrated significant high expression of tumoral GSTP1 protein expression in TNBC as compared to the other molecular subtypes (luminal A, luminal B and Her-2), indicating a useful target for TNBC patients.

**Conclusion**

Our preliminary data shows higher cytoplasmic and/or nuclear staining immunopositivity pattern of GSTP1 was observed in adjacent normal tissues as compared to tumor tissues, which was indicative of loss of GSTP1 protective function during the transition of malignant transformation. Observation of higher GSTP1 with

traditionally aggressive prognostic factors such as High BR score, presence of perinodal extension, ER PR negativity & TNBC, probably indicates that GSTP1 might be useful to identify patients with aggressive phenotype. In TNBC patients it may be a useful target. However, it needs to be confirmed by covering a larger number of patients.

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