

# Genetic Polymorphism of the Glutathione S-Transferase M1 and Development of Breast Cancer

Iraj Saadat<sup>1</sup>, Shahpour Omidvari<sup>2</sup> and Mostafa Saadat<sup>\*1</sup>

<sup>1</sup>Dept. of Biology, College of Sciences, Shiraz University, Shiraz; <sup>2</sup>Dept. of Radiotherapy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

## ABSTRACT

Glutathione S-transferases (GSTs) are encoded by a superfamily of genes and play a role in the detoxification of potential carcinogens. The human GSTs are divided into four classes: alpha, mu, pi and theta. Previous studies indicated that the absence of the Glutathione S-Transferase M1 (GSTM1) protein correlated with an increased risk of developing some types of cancers. Association between specific genotype and the development of breast cancer is still an open question. In the present study, the association between genetic polymorphism in the GSTM1 and susceptibility to breast cancer was investigated. The genetic polymorphism of the GSTM1 in exon 5 to exon 6 segments was detected by PCR in 59 patients with breast cancer and 59 normal subjects. The frequency of the GSTM1 null genotype in control and patient groups was 39.0% and 50.8% respectively. There was no significant association between null genotype of the GSTM1 and susceptibility to breast cancer ( $X^2 = 1.63$ ,  $df = 1$ ,  $p > 0.05$ ). *Iran. Biomed. J. 5 (1): 21-25, 2001*

**Keywords:** Breast cancer, Glutathione S-transferase M1, Polymorphisms

## INTRODUCTION

Both genetic and environmental factors are involved in the development of cancer. The environment gene interaction on carcinogenesis has been well demonstrated by phase I and phase II enzymes that are involved in the metabolism of the carcinogens. The phase I enzymes, cytochrome P450 (CYPs), activate many environmental procarcinogens by adding or exposing their functional groups [1]. The phase II enzymes, including glutathione S-transferase (GST) (E.C. 2.5.1.18) and N-acetyl transferase, are involved in the detoxification of activated metabolites of the carcinogens [2].

The human GST is a family of multifunctional enzymes that catalyzes the conjugation reaction between reduced glutathione and a variety of electrophiles [3]. The GSTs are divided into four classes: alpha, mu, pi and theta based on amino acid sequence similarity and antibody cross-reactivity [4]. In human, hereditary differences in the specific GST activities are due to the genetic polymorphisms [5, 6].

One member of the GST $\mu$  class, GSTM1, is involved in the detoxification of tobacco-related

carcinogens, such as epoxides and hydroxylated metabolites of benzo (a) pyrene [7]. The GSTM1 is polymorphic in human and three alleles have been described: two functional alleles (GSTM1\*A and GSTM1\*B) and a non-functional null-allele (GSTM1\*0) [8, 9]. The GSTM1\*0 allele has been shown to be the result of a deletion of the entire GSTM1 gene [5].

Several studies have shown that GSTM1 null genotype is associated with an increased risk of lung, bladder, gastric, colorectal, skin, breast and laryngeal cancers, and acute lymphocytic leukemia [10-18]. Whereas, other studies did not find a significant association between subjects lacking GSTM1 activity and the risk of developing breast cancer [17-19]. Thus the relationship between GSTM1 null genotype and susceptibility to breast cancer is still an open question.

In the present study, we employed PCR-based genotyping method to examine the GSTM1 polymorphism. We studied a control group of healthy blood donors to know the null genotype frequency in the general population and a group of breast cancer patients to evaluate the possible role of the GSTM1 null genotype in breast cancer susceptibility.

\*Corresponding Author; Tel. (98-711) 2220026; Fax: (98-711) 2220027

## MATERIALS AND METHODS

**Blood sampling and extraction of DNA.** Blood samples (5 ml taken into EDTA at venipuncture) were obtained from 59 patients bearing histologically proven breast cancer (mean age:  $46.0 \pm 10.6$ ; range: 27 to 68) in the Nemazi Hospital in Shiraz, Iran. Affected patients were recruited before or at the beginning of the radio- or chemotherapy treatment. Control blood samples were obtained from 59 healthy females (mean age:  $39.8 \pm 15.1$ ; range: 25 to 75). Both patients and control groups were unrelated Iranian Muslims. Immediately after collection, whole blood was stored at  $-20^{\circ}\text{C}$  until use. The genomic DNA for PCR was isolated from whole blood using the thawed blood samples by the standard procedure [20].

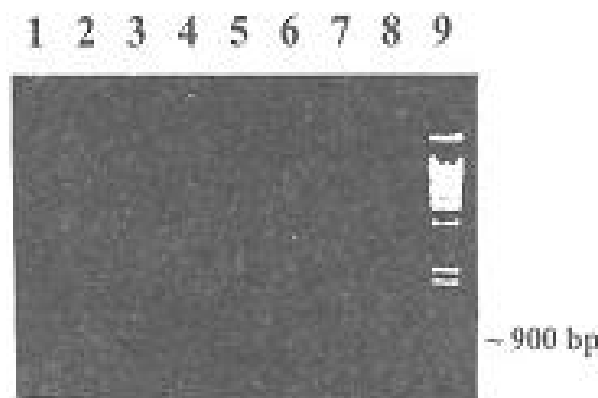
At the time of blood donation, participants completed a brief questionnaire that ascertained smoking status, medication taken, age, alcohol consumption, and family history for malignancies.

**Polymerase chain reaction (PCR).** Primers for amplifying the GSTM1 gene segment corresponding to exon 5, intron 5, and exon 6 were 5'AGACAGAAGAGGAGAAGATTC3' and 5'TCCAAGTACTTTGGCTTCAGT3' [5]. PCR was performed in 50  $\mu\text{l}$  reaction buffer containing 200  $\mu\text{M}$  dNTP, 1.5 mM, 1  $\mu\text{M}$  primers, about 1  $\mu\text{g}$  DNA and 2 units of thermostable *Taq* DNA polymerase using a programmable thermocycler (Progene, Techne, England). After 5 min of pretreatment at  $94^{\circ}\text{C}$ , 35 cycles of 1.5 min denaturation at  $94^{\circ}\text{C}$ , 1.5 min annealing at  $61^{\circ}\text{C}$  and 1 min extension at  $72^{\circ}\text{C}$  were performed. For evaluating the GSTM1 polymorphism, the amplification products were analyzed by gel electrophoresis (1.6% agarose). This technique does not distinguish between heterozygote and homozygote GSTM1-positive genotypes, but it conclusively identifies null genotype. To ensure laboratory quality control, two independent readers interpreted the gel photographs. Any sample with ambiguous results (generally due to low PCR yield) was retested, and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

**Statistical analysis.** The statistical significance of difference for frequency of the null genotype between patient and control groups was tested by chi-square analysis. A probability of  $p < 0.05$  considered statistically significant.

## RESULTS AND DISCUSSION

The amplified region between exons 5 and 6 including intron 5 clearly showed a single band of around 900 bp after 1.6% agarose gel electrophoresis, as reported previously [17, 18] (Fig. 1). The absence of amplified product was consistent with the null genotype.



**Fig. 1.** PCR for GSTM1 null and GSTM1 positive genotype identification: Agarose gel (1.6%) electrophoresis showing the 900 bp DNA fragment amplified from GSTM1. Lanes 1-3; healthy controls, Lanes 4-7; breast cancer subjects, Lane 8; no DNA; Lane 9; Hind III digested DNA marker. Lanes 1, 2, 4, and 7 showed the amplification products of GSTM1 nucleotide sequences between exon 5 to exon 6. Lanes 3, 5 and 6 showed no PCR product (= null genotype).

The results of the present study are summarized in Table 1. The prevalence of the GSTM1 null genotype is 39.0 % in the normal control group which is confirm our previous report [18]. The frequency of the null genotype has been shown to vary among different groups [17, 21-24]. About 50% of the individuals in most Caucasian populations and other populations are homozygous for the GSTM1\*0 allele and lack of the GST $\mu$  activity. However, the frequency of the null genotype in the studied population (Shiraz, Iran) is low, when compared with other studied populations. Because the GSTM1 located on human chromosome 1p13 [25], the lack of the GST $\mu$  activity inherited as an autosomal recessive phenotype. There is no statistically differences ( $X^2 = 0.12$ ;  $df = 1$ ;  $p > 0.05$ ) for the frequency of null genotype between males (35.7%) and females (39.0%) of healthy normal control groups (Table 1). The frequency of the null genotype was 50.8 % in patient group (Table 1). Comparison between the two studied groups showed that the observed difference was not significant ( $X^2 = 1.63$ ,  $df = 1$ ,  $p > 0.05$ ).

**Table 1.** Distribution of null- and non null-GSTM1 genotypes and development of breast cancer, stratified by selected characteristics

Subgroups (N)	GSTM1 null	Non-null
<b>Whole group</b>		
Female controls (59)	23	36
Male controls (56)      20	36	
Breast cancer patients (59)	30	29
<b>Age</b>		
Age <50		
Female controls (44)	17	27
Breast cancer patients (37)    20	17	
Age >50		
Female controls (15)	6	9
Breast cancer patients (22)	10	12
<b>History of cigarette smoking</b>		
Never		
Female controls (57)	22	35
Breast cancer patients (48)    25	23	
Ever		
Female controls (2)	1	1
Breast cancer patients (11)    5	6	
<b>Family history of malignancy in first degree of relatives</b>		
No		
Female controls (55)	22	33
Breast cancer patients (53)	27	26
Yes		
Female controls                      (4)	1	3
Breast cancer patients              (6)	3	3

Several studies have shown that GSTM1\*0 is associated with an increased risk of lung, bladder, stomach, larynx, and breast cancers and acute lymphocytic leukemia [10-18]. However, those studies did not find an association between GSTM1\*0 and breast cancer [17, 19].

Our data demonstrated that although the GSTM1 null genotype is more frequent in breast cancer cases than in community controls, this difference does not reach a statistical significant in the whole population under study. Our results are compatible to those obtained by other groups [17, 19], who studied on the large sample size of both control and patient groups [19].

The examination of GSTM1 genotypes in some stratified patient groups sometimes gave only weak statistical significance for some groups [12, 15, 26]; however, the overall pattern in those data indicated

that the null genotype may be associated with a slightly increased risk for developing breast cancer. As shown in Table 1, the risk of breast cancer associated with GSTM1 genotypes, stratified by age, cigarette smoking, and family history of malignancy in first degree relatives, was not statistically significant. A history of cigarette smoking was statistically associated with increased risk of breast cancer ( $X^2 = 7$ ;  $df = 1$ ;  $p < 0.05$ ).

In addition to polymorphism in phase II enzymes, genetic polymorphism in the phase I enzyme cytochrome P450 1A1 (CYP1A1) gene were correlated with an increased susceptibility to chemical carcinogenesis [27, 28]. CYP1A1 is involved in the activation of several procarcinogens [29]. Genetic variants in the CYP1A1 gene can result in an enhancement of the CYP1A1 activities [30].

There is a need to study biological and epidemiological aspects of breast cancer. This is required to elucidate the cooperative or possible antagonistic effect(s) of GSTM1 gene polymorphisms with other phase I or phase II enzymes, as well as the effect(s) of environmental exposure in breast carcinogenesis.

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