ASSOCIATION OF *GSTM1, GSTT1, AND GSTM3 GENE* POLYMORPHISMS AND SUSCEPTIBILITY TO CERVICAL CANCER IN A NORTH INDIAN POPULATION

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Introduction

- Cancer of the uterine cervix is the leading cancer among women in many developing countries including India and remains a major health problem
- The etiology of cervical cancer has been thought to be multifactorial
- Human papilloma virus (HPV) infection was identified as a primary causal agent in cervical dysplasia and carcinoma
- Tobacco smoking has been associated with risk of cervical malignancy

- Tobacco contains
  - carcinogens like polycyclic aromatic hydrocarbons
  - aldehydes
  - benzo-pyrene
  - ethylene oxide
  - 4-aminobiphenyl
  - Nitrosamines

- The genes of the glutathione S–transferase (GST)
- GSTs play an important role in conjugating glutathione to the products of endogenous lipid peroxidation and inactivating organic hydroperoxides
- protecting the cell from the deleterious effects of oxidative stress
- the GST family consists of 6genes
  - *GSTA (alpha) , GSTT1 (theta)*
  - *GSTM1 (mu) , GSTP1 (pi)*
  - *GST(kappa) , GST (sigma)*

- Inheritance of null alleles in the *GSTM1 and GSTT1 is associated with* loss of respective enzyme activity, which may result in higher risk of cytogenetic damage and May promote the development of cervical dysplasia
- Inherited differences in the effectiveness of the activation/detoxification of carcinogens play a crucial role in the host's cancer susceptibility. The altered biotransformation of xenobiotics including carcinogens may contribute toward the susceptibility to cervical cancer

MATERIALS AND METHODS

- **Subjects**
  - One hundred fifty consecutive cases of frank carcinoma cervix, confirmed by cervical biopsy (150)
  - One hundred sixty-eight healthy , All the controls underwent screening for cervical dysplasia in a Papanicolaousmear test and only cervicalcytology–negative (168)
  - Blood samples were collectedin EDTA and stored at 70°C

- **Genotyping**
  - ***Null alleles of GSTM1 and GSTT1 were*** **determined by using the multiplex polymerasechain reaction (PCR) with** ***CYP1A1 gene as an internal control***
    - **215 bp region between exon 4and 5 of the** ***GSTM1 gene***
    - **480 bp products for** ***GSTT1***
    - **312 bp size product of** ***CYP1A1***

- **Statistical analysis**
  - The genotypic risk as odds ratios (ORs) with 95% confidence intervals (CIs) were estimated
  - significant when the *P* - value was less than .05

RESULTS

- The mean age of the cancer patient was 45.2 8.8 years and healthy controls were 50.3 8.3 years

- Characteristics of patients

TABLE 1
Characteristics of patients

Variables	Cervical cancer (%)
Cervical cancer patients	150
Stages of cancer[a]	
IB	31 (20.7)
IIB	23 (15.3)
IIIB	88 (58.7)
IVB	8 (5.3)
Alcohol habit[a]	
Alcoholic	8 (5.3)
Tobacco habits[a]	
Total tobacco user	51 (37.22)
Tobacco chewer only	39 (28.46)
Chewing and smoking	9 (6.5)
Smoker only	3 (2.18)
Sexual partner[a]	
Single partner	120 (87.6)
Multiple partners	17 (12.4)
Pregnancy status[a]	
1-2 children	19 (13.9)
2-4 children	35 (25.5)
More than 4 children	83 (60.6)

[a] Data are missing.

Singh. Association of GSTM1, GSTT1, and GSTM3 gene polymorphisms and susceptibility to cervical cancer. Am J Obstet Gynecol 2008.

TABLE 2

Frequency distribution of *GSTM1*, *GSTM3*, and *GSTT1* genotypes in cervical cancer and healthy controls

Genotypes	Cervical cancer n (%)	Healthy controls n (%)	*P* value	OR (95% CI)
GSTM1 null	64 (42.7)	46 (28.0)	.009	1.52 (1.1 to 2.0)[a]
GSTT1 null	40 (26.7)	18 (11.1)	.0004	2.4 (1.4 to 4.0)[a]
GSTM3				
AA	115 (76.7)	139 (85.3)	.060	0.89 (0.8 to 1.0)[b]
AB	33 (22.0)	22 (13.4)	.053	1.64 (1.0 to 2.6)[b]
BB	2 (1.3)	2 (1.4)	1.000	1.0 (0.1 to 7.6)[b]

[a] Presence of *GSTM1* and *GSTT1* was taken as a reference group for statistical analysis.

[b] Presence of AB and BB for AA, AA, and BB for AB, AA, and AB for BB genotypes was taken as a reference group for statistical analysis.

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- Frequency of homozygous *GSTM1 null genotype was found to* be higher in cervical cancer patients (42.7%) as compared with healthy controls (28.0%), and the difference was significant
- Homozygous *GSTT1 null genotype* carrier frequency was also higher in the cancer patients (26.7%)as compared with healthy controls (11.1%) and significantly associated with the risk of cervical cancer
- The frequency of *AB genotype* of *GSTM3 was also higher in cervical* cancer patients, but the difference was of borderline significance

TABLE 3

Interaction of *GSTM1*, *GSTT1*, and *GSTM3* genotypes and association with cervical cancer

Combination of genotypes	Cervical cancer n (%)	Healthy controls n (%)	*P* value	OR (95% CI)[a,b]
GSTM1 null and *GSTM3 AA*	47 (31.3)	38 (23.2)	.127	1.35 (0.9 to 1.9)
GSTT1 null and *GSTM3 AA*	36 (24.0)	14 (8.5)	.0002	2.81 (1.6 to 5.0)
GSTT1 null, *GSTM1 null*, and *GSTM3 AA*	18 (12.0)	2 (1.2)	.0001	9.8 (2.3 to 41.6)
GSTM1 null and *GSTM3 AB*	15 (10.0)	5 (3.0)	.019	3.2 (1.2 to 8.8)
GSTM1 null and *GSTT1 null*	23 (15.2)	2 (1.2)	.000007	12.5 (3.0 to 52.4)
GSTM1 null, *GSTT1 null*, and *GSTM3 AB*	4 (2.7)	0 (0)	.051	—

[a] Presence *GSTM* and *GSTT1* were taken as reference group for statistical analysis.

[b] Presence of AB and BB for AA, AA, and BB for AB, AA, and AB for BB genotypes was taken as reference group for statistical analysis.

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- we expected the possible additive or synergistic effect of these genes in cervical carcinogenesis
- Interaction of *GSTM1 null with GSTT1 null* resulted in substantial increased risk as compared with individual contributions
- *GSTM3*AB also enhanced* the risk, particularly in the presence of *GSTM1 null*
- *GSTM3*AA interaction did not substantially* change the risk

TABLE 4

Frequency distribution of *GSTM1*, *GSTT1*, and *GSTM3* genotypes in tobacco-using cervical cancer patients

Genotypes	Tobacco user n (%)	Nonuser n (%)	*P* value	OR (95% CI)
GSTM1 null	20 (39.2)	36 (41.2)	.858	0.9 (0.6 to 1.4)[a]
GSTT1 null	13 (25.5)	21 (24.4)	1.000	1.0 (0.6 to 1.7)[a]
GSTM3				
AA	44 (86.3)	59 (68.6)	**.024**	2.1 (1.0 to 4.1)[b]
AB	7 (17.7)	26 (30.2)	.038	0.5 (0.2 to 1.0)[b]
BB	0	1 (1.2)	1.000	—

Bolding implies significant *P* value.

[a] Presence *GSTM1*, *GSTT1*, were taken as reference group for statistical analysis.

[b] Presence of AB and BB for AA, AA, and BB for AB, AA, and AB for BB genotypes was taken as reference group for statistical analysis.

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- The cancer patients with *GSTM1 null and GSTT1 null genotypes* did not show any elevated risk in the presence of a tobacco habit
- the frequency of *GSTM3 AA genotype* was found to be higher in patients using tobacco (86.3%) as compared with nonusers (68.6%) with significantly higher risk

Conclusion

- The GST play an active role in the detoxification and elimination of carcinogens by conjugating reduced glutathione to genotoxic intermediates
- The deficient (null) genotypes of *GST that are due to a homozygous* deletion of the *GSTM1 or GSTT1* genes are frequently observed in lung cancer and bladder carcinoma patients
- The association of *GSTM1 null genotype with cervical can* cer has been reported in previous studies but the *GSTT1 null association is* controversial.

- *GSTM3*B has been reported* as a risk allele in breast, esophagus, and colorectal cancers, but no significant association was found in gallbladder cancer
- *GSTM3*A* has been proposed to confer higher risk in oral cancer and leukoplakia among tobacco users
- *GSTM1 null and GSTT1 null in conferring* higher risk of cervical cancer. However, only *GSTM3*AB enhanced the risk* conferred by *GSTM1 alone. No enhancement* of risk was observed with *GSTM3*AA because the latter was not a* risk allele itself.

- It also suggests that environmental toxins with substrate specificity of all 3 gene products of the GST family may be involved in the higher risk of cervical cancer.
- Among tobacco users, the risk conferred by *GSTM1 null and GSTT1 null* was not enhanced . However, the presenceof *GSTM3*AA resulted in modulating*risk in tobacco users
- cervical cancer is believed to result from complex interactions between genetic and environmental factors.

- HPV infection is still considered a major causative factor in cervical cancer , the infection alone is not sufficient for complete progression to malignancy
- It has also been reported that HPV infection may modulate expression of cellular xenobiotic-metabolizing enzymes and affect the ability of cells to handle environmental carcinogens