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IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISM IN CHEMICAL METABOLIZING GENES AND EXPOSURE TO WOOD SMOKE ON RISK OF CERVICAL CANCER IN NORTH-INDIAN WOMEN

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***Aim*: In the present study, we investigated the hypothesis whether exposure to wood smoke increases the risk of cervical cancer (CC) in North-Indian women who inherit different polymorphic forms of chemical metabolizing genes (*GSTM1*, *GSTT1*, *GSTP1* and *CYP1A1*). *Materials and Methods:* One hundred fifty histologically confirmed CC patients and equal number of cancer-free age and ethnicity matched controls were genotyped for genetic polymorphism in chemical metabolizing genes by using polymerase chain reaction/restriction fragment length polymorphism method. The association of the different genotypes and exposure to wood smoke with the risk of CC in North-Indian women was estimated by doing statistical analysis using Statistical Package for the Social Science. *Results*: It was observed that the variant genotypes of *GSTM1*, *GSTT1*, *GSTP1* and *CYP1A1* did not significantly increase the risk of CC. However, statistically significant increased risk (odds ratio 3.6; 95% confidence interval, 1.34–9.78; $p = 0.008$) was observed for women who used wood for cooking and had *GSTM1* (null) genotype. *Conclusions*: The present study suggests that genetic differences in the metabolism of wood smoke carcinogens, particularly by *GSTM1*, may increase the risk of CC.**
***Key Words*: genetic polymorphism, metabolic genes, cervical cancer, risk, India, exposure to wood smoke.**

A number of studies [1, 2] have proved that infection of cervix with human papilloma virus (HPV) is a prerequisite for the development of cervical cancer (CC). However, for these infections to result into malignancy other risk factors also play important role [1, 3]. One such important risk factor is exposure to wood smoke. It has been reported that Colombian women who were exposed to wood smoke in the kitchen for a number of years had higher risk of developing high-grade squamous intraepithelial lesions (HSIL) [4]. A similar study [5] was carried out on HPV-infected women in Honduras, in which interaction of wood utilization and HPV was found to be significantly associated with risk of CC.

Wood smoke is composed of a number of gases and very small particles having variable composition and diameter [6, 7]. Zelkoff et al. [8] reported that smoke from burning wood contain a number of carcinogens such as polycyclic aromatic hydrocarbons, aromatic amines, and nitropolycyclic aromatic hydrocarbons, which are also present in tobacco smoke. However, it has been observed that not all individuals exposed to these carcinogens may develop cancer. This indicates the important role of carcinogen-metabolizing genes and genes involved in repair of DNA damage [9]. The main reason for this is that these carcinogens need to be metabolically activated in order to cause their carcinogenic effect in human body. Two major enzyme systems, which are involved in this metabolic process are phase I and phase II enzymes. Generally, phase I enzymes activate the carcinogen directly whereas phase II enzymes detoxify and process the activated metabolites for breakdown or excretion. Therefore, individuals having genotypes with high phase I enzyme and low phase II enzyme activity are at high risk of cancer development [10].

Sierra-Torres et al. [4] studied the combined effect of genetic polymorphisms in metabolic genes such as *CYP2E1*, *GSTM1*, and *GSTT1*, and exposure to wood smoke in increasing the risk of HSIL in Colombian women. It was observed that women, who had *CYP2E1* c2/c2 genotype and were exposed to wood smoke, were at an increased risk for HSIL. These findings indicate that polymorphisms in xenobiotic-metabolizing genes along with exposure to environmental carcinogens could affect the susceptibility of women to CC. The study was carried out on 183 women (91 cases and 92 controls). To further investigate these associations on larger population (150 cases and 150 controls), the present study was designed to analyze the impact of exposure to wood smoke and genetic polymorphism in chemical metabolizing genes on the risk of development of CC in North-Indian women.

MATERIALS AND METHODS

Sample collection. Histologically confirmed CC patients (n = 150) were recruited from the Post-Graduate Institute of Medical Education and Research (PGIMER), Chandigarh and Mohan Dai Oswal Cancer Hospital, Ludhiana, Punjab, India. This study was approved by human subject ethical committees of the involved institutions. None of the patients had received radiation or chemotherapy. Detailed data regarding age, education, menarche, menopausal status, number of children, age at marriage and birth of first child, exposure to wood smoke during cooking were ob-

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Abbreviations used: AC – adenocarcinoma; CC – cervical cancer; HPV – human papilloma virus; HSIL – high-grade squamous intraepithelial lesions; PCR – polymerase chain reaction; RFLP – restriction fragment length polymorphism; SCC – squamous cell carcinoma.

tained from an interviewer administered questionnaire. A written informed consent to participate in this study was obtained from all the participants before collection of their blood samples. To confirm exposure to wood smoke, the data were collected about the duration and regularity of the exposure. Since smoking by woman is considered a taboo in Indian culture, so there was no active smoker in the present study group. The control group consisted of 150 individuals who were free of any malignancy and well matched according to age and ethnicity. Genomic DNA was isolated from blood samples of CC patients and controls by SDS/proteinase K and phenol-chloroform method [11].

Genotyping of GSTM1, GSTT1, GSTP1 and CYPIA1 genes. The *GSTM1* and *GSTT1* genetic polymorphisms were analyzed using multiplex polymerase chain reaction (PCR) [12]. 50 µl PCR reaction mixture contained 100 ng of genomic DNA, 0.2 mM of each deoxynucleotide triphosphate, 2.5 mM of $MgCl_2$, 1 U of Taq polymerase (MBI, Fermentas), 5% DMSO and 10 pmol of each primer (*GSTM1-F*-GAACTCCCTGAAAAGCTAAAGC, R-GTTGGGCTCAAATATACGGTC; *GSTT1-F*-TTCCTTACTGGTCCTCACAATCT, R-TCACCGGATCATGGCCAGCA; Albumin-*F*-GCCCTCTGCTAACAAGTCCTAC, R-GCTAAAAAAGAAAATCGCCAATC). Amplification was carried out, using an initial denaturation at 95 °C for 2 min; 30 cycles of melting at 94 °C for 1 min, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min; followed by a final extension step at 72 °C for 5 min. The PCR products were then subjected to electrophoresis on a 2% agarose gel and observed under ultraviolet light after staining with ethidium bromide. Presence of bands of 480 and 215 bps indicates *GSTT1* and *GSTM1* genotypes, respectively, whereas the absence of one of them indicated the null genotype for that gene. Albumin indicated by a 350 bp product was used as an internal control (Fig. 1).

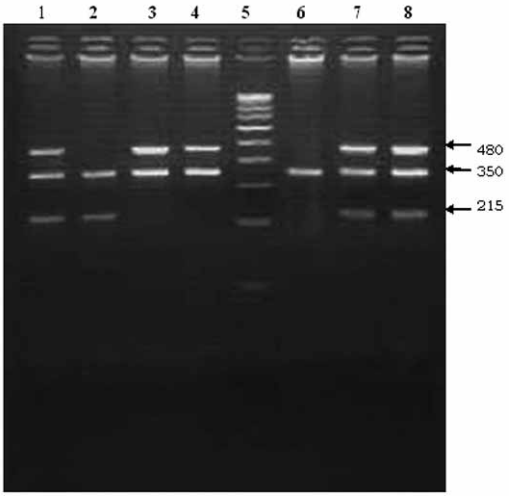

Fig. 1. Genotyping of *GSTM1* and *GSTT1* by multiplex PCR. Lanes 1, 7, 8 — *GSTM1* (present) (215 bp), *GSTT1* (present) (480bp); Lane 2 — *GSTM1* (present) (215 bp), *GSTT1* (null), Lanes 3, 4 — *GSTT1* (present) (480 bp), *GSTM1* (null); Lane 6 — *GSTM1T1* (null); Albumin as a positive control is detected as 350 bp fragment; Lane 5 — 100 bp DNA marker

PCR-RFLP (restriction fragment length polymorphism) analysis of *GSTP1* gene was carried out in exon 5 at codon 105 [13]. Genomic DNA was amplified using the primers F-GTAGTTTGCCCAAGGTCAAG, R-AGCCACCTGAGGGGTAAG (Sigma Aldrich, USA). PCR reaction mixture (50 µl) was prepared containing 10 pmol of each primer, 12.0 mM $MgCl_2$, 200 µM of each dNTPs, 1U Taq polymerase (MBI, Fermentas), 100–300 ng of genomic DNA. Cycling conditions included initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 90 s and extension at 72 °C for 90 s and final extension at 72 °C for 7 min to complete the process of elongation. PCR products were detected by electrophoresis using a 2% agarose gel and observed under ultraviolet light after staining with ethidium bromide. *GSTP1* PCR fragment was of 329 bp. PCR products were digested with 2 units of restriction enzyme *Alw261* (MBI, Fermentas) using 0.2 µl (2 unit) of enzyme, 1.8 µl of Y Tango buffer, 15 µl of PCR products at 37 °C for at least 3 h. The digested products were classified as wild (329; 113 bp bands), homozygous mutant (216; 113; 107 bp bands) and heterozygous mutant (329; 216; 113; 107 bp bands) types (Fig. 2).

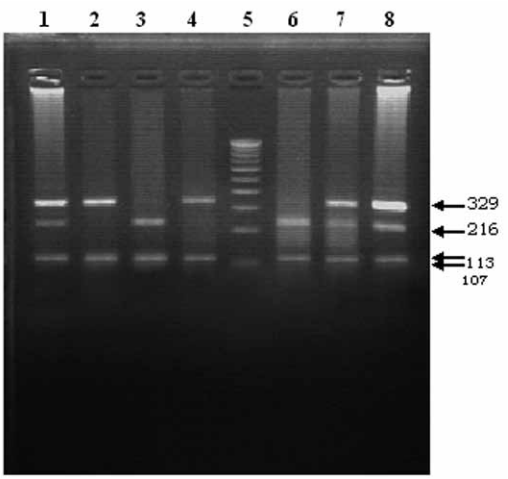

Fig. 2. RFLP analysis of *GSTP1*. Lanes 1, 7, 8 — heterozygous mutant (329; 216; 113; 107 bps); Lanes 2, 4 — homozygous wild (329; 113 bps); lanes 3, 6 — homozygous mutant (216; 113; 107 bps), Lane 5 — 100 bp DNA marker

The *MspI* polymorphism in the *CYP1A1* 3′-flanking region was determined using PCR and RFLP method of Wu et al. [14]. PCR reaction mixture (50 µl) consisted of 0.2 µg of genomic DNA, 0.5 µM of each primer (F-CAGTGAAGAGGTGTAGCCGC, R-TAGGAGTCTTGTCTCATGCC), 0.2 mM of each dNTPs, 2.5 mM $MgCl_2$, and 1.5 U of Taq polymerase (MBI, Fermentas). Amplification was performed in the thermal cycler (Biorad), at an initial denaturation at 94 °C for 5 min; 30 cycles of melting at 94 °C for 1 min, annealing at 61 °C for 1 min, and extension at 72 °C for 1 min; followed by a final extension step at 72 °C for 7 min. *CY1A1* PCR fragment was of 340 bp. These PCR products (10 µl) were digested with 3 units *MspI* (MBI, Fermentas) at 37 °C for 3 h. When *MspI* restriction site was present, 340 bp fragment was digested into two bands of 140 and 200 bp. Homozygous wild type in-

dividuals lacked the 140 and 200 bp fragments. Heterozygous individuals had 3 bands (340; 200; 140 bp) and individuals with homozygous rare allele lacked the large parent band, but had the smaller bands (140 and 200 bp) (Fig. 3).

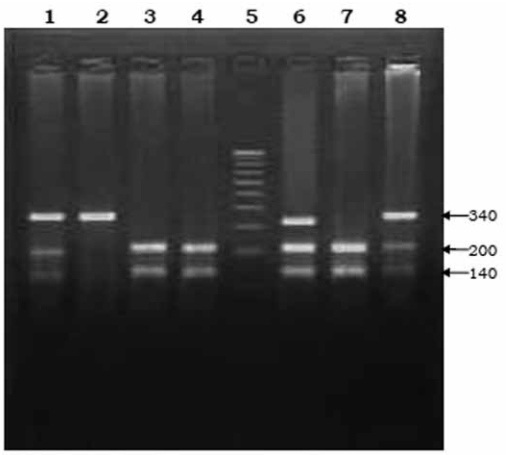

Fig. 3. RFLP analysis of *CYP1A1*. Lanes 1, 6, 8 — heterozygous mutant (340; 200; 140 bps), Lane 2 — homozygous wild (340 bps), Lanes 3, 4, 7 — homozygous mutant (200; 140 bps), Lane 5 — 100 bp DNA marker

Statistical analysis. The association between polymorphic forms of different genes with the risk of CC was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) using a multivariate logistic regression analysis which included several potential confounding variables (e.g., age, users and non-users of wood for cooking). Statistical analysis was performed using Statistical Package for the Social Science, version 10.0 and Epical Version 3.2. The probability level of less than 0.05 was used as the criterion of significance.

RESULTS

The study included 150 CC cases and an equal number of matching controls. The cases and controls were well matched according to age and ethnicity. Out of 150 cases, 131 (87.3%) had squamous cell carcinoma (SCC) and 19 (12.7%) adenocarcinoma (AC).

Baseline characteristics. Detailed analysis of various epidemiological risk factors of CC in North-Indian women has already been published by our research team [15]. Few factors out of them such as age, number of children, use of wood for cooking for cases and controls, are summarized in Table 1. The mean age (± SD) was found to be 48.5 ± 9.4 for cases and 46.1 ± 11.2 for controls. As compared with the controls, cases had young age at the time of marriage (16.4 ± 3.0) and birth of first child (18.4 ± 3.4) and had greater mean number of children (4.1). Mean age at menarche and menopause was found to be comparable between cases and controls.

Use of wood for cooking is more prevalent in case of cases, 130 (86.7%) as compared to controls, 116 (77.3%). Regarding the duration of exposure, 92.3% of the cases were exposed to wood smoke for > 15 years. This exposure to wood smoke occurs on daily basis. Apart from the exposure to wood smoke at the time of cooking, the subjects have not been exposed to smoke from furnaces and fireplaces. Individuals exposed to wood smoke were found to be at an increased risk (OR 2.0; 95% CI 1.03–3.49; $p = 0.05$) of CC.

Genotype, allele frequency and risk of CC. Distribution of *GSTM1*, *GSTT1*, *GSTP1* and *CYP1A1* genotypes among cases and controls and their relationship with the risk of CC are given in Table 2. The genotype distribution for both cases and controls was in Hardy — Weinberg equilibrium for *CYP1A1*. On the other hand, in case of *GSTP1* genotype distribution was not in Hardy — Weinberg equilibrium due to a statistically significant selection pressure against the homozygous mutant genotype and favoring the heterozygous. The Hardy — Weinberg equilibrium was not evaluated for the *GSTT1* and *GSTM1* polymorphisms because the PCR technique used in the study only recognizes the presence (wild-type) or absence (null genotype) of the genes, and does not distinguish between heterozygous and homozygous wild-type.

For *GSTM1* null genotype, the frequency was 42.0 and 34.7% in cases and controls, respectively. There was marginal increase in OR in case of individuals with *GSTM1* null genotype (OR 1.4; 95% CI 0.83–2.24). However, in case of *GSTT1*, the null genotype was found to be more prevalent in controls (24.7%) as compared to patients with CC (14.7%) and statistically significant decreased risk of CC was observed (OR 0.5; 95% CI 0.28–0.98; $p = 0.04$).

In case of *GSTP1*, there was no significant variation in frequencies of different genotypes between cases and controls and thus these genotypes were not found to increase the risk of CC. However, when

Table 1. Baseline characteristics of the study groups

No.	Variables	Cases	Control
1	Sample size	150	150
2	Age, years ± SD	48.5 ± 9.4	46.1 ± 11.2
3	Age at the time of marriage, years ± SD	16.4 ± 3.0	18.7 ± 3.8
4	Age at the birth of first child, years ± SD	18.4 ± 3.4	21.6 ± 3.6
5	Children, mean number	4.1	2.9
6	Age at menarche, years ± SD	14.9 ± 1.1	14.4 ± 0.8
7	Age at menopause, years ± SD	48.3 ± 3.6	47.5 ± 3.1
8	Use of wood for cooking		
	Non-user, n (%)	20 (13.3)	34 (22.7)
			1.0 (Ref.)
	User, n (%)	130 (86.7)	116 (77.3)
		OR 2.0; 95% CI 1.03–3.49; $p = 0.05$	
9	Duration of experience (years):		
	< 15	10 (7.6)	20 (17.2)
	> 15	120 (92.3)	96 (82.7)

Table 2. Distribution of *GSTM1*, *GSTT1*, *GSTP1* and *CYP1A1* genotypes among cases and controls and risk of CC

Genotype	Cases, n (%)	Controls, n (%)	All OR*	SCC, n (%)	OR* (95% CI)	AC, n (%)	OR* (95% CI)
GSTM1							
+	87 (58.0)	98 (65.3)	1.0 (Ref.)	76 (58.0)	1.0	11 (57.9)	1.0
–	63 (42.0)	52 (34.7)	1.4 (0.83– 2.24)	55 (42.0)	1.4 (0.82–2.28)	8 (42.1)	1.4 (0.47–3.97)
GSTT1							
+	128 (85.3)	113 (75.3)	1.0 (Ref.)	111 (84.7)	1.0	17 (89.5)	1.0
–	22 (14.7)	37 (24.7)	0.5 (0.28–0.98) (p = 0.04)	20 (15.3)	0.5 (0.29–1.05)	2 (10.5)	0.4 (0.09–1.62)
GSTP1							
Ile/Ile	44 (29.3)	46 (30.7)	1.0 (Ref.)	40 (30.5)	1.0	4 (21.0)	1.0
Ile/Val	97 (64.7)	96 (64.0)	1.1 (0.62–1.80)	82 (62.6)	1.0 (0.57–1.70)	15 (79.0)	1.7 (0.59–4.83)
Val/Val	9 (6.0)	8 (5.3)	1.2 (0.37–3.73)	9 (6.9)	1.3 (0.41–4.13)	–	–
Ile/Val/Val/Val	106 (70.6)	104 (69.3)	1.1 (0.63–1.80)	91 (69.4)	1.0 (0.59–1.73)	15 (79.0)	1.7 (0.59–4.83)
CYP1A1							
**1/*1*	61 (40.7)	67 (44.6)	1.0 (Ref.)	54 (41.2)	1.0	7 (36.8)	1.0
**1/*2A*	75 (50.0)	73 (48.7)	1.1 (0.68–1.86)	66 (50.4)	1.1 (0.67–1.89)	9 (47.4)	1.2 (0.38–3.76)
**2A/*2A*	14 (9.3)	10 (6.7)	1.5 (0.59–4.06)	11 (8.4)	1.4 (0.49–3.79)	3 (15.8)	2.4 (0.72–8.24)
**1/*2A/*2A/*2A*	89 (59.3)	83 (55.3)	1.2 (0.73–1.91)	77 (58.7)	1.1 (0.70–1.90)	12 (63.1)	1.4 (0.47–4.15)

Note: OR* represents Odds Ratio adjusted for age, age at marriage, age at birth of first child, number of children and use of wood for cooking; n – no. of cases/no. of controls.

Table 3. OR and corresponding 95% CI for the combined effect of *GSTM1*, *GSTT1*, *GSTP1* and *CYP1A1* genotypes on risk of CC

Genotypes			Case	Controls	OR* (95% CI)	SCC		AC	
			No. (%)	No. (%)		No. (%)	OR* (95% CI)	No. (%)	OR* (95% CI)
GSTM1	Present	Present	76 (50.7)	72 (48.0)	1.0 (Ref.)	66 (86.8)	1.0	10 (13.2)	1.0
and	Null	Present	52 (34.7)	41 (27.3)	1.2 (0.69–2.09)	45 (86.5)	1.2 (0.67–2.13)	7 (13.5)	1.2 (0.39–3.86)
GSTT1	Present	Null	11 (7.3)	26 (17.3)	0.4 (0.17–0.92) p = 0.02	10 (90.9)	0.4 (0.17–1.00) p = 0.04	1 (9.1)	0.3 (0.04–2.26)
	Null	Null	11 (7.3)	11 (7.3)	0.9 (0.36–2.53)	10 (90.9)	1.0 (0.36–2.72)	1 (9.1)	0.7 (0.10–4.87)
GSTM1	Present	*Ile/Ile*	25 (16.7)	30 (20.7)	1.0 (Ref.)	24 (96.0)	1.0	1 (4.0)	1.0
and	Null	*Ile/Ile*	19 (12.7)	16 (10.7)	1.4 (0.56–3.65)	16 (84.2)	1.2 (0.48–3.30)	3 (15.8)	4.9 (0.55–43.73)
GSTP1	Present	*Ile/Val/Val/Val*	62 (41.3)	68 (45.3)	1.1 (0.55–2.16)	52 (83.9)	1.0 (0.48–1.92)	10 (16.1)	4.0 (0.53–29.75)
	Null	*Ile/Val/Val/Val*	44 (29.3)	36 (24.0)	1.5 (0.70–3.10)	39 (88.6)	1.3 (0.63–2.91)	5 (11.4)	3.8 (0.46–30.74)
GSTM1	Present	*(*1/*1)*	36 (24.0)	42 (28.0)	1.0 (Ref.)	33 (91.7)	1.0	3 (8.3)	1.0
and	Null	*(*1/*1)*	25 (16.7)	25 (16.7)	1.2 (0.54–2.53)	21 (84.0)	1.2 (0.48–3.30)	4 (16.0)	2.1 (0.50–8.58)
CYP1A1	Present	*(*1/*2A/*2A/*2A)*	51 (34.0)	56 (37.3)	1.1 (0.57–1.99)	43 (84.3)	1.0 (0.48–1.92)	10 (15.7)	1.0 (0.51–1.87)
	Null	*(*1/*2A/*2A/*2A)*	38 (25.3)	27 (18.0)	1.6 (0.80–3.37)	34 (89.5)	1.3 (0.63–2.91)	5 (10.5)	1.6 (0.77–3.35)
GSTT1	Present	*Ile/Ile*	36 (20.0)	38 (25.3)	1.0 (Ref.)	32 (88.9)	1.0	4 (11.1)	1.0
and	Null	*Ile/Ile*	8 (5.3)	8 (5.3)	1.1 (0.32–3.52)	8 (80.0)	1.2 (0.35–4.00)	–	–
GSTP1	Present	*Ile/Val/Val/Val*	92 (61.3)	75 (50.0)	1.3 (0.72–2.32)	79 (85.9)	1.2 (0.68–2.29)	13 (14.1)	1.5 (0.54–4.47)
	Null	*Ile/Val/Val/Val*	14 (9.3)	29 (19.3)	0.5 (0.22–1.20)	12 (85.7)	0.5 (0.20–1.20)	2 (14.3)	0.7 (0.13–3.47)
GSTT1	Present	*(*1/*1)*	51 (34.0)	54 (36.0)	1.0 (Ref.)	46 (90.2)	1.0	5 (9.8)	1.0
and	Null	*(*1/*1)*	10 (6.7)	13 (8.7)	0.8 (0.30–2.20)	8 (80.0)	0.7 (0.25–2.08)	2 (20.0)	1.6 (0.34–7.33)
CYP1A1	Present	*(*1/*2A/*2A/*2A)*	77 (51.3)	59 (39.3)	1.4 (0.80–2.38)	65 (84.4)	1.3 (0.74–2.27)	12 (15.6)	2.0 (0.75–5.34
	Null	*(*1/*2A/*2A/*2A)*	12 (8.0)	24 (16.0)	0.5 (0.22–1.25)	12 (100.0)	0.6 (0.24–1.39)	–	–

Note: OR* represents OR adjusted for age, age at marriage, age at birth of first child, number of children and use of wood for cooking.

the genotypes were classified according to histological subtypes, individuals having *Ile/Val* and *Ile/Val/Val/Val* genotypes had elevated risk of AC (OR 1.7; 95% CI 0.59–4.83).

For *CYP1A1*, the frequency of homozygous mutant genotype (**2A/*2A*) was more in patients with CC as compared to controls (9.3% *vs* 6.7%, respectively) and this genotype was found to be associated with 1.5 fold (95% CI 0.59–4.06) increased risk of CC. When stratified according to histology this risk was further elevated to 2.4 fold (OR 2.4; 95% CI 0.72–8.24) for AC.

Gene-gene interactions. The analysis of combined effect of different genotypes of metabolizing genes on risk of CC is given in Table 3. *GSTM1* (present) and *GSTT1* (null) genotype had statistically significant inverse relationship with risk of CC (OR 0.4; 95% CI 0.17–0.92; $p = 0.02$), that is they had a protective effect.

However, individuals having *GSTM1* (null) and *GSTP1* (*Ile/Ile*) genotype, had 4.9 fold (95% CI 0.55–43.73) increased risk of developing AC. Similarly, risk for AC was elevated for genotypic combinations such as *GSTM1* (present) and *GSTP1* (*Ile/Val/Val/Val*) (OR 4.0; 95% CI 0.53–29.75), *GSTM1* (null) and *GSTP1* (*Ile/Val/Val/Val*) (OR 3.8; 95% CI 0.46–30.74). However, none of these associations were statistically significant.

Interaction between genotype and wood smoke exposure. Combined effect of wood smoke exposure and genotype on the risk of CC was also studied (Table 4). Significant association (OR 3.6; 95% CI 1.34–9.78; $p = 0.008$) was observed between *GSTM1* null genotype and risk of CC in those women who used wood for cooking.

DISCUSSION

The present study indicates that the interaction between exposure to wood smoke and polymorphic forms of chemical metabolizing genes plays an important role in the development of CC in North-Indian women. Increased risk of CC with borderline significance (OR 2.0; $p = 0.05$) was observed in case of women who used wood for cooking. The carriers of *GSTM1* (null) genotype when exposed to wood smoke showed statistically significant high risk of developing CC.

It has been reported that individuals exposed to wood smoke for long time accumulate inhaled constituents of wood smoke in their cervical epithelial

Table 4. Combined effect of genotype and use of wood on risk of CC

Genotype		User of wood		Non-user of wood	
		Cases, n (%), OR*(95% CI)	Controls, n (%)	Cases, n (%)	Controls, n (%) OR
GSTM1	+	79 (60.8)	75 (64.6)	8 (40.0)	23 (67.6) OR = 1.0 (Ref.)
	–	51 (39.2) OR = 3.6 (1.34–9.78) p = 0.008	41 (35.4)	12 (60.0)	11 (32.4)
GSTT1	+	113 (86.9)	87 (75.0)	15 (75.0)	26 (76.5) OR = 1.0 (Ref.)
	–	17 (13.1) OR = 1.0 (0.39–2.67)	29 (25.0)	5 (25.0)	8 (23.5)
GSTP1	*Ile/Ile*	38 (29.2)	35 (30.2)	6 (30.0)	11 (32.3) OR = 1.0 (Ref.)
	Ile/Val	85 (65.4) OR = 1.5 (0.78–2.91)	75 (64.6)	12 (60.0)	21 (61.8)
	Val/Val	7 (5.4) OR = 2.1 (0.39–12.33)	6 (5.2)	2 (10.0)	2 (5.9)
CYP1A1	*(*1/*1)*	52 (40.0)	52 (44.8)	9 (45.0)	15 (44.1) OR = 1.0 (Ref.)
	*(*1/*2A)*	67 (51.5) OR = 2.0 (0.74–5.29)	57 (49.1)	8 (49.1)	16 (47.1)
	*(*2A/*2A)*	11 (8.5) OR = 2.6 (0.63–11.28)	7 (6.1)	3 (6.1)	3 (8.8)

Note: OR* represents OR adjusted for age, age at marriage, age at birth of first child, number of children and use of wood for cooking.

cells [5]. These inhaled constituents get converted by metabolizing enzymes into their reactive forms which may ultimately initiate carcinogenic process. Researchers have already reported such susceptibilities to cancer in cases with genetically determined differences in metabolism related to CYP and GST [16, 17].

In the present study significant association (OR 3.6; 95% CI 1.34–9.78; $p = 0.008$) was observed between *GSTM1* (null) genotype and risk of CC in the individuals who used wood for cooking. A similar study was carried out in Colombia and it was observed that women, who were exposed to wood smoke and had *GSTT1* and *GSTM1* null genotype, had a small but non-significant risk of developing HSILs [4]. Variation in results from the present study might be due to difference in the ethnicity of the population studied.

When the impact of these chemical metabolizing genes only on risk of CC was analyzed it was observed that *CYP1A1* (*MspI*) polymorphism does not play an important role in modulating the risk of CC. These results are in co-ordinance with those of Kim et al. [18]. Similarly, in case of *GSTs*, *GSTM1* and *GSTT1* null genotypes and variant forms of *GSTP1* were not found to be significantly increasing the risk of CC. Similar results have been reported by earlier studies [19–25].

Relatively fewer studies [18, 19, 23, 26–28] have analyzed the combined effect of *CYP1A1* (*MspI*), *GSTM1* and *GSTT1* polymorphic forms on risk of developing CC. In the present study, the combination of *GSTM1* (present) and *GSTT1* (null) genotype was associated with statistically significant decreased risk of CC (OR 0.4; 95% CI 0.17–0.92; $p = 0.02$). This suggests that this genotypic combination exert a protective effect. This is the first report of protective effect of *GSTM1* (present) and *GSTT1* (null) genotype in relation to risk of CC. Similar findings have been reported in oral precancerous conditions [29], breast cancer [30] and bladder cancer [31]. These observations may be due to the reason that *GSTM1* helps in the excretion of a number of carcinogens, reactive oxygen species and chemotherapeutic agents from the body [32] and *GSTT1* has been reported to be involved in the bio-activation of certain halogenated compounds [33]. A decreased risk observed for the combined genotype of *GSTM1* (present) and *GSTT1* (null) in the present study could imply that *GSTT1* activates certain known or yet to be identified environmental pro-carcinogens.

CONCLUSION

From the present study, it can thus be concluded that inheritance of either the *GSTM1* or *GSTT1* null genotype or variant forms of *CYP1A1* had no effect on the risk of CC. However, when these genotypes were analyzed in combination with wood smoke exposure it was observed that individuals having null genotype of *GSTM1* had increased risk of CC. These findings will highly be beneficial for CC preventive programs, especially in India where there is a high incidence of CC particularly in rural areas, where a vast majority of women burn wood as an energy source to cook food. They use homemade clay-stoves, which generate a variety of airborne products along with polycyclic aromatic hydrocarbons in an uncontrolled manner. Thus, these women are exposed daily to high concentrations of carcinogenic polycyclic aromatic hydrocarbons while cooking food. Because of higher incident rate of CC and use of wood for cooking especially in rural areas of India this risk factor deserves further study.

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CONFLICT OF INTEREST

No potential conflict of interests relevant to this article.

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