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Original Research Article

Genetic polymorphisms in carcinogen detoxifying genes and risk of cervical cancer in Maharashtra, India: a case control study

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Abstract

Objectives: To explore the association of genetic polymorphisms in individual or combined genotypes of GSTM1, GSTT1 and GSTP1 with cervical cancer (CC) susceptibility in women from Maharashtra.
Methods: The polymorphisms in GSTM1 and GSTT1 were analyzed by polymerase chain reaction (PCR) and GSTP1 by polymerase chain reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) in 350 CC cases and 400 healthy controls.
Results: In this study we found GSTM1 and GSTT1 as risk factors with significant association with null (-/-) genotypes of M1 and T1 with increasing the risk of CC by 1.41 and 1.46 folds respectively. *GSTM1* (-/-) null genotype was prominent among cases (36.29%) indicating contributory risk factor for development of CC (OR=1.41; 95% CI: 1.03-1.91; p=0.02), GSTT1 null increases in CC cases (OR=1.46, 95% CI: 1.04-2.06; p=0.02). The combined analysis of GSTM1 (-/-) / GSTT1 (-/-) showed 2.27 folds higher risk of development of CC (OR=2.27, 95% CI: 1.25-4.09; p=0.005), whereas, combinations of GSTP1 genotype (A/G +G/G) of exon 5 with GSTM1 null, which showed 1.92 fold risk of CC (OR=1.92, 95% CI: 1.21-3.04; p=0.005) and combination of (C/T +T/T) genotype of GSTP1 exon 6 with GSTM1 (-/-) null did not show any association with development of CC (OR=0.81, 95% CI: 0.52-1.25; p=0.35). Triple combinations of GSTM1, T1 null and P1 (A/G or G/G) genotypes showed significant association (p=0.005) with a 3.04 times risk of developing CC.
Conclusions: This investigation affirms the conceivable interactions between GST polymorphisms and development of cervical cancer in rural population of south western Maharashtra from India.
Keywords: Cervical Cancer, GSTM1, GSTT1, GSTP1, Genetic Polymorphism, PCR-RFLP.

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1. Introduction

Cervical cancer is the fourth most malignancy in women around the world, with roughly 85% of the global burden of wellbeing illness in low income nations [1]. In India, CC is the second most common cancer in women representing about 10 % of all cancer related deaths in the country [2,3]. Rural women are at higher risk of developing CC as compared to the urban counterparts [4]. Earlier, epidemiologic studies have demonstrated that CC is caused by numerous etiological factors predominantly, early marriages, early child birth, early premenopause, sexual history, parity, multiple pregnancies and sex accomplices, intake of oral contraceptives, certain life style factors as well as environmental elements including tobacco smoking, latent smoking and introduction to pollutants including kitchen smoke [5-7]. Likewise, time to time studies showed that the most vital hazardous factor for consequent advancement of CC is sexually transmitted agent, human papilloma virus (HPV) infection [8,9]. In spite of, not all the women exposed to the said risk factors develop CC, indicating some other factors are associated with cervical carcinogenesis. In this regard, the growing confirmations on the genetic determinants and their relationship with carcinogenesis have been considered in numerous reports, proposing that the genetic factors may play an imperative role in development of CC.

The cancer-causing agents derived from tobacco smoke and other environmental contaminants such as polycyclic aromatic hydrocarbons including nicotine and nitrosamines are recognized in cervical bodily fluid of smokers or passive smokers with irregular cervical cellularity [5]. Such xenobiotics are known to be metabolized by a group of detoxifying catalysts such as glutathione S transferase (GST) enzymes and protecting the macromolecules inside the cells against deleterious and oxidative damage [10]. GST enzymes are imperative candidates of intracellular detoxification process which are encoded by GST genes, secure the cells against cytotoxic and genotoxic impacts of both exogenous just as endogenous reactive metabolites and carcinogens. Among the cytosolic GSTs, GSTM1, GSTT1 and GSTP1 are the significant elements of detoxification process of carcinogens predominantly evolved from tobacco smoke and other pollutants. Genetic polymorphisms in GST genes can result in nonappearance or a lack of enzyme activity may lead to diminished capacity to detoxify mutagens and carcinogens which at last present an expansion in malignant growth vulnerability. The polymorphism in GSTM1 and GSTT1 genes caused by a gene deletion which results in the absence of enzyme activity in individuals with the GSTT1 and GSTM1 null genotypes. These GSTM1 and GSTT1 homozygous null polymorphisms may lead to variations in the metabolic activation of synthetic cancer causing agents. GSTP1 is a polymorphic gene with two single nucleotide substitutions in exon 5 and exon 6 which give rise to Ile105 Val and Ala114Val amino acid substitutions.

In the course of past few years, number of studies has revealed the relationship of polymorphisms of GSTs with the risk of several malignancies including lung, bladder, breast and colon cancer [11-16]. However, the results of a few studies are divergent to some extent with conflicting outcomes [17-20]. Similarly, connection of GST gene polymorphism with CC development in different populations has been demonstrated by a series of studies [10,21-24], however other studies challenge their relationship with CC [25] subsequently, remained a proviso for further research in this field.

Consequently, in the view of literature, when we considered polymorphisms in xenobiotics detoxifying genes and their association with CC development, we find barely of any studies announced the association of polymorphisms in GST genes with cervical carcinogenesis in Maharashtrian population. Therefore, we conjectured that the polymorphism in GSTM1, GSTT1 or GSTP1 gene may add to the etiology of CC in a rural population, and in this way we performed a hospital based case-control study to explore the effect of genetic polymorphisms of these genes on the risk of CC in Maharashtrian women subjects. Thus we choose to explore the association of individual or combined genotypes GSTM1, GSTT1 and GSTP1 polymorphisms with CC in general. Furthermore, it was attempted to see if the polymorphisms in GST genes could influence the risk of developing CC in women either with risk factors like tobacco smoking habit. The homozygous null polymorphisms of GSTM1, GSTT1 from 350 patients with CC and 400 controls were assessed to see their association with CC development in a population from south-western Maharashtra region of India. Additionally, GSTP1 Ile105Val of exon 5 and GSTP1 Ala114Val of exon 6 polymorphism were precisely assessed to find its association with CC risk.

2. Materials and Methods

2.1 Selection of study subjects

This hospital based case-control study was conducted in Krishna Institute of Medical Sciences "Deemed to be University" from South-Western Maharashtra of India. In this investigation, study subjects included 350 newly diagnosed CC patients and 400 healthy, disease free, age matched females as controls. Occurrence instances of CC were recognized using colposcopy at Department of Obstetrics and Gynecology of the Krishna Hospital & Medical Research Centre (KH&MRC) and cell cytology at Department of Pathology of Krishna Institute of Medical Sciences. Controls were haphazardly chosen from a group of women visiting to KH & MRC for blood donation and other purposes. All cases ranged in age from 20-80 years (Mean ± SD) (48.67 ±13.78) were enlisted immediately after being diagnosed during the year 2014-2018. Trained interviewers used a structured questionnaire to collect personal interview data from the participants regarding demographic factors and known risk factors. The study protocol was approved by Institutional Ethics Committee of Krishna Institute of Medical Sciences "Deemed to be University" for the utilization of human subjects in the research.

2.2 Genomic DNA isolation from whole blood

Five milliliter (mL) of intravenous blood sample from patients and normal controls was collected in sterile ethylenediaminetetraacetic acid (EDTA) containing vacutainer after receiving written informed consent. Genomic DNA extraction was carried out from the peripheral blood samples by method where red blood cells are processed with red cell lysis buffer (10mM Tris-HCl pH 7.6, 320 mM sucrose, 5mM $MgCl_2$, 1% Triton X-100, pH 7.6), thereafter treated with nucleic lysis buffer (10mM Tris-HC1, 11.4 mM sodium citrate, 1 mM EDTA, 1 % SDS, pH 8.0). After treatment with 100 µg/mL concentration of proteinase K at 55^0C and subsequently RNase A (100 µg/mL) at 37^0C, precipitated and purified DNA was checked on 1% agarose gel for its quality as well as quantity. The pure DNA was used for genotyping by polymerase chain reaction (PCR) and Restriction fragment Length Polymorphism (RFLP).

2.3 Genotyping Assays

The genotyping of GSTM1 and GSTT1 were performed by PCR. The PCR amplification of GSTM1 and GSTP1 were carried out separately in 20 micro liter (µL) reaction mixtures containing 1X PCR buffer 0.2 mM each dNTP, 10 picomole (pmol) of each primers (Xcelris Genomics) , 1U Taq DNA polymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA. The primers selected to amplify the GSTM1; forward primer: 5′- caaattctggattgtagcagatcatgc-3′, reverse primer: 5′-cacagctcctgattatgacagaagcc-3′ and GSTT1; forward primer: 5'-ttccttactggtcctcacatctc-3', reverse primer: 5'-tcaccggatcatggccagca-3'. The PCR conditions for amplification of 625 bp fragment (Figure 1-a) of GSTM1 were initial denaturation at 95^0C for 5 minutes (min) followed by 30 cycles of 95^0C- 30 seconds (sec) , 56^0C- 30 sec, 72^0C- 30 sec and final extension at 72^0C for 10 min. The conditions for GSTT1 of 480 bp (Figure 1-b) were initial denaturation at 95^0C for 5 min followed by 30 cycles of 95^0C- 30 sec, 60^0C- 30 sec, 72^0C- 30 sec and final extension at 72^0C- 10 min. After performing PCR program for each of the reactions with a Master Cycler Gradient PCR (Eppendorf), the PCR products were analyzed by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer thereafter stained with ethidium bromide (10 mg/mL) and visualized under UV-transilluminator and photographed in gel documentation system (BioRad Laboratories). The nonfunctional allele homozygous null for GSTM1 and GSTT1 was evidenced by the absence of gene fragment, and presence of gene was indicated by amplification gene fragment in the PCR. The genotyping of GSTP1 was performed by PCR followed by RFLP. The PCR reaction for analyzing GSTP1 Ile105Val of exon 5 and GSTP1 Ala114Val of exon 6 polymorphism was carried out using 10 pmole of each forward and reverse primer, 0.2 mM each dNTP, 1U Taq DNA polymerase and 100ng of purified DNA. The primer sequences for amplification of GSTP1 Ile105Val of exon 5 are (forward primer: 5'-agccacctgaggggtaag-3'reverse primer: 5'-gggagcaagcagaggagaat-3') forward and the primers used for GSTP1 Ala114Val of exon 6 are (forward primer: 5'-gtagtttgcccaaggtcaag-3'reverse primer: 5'-caggttgtagtcagcgaaggag-3'). The PCR conditions for amplification of 433 bp fragment of GSTP1 Ile105Val of exon 5 are (initial denaturation at 95^0C for 5 minutes (min) followed by 30 cycles of 95^0C- 20 seconds (sec) , 55^0C- 20 sec, 72^0C- 20 sec and final extension at 72^0C for 10 min) and 420 bp of GSTP1 Ala114Val of exon 6 (initial denaturation at 95^0C for 5 minutes (min) followed by 30 cycles of 95^0C- 30 seconds (sec) , 57^0C- 20 sec, 72^0C- 30 sec and final extension at 72^0C for 10 min) respectively. After PCR amplification, RFLP analysis for both exon 5 and exon 6 were carried out with the help of 1 unit of restriction enzymes BsmAI and AciI respectively. Following the digestion of PCR products at 37^0C, the digested products were separated on 3% low EEO agarose (GeNei, Merck Biosciences) gel, stained with ethidium bromide and photographed with gel documentation system. Complete digestion of GSTP1 exon 5 with BsmAI yield (wild type allele with two bands 328 bp & 105 bp; heterozugous allele with 4 bands 328 bp, 222 bp, 106 bp & 105 bp and variant allele with 222 bp,106 bp & 105bp) (Figure 1-c) where as GSTP1 exon 6 digestion with AciI (wild allele with three bands 246 bp,116 bp & 58 bp; heterozugous allele with 4 bands 362 bp, 246 bp,116 bp & 58 bp and variant allele with 362 bp & 58 bp) (Figure 1-d).

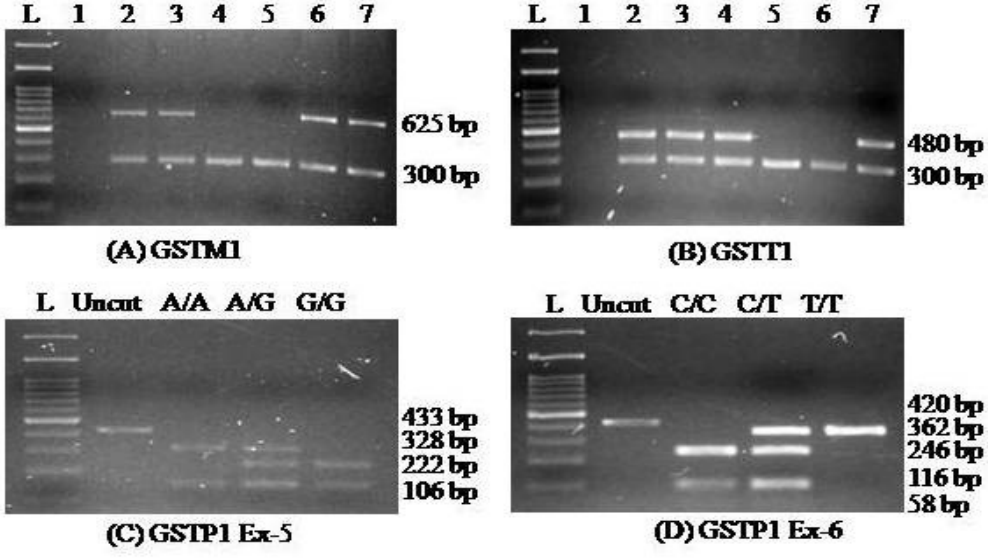

Figure 1: Representative Agarose gel image showing genotype distribution of GSTM1, GSTT1 and GSTP1

(A) GSTM1 (625bp) polymorphism (Lane 1: 100bp DNA ladder, lane 2: Negative control, lane 3, 5, 6: GSTM1 (+/+), lane 3 & 4: GSTM1 (-/-), (B) GSTT1 (480bp) (Lane 1: 100bp DNA ladder, lane 2: Negative control, lane 3, 5, 6: GSTT1 (+/+), lane 3 & 4: GSTT1 (-/-), (C) GSTP1 ex-5 (433bp) (Lane 1: 100bp DNA ladder, lane 2: Uncut PCR product, lane 3: (A/A) genotype, lane 3: (A/G) genotype and lane 4: (G/G) genotype and (D) GSTP1 ex-6 (420bp) (Lane 1: 100bp DNA ladder, lane 2: Uncut PCR product, lane 3: (C/C) genotype, lane 3: (C/T) genotype and lane 4: (T/T) genotype.

2.4. Statistical Analysis

Chi-square test was used to compare the frequency distribution of genotypes between selected demographic variables and frequencies of allele and genotype of polymorphism between cases and controls which are summarized as Mean ± SD.

The association between the GSTM1, GSTP1 and GSTT1 genotypes and risk of developing CC were studied by odds ratio (OR). Logistic regression model was used to calculate the OR and 95% confidence intervals (CI) with adjustment of variables to determine the CC risk associated with genotypes. All *P* values were two-sided and differences were considered statistically significant for $p \leq 0.05$ and $p \leq 0.005$. All statistical analyses were performed with SPSS (Version 11.0).

3. Results

3.1 Characteristics of selected demographic variables.

350 patients and 400 cancer-free controls included in the study. The characteristics of the study participants are summarized in Table 1. Most of the cases were in stage III, well differentiated squamous cell carcinoma. The demographic characteristics of the study population such as age at cancer occurrence, age at parity and age at first pregnancy, diet, tobacco chewing status, education and economic status were recorded. There was no difference in age distribution where the mean ± SD age of cases and controls was 48.67±13.78 (median: 50, range 25-75) and controls 46.37±13.90 (median: 33.5 range 24-75) years respectively (p=0.03). It was also observed that CC occurred in patients (78.90 %) who were married at younger age and pregnant soon (15-20) which showed significant association when compared to the controls (p=0.005).

Table 1: Distribution of selected demographic characteristics of cervical cancer cases and healthy controls

Variable	Cases N=350		Controls N=400		*P* Value based on $\chi 2$
Age (Mean ± SD) years	48.67 ±13.78		46.37 ± 13.90		(0.03)
	No.	(%)	No.	(%)	
≤ 50	215	61.40	284	71.00	
51-60	59	16.90	69	17.20	
61-70	57	16.30	34	08.50	
>70	19	05.40	13	03.20	
Tobacco status					<0.01
Tobacco users	189	54.00	113	28.20	
Tobacco no users	161	46.00	287	71.80	
Age @ Ist Pregnancy (yrs)					<0.001
15-20	276	78.90	181	45.25	
21-25	73	20.90	178	44.50	
26-30	00	0.00	36	9.00	
31-35	01	0.20	05	01.25	
Diet					0.59
Vegetarian	97	27.70	118	29.50	
Mixed	253	72.30	282	70.50	
Education					<0.01
High School	140	40.00	108	27.00	
High School graduate (12 y)	24	06.90	50	12.50	
College /Graduate	43	12.20	128	32.00	
No School	143	40.90	114	28.50	
Economic Status					<0.01
Rich	55	15.70	129	32.20	
Middle	97	27.70	140	35.00	
Poor	198	56.60	131	32.80	
Family history of Cancer					<0.01
Yes	62	17.70	10	02.50	
No	288	82.30	390	97.50	

Significance $p < 0.05$

3.2 Genotyping of GSTM1, GSTT1 and GSTP1gene polymorphisms

We determined the frequency distribution of GSTM1 and GSTT1 genotypes in cases and matched cancer free controls in order to evaluate find their association with CC is presented in Table 2. The frequency of GSTM1 null (-/-) genotype was comparatively greater in cases (36.29) than controls (29 %) which indicating a contributory increase in risk of CC(OR=1.41; 95% CI: 1.03-1.91; p=0.02) in rural population. Similarly when we compared the frequency distribution of GSTT1 of CC cases with normal controls, we observed GSTT1 null (-/-) increases in cases (OR=1.46, 95% CI: 1.04-2.06; p=0.02). The GSTM1 and GSTT1 were found to be a risk factors for CC which showed significant association with CC cases (p=0.02) with null (-/-) genotypes increasing the risk of CC by 1.41 and

1.46 folds respectively. When we studied A/G and G/G genotypes of exon 5 and C/T and T/T genotypes of exon 6 of GSTP1, we observed that GSTP1 G/G genotypes showed significant risk of CC (OR=2.49, 95% CI: 1.31-4.47; *p*=0.005) as compared to C/C genotypes of GSTP1(OR=0.02, 95% CI: 0.001-0.45; *p*=0.01). The A/G and G/G genotypes of GSTP1 exon 5 found to be a risk factors for CC which showed significant association with CC cases (*p*=0.02) which increasing the risk of CC by 2.49 fold whereas C/C or C/T genotypes of exon 6 did not show any functional association with development of CC in rural population as presented in Table 2.

Table 2: The genotype frequencies of *GSTM1, GSTT1 and GSTP1* gene variants and their association with cervical cancer in untreated CC patients and healthy controls

GENE	Genotype	CASES (n= 350)(%)	CONTROL (n = 400)(%)	Odds' Ratio (95% CI)	*P* value	Adjusted Odds Ratio (95% CI)	*P* value
GSTM1	+/+	223 (63.71)	284 (71.00)	1 (Reference)		1 (Reference)	
	−/−	127(36.29)	116 (29.00)	1.41 (1.03-1.91)	**0.02****	1.44 (1.05-1.98)	0.02
GSTT1	+/+	256 (73.14)	320 (80.00)	1 (Reference)		1 (Reference)	
	−/−	94 (26.86)	80 (20.00)	1.46 (1.04-2.06)	**0.02****	1.42 (1.02-2.01)	0.04
GSTP1 Exon 5 A>G	Ile/Ile	184 (52.58)	253 (63.25)	1 (Reference)		1 (Reference)	
	Ile/Val	137 (39.14)	131 (32.75)	1.43 (1.05-1.95)	0.01	1.46 (1.07-2.00)	0.01
	Val/Val	29 (8.28)	16 (04.00)	2.49 (1.31-4.47)	**0.005***	2.56 (1.30-5.02)	0.006
	Ile/Val+ Val/Val	166 (47.42)	147 (36.75)	1.55 (1.15-2.07)	0.003	1.58 (1.17-2.12)	0.002
GSTP1 Exon 6 C>T	Ala/Ala	226 (64.57)	214 (53.50)	1 (Reference)		1 (Reference)	
	Ala/Val	124 (35.43)	169 (42.25)	0.69 (0.51-0.93)	0.01	0.60 (0.44-0.81)	0.001
	Val/Val	0 (00)	17 (04.25)	0.02 (0.001-0.45)	0.01	0.018 (0.001-0.42)	0.01
	Ala/Val + Val/Val	124(35.43)	186 (46.50)	0.63 (0.47-0.84)	0.002	0.60 (0.44-0.81)	0.001

(+/+)= Present, (-/-) = null, * Indicates significance (p<0.005), ** significance ((p<0.05), p value determined based on $\chi 2$, 1.0 (Reference)

The distribution of double and triple combinations of GSTM1, GSTT1 and GSTP1 genotypes and their association with CC is shown in Table 3. The combinations of two genotypes GSTM1 (-/-) / GSTT1 (-/-) showed 2.27 folds higher risk of development of CC (OR=2.27, 95% CI: 1.25-4.09; *p*=0.005) in women of rural population. When we considered combinations of GSTP1 genotype (A/G +G/G) of exon 5 with GSTM1 (-/-) null, which showed 1.92 fold risk of CC (OR=1.92, 95% CI: 1.21-3.04; *p*=0.005) whereas combination of (C/T +T/T) genotype of GSTP1 exon 6 with GSTM1 (-/-) null did not show any association with development of CC (OR=0.81, 95% CI: 0.52-1.25; *p*=0.35). Similarly, when we looked into the combinations of GSTP1 (A/G +G/G) of exon 5 with GSTT1 (-/-) null, we found significant association genotype distribution with development of CC (OR=2.0, 95% CI: 1.21-3.29; *p*=0.005), where as a combination of GSTP1 (C/T+T/T) of exon 6 and GSTT1 (-/-) null genotypes were negative for any association with CC (OR=0.64, 95% CI: 0.37-1.10; *p*=0.11). When we studied a combinations of GSTM1 (-/-) null, GSTT1 (-/-) null and GSTP1 (A/G + G/G) genotypes, we found 3.04 fold increased risk of developing CC (OR=3.04, 95% CI: 1.37-6.76; *p*=0.005) in a rural population of south western Maharashtra.

Table 3: Distribution of double combination1 of *GST* genotypes and their association with cervical cancer

GENE	Genotype	Cases (n= 350) (%)	Controls (n = 400) (%)	Odds' Ratio (95% CI)	*P* value
	Double				
GSTM1 and GSTT1	Both present (+/+)	165 (47.14)	227 (56.75)	1 (Reference)	
	M1 null −/+	92 (26.29)	92 (23.00)	1.37 (0.96-1.95)	0.07
	T1 null (+/−)	60 (17.14)	61 (15.25)	1.35 (0.89-2.03)	0.14
	Both null (−/−)	33 (9.43)	20 (05.00)	2.27 (1.25-4.09)	**0.005***
GSTM1 and GSTP1 (Ex5)	M1 (+/+), P1 (A/A)	114 (32.58)	186 (46.50)	1 (Reference)	
	M1 (+/+), P1 (A/G+ G/G)	111 (31.71)	102 (25.5)	1.77 (1.24-2.53)	0.001
	M1 (−/−),P1 (A/A)	72 (20.57)	67 (16.75)	175 (1.16-2.63)	0.005*
	M1 (−/−), P1 (A/G+ G/G)	53 (15.14)	45 (11.25)	1.92 (1.21-3.04)	0.005
GSTM1 and GSTP1 (Ex 6)	M1 (+/+), P1 (C/C)	145 (41.43)	163 (40.75)	1 (Reference)	
	M1 (+/+), P1 (C/T+ T/T)	77 (22.00)	123 (30.75)	0.70 (0.48-1.01)	0.05
	M1 (−/−),P1 (C/C)	81 (23.14)	49 (12.25)	1.85 (1.22-2.82)	0.003*
	M1 (−/−), P1 (C/T+ T/T)	47 (13.43)	65 (16.25)	0.81 (0.52-1.25)	0.35
GSTT1 and GSTP1 (Ex5)	T1 (+/+), P1 (A/A)	139 (39.71)	204 (51.00)	1 (Reference)	
	T1 (+/+), P1 (A/G+ G/G)	118 (33.71	113 (28.25)	1.53 (1.09-2.14)	0.01
	T1 (−/−),P1 (A/A)	48 (13.72)	50 (12.50)	1.40 (0.89-2.21)	0.13
	T1 (−/−), P1 (A/G+ G/G)	45 (12.86)	33 (08.25)	2.0 (1.21-3.29)	0.005*
GSTT1 and GSTP1 (Ex 6)	T1 (+/+), P1 (C/C)	163 (46.57)	177 (44.25)	1 (Reference)	
	T1 (+/+), P1 (C/T+ T/T)	96 (27.43)	143 (35.75)	0.72 (0.52-1.01)	0.06
	M1 (−/−),P1 (C/C)	66 (18.86)	38 (09.50)	1.88 (1.19-2.96)	0.006
	M1 (−/−), P1 (C/T+ T/T)	25 (7.14)	42 (10.50	0.64 (0.37-1.10)	0.11

(+/+)= Present, (-/-) = null, * Indicates significance ($p \leq 0.005$), p value determined based on $\chi 2$, 1.0 (Reference)

3.3 Effect of age of cancer occurrence, tobacco smoke status and age at 1st pregnancy on the association of GSTM1, GSTT1 and GSTP1 with cervical cancer risk

The association between GSTM1, GSTT1 and GSTP1 and the risk of CC was further examined after stratification of confounding factors such as age, age at first pregnancy and tobacco chewing status. The genotype distributions of the selected GSTM1, GSTT1 and GSTP1 gene polymorphisms in cases and controls and their associations with CC risk are summarized in Table 4.

The logistic regression analysis showed that none of the polymorphisms including GSTM1 or GSTP1 Null genotype associated with CC risk after being adjusted for age, tobacco smoking status and the earlier age at first pregnancy. In Maharashtrian patients, the age of CC beginning is 30 years, considerably lesser than reported in other reports. Also, the association of CC with first delivery age was reviewed in this study which showed that 15-20 yrs age of first delivery, considerably associated with increased CC risk.

Table 4: Association of GSTM1, GSTT1 and GSTP1 with demographic factors including age, tobacco smoking, age at first pregnancy in patients with CC and control group from Population of Maharashtra

		Demographic Factors							
Gene	**Genotype**	**Age (Cases/Control)**		**Tobacco status (Cases/Control)**		**Age @ 1st pregnancy (Cases/Control)**			
		≤ 50 N=215/284	> 50 N=135/116	Users N=189/113	Non-Users N=161/287	15-20 N=276/181	21-25 N=73/178	26-30 N=0/36	31-35 N=1/5
GSTM1	+/+	131/212	92/72	95/74	98/210	178/119	44/130	0/30	1/5
	−/−	84/72	43/44	94/39	63/77	98/62	29/48	0/6	0/0
	OR (95% CI)	1.88 (1.28-2.76)	0.76 (0.45-1.28)	1.87 (1.16-3.03)	1.75 (1.16-2.64)	1.05 (0.71-1.56)	1.78 (1.00-3.16)	4.69 (0.08-258.87)	3.66 (0.04-274.53)
	P value	0.001	0.31	0.01	0.007	0.78	0.04	0.44	0.55
GSTT1	+/+	164/231	92/89	139/95	117/225	199/137	57/146	0/34	0/3
	−/−	51/53	43/27	50/18	44/62	77/44	16/32	0/2	1/2
	OR (95% CI)	1.35 (0.87-2.09)	1.54 (0.87-2.70)	1.89 (1.04-3.45)	1.36 (0.87-2.13)	1.20 (0.78-1.85)	1.28 (0.65-2.51)	13.80 (0.22-853.64)	4.20 (0.11-151.97)
	P value	0.16	0.13	0.03	0.17	0.39	0.47	0.21	0.43
GSTP1 Exon 5 A>G	Ile/Ile	125/183	59/70	99/71	85/182	141/107	42/118	0/24	1/4
	Ile/Val+ Val/Val	90/101	76/46	90/42	76/105	135/74	31/60	0/12	0/1
	OR (95% CI)	1.30 (0.90-1.87)	1.96 (1.18-3.24)	1.53 (0.95-2.47)	1.54 (1.04-2.29)	1.38 (0.94-2.02)	1.45 (0.83-2.53)	1.96 (0.03-104-76)	1.00 (0.02-40.27)
	P value	0.15	0.008	0.07	0.02	0.09	0.19	0.74	1.00
GSTP1 Exon 6 C>T	Ala/Ala	139/155	87/59	113/76	113/138	174/91	51/99	0/21	0/3
	Ala/Val + Val/Val	76/129	48/57	76/37	48/149	102/90	22/79	0/15	1/2
	OR (95% CI)	0.65 (0.45-0.94)	0.57 (0.34-0.94)	1.38 (0.84-2.25)	0.39 (0.26-0.59)	0.59 (0.40-0.86)	0.54 (0.30-0.96)	1.38 (0.026-73.79)	4.20 (0.11-151.97)
	P value	0.02	0.03	0.19	<0.0001	0.007	0.03	0.87	0.43

(+/+)= Present, (-/-) = null, *p* value determined based on $\chi 2$, 1.0 (Reference)

4. Discussion

CC is a polygenic and multifactorial disease where several pathways are related with its pathogenesis. Present study was intended to create information on prevalence of polymorphism of GST in routine cervical disease scrapings from the women of rural population. The study was additionally extended to explore whether GST polymorphisms could impact the risk to develop CC, exclusively or in blend with other demographic risk factors in these women. When we considered the homozygous null polymorphisms in GST genes in the present study, a noteworthy relationship of GSTM1 and GSTT1 homozygous null genotype with CC cases was observed when compared with those with ordinary cervical cytology. We observed that individuals conveying GSTM1 homozygous null genotype were roughly twice at risk of having CC as compared to GSTM1 non-null individuals which are similar to other studies [26]. *GSTP1* Ile105Val of exon 5 and GSTP1 Ala114Val of exon 6 genotypes did not show significant association with development of CC in the studied individuals.

The polymorphisms in GST family genes have been found to be associated with increased risk of developing bladder, colon, breast and lung cancer [11-16]. The majority of studies cited in literature did not find any interaction between smoking habits and the polymorphisms of GSTM1 or GSTT1 [10,24,27] and GSTP1 genes [28]. These variations found in different populations may be due to different lifestyles, diet, environmental factors and other genetic factors [29], as well as progressively efficient metabolic and detoxifying activity of tobacco compounds in some individuals than in others [24]. Barely, any studies have been done to know the relationship of age with GSTM1 and GSTT1 homozygous null genotypes in women with CC. A significant difference was found between the CC cases and controls in the distribution of the null genotype of GSTM1 in women aged above 50 years but this difference was not significant in individuals aged less than

50 years [30]. We did not observe any association between GSTM1, GSTT1 or GSTM1T1 homozygous null genotypes and ≤50years or >50 years of age groups. When we compared our outcomes with studies reported worldwide it was observed that GSTM1 homozygous null genotype is associated with the development of CC, especially in Chinese [29,31] and other Indian population [10,21], whereas only two studies from American population [23, 32] reported an association of GSTM1 homozygous null genotype with CC. No connection between GSTM1 homozygous null genotype and CC risk was accounted in studies of the American population [24,28,30] and in Asian population [5]. Few studies have reported a noteworthy connection between GSTT1 null genotypes and CC [10,33], whereas no risk of CC was observed in GSTT1 homozygous null genotype in other studies [5,24,28,30] alongside two meta-analyses [22,25]. Recent publications on meta-analyses found a significant association between GSTM1 and GSTT1 genotypes with increased risk of CC in Asian population [10,22,25,33], however, different investigation did not affirm this correlation [28]. It is evident from other Indian studies that the CC risk may increases significantly with GSTM1 and GSTT1 null genotypes in response to exposure to passive smoking [5, 34].

In any case, non accessibility of information on the association of genetic polymorphisms in GST isoforms mainly GSTM1, GSTT1 and GSTP1 and their susceptibility to advancement of CC allowed us to survey the impact of functional polymorphisms in GSTs to the risk of CC in susceptible individuals from rural population of Maharashtra. Accordingly, this is the primary report of its own kind to examine the polymorphisms in carcinogen detoxifying genes and their conceivable relationship with development of CC from a pool of unexplored rural Maharashtrian population and showed that GSTM1 and GSTT1 homozygous null may contribute in cervical carcinogenesis in Maharashtrian women. In spite of the fact that this study is a first in medical literature to show association of polymorphism in GSTM1 and GSTT1 genes and cervical cancer occurrence, it showed similar outcome as mentioned in above studies. It would be of any importance to further assess whether these polymorphisms could be utilized as a disease marker for the normal history of cervical neoplasms in setting of larger patient and control groups.

5. Conclusion

This investigation confirms the interactions between polymorphisms of GSTM1 (-/-), GSTT1 (-/-) and GSTP1 (G/G) of exon 5 may be associated with development of cervical cancer in rural population of south-western Maharashtra from India. However, large sized cohort study required to confirm the believable interactions between GST polymorphisms for their association with cervical cancer development in rural women.

Conflict of Interest: None declared.

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