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ORIGINAL ARTICLE

Risk and Frequency of Mutations in the BRCA1 in Relation to GSTM1 and GSTT1 Genotypes in Breast Cancer

Jagadish Hansa[1, 3, *], Sankar K Ghosh[3], Sunil Kumar Agrawala[1] and Manash P Kashyap[2]
1-Department of Surgical Oncology,
IMS & SUM Hospital, SOA University, Kalinganar, Odisha, India
2-Department of Business Administration, Assam University, Silchar, India
3- Department of Biotechnology, Assam University, Silchar, India
*Corresponding Author- jagadish.hansa@gmail.com

ABSTRACT
Polymorphism of xenobiotic metabolizing genes (GSTM1 and GSTT1) induces mutations in several cancer types but its effect in inducing mutation in the BRCA1 gene is unclear. Earlier to this, in a study, we tested patients for their genomic alteration in BRCA1 gene through genetic analysis including PCR and Sequencing. As GSTM1 and GSTT1 genes are factors for the inducing mutations, we came forward with hypothesis to relate the sequenced data with the multiplex PCR results of same individuals for their variations in xenobiotic metabolizing genes. Further Hosmer-Lemeshow statistical test was used to validate, whether or not the observed event rates match expected event rates in samples of the model population. A significant relation was found in the polymorphism of GSTs (GSTM1 and GSTT1) and mutation of the BRCA1 gene in breast carcinoma patients. The logistic regression for mutation is positive:- $\log it(Y) = -1.933 + 2.086 g_1 + 3.366 g_2$. *The lack of GSTM1 (OR= 28.98, p=0.007) and GSTT1 (OR=8.056, p= 0.04) gene was significantly associated with the mutation rate in breast carcinoma patients. This contribution was significantly higher in patients carrying both null GSTM1 and GSTT1 genotypes. In conclusion, this study suggests that the GSTM1 gene deletion may be an attractive susceptibility marker for the mutation of breast cancer gene1. Hence there is need of genetic profiling for breast cancer gene1 in breast carcinoma patients after recognized polymorphism of xenobiotic metabolizing genes.*
Key words: - *Breast cancer, Polymorphism, GSTT1, GSTM1, BRCA1 gene*

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INTRODUCTION

Nowadays breast carcinoma is the most frequent malignancy among women. In India, almost 100,000 women are diagnosed every year with breast cancer, and a rise to 131 000 cases is predicted by 2020 [1]. Owing to genotoxic stress from tobacco exposure, North-East India breast cancer has always been a hot spot in comparison to rest part of the country [2].Breast cancer is triggered in several ways; the best understood causal mechanism being due to mutations in tumor suppressor genes and these mutations drive oxidative stress and glycolysis in the tumor environment [3]. Polycyclic aromatic hydrocarbons (PAHs), aromatic and heterocyclic amines present in the diet and environmental exposures are the potent carcinogens involved in breast carcinogenesis [4]. Polycyclic aromatic hydrocarbons are detoxified by glutathione-S-transferases (GSTs), which is activated by cytochrome P-4501A1 (CYP1A1). Gluthatione-S-transferases (GSTs) enzymes are expressed in tumor breast tissue as well as in normal breast tissue [5]. The presence of these enzymes have active roles in the elimination of several products resulting from reactive oxidant damage to DNA and prevent further oxidant damage to cells. Individuals are at risk of cancer, as reduction of removal of secondary organic oxidation products reduced, when they are homozygous for the null-GSTM1 or null-GSTT1 genotypes [6]. The various arguments in favor and against the role of xenobiotic metabolizing genes in breast cancer are an interesting area of research for breast

carcinoma [7, 8]. The reason may be due to differences in analyzed populations as well as the presence of different environmental factors [9-11].In this study, we investigated whether the polymorphism of Glutathione-S-transferase enzyme genes have any significant relation in breast cancer1 gene mutation from southern Assam breast cancer patients? We tried to find out the relationship of variation of GSTT1 and GSTM1 genes with the sequenced data of same thirty two breast cancer patients. Further Hosmer-Lemeshow statistical test was used to draw a logical conclusion and validate the data.

MATERIALS AND METHODS

Collection of samples

Patient samples were collected from the Cachar Cancer Hospital and Research Centre, living in the southern part of North-East India. All thirty two patients included in this study had primary breast carcinoma, with unilateral breast tumors. The patients had a mean age of 52 years.

DNA extraction and PCR reactions

Genomic DNA was extracted from peripheral blood as well as fresh tissue by phenol chloroform and isoamylalcohol methods [12]. Polymorphism analysis of GSTT1 and GSTM1 genes was detected by Multiplex PCR based assays with CYP1A1 as an internal control gene [13](Mondal et al., 2013). The CYP1A1 gene primer pair were; Forward- 5'-GAA CTG CCA CTT CAG CTG TCT-3', and Reverse- 5'-GCT GCA TTT GGA AGT GCT C-3'). In addition to the CYP gene, two sequence specific oligonucleotide primers (GSTT1 or GSTM1) were used for multiplex PCR. For GSTT1, Forward Primer 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and Reverse Primer 5'-TCA CGG GAT CAT GGC CAG CA-3' and for GSTM1, Forward Primer 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and Reverse Primer 5'-GTT GGG CTC AAA TAT ACG GTG G-3' were used. Earlier to this study we had generated sequence chromatograms of BRCA1 gene from these thirty two breast cancer patients. The rate of mutation is high (> 40%) within this population, three variant type of mutation (3889DelAG, 1014DelGT and 185DelAG) was found [14].

Statistical Analysis

Hosmer–Lemeshow statistical test was used to assess whether or not the observed event rates match expected event rates in samples of the model population. Usually this test is used to test for goodness of fit for logistic regression models. It is used frequently in risk prediction models.

Let, Y be the response variable, which is binary (i.e. Mutation is positive or negative) and g_1 and g_2 are independently variable that is also dichotomous in nature.

Y= 1, Mutation is Positive
0, Mutation is Negative

P(Y=1) =1-P(Y=0)

g_1 = 1, GSTT1 present
0, GSTT1 not present

g_2 = 1, GSTM1 present
0, GSTM1 not present

Therefore, the probability that Mutation is positive

$$P(Y=1)=\frac{\exp\left(\beta_0+\beta_1 g_1+\beta_2 g_2\right)}{1+\exp\left(\beta_0+\beta_1 g_1+\beta_2 g_2\right)} \quad \ldots(1)$$

Where, β_0, β_1, β_2 are the parameters of the model to be estimated from the data. More conveniently the equation (1) can be written as

$$P(Y=1)=P(Y=1|\,g_1+g_2)=P(\delta),$$

Where, $\delta=(g_1,g_2)$ ... (2)

Thus,

$$\log it(Y)=\log_e\left[\frac{P(Y=1)}{1-P(Y=1)}\right]$$

$$=\beta_0+\beta_1 g_1+\beta_2 g_2 \quad \ldots(3)$$

Thus the logistic regression for mutation is positive

$$\log it(Y)=-1.933+2.086\,g_1+3.366\,g_2 \quad \ldots(4)$$

RESULTS AND DISCUSSION

Results

The distribution of the GSTM1 and GSTT1 genotypes in the patient is shown in Table 1. From the equation (4), it is clear that the absence of GSTM1 is more effect on the mutation positive than the GSTT1, when we stratified the patients according to their mutation status of the BRCA1 gene. This signifies an association between the presence of the null GSTM1 genotype and the mutation of the BRCA1 gene in breast carcinoma patients. The result shown in Table-1 signified that the null GSTM1 genotype frequency was significantly higher in cancer patients carrying mutation in breast cancer1 gene in comparison with null GSTT1 (Table-1). Significantly mutation was higher when patients carrying both null GSTM1 and GSTT1 genotypes. The Hosmer and Lemeshow test that is used to test the goodness of fit that the model defined in (1) adequately fits the data provides a p-value is 0.880, which approves the model for the data. The following table summarizes the roles of parameters in the model Table 1.

Table 1 : Variable in the Equation

	Odds Ratio	Coefficient	Significant	95 % Confidence Interval
GSTT1	8.056	2.086	0.045	1.047977 - 61.93428
GSTM1	28.980	3.366	0.007	2.489996 - 337.2973
Constant	0.1447	-1.933	0.012	0.0321085 - 0.6523912

Table 1, shows the estimate of parameters corresponding to the exploratory variable. From the significant column corresponding to GSTT1 and GSTM1 ($P_{GSTT1} = 0.045$ and $P_{GSTM1} = 0.007 < 0.05$) indicates that the absence of GSTT1 and GSTM1 has a negative impact on mutation i.e mutation positive.

DISCUSSION

We have found a substantial number of reports of studies that have investigated xenobiotic metabolizing genes for low-penetrance breast cancer susceptibility alleles, but the results are conflicting [15, 17, 18]. The findings, which showed that GSTs enzymes play crucial role in the detoxification of numerous products induced by cancer therapy, prompted us to evaluate the prognostic significance of GSTs deletions in breast carcinoma. There were studies showed a borderline significant increase in the risk of breast carcinoma in unselected subjects carrying the null-GSTM1 genotype [19]. This association becomes clearly significant for premenopausal women. Rather, the GSTT1 deletion seems to be associated specifically with the early onset of breast carcinoma. The GSTT1 and GSTM1 enzymes have been shown to have removal activity toward lipid hydroperoxides. In this study, we initiated the significance evaluation of the GSTs variations by investigating the association between GSTT1, GSTM1 and BRCA1 gene deletion.

CONCLUSION

In conclusion, this study suggests that the GSTM1 gene deletion may be an attractive susceptibility marker for the mutation of breast cancer gene1 in breast cancer patients. Hence there is need of genetic profiling for breast cancer gene1 in breast carcinoma patients after recognized polymorphism of low penetrance candidate genes.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest is declared.

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