

RESEARCH COMMUNICATION

Lack of Influence of Glutathione S-Transferase Gene Deletions in Sporadic Breast Cancer in Pakistan

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Abstract

Glutathione S-transferases constitute the phase II detoxification enzymes involved in the metabolism and detoxification of a wide range of potential environmental carcinogens. GSTM1 and GSTT1 are polymorphic and their deletions have been found to be associated with breast cancer risk in some of the world populations. The current study was aimed at evaluation of GSTM1 and GSTT1 gene deletions in 150 unrelated breast cancer patients and 150 healthy controls from Pakistani population. Multiplex PCR assay along with CYP1A1 exon 7 as an internal control was used. Our sampled patients and controls had a mean age of 48 (+11.8) and 45 (+7.9) years respectively. The analysis suggested that only 2% breast cancer patient and 8% controls had homozygous GSTM1 gene deletions (OR 0.23, 95% CI 0.06- 0.85). A total of 8.7% patients and 18.6% controls had homozygous GSTT1 deletion (OR 0.41, 95% CI 0.25- 0.83). The statistical analysis suggest that a non significant number ($P>0.05$) of individuals compared to controls have GSTM1 and GSTT1 gene deletions. Deletion in both genes was not observed in any of the patients or controls. The present case control study suggests no association of GSTM1 and GSTT1 gene deletions with sporadic form of breast cancer in Pakistani population.

Keywords: Breast cancer - GSTM1 - GSTT1 - deletions - Pakistan

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Introduction

Breast cancer represents a major health problem in many countries (Henderson, 1993; Ghardirian et al., 1998) and is the most common type of cancer among Pakistani women (Faheem, 2007; Hanif et al., 2009). Epidemiological studies have suggested that environmental factors along with genetic susceptibility may play a major role in the development of breast carcinoma (Haris et al., 1992; Henderson, 1993).

Glutathione S-transferase (GST) is a family of genes with a critical function in the protection against electrophiles and the products of oxidative stress (Hayes, 1995). GSTs are widely distributed in nature and are found in essentially all eukaryotic species. The four major families of GSTs, based on their primary structure, are designated as α , μ , π , and θ and are encoded by the GSTA, GSTM, GSTP, and GSTT genes, respectively. Epoxides formed from PAHs are substrates for GSTM1 and GSTT1 enzymes. GSTM1 and GSTT1 are involved in the conjugation of electrophilic compounds with glutathione and making them water soluble and in turn excreted from the body. Mainly GSTM1 and GSTT1 are found in breast tissue (Forrester et al., 1990; Kelley et al., 1994; Salinas and Wong, 1999). These enzymes are expressed in normal breast tissue as well as in breast tumors (Sadreih et al., 1996; Lee et al., 1997; Stone et al., 1998).

At least 20 isoenzymatic forms of GST have been

identified, and many of them show genetically based individual variability of enzyme activity. The GSTM1 and GSTT1 genes both exhibit deletion polymorphisms (Seidegard et al., 1988; Pemble et al., 1994). Deletions of these genes, referred to as GSTM1 null and GSTT1 null genotype respectively, result in complete absence of enzymatic activity (Seidegard et al., 1988; Pemble et al., 1994). The null GSTM1 genotype appears to be common in several populations, whereas the null GSTT1 genotype exhibits population frequencies that depend on ethnicity (Bell et al., 1993; Nelson et al., 1995; Lin et al., 1998). The GSTM1 and GSTT1 defects seem to be associated with increased risk of breast cancers (Rebbeck, 1997; Strange et al., 1998); however, conflicting results have been observed in other cancers (Chen et al., 1996; Baily et al., 1998; Houston, 1999) which may be attributed to differences in study designs and the analyzed populations. The deletion mutations in GSTM1 and GSTT1 have been investigated for associations with breast cancer in a large number of studies. The GSTT1 polymorphism has also been investigated in many studies, also with conflicting results (Gudmundsdottir et al., 2001; Krajcinovic et al., 2001; Mitrunen, 2001; Xiong et al., 2001; de Fonte et al., 2002; Matheson et al., 2002; Siegelmann and Buetow, 2002; Zheng et al., 2002; Zheng et al., 2002).

In this study we have analyzed the relationship between gene deletions of GSTM1 and GSTT1 and the susceptibility to breast carcinoma in Pakistani population.

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Materials and Methods

The present case-control study consisted of 150 cases with pathologically confirmed breast cancer along with age and sex matched 150 cancer free normal individuals as controls. They were recruited from National Oncology and Radiotherapy Institute (NORI) and Institute of Medical Sciences (PIMS) Pakistan from March 2009 to April 2010 with a prior approval from Ethical Committees of both CIIT and hospitals.

All study subjects participated on a volunteer basis with informed consent. All subjects were personally interviewed according to a structured questionnaire. Blood was collected from subjects with their informed consent. Subjects' blood was sampled before starting the therapy. Blood samples were collected into EDTA-containing tubes and stored at -20°C until further use. DNA was isolated, using organic protocol with phenol-chloroform extraction as previously described (Baumgartner et al., 2001; Vierhapper et al., 2004). Electrophoresis was performed on isolated DNA in 1% ethidium-bromide stained agarose gel and photographed (BioDocAnalyze Biometra). 5ng dilutions were made of each DNA isolated and stored at 4°C until use.

Primers for GSTM1, GSTT1 and CYP1A1 exon 7 were synthesized by using primer 3 input software version 0.4.0 (Table 1) and BLAST using NCBI PRIMER BLAST. $2\mu\text{l}$ DNA (10 ng/ μl) was added to a $20\mu\text{l}$ PCR mixture composed of $2\mu\text{l}$ PCR buffer, $2\mu\text{l}$ of each primer (10mM), $0.24\mu\text{l}$ deoxynucleotide triphosphate (25mM) and $0.2\mu\text{l}$ Taq polymerase (5u/ μl). The reaction mixture was placed in 9700 thermal cyclor of ABI systems for 5 min at 94°C and subjected to 30 cycles at 94°C for 25 sec, annealing temperature for 1min and 72°C for 1 min, followed by a final step at 72°C for 10 min and hold at 4°C .

Amplification products were resolved on a 2% ethidium bromide-stained agarose gel along with 100bp DNA ladder. Amplification of CYP1A1, but no amplification of either GSTM1 or GSTT1 gene means deleted genotype of respective gene. All of the photographs of gel electrophoresis were read by two technicians blind to each other's assessments. Statistical analysis was performed by using SPSS statistics 17.0 software and GraphPad Prism 5 Demo for calculating Odds ratio, 95% confidence interval and standard deviation.

Results

Mean age of the breast cancer patients and controls was calculated as 48 (+11.8) and 45 (+7.9) years

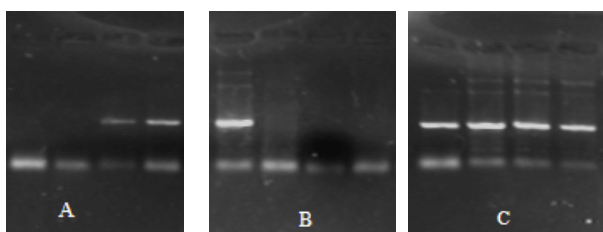


Figure 1. Agarose Gel Electrophoresis showing Deletion of GSTM1 and GSTT1 genes. A, GSTM1 deletion, B, GSTT1 deletion and C, positive control

Table 1. Primer Sequences Used for Amplification

Primer	Sequence
GSTM1 Forward	TCTGGGGAGGTTTGTTTTCA
GSTM1 Reverse	TGGACACAGAACATCATGGAA
GSTT1 Forward	GGCGAGAGAGCAAGACTCAG
GSTT1 Reverse	GGCAGCATAAGCAGGACTTC
CYP1A1 Forward	TGTCTACCTGGTCTGGTTGG
CYP1A1 Reverse	CCTCCAGGACAGCAATAAGG

Table 2. Statistical Analysis of GSTM1 and GSTT1 Gene Deletions

Variables	Patients	Controls	OR (CI 95%)
GSTM1 deletion	3	12	0.23 (0.06-0.85)
GSTT1 deletion	13	28	0.41 (0.25-0.83)

respectively. 2% breast cancer patients and 8% controls showed homozygous GSTM1 deletions. The percentage of GSTM1 null genotype was lower in the breast cancer cases compared with the controls (Odds Ratio [OR] 0.23, 95% [CI] 0.06- 0.85). The mean ages of patients and controls showing deletion of GSTM1 gene was 42 (+9.2) and 45.9 (+13.4) years respectively.

Deletion bands for GSTM1 and GSTT1 are shown in figure 1. For GSTT1 null genotype percentage of breast cancer cases (8.7%) was lower as compared to controls (18.7%) (Odds Ratio [OR] 0.41, 95% [CI] 0.25- 0.83) (Table 2). The mean ages of patients and controls with GSTT1 deletion genotype was calculated as 44.75(+12.5) and 43.5(+13.8) years respectively.

The difference of gene deletions between breast cancer patients and healthy controls were statistically non significant {GSTM1 ($P>0.05$) and GSTT1 gene deletions ($P>0.05$)}. Gene deletions for both genes were not observed for either cancer patients or healthy cancer free patients.

Discussion

The homozygous deletion genotypes of GSTM1 and GSTT1 gene have been reported with conflicting results in different populations. The present case control study was designed to find out the association of these deletions in Pakistani population. 150 cases and controls were studied. GSTM1 null genotype has been found associated with increased risk of breast cancer in many populations (Zhong et al., 1993; Mitrunen, 2001; Matheson et al., 2002), but in our study GSTM1 deletion polymorphism had no association with breast cancer risk. Our findings are in accordance with many populations such as China (Lori et al., 2008), Iran (Iraj and Mostafa, 2003), South Korea (Park et al., 2000), Australia (Curran et al., 2000), USA (Xiong et al., 2001), Iceland (Gudmundsdottir et al., 2001), Chinese Caucasians (Zhong et al., 2006) and Itay (Vogl et al., 2004; Linhares et al., 2005). Previously we have already reported GSTM1 and GSTT1 gene deletions to be associated with head and neck cancer (Nosheen et al., 2010)

GSTT1 gene deletion was also non-significantly associated with breast cancer risk. Studies reported earlier have found conflicting results regarding GSTT1

gene deletion in different populations. GSTT1 deletion genotype was found to be not associated with breast cancer development in our case control study and similar results have also been reported in other studies (Curran et al., 2000; Millikan et al., 2000; Park et al., 2000; Gudmundsdottir et al., 2001; Krajinovic et al., 2001; Mitrunen, 2001; Xiong et al., 2001; da Fonte et al., 2002; Matheson et al., 2002; Siegelmann and Buetow, 2002; Zheng et al., 2002; Iraj and Mustafa, 2003; Vogl et al., 2004). Nevertheless some studies has opposite trends where association has been seen with breast cancer risk (Rebeck, 1997; Strange et al., 1998).

The GSTM1 gene deletion is caused by a homologous recombination involving the left and right 4.2-kb repeats (Xu et al., 1998). These repeats result in a 16-kb deletion containing the entire GSTM1 gene. The GSTM1 gene is excised relatively precisely leaving the adjacent genes intact. Therefore, one can rule out recombination with neighboring GSTM genes as a possible mechanism for the GSTM1 gene deletion, despite extensive homologies in certain regions. Similar to GSTM1 null genotype, the GSTT1 gene deletion is most likely caused by a homologous recombination event involving the left and right 403-bp repeats. The recombination results in a 54-kb deletion containing the entire GSTT1 gene (Fritz, 2004).

In this study our cancer free controls have also shown GSTM1 and GSTT1 gene deletions. Asian population and Caucasians have nearly 50% population with deletion of these genes (Mitrunen, 2001). In Pakistan also 45% normal population have GSTM1 deletion and 23% normal population have GSTT1 gene deletions (Rehan et al., 2010). This study confirms the results of earlier studies regarding the deletion polymorphisms of these genes in our normal population.

The current study reports a statistically non-significant difference of gene deletions for GSTs (GSTM1 and GSTT1) between breast cancer patients and healthy controls. Thus GSTM1 and GSTT1 gene deletions cannot be considered as a risk factor for developing breast cancer specially in Pakistani population. However, more extensive studies involving many genes interactions with much larger number of cases and controls are needed in Pakistani population.

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