Role of Glutathione S-Transferase M1 and Glutathione S Transferase Theta 1 Gene Polymorphism, Histopathological, and Immunohistochemistry in Carcinoma Breast

Abstract

Background: Breast cancer is the most common invasive cancer in females in developing countries such as India. It is the most common malignancy in females in the Punjab state. Objectives: (1) The purpose of this study was to calculate the prevalence of the four subtypes of breast cancer based on molecular classification and (2) to determine the association of polymorphisms in Glutathione S-Transferase M1 (GSTM1) and Glutathione S transferase theta 1 (GSTT1) gene in carcinoma of the breast with histopathological grading. Materials and Methods: This study analyzed histologically confirmed 100 cases of carcinoma breast; immunohistochemistry and reverse transcription polymerase chain reaction molecular tests were performed for further grading, molecular typing, and gene polymorphism. Results: Out of 24 Grade I tumors, 18 (75.00%) expressed the GSTM1 gene and 6 (25.00%) were negative. Out of 48 Grade II tumors, 30 (62.50%) expressed the GSTM1 gene and 18 (37.50%) were negative. Out of 28 Grade III tumors, 8 (28.57%) expressed the GSTM1 gene and 20 (71.43%) were negative. Out of 24 Grade I tumors, 17 (70.83%) expressed the GSTT1 gene and 7 (29.17%) were negative. Out of 48 Grade 2 tumors, 28 (58.33%) expressed the GSTT1 gene and 20 (41.67%) were negative. Out of 28 Grade III tumors, 8 (28.57%) expressed the GSTT1 gene and 20 (71.43%) were negative. Conclusion: Our study shows that polymorphism of both GSTM1 and GSTT1, either individually or in combination, influences the risk of developing carcinoma due to DNA damage caused by many factors including environmental and genetic.

Keywords: Carcinoma breast, Glutathione S-Transferase M1 and Glutathione S transferase theta 1 gene, immunohistochemistry

Introduction

Breast cancer is the most common cancer, with an estimated 2 million new cases and is preceded by lung cancer.[1] It is rare before 25 years of age and increases rapidly after 30 years.[2] Common genes associated with familial breast cancer include BRCA1, BRCA2, TP53; responsible for DNA repair and maintaining genomic integrity.[3] Glutathione-S-transferases (GSTs) are enzymes that have a role in the detoxification of various carcinogens, environmental toxins, reactive oxygen species, hence, DNA protection.[4] The Glutathione-S-transferase theta 1 (GSTT1) and Glutathione-S-transferase M1 (GSTM1) genes family encode major proteins involved in detoxification. They can be present or deleted in homozygous form.[4]

Materials and Methods

The present study analyzed the frequency of the four immunohistochemistry (IHC) subtypes of breast cancer based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression as well as the role of GSTM1 and GSTT1 gene polymorphism between June 2016 and December 2018. Hundred histopathological confirmed breast cancer patients and 100 controls in Punjabi patients were taken. Patients who had previously undergone any type of treatment for carcinoma breast were excluded.

All the specimens received were placed in 10% formalin and allowed to fix for at least 4 h in 10% formalin. Then, after grossing and processing, thin sections were made from the suspicious area and the

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adjoining areas. The tissue section was stained with routine hematoxylin and eosin stain. In histologically confirmed cases of carcinoma breast, paraffin blocks-containing cancer tissues were selected. Slides were prepared from these paraffin blocks. The standard operating procedure was followed for immunohistochemical staining for ER, PR, and HER2. Scoring for ER and PR was done on basis of the Allred scoring system. Scoring for HER2 was done on pro forma.

For GSTT1 and GSTM1 gene polymorphism—Took 5 ml of venous blood sample of a breast cancer patient from the peripheral vein and placed it in a sterile vial containing 10% ethylenediaminetetraacetic acid as an anticoagulant. This sample was used for DNA extraction and simultaneously amplifying multiple sequences in a single reaction, as multiplex polymerase chain reaction (PCR) for saving time. An optimal combination of annealing temperature and buffer concentration was essential in multiplex PCR to obtain highly specific amplification products. Magnesium chloride concentration was proportional to the amount of dNTPs while adjusting primer concentration for each target sequence.

This study was conducted after clearance from the Ethical Committee of our institute and informed consent was obtained in the local language from patients and relatives on pro forma.

Statistical analysis

Medcalc software for Windows, version 15.5.5 (Belgium) was used for the statistical analysis. The different distributions of the GSTM1 and GSTT1 variants of the two groups were examined by the Chi-square test. Based on grade criteria, among 100 breast cancer patients, 71.43% (20) were categorized in Grade III and 37.5% (18) in Grade II, as shown in Table 2. Based on the genotypic distribution of the two genes and the regression analysis, it was found that the breast cases having the null GSTM1 genotype had a 7.5-fold increased susceptibility of being in Grade III tumor (odds ratio [OR] = 7.15; 95% confidence interval [CI] = 2.18‑25.5; P = 0.0014). Parameters such as patient age, histopathological type of carcinoma breast, lymph node involvement, family history, parity, and breastfeeding were studied. The frequency of the four IHC subtypes of breast cancer based on ER, PR, and HER2 expression was then calculated. The frequency of GSTM1 and GSTT1 genes was determined, and their correlation with the grade of cancer was studied.

In the current study, the maximum number of cases (84%) were of infiltrating duct carcinoma Not Otherwise Specified (IDC-NOS) followed by 6% cases of Infiltrating Lobular Carcinoma (ILC), 5% cases of apocrine carcinoma, 3% cases of medullary carcinoma, 1% case of malignant phylloides, and 1% invasive papillary.

The left breast was observed to be more commonly involved (58%) as compared to the right breast (42%) in this study. In the present study, axillary lymph nodes showed metastases from breast cancer in 68% of cases. Thirty-two percent of cases showed no nodal involvement or reactive changes only.

Out of 24 Grade I tumors [Table 2], 18 (75.00%) expressed the GSTM1 gene and 6 (25.00%) were negative for this gene. Out of 48 Grade II tumors, 30 (62.50%) expressed the GSTM1 gene and 18 (37.50%) were negative for GSTM1. Of the 28 Grade III tumors, 8 (28.57%) expressed the GSTT1 gene and 20 (71.43%) were negative. Based on grade criteria, among 100 breast cancer patients, 71.43% (20) were categorized in Grade III and 37.5% (18) in Grade II, as shown in Table 2. Based on the genotypic distribution of the two genes and the regression analysis, it was found that the breast cases having the null GSTM1 genotype had a 7.5-fold increased susceptibility of being in Grade III tumor (odds ratio [OR] = 7.15; 95% confidence interval [CI] = 2.18‑25.5; P = 0.0014).

Out of 24 Grade I tumors [Table 3], 17 (70.83%) expressed the GSTT1 gene and 7 (29.17%) were negative for the GSTT1 gene. Out of 48 Grade 2 tumors, 28 (58.33%) expressed the GSTT1 gene and 20 (41.67%) were negative for the GSTT1 gene. Out of 28 Grade III tumors, 8 (28.57%) expressed the GSTT1 gene and 20 (71.43%) were negative for this gene. It was seen that among 100 patients having the GSTT1 gene, 29.17% (7) were in Grade I, 41.67% (20) were in Grade II, and 71.43% (20) were categorized in Grade III carrying the null GSTT1 genotype. This relationship was supported by regression analysis showing that the patients carrying the null

### Table 1: Histopathologic type diagnosed in patients of carcinoma breast

| Histopathologic subtype            | Number of cases, n (%) |
|------------------------------------|------------------------|
| Infiltrating duct carcinoma-NOS    | 84 (84)                |
| Infiltrating lobular carcinoma     | 6 (6)                  |
| Apocrine carcinoma                 | 5 (5)                  |
| Medullary carcinoma                | 3 (3)                  |
| Malignant phylloides               | 1 (1)                  |
| Invasive papillary carcinoma       | 1 (1)                  |
| Total                              | 100 (100)              |

NOS: Not otherwise specified

### Table 2: Comparison of glutathione S-transferase M1 with grade (n=100)

| GSTM1 status | Grade I, n (%) | Grade II, n (%) | Grade III, n (%) | Adjusted OR (95% CI) | P       |
|--------------|----------------|-----------------|------------------|----------------------|---------|
| Absent       | 6 (25.00)      | 18 (37.50)      | 20 (71.43)       | 7.5 (2.18‑25.5)      | 0.0014  |
| Present      | 18 (75.00)     | 30 (62.50)      | 8 (28.57)        |                      |         |
| Total        | 24 (100)       | 48 (100)        | 28 (100)         |                      |         |

OR: Odds ratio, CI: Confidence interval, GSTM1: Glutathione S-Transferase M1
The age of the patients with carcinoma breast in the current study varied from 23 to 80 years. The mean age was 51 years. Only 10% cases were seen below 35 years of age, 24% cases were seen in the age group of 35–44 years, 32% in the age group of 45–54 years, 18% in the age group of 55–64 years, 10% cases in the age group of 65–74 years, and 6% cases were 75 years or older. Hence, the highest number of patients was seen in the 45–54 years age group and cases were less frequent in the extremes of age.

Among Grade I tumors (24 cases), 22 cases (39.29%) were positive for ER and only 2 cases (4.54%) were negative for ER. Among Grade II tumors (48 cases), 24 cases (42.86%) were ER-positive and 24 cases (54.54%) were ER-negative. Among Grade III tumors (28 cases), 10 cases (17.86%) were ER-positive and 18 cases (40.90%) were ER-negative [Figure 1]. These differences were highly significant statistically with $P = 0.0014$. Among Grade I tumors (24 cases), 22 cases (39.29%) were positive for ER and only 2 cases (4.54%) were negative for ER. Among Grade II tumors (48 cases), 24 cases (42.86%) were ER-positive and 24 cases (54.54%) were ER-negative. Among Grade III tumors (28 cases), 10 cases (17.86%) were ER-positive and 18 cases (40.90%) were ER-negative [Figure 1]. These differences were highly significant statistically with $P = 0.0014$.

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Among Grade I tumors (24 cases), only 1 case (5%) was positive for HER2 and 23 cases (28.75%) were negative for HER2. Among Grade II tumors (48 cases), 18 cases (39.29%) were positive for HER2 and only 2 cases (4.54%) were negative for HER2. Among Grade III tumors (28 cases), 11 cases (46.43%) were positive for HER2 and only 4 cases (14.29%) were negative for HER2 [Figure 1]. These differences were highly significant statistically with $P = 0.0014$.

5 cases (25%) were HER2 positive and 43 cases (53.75%) were HER2 negative. Among Grade III tumors (28 cases), 14 cases (70%) were HER2 positive and 14 cases (17.50%) were HER2 negative [Figure 1]. The differences between the three grades were highly significant statistically with $P = 0.0000$.

Of the 84 cases [Figure 2] of IDC-NOS, 42 (50.00%) cases were of Luminal A type, 6 (7.14%) cases were of Luminal B type, 11 (13.09%) cases were of HER2 over-expression type and 25 cases (29.76%) were basal-like. Of the 6 ILC cases, 4 (66.66%) cases were of Luminal A-type and 2 (33.33%) cases were basal-like. There were no cases of ILC in the Luminal B type or the HER2 overexpression type. Hence, no case of ILC over-expressed HER2. Of the 5 cases of apocrine carcinoma, 4 (80.00%) cases were luminal A and 1 (20.00%) was luminal B. Of the three cases of medullary carcinoma, two cases (66.66%) were Basal-Like and 1 (33.33%) case was of HER2 positive type. The single case of malignant phylloides was of the Luminal A-type. The single case of invasive papillary carcinoma was of the Luminal A-type.

**Discussion**

The current study aimed to determine the association of polymorphisms in GSTM1 and GSTT1 gene in carcinoma of the breast with histopathological grading and to calculate the prevalence of the four subtypes of breast cancer based on the molecular classification. This study was conducted in the department of pathology, in our institution, to categorize 100 cases of histologically proven breast cancer into the four immunohistochemical surrogate subtypes based upon ER, PR, and HER2 over-expression and to compare the expression of these three receptors with histopathological types and grade. The present study results were following previous such studies. GSTT1, GSTM1, and GSTP1 gene
changes, in the population have a high probability of having cancers of the bladder, breast, large and narrow intestines, brain, and lung.[7] IDC-NOS was the predominant subtype, seen in 84% of cases in our study and varied from 70% in the study done by Engstrom et al.[9] to 89% in the study done by Kaul et al.[9] in previous studies. ILC was seen in 9% of cases in our study and this was similar to 13.6% cases seen in the study by Engstrom et al.[9] and 12.1% cases seen in the study by Onitilo et al.[10] However, 2% in the study done by Azizun-Nisa et al.[11] and 1.8% in the study done by Kaul et al.[9] were a much smaller number of ILC cases.

Luminal A: The Luminal A subtype (ER/PR positive, HER2 Negative) showed a variable frequency in previous studies. This could be due to differences in the populations studied and varied from 59% luminal A cases in the study by Wong et al.[12] to 75% luminal A cases in the study by Nguyen et al.[13] The results of the present study (50%) most closely matched those of Wong et al.[12] who found 59% luminal A. Luminal B: The Luminal B subtype (ER/PR positive and HER2 positive) too showed a variable frequency in different studies. It varied from just 6.3% of cases seen by Blows et al.[14] to 18.4% cases reported by Wong et al.[12] The results of the present study (79%) most closely matched those of 6.3% reported by Blows et al.[14] and 7.7% found by Engstrom et al.[9] HER2 Overexpressing: The HER2 over-expressing or HER2 Positive subtype (ER and PR negative and HER2 positive) subtype varied from 4% cases reported by Nguyen et al.[13] to 8.2% cases by Wong et al.[12] The result of the present study (12%) most closely matches the result of Wong et al.[12] who reported 8.2% of cases that overexpressed HER2. Triple-Negative: The triple-negative or basal-like subtype (ER, PR, and HER2 negative) in previous studies varied from 11.3% cases seen by Nguyen et al.[13] to 16.2% cases found by Blows et al.[14] The result of the present study (31%) most closely matches the result of Blows et al.[14] who found 16.2% triple-negative cases. The higher number of triple-negative results in the present study may be because only tumors with 3 + membrane positivity were considered HER2 positive. This could also be the reason for a relatively low frequency of the Luminal B subtype. A study conducted by Janchiv et al.[17] to determine the proportion of deleted alleles of both GSTT1 and GSTM1 genes in the patients was slightly higher, i.e. (35%) than GSTT1 and GSTM1 seen individually which was 25% and 26.6%, respectively, whereas the frequency of both alleles without deletions is 13.3%. Kimi et al.[15] have studied GSTM1, GSTT1, and GSTP1 gene polymorphism to breast cancer susceptibility in Mizoram population, North-East India, and found that GSTM1 was 91% and GSTT1 was 63.6% as compared to the healthy population. While GSTM1 and GSTT1 null genotypes both simultaneously were found to be present in 30%. The study of Sharma et al.[16] compared the Delhi population with other Indian and global populations found that null genotypes of GSTM1 were 33.6% and GSTT1 was 12.4%, and 10.8% of individuals were homozygous null for both the genes simultaneously. In the current study, the GSTM1 and GSTT1 gene polymorphism in breast carcinoma in the Punjab population was determined separately as per Grades 1, 2, and 3 of tumors not done previously. Grade I tumors, 18 (75.00%) expressed the GSTM1 gene and 17 (70.83%) expressed the GSTT1 gene. Grade II tumors, 30 (62.50%) expressed the GSTM1 gene and 28 (58.33%) expressed the GSTT1 gene. Grade III tumors, both GSTM1 and GSTT1 genes were expressed in 8 (28.57%). The significance of GSTM1 genotype varied from P < 0.001 in their studies by Kimi et al. (2015),[15] Sharma et al.,[16] Ada et al.,[17] and Millikan et al.[18] to P = 0.1125 in a study by Mishra et al.[19] The present study similarly had a significant P value. The significance of GSTT1 genotype varied from P < 0.001 in their studies by Kimi et al. (2015,[15] Sharma et al.,[16] Mishra et al.,[19] to P = 0.85 according to Ada et al.[17] and Millikan et al.[18] in our study also P value was significant. Hence, polymorphism of GSTT1 and GSTM1 was both significant and risk factors for causing high grade of breast carcinoma in the Punjab population.

Conclusion

This current study concludes that the different histopathological subtypes were found to have distinct immunohistochemical profiles in the Punjab population. In general, low-grade breast cancers tended to be Luminal A or B. HER2 over-expressing and basal-Like subtypes were seen in tumors of higher grades. This showed that deletion of GSTT1 and GSTM1 is associated with higher tumor grade and poor outcomes. Therefore, the study shows, the role of HER2 overexpression and polymorphism of GSTT1 and GSTM1 in determining the tumor aggressiveness, and accordingly, target therapy for treatment and make strategies for prevention of carcinoma breast.

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Ethical clearance

Approved by Institutional Ethics Committee.

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Conflicts of interest

There are no conflicts of interest.

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