Prospective Case–Control Study to Evaluate the Role of Glutathione S Transferases (GSTT1 and GSTM1) Gene Deletion in Breast Carcinoma and Its Prognostic Significance

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Abstract Breast cancer is the most common cause of cancer death in women with the incidence rising in young women. GST gene polymorphisms are significant because of their role in the detoxification of both environmental carcinogens and also cytotoxic drugs used in therapy for breast cancer. The present study has been designed to identify the role of polymorphisms in GSTT1 and GSTM1 genes in the risk of development of breast cancer, in the prognostication of breast cancer, and in the prediction of response towards chemotherapy. Ninety-nine patients with breast cancer and 100 healthy controls with no history of cancer were taken from blood donors after informed consent. Epidemiological and clinical data was collected from participants and 5 ml of peripheral venous blood was collected for genotype analysis. Null genotype of GSTT1 was detected in 51.04 % of the controls in comparison to 20.2 % of patients with carcinoma breast, which was found to be statistically significant (OR 4.18; 95 % CI 2.01–8.75; $P=0.0001$). GSTM1 gene deletion was also significantly more common among controls (60 %) than in patients with breast cancer (33 %) (OR 4.57; 95 % CI 2.20–9.51; $P=0.0001$). Tumors more than 5 cm in size had greater tendency for GSTM1 gene expression (P value=0.019), but other clinicopathological parameters did not show any correlation. GSTT1 and GSTM1 genes status did not show any association with response to chemotherapy. The results indicated the null genotype of both GSTT1 and GSTM1 to be protective for the development of carcinoma breast. None of

the known etiological factors have any correlation with GSTT1 and GSTM1 gene deletion. Patients with small tumor size expressed GSTM1 gene deletion. Other tumor characteristics and clinicopathological parameters did not have any correlation with gene deletion.

Keywords Breast cancer · GST · Polymorphisms

Introduction

Breast cancer is the most common cause of death in women with the higher incidence in young women [1]. Hereditary factors play an important role in the carcinogenesis of breast cancer in more than one fourth of cases [2]. However, among the hereditary factors, high penetrance genes like BRCA1 and BRCA2 account for only 5 % of all breast cancer cases [3, 4].

The role of environmental carcinogens has been implicated in lung, bladder, breast, colon, and ovarian cancer in various epidemiological studies [5, 6]. It is now being suggested that endogenous, lifestyle and environmental factors acting together with the low-penetrance genes like the Glutathione S transferases (GST) and some other yet unknown genes are responsible for the majority of breast cancer cases [7, 8]. This also seems to explain the different susceptibilities of various individuals exposed to the same factors and also the different response of patients to the same treatment.

Glutathione S transferases are a family of phase II detoxification enzymes which act by catalyzing the conjugation of glutathione to the electrophilic intermediates of both endogenous and exogenous compounds [9]. They are divided into two superfamilies, namely the microsomal and the cytosolic forms.

Cytosolic GST's are divided into six classes, namely alpha (α), mu (μ), omega (Ω), pi (π), theta (θ), and zeta (ζ) [9]. The

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GSTs are induced under conditions of oxidative stress, with the α , μ , π , and θ classes especially active in the detoxification of numerous agents that cause reactive oxidant damage, protecting cells from a wide variety of environmental carcinogens [10].

GSTM1, GSTT1, and GSTP1 iso-enzymes are present in both normal and breast tumor tissues and belong to the best characterized classes, along with class alpha and omega. GSTM1 is coded by a gene located on chromosome 1p13.3 [11]. The homozygous deletion (null genotype) of the GSTM1 gene results in the total absence of a functional gene product, and thus a total absence of the respective enzyme activity. Like GSTM1, GSTT1 gene, located at 22q11.2, has a genetic variant that consists of a complete deletion of the whole gene, resulting in the lack of the GSTT1 form of enzyme.

Cytotoxic drugs act on tumor cells through various mechanisms, one of them being through the generation of reactive oxygen intermediates that cause oxidative damage to the tumor cells, thereby killing them. The GST has been shown to have activity both by direct detoxification of the drugs themselves or through detoxification of lipid hydro peroxides generated by the cytotoxic drugs [12]. In addition, the GSTs are also involved in cell signaling pathways that control stress response, apoptosis, and cellular proliferation [13]. Thereby, the polymorphisms in the genes of these enzymes could contribute to tumor cell survival and drug resistance by causing detoxification of products of cancer chemotherapy.

Several previous studies have been done to identify the association between GSTM1 and GSTT1 polymorphisms and the risk of development of breast cancer with inconsistent results [14, 15]. Literature is lacking in studies that highlight the prognostic significance of these polymorphisms. Khedhaier et al. had found a significant association between lack of GSTT1 deletion and poor response to chemotherapy [10]. The present study was done with the aim to identify the role of GSTT1 and GSTM1 genes polymorphism in the risk of development of breast cancer, and whether this polymorphism has any prognostic significance and any role in predicting the response to chemotherapy.

Materials and Methods

Study Population

The Institute Ethics Committee approved the study protocol. Ninety-nine patients of breast cancer were included after informed consent. One hundred healthy, age-matched controls having no history of cancer were taken from blood donors during the study period. Variables collected include age, marital status, menopausal status, age at menarche, age at first child birth, parity, history of breast feeding, use of hormonal

contraception, history of smoking and alcohol consumption, and family history of breast cancer.

The preoperative clinical stage and the treatment given in the form of chemotherapy were recorded. The response to chemotherapy (partial response, complete response, no response) was recorded according to the UICC criteria.

The operative details and the pathological stage of the disease were recorded. Details of post-operative treatment received by the patients in the form of chemotherapy, radiotherapy, and/or hormonal therapy were noted. The receptor status of the tumor (ER/PR/Her2neu) was recorded. The patients were followed up for 6 months after completion of therapy and any local recurrence or metastasis was recorded.

Genotype Analysis

Five milliliters of peripheral blood sample was collected. DNA isolation using standard salting out method was done. DNA was subjected to amplification by polymerase chain reaction (PCR) to look for the presence or absence of null genotype using primer pair [10]. GSTM1- and GSTT1-specific primer pairs were used in separate set of reaction along with β -integrin serving as an internal control.

Statistical Analysis

Pearson's Chi-square test was used to assess phenotypic differences across different groups of women with GSTT1 and GSTM1 genotype. Odds ratios (ORs) at 95 % confidence intervals (CIs) were obtained for different parameters with presence or absence of GSTT1 and GSTM1 allele using conditional logistic regression. Odds ratios and 95 % CIs were used to estimate the association between GSTT1 and GSTM1 genotypes and breast cancer risk. Odds ratios adjusted for potential confounders were estimated with logistic regression models for matched sets. Age at menarche, family history of breast cancer among mother or sisters, parity, age at first live birth, body mass index (weight in kilograms divided by [height in meters]²), and benign breast disease were considered as potential confounders. Gene–gene and gene–environment interactions were assessed in logistic regression models by Wilcoxon rank-sum (Mann–Whitney) test for variation in pre and postmenopausal status, number of pregnancies, hormonal contraception, axillary nodes, recurrent primary pathological staging, radiotherapy, and hormonal treatment.

Results

The mean age of the study population was 48.9 ± 11.6 years (range, 25–80 years). Majority of patients presented to the

Table 1 Incidence of gene deletions

	Cases, <i>n</i> (%) (<i>n</i> =99)	Controls, <i>n</i> (%) (<i>n</i> =100)	OR (95 % CI)	<i>P</i> value
Age	48.9±11.6 (25–80)	46.8±12.8 (22–65)		0.3
Pre-menopausal	49 (49.5 %)	55 (55.0 %)		0.4
Post-menopausal	50 (50.5 %)	45 (45.0 %)		
GSTT1	20 (20.2 %)	51 (51 %)	4.18 (2.01–8.75)	0.0001
GSTM1	33 (33.3 %)	60 (60 %)	4.57 (2.20–9.51)	0.0001

hospital with history of lump in the breast (96.7 %) and only one patient presented with metastatic symptoms (Table 1).

Null genotype of GSTT1 was found in 51.04 % of the controls in comparison to 20.2 % of patients with breast cancer that was statistically significant (OR 4.18; 95 % CI 2.01–8.75; *P*=0.0001). GSTM1 gene deletion was also significantly more common among controls (60 %) than in the patients with breast cancer (33 %) (OR 4.57; 95 % CI 2.20–9.51; *P*=0.0001) (Table 1).

There was no correlation between the GST gene polymorphism and the presumed risk factors for development of breast cancer. Also, there was no association between use of hormonal contraceptives with the GST gene polymorphisms (*P*=0.3) (Table 2).

The size of the lump did not show any correlation with GSTT1 gene polymorphisms but tumors more than 5 cm in size had a greater tendency toward expression of GSTM1 gene (*P*=0.019). Number of lymph nodes and pathological stage of the disease did not show any significant association with GST gene polymorphisms (Table 3). Hormonal receptor

(ER and PR) status and Her2neu receptor status did not show any significant association with polymorphisms in the GSTT1 and GSTM1 genes (Table 4).

Ninety-eight patients received chemotherapy, 44 patients (44.4 %) had neoadjuvant chemotherapy and 54 patients (54.5 %) had adjuvant chemotherapy. However, there was no significant association found between the status of GSTT1 and GSTM1 genes and the response to chemotherapy. The patients were also analyzed according to the type of chemotherapy received, namely the regimen containing cyclophosphamide, etoposide and 5-fluorouracil (CEF) or the cyclophosphamide, adriamycin and 5-fluorouracil (CAF) regimen. It was found that response to neither regimen had any significant association with the genotype of GSTT1 or GSTM1 gene (Table 5).

Patients were followed up for a mean duration of 23.7 months (SD, 2.3 months; range, 3–24 months). Only one patient had a recurrence on follow-up and this patient had a null genotype of GSTT1 with normal expression of GSTM1.

Table 2 Presumed risk factors and gene deletion

	GSTT1		<i>P</i> value	GSTM1		<i>P</i> value
	Null (<i>n</i> =20)	Present (<i>n</i> =79)		Null (<i>n</i> =33)	Present (<i>n</i> =66)	
Age at presentation	45.9±10	49.7±12		49.5±12.9	48.7±11.1	
Age at menopause	43.9±2.4	45.6±6.4		45±4.2	45.4±6.5	
Age at menarche	13.7±0.9	13.9±1.2		13.7±0.9	13.9±1.2	
Age at first child birth	20.8±2.8	21.3±2.8		20.2±2.4	21.7±2.9	
Menopausal status, <i>n</i> (%)			1			0.7
Pre-menopausal	10 (20.4 %)	10 (20 %)		17 (34.7 %)	16 (32 %)	
Post-menopausal	39 (79.6 %)	40 (80 %)		32 (65.3 %)	34 (68 %)	
Family history	1 (25 %)	3 (75 %)		1 (25 %)	3 (75 %)	
Hormonal contraception	20 (21.5 %)	73 (78.5 %)	0.3	29 (31.8 %)	64 (68.8 %)	0.1
Number of pregnancies, <i>n</i> (%)			1			0.4
<2	8 (40 %)	30 (38 %)		15 (45.5 %)	23 (34.8 %)	
2–5	12 (60 %)	44 (55.7 %)		16 (48.5 %)	40 (60.6 %)	
>5	0	5 (6.3 %)		2 (6 %)	3 (4.5 %)	

Table 3 Clinical factors and gene deletion

	GSTT1, <i>n</i> (%)		<i>P</i> value	GSTM1, <i>n</i> (%)		<i>P</i> value
	Null (<i>n</i> =20)	Present (<i>n</i> =79)		Null (<i>n</i> =33)	Present (<i>n</i> =66)	
Size of lump			0.302			0.019
T1	0	10 (12.7 %)		4 (12.1 %)	6 (9.1 %)	
T2	3 (15 %)	16 (20.3 %)		5 (15.2 %)	14 (21.2 %)	
T3	11 (55 %)	25 (31.6 %)		12 (36.4 %)	24 (36.4 %)	
T4	6 (30 %)	28 (35.4 %)		12 (36.4 %)	22 (33.3 %)	
Axillary nodal status	14 (22.2 %)	49 (77.8 %)	0.5	25 (39.7 %)	38 (60.3 %)	0.1
Pathological stage			0.9			0.8
Stage 1	2 (20 %)	8 (80 %)		3 (30 %)	7 (70 %)	
Stage 2	11 (19.3 %)	46 (80.7 %)		20 (35.1 %)	37 (64.9 %)	
Stage3	5 (19.2 %)	21 (80.8 %)		10 (38.5 %)	16 (61.5 %)	
Stage <i>x</i>	2 (40 %)	3 (60 %)		0	5 (100 %)	

Discussion

GST are a family of enzymes involved in the detoxification of benzopyrene and other carcinogens found in tobacco smoke, cytotoxic drugs, and chemical solvents [16]. These enzymes are induced in the presence of oxidative stress [10].

Chemotherapy and radiotherapy exert their effects by generating reactive oxygen species (ROS) and their by-products [17]. Since the ROS are the cause of tumor cell death, the amount of reactive species that reach the tumor cells and have direct cytotoxic effects or trigger intracellular apoptotic pathways are likely to have initial and immediate impact on treatment efficacy. Thus, inter-individual variability in enzymes that will affect ROS levels is likely to impact patient prognosis after treatment.

Numerous clinical studies have shown that patients treated with a wide variety of cytotoxic agents have marked increase in the lipid peroxidation products [18]. There are data that all the agents used in the treatment of breast cancer, but particularly cyclophosphamide and adriamycin, result in an increase in the lipid peroxidation products [19]. GSTs M1 and T1 have been shown to have activity toward lipid hydroperoxides, and individuals lacking each of these enzymes may have reduced removal of secondary organic oxidation products produced by cancer chemotherapy and thus may have better prognoses.

Ambrosone et al. [20] found that genetic polymorphisms in GSTs M1 and T1, known to be involved in response to ROS and products of lipid peroxidation resulting from chemo and radiation therapy, were associated with significantly reduced hazard of death and risk of recurrence following treatment of breast cancer. Women with null genotypes for both GSTM1 and GSTT1 had one third the hazard of death than those with alleles for both genes present. Though the hazard ratios were not calculated in the present study, there was no significant difference in the risk of recurrence in our study and response to chemotherapy also did not show any correlation with GSTT polymorphism.

In the Carolina Breast study [21], the relation of GSTM1, GSTT1, and GSTP1 genotypes and breast cancer risk in a population-based case-control study of African-American and White women in North Carolina was studied, which showed GSTM1, GSTT1, and GSTP1 genotypes were not associated with breast cancer risk in both races. Khedhaier et al. have found a significant association between gene deletion of GSTT1 and the risk of early onset of breast carcinoma [10]. Garcia et al. [22] reported no association between null genotype of both GSTT1 and GSTM1 and breast carcinoma, with null GSTT1 being protective in pre-menopausal women. Park et al. [23] showed a protective effect for null GSTM1. We found that null genotype of both GSTM1 and GSTT1 had

Table 4 Tumor markers and gene deletion

	ER, <i>n</i> (%)		<i>P</i> value	PR, <i>n</i> (%)		<i>P</i> value	Her 2 neu, <i>n</i> (%)		<i>P</i> value
	Positive	Negative		Positive	Negative		Positive	Negative	
GSTT1 <i>n</i> =20	4 (17.4 %)	16 (21.1 %)	1	16 (21.9 %)	4 (15.4 %)	0.6	0 %	13 (20.6 %)	0.5
GSTM1 <i>n</i> =33	11 (47.8 %)	22 (28.9 %)	0.1	24 (32.9 %)	9 (34.6 %)	0.9	2 (29 %)	23 (36.5 %)	0.6

Table 5 Adjuvant therapy and gene deletion

	GSTT1, <i>n</i> (%)		<i>P</i> value	GSTM1, <i>n</i> (%)		<i>P</i> value
	Null (<i>n</i> =20)	Present (<i>n</i> =79)		Null (<i>n</i> =33)	Present (<i>n</i> =66)	
Type of therapy						
Chemotherapy	20 (20.6 %)	77 (79.4 %)	1	32 (33 %)	65 (67 %)	1
Radiotherapy	8 (19.1 %)	26 (80.9 %)	0.6	10 (34.9 %)	24 (65.1 %)	0.6
Hormonal therapy	4 (12.1 %)	29 (87.9 %)	0.2	11 (33.3 %)	22 (66.7 %)	1.0
Type of chemotherapy			0.7		0.4	
Neoadjuvant	8 (18.6 %)			16 (37.2 %)		
Adjuvant	12 (22.2 %)			16 (29.6 %)		
Regimen of chemotherapy			NS			NS
CEF	25 (25.3 %)	74 (74.7 %)		39 (39.4 %)	60 (60.6 %)	
CAF	19 (19.2 %)	80 (80.8 %)		30 (30.3 %)	69 (69.7 %)	

a protective effect for the development of carcinoma breast. The variation in these results could be because of the variations in the population in the study group in each of these studies which could have caused the influence of other environmental factors on the incidence of cancer.

There are conflicting reports of GSTT polymorphism and menopausal status at which breast cancer manifests in the literature. Helzlsouer et al. [14] and Charrier et al. [24] reported positive associations for the GSTM1 null genotype and post-menopausal women, whereas Ambrosone et al. [20] reported a positive association between null GSTM1 and younger pre-menopausal women. Helzlsouer et al. [14] reported no association for GSTT1 null genotype and breast cancer in pre- or post-menopausal women, whereas Garcia-Closas et al. [22] observed an inverse association for GSTT1 null genotype among pre-menopausal women. We did not find any association between GSTT1 and GSTM1 gene deletion and menopausal status in the development of breast carcinoma.

With regard to clinicopathological factors, Sigelmann et al. [25] reported that GSTM1 gene was overexpressed among large primary tumors and axillary lymph nodes which concur with the results of our study, whereas Millikan et al. [21] reported that stage of the disease had no association with the GST genotype.

There have been few studies of GST genetic polymorphisms and survival. Two studies have evaluated the associations between GST genetic polymorphisms and survival with ovarian cancer [26, 27]. There was no effect of GSTM1 or GSTT1 phenotypes alone, but the combined null genotypes for GSTT1 and GSTM1 were associated with poorer survival. In studies of hematopoietic cancers, reduced risk of disease recurrence was noted among children with acute lymphoblastic leukemia who had alleles encoding no or lower activity for GSTM1, GSTP1, and GSTT1 [28]. The present study showed

no association between the survival and disease recurrence and GSTM1 and GSTT1 gene polymorphisms.

Our study showed a protective effect for the null genotype of both GSTM1 and GSTT1 genes for the development of breast cancer. There was also no relation between the existing risk factors and GST gene polymorphisms. There was also no prognostic significance of the status of the GST gene polymorphisms. The statistical power of the study is our main limitation because of the relatively small number of participants in the study.

The existing literature suggests that there is a definite association between the GST gene polymorphisms and breast carcinoma. It is possible that detection of this genetic polymorphism could be used as a predictor for response to chemotherapy and aid in the tailoring of therapy according to the genetic build up, though further studies with larger sample sizes and well-matched controls are needed for a comprehensive understanding of the gene–environment interactions and breast cancer risk.

Conclusion

The present study examined the association of GSTM1 and GSTT1 gene polymorphisms in a hospital-based case–control study with not only incidence of breast cancer but also with the response to chemotherapy. The results indicated the null genotype of both GSTT1 and GSTM1 to be protective for the development of carcinoma breast. All known etiological factors do not have any correlation with GSTT1 and GSTM1 gene deletion. It was found that patients with GSTM1 gene deletion tend to present with smaller tumors. Other tumor characteristics and clinicopathological parameters do not have any correlation with gene deletion.

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Conflict of Interest Authors Dr Virinder Kumar Bansal, Dr Karthik Rajan, Dr Arundhati Sharma, Dr Gaurav Charbal, Dr Vikas Jindal, Dr Mahesh C Misra, and Dr Kiran Kucheria have no competing conflict of interests or financial disclosures to make.

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