Polymorphisms at GSTM1 and GSTT1 gene loci and susceptibility to cervical cancer in Indian population

A. SHARMA[1], J.K. SHARMA[1*], N.S. MURTHY[2], A.B. MITRA[1]

[1]Division of Molecular Genetics, e-mail: jksharma55@hotmail.com, and [2]Division of Biostatistics, Institute of Cytology and Preventive Oncology (ICMR) Bahadur Shah Zafar Marg, New Delhi-110002, India.

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Multiple allelism at loci encoding detoxifying enzymes is associated with cancer risk. Glutathione S-transferase (GSTs) catalyzes the conjugation of glutathione to numerous potentially genotoxic compounds. This study evaluates the influence of genetic polymorphisms of GST M1 and GST T1 on susceptibility to cervical cancer. A multiplex polymerase chain reaction method was used to detect the presence or absence of the GSTM1 and GSTT1 genes in genomic DNA isolated from cases with cervical cancer (n=142) and normal controls (n=96). The results showed that the frequency of homozygous GSTM1 null genotype was higher in cervical cancer cases (57.0%) as compared to controls (34.4%) and the differences were significant ($p<0.05$), OR=2.5, 95% CI: 1.4–4.5. The frequency of homozygous GSTT1 null genotype in cancer cases was 19.7% in comparison to 12.5% in controls, however, the difference was not statistically significant (OR=1.7, 95% CI: 0.8–3.8). Significant difference was found between the cases and controls in the distribution of the null genotype of GST M1 in individuals aged above 45 years ($p=0.04$), but this difference was not significant in individuals aged below 45 years ($p=0.06$). No significant differences were found in cervical cancer cases and controls when data were analyzed according to age group for GSTT1 null genotype. Further, the combined analysis of both GSTM1 null and GSTT1 null genotypes did not appear to influence the susceptibility to cervical cancer, suggesting that polymorphisms of other detoxifying enzymes may play a significant role in cervical carcinogenesis.

Key words: cervical cancer, epidemiology, glutathione S-transferase, GSTM1, GSTT1, polymorphism

Cancer of the uterine cervix is the leading cancer among women in many developing countries including India, and remained a major world wide health problem [22]. The etiology of cervical cancer has been thought to be multifactorial [8]. Various risk factors include genital, sexual, chemical, dietary, HPV and life factors, but there is no consistency in the independent effect of these factors in the development of cervical cancer [20]. There is likely to be a complex interaction between environmental and genetic factors in the development of cervical cancer.

The Glutathione S-transferases (GSTs) are a family of enzymes which are involved in xenobiotic detoxification by means of conjugating glutathione to the electrophilic centre of the compound. GSTs contribute to the protection against a broad range of compounds including carcinogens, pesticides, antitumor agents and environmental mutants [2]. GST M1 and T1 are among the most studied phase II enzymes regarding cancer susceptibility. GSTM1 detoxifies more specifically active metabolites of polycyclic aromatic hydrocarbons, whereas GSTT1 is known to be involved in the metabolism of small compounds found in tobacco smoke like mono halo methanes and ethylene oxide [13]. In humans, the GSTM1 is situated in the GSTμ cluster, which has been localized to chromosome 1 in region 1p13.3 [19]. GSTM1 has three alleles. GSTM1a and GSTM1b differ by a substitution in one base pair and seem

*Author to whom correspondence should be sent.

Abbreviations: CI – confidence interval; GST – glutathione S-transferase; OR – odds ratio; PCR – polymerase chain reaction.

to be functionally identical. The GSTM10 allele has been shown to be result of deletion of the entire GSTM1 gene [15]. The GSTT1 gene is located on chromosome 22q11.2. Two functionally different genotypes have been identified at the GSTT1 locus. The first GSTT1-0 is a homozygous deletion and the second GSTT1-1 comprises genotypes with one or two functional alleles. Individuals with homozygous deletion of GSTM1 or GSTT1 genes have no GST activity and, therefore, may be unable to eliminate electrophilic carcinogens as efficiently, what may increase the risk of somatic mutations leading to tumor formation. There is no evidence whether heterozygosity in either GSTM1 or GSTT1 affects gene function. There is geographical and ethnic variation in genotype frequencies for both GSTM1 and GSTT1 alleles [5]. Homozygous null GSTM1 genotype has been reported to have some association with the pathogenesis of cancer of the lung [12], bladder [24], laryngx [11], colon [5], and GSTT1 null type allele has been suggested as a risk factor in colon cancer [5], astrocytoma [7] and colorectal cancer [6]. Although several studies have been undertaken to examine the association between susceptibility to some types of cancer and genetic polymorphisms in GSTs, there are limited data on their association with cancer of the uterine cervix. In the present study an attempt has been made to study an association between genetic polymorphism in GSTM1 and GSTT1 and the risk of developing cervical cancer.

Material and methods

Selection of cases and controls. Peripheral blood samples from 142 incident and untreated cervical cancer cases were collected from the cancer clinic of Lok Nayak Hospital, New Delhi, India after appropriate informed consent, during a period from February to December, 1994. All the cases reported to the hospital during the above period were included in the study. The majority of cases belonged to stage II and III. Histologically, all the tumors were squamous cell carcinoma. Only five cases were poorly differentiated, while the remaining samples were moderately to well differentiated carcinomas. The ages of the patients ranged from 35 to 71 yrs., with mean $\pm$ standard deviation (SD) of age 49.2 ± 8.8 and a median of 48 years. The controls selected for the study were normal volunteers, who were not related to the cases. They were in the same age range as that of cases and without any history of chronic disease. The mean $\pm$ SD of age was 41.4 ± 8.4 and median age was 39 yrs.

DNA isolation. High molecular weight DNA from peripheral blood samples was isolated using standard procedures of proteinase K digestion and phenol-chloroform extraction [18].

Genotyping assays. The homozygous null polymorphisms of GSTM1 and GSTT1 genotypes were determined by using three sets of primers to amplify a 215 bp sequence of the GSTM1 gene, a 480 bp sequence of GSTT1 and a 350 bp sequence of albumin gene fragment, which served as an internal positive control [1]. The PCR primers were as follows:

GSTM1,
5'-GAACTCCCTGAAAAGCTAAAGC-3'and
5'GTTGGGCTCAAATATACGGTGG-3';
GSTT1,
5'-TTCCTTACTGGTCCTCACATCTC-3'and
5'-TCACCGGATCATGGCCAGCC-3';
Albumin,
5'-GCCCTCTGCTAACAAGTCCTAC-3'and
5'-GCCCTAAAAAGAAAATCGCCAATC-3'.

PCRs were performed in 25 μl reaction volume containing 50–100 ng of genomic DNA, 50 mM KCl 2.5 mM $MgCl_2$, 200 mM Tris-Hcl (pH 8.4), 200 mM of dNTPs, GSTM1 primers at 3 μg each, GSTT1 primers at 1 μg each, albumin primers at 600 ng/ml. each and 1.5 units of DNA AmpliTaq (Cetus) in a Perkin-Elmer thermal cycler. After an initial denaturation at 96 °C for 5 min., amplification was carried out for 35 cycles at 94 °C for 1 min., 56 °C for 1 min. and 72 °C for 1 min. followed by final elongation at 72 °C for 7 min.

The products of multiplex PCR (215 bp for GSTM1, 480 bp for GSTT1 and 350 bp for albumin) were separated by electrophoresis with ethidium bromide stained 3% agarose gel. The GSTM1 homozygous null was evidenced by the absence of a 215 bp fragment and of GSTT1 homozygous null by the absence of a 480 bp fragments. The presence of 350 bp albumin fragment was indicator of a successful PCR (Fig. 1).

Statistical analysis. The data were tabulated and analyzed. The mean $\pm$ SD were estimated for quantitative data. In order to test the significance in the proportion of GSTM1 and GSTT1 homozygous null genotype between cases and controls, chi-square test of significance was employed. Probability value of <0.05 was considered for statistical sig-

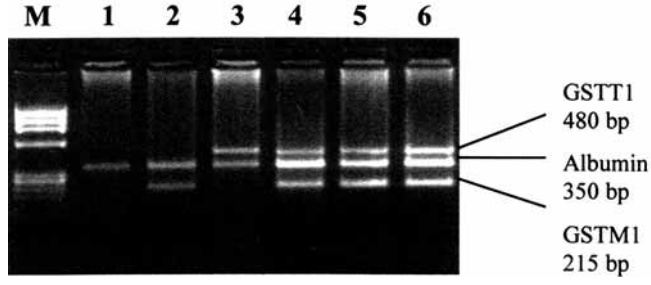

Figure 1. Representative multiplex PCR analysis of GSTM1 and GSTT1 gene products resolved by 3% agarose gel electrophoresis. A 350 base pair DNA fragment corresponding to Albumin gene product provides an internal positive control, seen in all lanes. A 215 base pair product only seen in lanes 2, 4, 5 and 6 containing the GSTM1 gene, while a 480 base pair product of GSTT1 gene is seen in lanes 3, 4, 5 and 6. Both GSTM1 and GSTT1 genes are absent in lane 1. M represents Phi X Hae III digested marker.

nificance. Odds ratio (OR) and 95% confidence intervals (95% CI) were also estimated.

Results

Over all prevalence of GSTM1 and GSTT1 null genotypes. The study comprised 142 cervical cancer cases and 96 controls. The age distribution was found to be slightly different between cases and controls. Controls were younger than cases. The median age being 39 years and 48 years for controls and cases, respectively. The distribution of GSTM1 and GSTT1 genotypes in patients with cervical cancer cases and controls is shown in Table 1.

Regarding GSTs genotype, 57.0% (81/142) of cervical cancer cases were GSTM1 null in comparison to 34.4% (33/96) in controls (Tab. 1). An increased risk for null type was noted in the cervical cancer cases with an odds ratio of 2.5 (95% CI: 1.4–4.5), which was found to be statistically significant (p=0.001). A total of 19.7% (28/142) of the cases presented homozygous deletion of GSTT1 genotype as compared to controls 12.5% (12/96) (Tab. 1). The OR was found to be 1.7, (95% CI: 0.8–3.8). No significant difference in the proportions of GSTT1 null genotype was observed between the two groups. An attempt was made to evaluate the proportion of the cases that were null for both genotypes GSTM1 and GSTT1. It was observed that cervical cancer cases had marginally higher proportion of subjects who had the homozygous null genotypes of both GSTM1 and GSTT1 as compared to controls. However, differences were not statistically significant (p>0.17).

Prevalence of GSTM1 and GSTT1 according to age. The mean age of cases and controls was found to be 49.2 ± 8.8 and 41.4 ± 8.4, respectively. It could be noted that the cases were of nearly one decade higher in age, as compared to controls. Hence a stratified analysis according to age group was carried out. The individuals were categorized into two groups, up to 45 years and above 45 years of age. The proportion of GSTM1 null genotype up to 45 years of age was found to be higher in cancer cases 54% (27/50) than the controls 34.8% (24/69) OR=2.2, 95% CI: 0.9–4.9 (Tab. 2). The difference in the proportions of null type of genotype up to 45 years was not significant at 5% level (p=0.06). Similarly, above 45 years of age, the proportion of GSTM1 null genotype was 58.7% (54/92) in cancer cases compared to controls 33.7% (9/27), OR=2.8, 95% CI: 1.1–7.7 (Tab. 2). In the above 45 years age group, the difference in the proportion of the null genotype was found to be statistically significant between cases and controls p=0.04.

No significant difference was detected when the data were compared in cancer cases and controls for GSTT1 genotype according to age group. Up to 45 years of age the prevalence of homozygous GSTT1 null gene was 16.0% (8/50) in cervical cancer cases as compared to controls 8.7% (6/69). The OR was found to be 2.0, (95% CI: 0.6–7.1) (Tab. 2) which was not statistically significant (p=0.35). Similarly above 45 years of age, the proportion of GST T1 null genotype in patients with cervical cancer was identical 21.7% (20/92) to that found in healthy controls 22.2% (6/27). The OR was 0.98, (95% CI: 0.3–3.1) (Tab. 2).

Table 1. Genotype frequency of GSTM1 & GSTT1 in cases with cervical cancer and controls

Genotypes	Controls (N=96)	Cervical cancer cases (N=142)	OR	95% CI
GSTM1 0/0	33(34.4)	81(57.1)	2.5	1.4–4.5
GSTM1 +/+	63(65.6)	61(42.9)	1.0	
GSTT1 0/0	12(12.5)	28(19.7)	1.7	0.8–3.8
GSTT1 +/+	84(87.5)	114(80.3) 1.0		
GSTM1 0/0 & GSTT1 0/0	11(11.4)	27(19.0)	0.55[a]	0.2–1.2

Figures in parentheses indicate percentages. [a]The other groups comprising of GSTM1 and GSTT1 +ve individuals formed as reference category.

Table 2. GSTM1 and GSTT1 genotypes in cervical cancers and controls according to age

Age (yrs)	No. of Cases	Cont	GSTM1 Cases	Null Cont	OR (95% CI)	GSTT1 Cases	Null Cont	OR (95% CI)
≤45	50	69	27 (54.0)	24 (34.8)	2.2 (0.9–4.9)	8 (16.0)	6 (8.7)	2.0 (0.6–7.1)
>45	92	27	54 (58.7)	9 (33.7)	2.8 (1.1–7.7)	20 (21.7)	6 (22.2)	0.98 (0.3–3.1)

Figures in parentheses indicate percentages.

Discussion

Epidemiological studies have suggested multiple risk factors including HPV in the etiology of cervical cancer [28]. It is also known that only a certain percentage of cervical intraepithelial neoplasias develop into cervical cancer suggesting that in this complex disease, probably multiple genes are operating on the coincidence of several gene polymorphisms acting together by imparting on a small relative risk of each polymorphism [14]. Recent reports have suggested that genetic polymorphisms of the phase II detoxifying agents may be the risk factors in the pathogenesis of cervical cancer, as GSTs are involved in the metabolism of many carcinogens and environmental pollutants [4]. The genes coding for the enzymes GSTM1 and GSTT1 and GSTP1 are polymorphic, but GSTM1 and GSTT1 are among the most studied enzymes [9].

There are substantial differences in the baseline frequencies of null genotypes for GSTM1 and GSTT1 in different

ethnic groups [5]. The prevalence of GSTM1 null genotype has been reported to vary between 39–62% in Europeans, 33–63% in East Asians and 23–45% in Africans [23]. The highest frequencies have been reported in studies involving small number of subjects from parts of the South Pacific i.e. 64–100% [17].

The prevalence of GSTMI null genotype has been reported to be 17% and 24% in Indian population from Bombay and Trivandrum regions, respectively [3, 21]. The prevalence of GSTMI null genotype was 16% in Indians from Malaysia and Singapore, while in Asian Indians from Los Angeles or Malaysia it was 36 and 33%, respectively [27]. The frequencies of the GSTMI null genotypes among controls in the present study were comparable with data that has been reported in various studies from different ethnic groups. We observed the prevalence of 34.4% for GSTMI null genotype which was relatively lower than in Caucasian populations 50% [25] and higher than Indian population from Bombay (17%) and Trivandrum (24%) [3, 21]. In the present study the prevalence of GSTTI null genotype was found to be 12.5% in Indian population. The presence of GSTT1 null genotype was less in Indian population as compared to GSTM1 null. Our results are comparable to those of others [3, 21] reported by other studies, 12.3%, 22% for GSTT1 null genotypes in controls. Studies of GSTT1 null genotype from various geographical regions have demonstrated the range of frequencies from 16% to 64% in Asia, 44% or higher in China and Japan [5]. It has been suggested that in Asian countries the frequency of GSTT1 null is similar to that of GSTM1, whereas in Africans, African-Americans and white populations, the frequency of GSTT1 null genotype is lower than for GSTM1 null genotype. NAIR et al [21] did not find any subject with homozygous null genotype for both GSTM1 and GSTT1 in 82 controls in Indian population from Trivandrum, suggesting that this combination is rare in Indian ethnic population, but we have found 11.4% (11/96) subjects with null genotype for both GSTM1 and GSTT1 in controls and 19.0% (27/142) from cervical cancer cases from New Delhi, India.

Till now, there are not many studies on the role of GSTs in cervical carcinogenesis. This is the first report from Indian population on the role of GSTs in cervical carcinogenesis. The results available from these case control studies of GSTM1 and GSTT1 null genotype and cervical cancer are inconsistent. We found significant correlation between homozygous null genotypes of GSTM1 null genotypes and cervical cancer cases (p=0.001) (Tab. 1), where as others did not find any correlation between GSTM1 and GSTT1 null genotypes and cervical cancer [10, 26]. KIM et al [16] however, reported that HPV positive women carrying both null genotypes of GSTM1 and GSTT1 have an increased risk of cervical cancer development before the age of 40 years. In the present study, the genotype combination of GSTM1 and GSTT1 did not confer significant association with cervical cancer. Further, we did not find any significant difference at the conventional level of 5 percent in individuals below 45 years (p=0.06) but above 45 years of age the differences between the two groups were significant (p=0.04) for homozygous GSTM1 null genotype. The reason for this discrepancy may be attributed to less number of cancer cases in the group <45 years of age. However, prevalence of homozygous GSTT1 null genotype was not significant in cancer cases as compared to controls.

The influence of GSTT1 null genotypes on the susceptibility to cervical cancer, thus, remains unpredictable. The data presented here indicate that the incidence of homozygous GSTM1 null genotype is significant in cancer cases as compared to controls, but GSTT1 null genotype results showed that this is not a critical factor in cervical cancer development. In conclusion, our results revealed that null genotypes of only GSTM1 individually, but not in combination with GSTT1 play an important role in carcinogenesis. Further studies are needed to investigate more number of cases and also examining polymorphisms of other detoxifying enzymes.

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