Original Research Article

Evaluation of common polymorphisms (GTSM1, GSTT1 and GSTP1) in S-glutathione transferase family and susceptibility to basal cell carcinoma (BCC) in Iranian population

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ABSTRACT

The glutathione S-transferase (GST) is a polymorphic supergene family of detoxification enzymes that are involved in the metabolism of potential carcinogens. To evaluate the association between polymorphisms in the GSTs (GSTT1, GSTM1 and GSTP1) and basal cell carcinoma (BCC), 50 BCC patients and 50 healthy controls were studied. Based on our findings, the frequencies of GSTM1 null genotype in patients and controls were 34 % and 6 %, respectively. Furthermore, the overall frequency of GSTT1 null genotype was higher than in the BCC patients compared to the control samples. The strongest association was observed between GSTP1 polymorphism and BCC in the population. Genotype mixture of GSTM1 and GSTT1 showed a higher risk of developing BCC in subjects with one null genotype. This study suggested a reasonable association between GST polymorphisms and BCC susceptibility among Iranian population.

***Keywords*:** Basal Cell Carcinoma, polymorphisms, S-glutathione transferase, Iranian population

INTRODUCTION

Cancers are a group of disease with abnormal cell growth and potential to spread to different tissues of body [1-5]. Despite much progress in the management of cancer, cancer is still a major public health problem and one of the deadliest diseases worldwide, with approximately 14 million new cases and 8.2 million death each year [6].

Basal cell carcinoma (BCC) is the most prevalent malignancy in caucasians and its incidence is increasing worldwide. Although the majority of BCC have occurred sporadically, there are some scarce syndromes associated with susceptibility to BCC [7,8]. BCC frequently happens by sun-exposure to the head and neck. Several evidence indicates that different genetic and environmental factors are contributed to the etiology of basal cell carcinoma [9]. It was previously demonstrated that variations in the glutathione S-transferase (GST) family increases the risk of susceptibility to multiple basal cell carcinoma [10,11]. Glutathione S-transferases (GSTs) constitute a superfamily of ubiquitous, multifunctional enzymes that have protective role against chemical toxins and carcinogens by catalyzing the conjugation of glutathione to electrophiles [10]. This supergene family are located on seven chromosomes encoding the glutathione-S-transferase (GST) enzymes. Approximately 16 genes encode the enzymes in the cytosols and the product of six of them is expressed on the membranes [12]. In mammals, the GSTs comprise several isoenzymes, including alpha (A), mu (M), pi (P), theta (T), and zeta (Z) gene families [13]. Among them, GSTM1, GSTT1 and GSTP1 polymorphisms have been extensively studied. GSTM1 is situated in the GSTμ cluster, which is localized in chromosome 1 in region 1p13.3 and catalyzes the metabolism of epoxides formed from pollutants such as polycyclic aromatic hydrocarbons [14]. GSTT1 gene is located on chromosome 22q11.2 and is involved in the metabolism of small compounds found in tobacco smoke like mono halo methanes and ethylene oxide [15-17]. GSTT1 also participates in the metabolism of oxidized lipid, DNA and epoxides [15]. GSTP1 function were found to detoxify some compounds and some drug-resistance tumor, because this polymorphism was conjugated with amines and other carcinogens [18]. Changes in susceptibility to certain types of cancer observed in a group of individuals are often linked to the genetic polymorphism observed in enzymes that play important role in the detoxification of Xenobiotics [19-21]. Alteration in GST enzyme activities are supposed to affect the individual metabolism

of carcinogenic aromatic amines, thereby modifying the vulnerability to certain cancers. The GST gene family in human is highly polymorphic, however, GSTM1 and GSTT1 genes are deleted in approximately 50-20 % of the Caucasian population, respectively with a significant loss of enzyme function [21,22]. Inheritance of null GST alleles are shown to be associated with increased susceptibility to non-melanoma skin cancers [22,23]. GST null genotype has been shown to be associated with the increased sunburn sensitivity and reduced minimal erythemal dose (MED) after broadband UV radiation exposure in healthy volunteers and with susceptibility to skin cancer [24,25]. In particular, individuals with the null genotype at both loci may be vulnerable, especially for oxidative or chemical stress [7]. Also, GSTP1 Ile105Val polymorphism was recommended to be associated with risk in progressing cancer [26,27]. In this study, we analyzed three polymorphisms (GSTM1, GSTT1 and GSTM1) are associated with the risk of skin cancer in Iranian population.

MATERIALS AND METHODS

Sample collection

In this study, 50 patients (17 females and 33 males) with histologically proven BCC were recruited from skin research institute of Shahid Beheshti university of medical sciences in Tehran.

A control group comprising 50 sex-matched individuals referred to this center for dermatological problems were also recruited. Controls had no clinical or histological evidence of any malignancy. This study was approved by the ethics review committee of Guilan University. Paraffin embedded skin biopsies of patients were cut into slices by microtome (12µ diameter), placed in 1.5 ml Microtubes and kept at room temperature for DNA extraction. Blood samples were obtained from controls and reserved at 4°C until implementation. DNA were extracted from skin specimens by the manufacturer protocol using tissue DNA Mini kit (Bioron GmbH, Germany) and genomic DNA were extracted from whole blood sample using an established protocol from nucleated blood cells [28].

Allele assay and polymorphism genotyping

Gap-PCR *(*Polymerase chain reaction) was carried out for GSTM1 and GSTT1 gene amplification. The sequences of the primers used in this study are shown in table 1. The amplification was performed in the following mixture (total volume 15 µL): 7.5µl Taq DNA Polymerase 2x Master Mix, Red containing dNTP mix and MgCl2 (Ampliqon, Denmark), 1.5 µl from 10 µM primers of GSTM1 and GSTT1, 0.8 µl of 10

μM β-gol primers, with 5 μl (approximately 30 ng) isolated DNA. The reaction mixture for GSTM1 was first subjected to initial denaturation at 95 °C for 5 min; 30 cycles consisting of denaturation at 94 °C for 30 sec, primer annealing at 59 °C for 40 sec and DNA extension at 72 °C for 30 sec; the final DNA extension was at 72 °C for 3 min. The amplification conditions for GSTT1 were: initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 45 sec, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The products of the PCR amplification (GSTM1-267bp, GSTT1-434 bp and β-gol -102 bp) were visualized by electrophoresis on 2 % agarose gels. Both GSTM1 and GSTT1 products were categorized as having either a non-null or null (homozygous deletion) genotype. β-globin primers were used as internal controls. ARMS-PCR was used to determine GSTP1 Ile105Val genotype. Primers feature are provided in table 1. PCR was performed in a 15 μL (final volume) containing 7.5 μl Taq DNA Polymerase 2x Master Mix, Red containing dNTP mix and $MgCl_2$ (Ampliqon, Denmark), 1.5 μL from of primers, and 5 μL (approximately 30 ng) of extracted DNA.

PCR amplification was carried out by denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 63 °C for 50 sec and 72 °C for 30 sec and a final extension at 72 °C for 5 min. PCR products were analyzed by 2 % agarose gel electrophoresis.

Statistical analysis

All data analyses were performed using SPSS 16 (SPSS Inc. Chicago, IL, USA). P-values less than 0.05 were considered statistically significant. The chi-square ($\chi 2$) and Fischer (F) exact test were used to compare variables between groups. All tests were two-sided. Continuous variables were reported as mean ± SD or as medians. The studied individuals were divided into two groups: BCC patients (Micronodular and superficial subtypes) and other subtypes. The association between GST genotypes and disease status (BCC patients and healthy controls) was estimated by computing odd ratio (OR) and 95 % confidence intervals (CI) from logistic regression analyses.

RESULTS

In this study, the GSTM1, GSTT1 and GSTM1 genotypes were evaluated in 50 suspected BCC patients and 50 healthy controls. Based on the findings, considering baseline demographics and clinical features of participants, there were no significant differences between the two groups regarding the age and gender of participants (table 2). Classification of BCC in patients

showed that most lesions were solid (46 %), micronodular (18 %) and pigmented (16 %). Other less common histological forms were adenoid (8 %), superficial (8 %) and keratotic (4 %). Distribution of GST genotypes in patients with BCC and healthy individuals was summarized in table 3. Frequency of polymorphism alleles (Val in GSTP1 and null in GSTT1 and GSTM1) was more prevalent among patients with BCC than healthy controls ($P < 0.001$ for all comparisons). The results revealed that GSTM1 null genotype (GSTM1*0/0) was significantly more frequent in BCC patients compared to control subjects (34 % and 6 %, , respectively) and subjects with GSTM1 null genotype were more likely to develop BCC than healthy individuals (OR=8.07, 95 % CI: 2.19 to 29.78, P=0.002). Also, GSTT1 null genotype (GSTT1*0/0) was significantly more common in patients with BCC than healthy subjects (34 % and 12 %, respectively). In addition, subjects with GSTT1 null genotype were significantly at higher risk of developing BCC compared to the controls (OR=3.78, 95 % CI: 1.34 to 10.63, P=0.01). The results depicted that Ile/Val of GSTP1 polymorphism was enhanced the risk of BCC dramatically (OR=12.67, 95 % CI: 4.90 to 32.73), and this association was the strongest point in this study. According to genotype combinations between GSTM1 and GSTT1, subjects with at least one null genotype in combined GST were more likely to develop BCC than subjects with both null genotype (-/-) (OR=4. 47, 95% CI: 1.75 to 11.43, P=0. 002). Also the combined genotypes of GSTM1 present (+) / GSTP1 Ile/Val or Val/Val or GSTT1 present (+) / GSTP1 Ile/Val or Val/Val were more frequent in control samples than patients with BCC.

Founded on our results, the frequency of polymorphisms (GSTM1, GSTT1 and GSTP1) genotypes were not significantly different between patients with aggressive (micronodular and superficial types) and non-aggressive (other subtypes) BCC types. In addition, the proportions of patients with at least one null genotype in combined GST were not significantly different between aggressive and non-aggressive BCC subtypes (P=0.21). Thus, GSTT1 null genotype is considerably associated with the BCC occurrence in Iranian population (P=0.01), is shown in table 4.

Table 1. Primer sequences were used for genotyping in this study

Primer	Locus	Size of Product (bp)	Primer Sequence (5′ to 3′)
GSTM1-F GSTM1-R	GSTM1	267	TACTTGATTGATGGGGCTCAC CTGGATTGTAGCAGATCATGC
GSTT1-F GSTT1-R	GSTT1	434	CTTACTGGTCCTCACATCTC CAGGGCATCAGCTTGTGCTTT
β-gol-F β-gol-R	β-gol	102	GTGCACCTGACTCCTGAGGAG CCTTGATACCAACCTGCCCAG
GSTP1-105-FC GSTP1-105-RC	GSTP1	401	TCCTCTTCCCCTCCTCCA CTGCAGGTTGTGTCTTGTCC
GSTP1-105-RM GSTP1-105-RN		226	ACATAGTTGGTGTAGATGAGGGTGAC ACATAGTTGGTGTAGATGAGGGTGAT

Table 2. Baseline demographics and clinical features of the participants in this study

Characteristic	Patients with BCC (n=50)	Healthy controls (n=50)
Gender, no. (%)		
Female	17 (34 %)	17 (34 %)
Male	33 (66 %)	33 (66 %)
Age, years		
Mean ± SD	62.54±12.27	58.78±11.39
Median (range)	65 (29-84)	62.5 (30-80)
Types of BCC		
Solid	23 (46 %)	
Micronodular	9 (18 %)	
Pigmented	8 (16 %)	
Adenoid	4 (8 %)	
Superficial	4 (8 %)	
Keratotic	2 (4 %)	

Table 3. Distribution of glutathione S-transferase genotypes in patients with BCC and healthy individuals

Genotypes	**Number (%)**		**OR (95% CI)**
	Patients with BCC (n=50)	Healthy controls (n=50)	
GSTM1			
Null	17 (34 %)	3 (6 %)	8.07 (2.19-29.78)
Present	33 (66 %)	47 (94 %)	1.00 (reference)
GSTT1			
Null	17 (34 %)	6 (12 %)	3.78 (1.34-10.63)
Present	33 (66 %)	44 (88 %)	1.00 (reference)
GSTP1			
Ile/Ile	12 (24 %)	40 (80 %)	1.00 (reference)
Ile/Val	38 (76 %)	10 (20 %)	12.67 (4.90-32.73)
Val/Val	0 (0 %)	0 (0 %)	
GSTM1/ GSTT1			
One null	12 (24 %)	7 (14 %)	4.47 (1.75-11.43)*
Both null	11 (22 %)	1 (2 %)	
Both present	27 (54 %)	42 (84 %)	1.00 (reference)
GSTM1/GSTP1			
M1 (+/+)/P1 (Ile/Ile)	8 (16 %)	37 (74 %)	
M1(+/+)/P1(Ile/Val)	25 (50 %)	10 (20 %)	
M1 (-/-) /P1 (Ile/Ile)	4 (8 %)	3 (6 %)	
M1(-/-) /P1 (Ile/Val)	13 (26 %)	0 (0 %)	
GSTT1/GSTP1			
T1 (+/+)/ P1 (Ile/Ile)	8 (16 %)	34 (68 %)	
T1(+/+)/ P1 (Ile/Val)	25 (50 %)	10 (20 %)	
T1 (-/-) /P1 (Ile/Ile)	4 (8 %)	6 (12 %)	
T1 (-/-)/P1 (Ile/Val)	13 (26 %)	0 (0 %)	

OR, Odds Ratio; 95 % CI, 95 % Confidence Interval *The two categories of one null and both null were pooled for calculating OR (95 % CI), because of few observations in category of both null.

Table 4. Distribution of glutathione S-transferase genotypes according to BCC subtypes

Genotypes	BCC subtypes		OR (95% CI)
	Micronodular+Superficial (n=13)	Other subtypes (n=37)	
GSTM1			
Null	2 (15.4 %)	15 (40.5 %)	0.27 (0.05-1.38)
Present	11 (84.6 %)	22 (59.5 %)	1.00 (reference)
GSTT1			
Null	2 (15.4 %)	15 (40.5 %)	0.27 (0.05-1.38)
Present	11 (84.6 %)	22 (59.5 %)	1.00 (reference)
GSTP1			
Ile/Ile	1 (7.7 %)	11 (29.7 %)	1.00 (reference)
Ile/Val	12 (92.3 %)	26 (70.3 %)	5.08 (0.59-43.95)
Val/Val			
GSTM1/GSTT1			
One null	4 (30.8 %)	8 (21.6 %)	0.42 (0.11-1.61)*
Both null	0 (0 %)	11 (29.7 %)	
Both present	9 (69.2 %)	18 (48.6 %)	1.00 (reference)

The values were number of subjects, unless otherwise stated.
OR, Odds Ratio; 95 % CI, 95 % Confidence Interval, other subtypes included solid, pigmented, adenoid and keratotic BCC. *The two categories of one null and both null were pooled for calculating OR (95 % CI)

DISCUSSION

In human body a variety of antioxidant enzymes, including glutathione S-transferase enzymes and different mechanism protect the cells from the dangerous oxidative process. The glutathione S-transferase is a polymorphic supergene family of detoxification enzymes that show a protective role against chemical toxins and carcinogens by catalyzing the conjugation of glutathione to electrophiles in the substrate. However, the mechanism of the association between BCC occurrence and polymorphisms still remains unclear [29,30]. Considering the critical role of GST family genes in cells detoxification, it is assumed that the polymorphisms of the GST genes may influence the susceptibility to BCC occurrence. Therefore, we evaluated the association of the polymorphisms of GST gene (GSTM1, GSTT1 and GSTM1) with the risk of the development of BCC in a case-control study.

In the present study, both GSTM1 and GSTT1 null genotypes revealed a significant association with susceptibility to BCC. In the patient group, the frequencies of the null genotypes for GSTM1 and GSTT1 had higher percentage compared to the controls, hence indicating that these null genotypes were associated with the risk of developing BCC in Iranian population (*GSTM1*: OR=8.07, 95 % CI: 2.19-29.78, *P*=0.002; for *GSTT1*: OR=3.78, 95 % CI: 1.34-10.63, *P*=0.01). These results were revealed evidence about the association between UV radiation, the most important cause of basal cell carcinoma, and pathogens for development of BCC [31]. Thus, host genetic factors could be important in this process [32]. The relationship between the GSTM1 or GSTT1 polymorphisms and the risk of different kinds of cancer has been previously reported [33-38]. There were numerous evidences presenting a correlation between GST gene polymorphisms, particularly, GSTM1 and GSTT1, with vulnerability to non-melanoma cancers including BCC [22,23,39]. Furthermore, the frequencies of homozygous null genotypes of GSTM1 and GSTT1 in the control group were 6 % and 12 %. Interestingly, our results were different from other studies which probably were the result of genetic features in our population. In other words, the frequency of the GSTM1 null genotype is greater than GSTT1 null genotype in other populations [40].

Moreover, in a study carried out on 222 English patients after renal transplantation, GSTM1 and GSTT1 genes were shown no association with the risk of BCC [41], which was in contrast with the findings of the current study. The discrepancy between results could be explained by ethnic and racial variations or it could be the result of small size of the samples or genotyping technique.

Present study was in agreement with those studies that found a significant correlation between both GSTM1 and GSTT1 polymorphism and higher risk of BCC. Lear and his colleagues demonstrated that glutathione S-transferase enzyme (GSTM1, GSTT1) influenced tumor [7]. Besides, in a study which was carried out in Turkey, a 2.12 fold increased risk of BCC cancer was recorded in individuals with the GSTM1 null genotype compared to the control group [42]. Leite and his colleagues indicated that BCC patients with the combined GSTM1-GSTT1^{+} genotype were more frequent (49.1 %) than controls among Brazilians (29.8 %) (P =0.04) [22]. The obtained data were compatible with the findings of Lira and his colleagues, who revealed a higher risk of BCC in Italian patients with GSTM1 null/null (P=0.003) [23]. Heagerty and his

colleagues concluded the association of heterozygote (GSTM1 A/B) genotype with a reduced risk of multiple BCC [43]. The mechanism for this protective effect against multiple BCC is unclear, but might be related to the ability of these enzymes to catalyze the metabolism of a variety of products of oxidative stress [22]. Some investigations on antioxidant function of GSTP1 in skin cancer, were suggested this enzyme plays an important role in the etiology of skin malignancies [44]. Nevertheless, these data were contrary to data obtained from Brazilian patients with BCC [22].

Previous studies emphasized that the null genotypes of GSTM1 and GSTT1 lead to lack of enzyme activity [45]. Furthermore, the heterozygous genotype of GSTP1 (Ile105Val) are related to decreased function of enzymes [46]. Some reports suggest that the null genotypes of GSTM1 and GSTT1 or heterozygote genotype of GSTP1 lessen GST detoxification function, leads to grow toxic metabolites [38,47]. The relationship between GST genotypes and BCC was confirmed the prior observations. It was suggested that GSTP1 Ile105 allele was connected to the risk of basal cell carcinoma [39,48]. It was demonstrated that there was a logical relationship between Val/Ile and Val/Val genotypes of GSTP1 and lack of TP53 expression in BCC [49].

We illustrated the genotype combination of (+) GSTT1 or GSTM1 and Ile/Val of GSTP1 play a protective role against BCC. Furthermore, the genotype of GSTP1Val/Val was not observed in any of the studied patients as well as controls. Previous studies disclosed that GSTP1 Val/Val was correlated with the phenotype multiple presentation (MPP) in the BCC [10]. The BCC patients in current study had only a single lesion at any presentation (SPP). And also MPP phenotype was not observed in any of the studied patients. The union of three polymorphisms GSTM1, GSTT1 and GSTP1 influenced by different cancers in Iranian patients. For example, null genotypes of GSTM1, GSTT1 and GSTP1 were related to the risk of prostate cancer and uterine leiomyoma risk in Iranian patients [36,38]. Also the GSTM1 polymorphism had a strong impact on breast cancer development in Iranian women [47]. Since GST enzymes were known to regulate pathways that prevent damages caused by several carcinogens, individuals with homozygous deletions of GSTM1 or GSTT1 alleles may be unabled to eliminate electrophilic carcinogens efficiently, lead to increase risk of generating somatic mutations that produce tumor formation.

CONCLUSION

In this study, a significant association was found between both GSTM1*0/0 and GSTT1*0/0 genotypes and BCC. Therefore, further extensive research with a large number of samples from different populations will be required to validate the relationship between the studied polymorphisms and BCC risk. Moreover, as a multifactorial disease, BCC should be studied effects of different environmental and genetic factors. A better understanding of factors that predispose single and multiple BCC could help devise better preventative strategies for increasing public health problem. Accordingly, the GST genotypes could be assumed as a risk factor for the BCC prevalence in this population. So GST polymorphisms profile could help identification of Iranian individuals with higher risk for BCC.

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