RESEARCH ARTICLE

Glutathione S-transferase M1 and T1 Polymorphisms, Cigarette Smoking and HPV Infection in Precancerous and Cancerous Lesions of the Uterine Cervix

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Abstract

Glutathione S-transferases (GSTs) play an important role in detoxification of carcinogenic electrophiles. The null genotypes in *GSTM1* and *GSTT1* have been implicated in carcinogenesis. Present study was planned to evaluate the influence of genetic polymorphisms of *GSTM1* and *GSTT1* gene loci in cervical carcinogenesis. The study was conducted in Lok Nayak hospital, New Delhi. DNA from clinical scrapes of 482 women with minor gynaecologic complaints attending Gynaecology OPD and tumor biopsies of 135 cervical cancer cases attending the cancer clinic was extracted. HPV DNA was detected by standard polymerase chain reaction (PCR) using L1 consensus primer pair. Polymorphisms of *GSTM1* and *GSTT1* were analysed by multiplex PCR procedures. Differences in proportions were tested using Pearson's Chi-square test with Odds ratio (OR) and 95% confidence interval (CI). The risk of cervical cancer was almost three times in women with *GSTM1* homozygous null genotype (OR-2.62, 95%CI, 1.77-3.88; p<0.0001). No association of *GSTM1* or *GSTT1* homozygous null genotypes was observed in women with normal, precancerous and cervical cancerous lesions among ≤35 or >35 years of age groups. Smokers with null *GSTT1* genotype had a higher risk of cervical cancer as compared to non-smokers (OR-3.01, 95% CI, 1.10-8.23; p=0.03). The results further showed that a significant increased risk of cervical cancer was observed in HPV positive smoker women with *GSTT1* (OR-4.36, 95% CI, 1.27-15.03; p=0.02) and *GSTM1T1* (OR-3.87, 95% CI, 1.05-14.23; p=0.04) homozygous null genotypes as compared to HPV positive non smokers. The results demonstrate that the *GST* null genotypes were alone not associated with the development of cervical cancer, but interacted with smoking and HPV to exert effects in our Delhi population.

Keywords: Cervical cancer - GST polymorphisms - GSTM1 - GSTT1 - smoking - Pap smear - HPV

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Introduction

Cervical cancer is the fourth most common cancer affecting worldwide, after breast, colorectal, and lung cancers with 528, 000 new cases every year. It is also the fourth most common cause of cancer death (266,000 deaths in 2012) in women worldwide. Almost 70% of the global burden fall in areas with lower levels of development and more than one fifth of all new cases are diagnosed in India (Ferlay et al., 2013). Cervical cancer is preventable and curable disease, especially if identified at an early stage. Pap smear test is the main screening method used for the detection of precancerous cells easily (Kotaniemi-Talonen et al., 2008). Pap smears have effectively reduced the incidence of cervical cancer by 75-90% in developed countries (Risendal et al., 1999). Cervical cancer is a polygenic and multifactorial disease, indicating that multiple distinct pathways may be involved in its pathogenesis. Epidemiological studies have established various risk factors, i.e., age at menarche, age at consummation of marriage, sexual history, including multiple sexual partners, parity, oral contraceptive intake, smoking, passive smoking for cervical cancer, but none has been shown to be a significant independent risk factor (Schoell et al., 1999).

HPV infection has been identified as the strongest risk factor, but is still an insufficient event for the development of cervical cancer (Walboomers Meijer 1997, Schiffman et al., 2007). HPV is detected at a certain frequency among women with normal cytology, but not all HPV-infected individuals develop cervical cancer, thus suggesting that environmental and genetic factors also play an important role in cervical carcinogenesis.

In order to understand the molecular pathways of diseases, polymorphisms of phase I and phase II enzymes involved in detoxification and cellular protection have

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long been assessed. GSTs are a family of phase II enzymes involved in the detoxification of various exogenous as well as endogenous reactive species (Hengstler et al., 1998). The GSTs conjugate glutathione to various potentially carcinogenic compounds, reactive oxygen species, and chemotherapeutic agents, with a variety of substrate specificities (Rebbeck 1997). The mode of action of GSTs is thought to involve simultaneous enzyme activation and detoxification. In mammals, eight classes of GSTs namely, alpha (α), mu (μ), Pi (π), Theta (θ), sigma (σ), Kappa (κ), Omega (ω) and Zeta (ζ) have been identified based on sequence homology and substrate specificity (Mannervik et al., 1992). *GSTM1* and *GSTT1* are extensively studied among all. The polymorphisms of *GSTM1* and *GSTT1* gene loci is caused by a gene-deletion which results in the absence of enzyme activity in individuals with the *GSTM1* and *GSTT1* homozygous null genotypes. The polymorphisms of *GSTM1* and *GSTT1* have been associated with cancers of the lung, bladder, breast and colon (Anantharaman et al., 2007). A large number of epidemiological studies have investigated the association between *GSTM1* and *GSTT1* and risk of cervical cancer in different populations (Song et al., 2006; Singh et al., 2008; Agodi et al., 2010; Palma et al., 2010). Yet there is still not any conclusive data concerning the effect of these factors in cervical carcinogenesis. The purpose of this study was to investigate the prevalence of *GSTM1* and *GTT1* homozygous null polymorphism in women with normal, precancerous and cancerous lesions of uterine cervix. It was attempted further to find out whether *GSTM1* and *GSTT1* polymorphisms could influence the risk of developing cervical cancer in these women either independently or in combination with other risk factors like smoking and HPV infection.

Materials and Methods

Sample collection

A total of 482 women with minor gynecologic complaints attending the Gynaecology OPD of Lok Nayak Hospital, New Delhi, were screened cytologically under a programme to study the biological behaviour of HPV infection of the cervix. Biopsies from 135 cases of carcinoma of the cervix were collected from the cancer clinic of Lok Nayak Hospital, New Delhi. Institutional review and ethical committee approved the study and written consent was obtained from all the subjects. Epidemiological data relating to age, history of smoking, etc. was obtained from all women and was recorded.

While these women underwent routine Pap test for cytological evaluation, a sample of cervical epithelial cells was also obtained at the same time in a container with phosphate-buffered saline for molecular studies. The pap smears were reported, according to the Bethesda 2001 system (Solomon et al., 2002). After cytological evaluation and review of medical records, 25 (5.2%) women with abnormal cytology [cases of Atypical Squamous cells (ASC), Low grade Squamous intraepithelial lesions (LSIL), High grade Squamous intraepithelial lesions (HSIL)] in their Pap smears were categorized in 'precancerous group' and the remaining 457 (94.8%) women with normal cytology and no previous abnormality in their records were categorized in 'normal group'. All patients, in the cancer group, were diagnosed as Cervical Squamous Cell Carcinoma on histopathology. Their age ranged from 20-56 yrs. The association of cervical cancer with *GSTM1* and *GSTT1* homozygous null genotype alone and in combination with risk factors like age, smoking and HPV infection was evaluated in these women.

Determination of HPV infection and genotyping of GSTM1 and GSTT1

DNA was extracted from the scrape samples and biopsies by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. HPV DNA was detected by enzymatic amplification of DNA by standard polymerase chain reaction (PCR) protocol (Arora et al., 2005) using an L1 consensus primer pair MY09 and MY11, which promotes amplification of an approximately 450 base pair product and can detect more than 40 distinct low and high risk genital HPV types. β globin gene primers were used as internal controls.

The *GSTM1* and *GSTT1* genotypes were determined by a multiplex PCR using three sets of primers for *GSTM1*, *GSTT1*, and the albumin gene (internal control) (Arand et al., 1996) with slight modifications described earlier (Sharma et al., 2012). The products of multiplex PCR were separated by electrophoresis with ethidium bromide stained 3% agarose gel. Presence of amplicons of 218bp, 460bp, and 350bp revealed the presence of *GSTM1*, *GSTT1*, and Albumin (internal control) respectively.

Statistical analysis

The data were tabulated and analyzed. The mean±S.D. were estimated for quantitative data. In order to test the significance in the proportion of *GSTM1*and *GSTT1* null genotypes in women with normal, precancerous and cancerous lesions of uterine cervix, chi square test of significance and Fisher's exact test were employed. The probability value of <0.05 was considered for statistical significance. Odds ratio (OR) and 95% confidence interval (95%CI) were also estimated.

Results

Demographic Data

The study group consisted of 482 women who underwent routine Pap smear examination and 135 cervical cancer cases. The description of cases, controls and other variables is given in Table 1. We conducted GST genotype analysis together with HPV status in the all above stated individuals. The mean age of women with normal cervical cytology, precancerous and cancerous lesions of uterine cervix was 41.1±8.9, 40.7±9.5 and 42.1±11.7 respectively. 272 (59.5%) women with normal cervical cytology were in ≤35 years of age and 185 (40.5%) were in >35 years of age group. As the number of women above 50 years was less, it was decided to compare the results in age groups ≤35 years and > 35 years of age. There were 14 (56.0%) women with precancerous lesions in ≤ 35 years age group and 11 (44.0%) in >35 years age group. In cervical cancer

Table 1. Various Risk factors in Women with Normal, Precancerous and Cervical Cancer Lesions

	Age					Smoking				
	In years									
Cervical cytology (N)	≤35	>35	P	OR	95% CI	Smokers	Non-smokers	P	OR	95% CI
Normal[a] (N)	272	185	Reference			39	418			
%	59.5	40.5				8.5	91.5			
Precancerous[b] (N)	14	11	0.73	1.56	0.51-2.60	4	21	0.21	2.04	0.67-6.25
%	56.0	44.0				16.0	84.0			
Cervical cancer[c] (N)	74	61	0.33	0.83	0.56-1.21	22	113	0.01*	2.09	1.19-3.67
%	54.8	45.2				16.3	83.7			
	HPV					HPV and Smoking				
Cervical cytology (N)	Positive	Negative	P	OR	95% CI	HPV +ve & smokers	HPV +ve & Non smokers	P	OR	95%CI
Normal[a] (N)	91	366				15	76			
%	19.9	80.1				16.5	83.5			
Precancerous[b] (N)	6	19	0.62	1.27	0.49-3.27	3	3	0.06	5.07	0.93-27.55
%	24.0	76.0				50.0	50.0			
Cervical cancer[c] (N)	89	46	<0.0001*	7.78	5.09-11.89	17	72	0.65	1.20	0.56-2.57
%	65.9	34.1				19.1	80.9			

*significance <0.05, Frequencies of [a] compared to [b] and [c]

Table 2. Association of GSTM1,GSTT1 and GSTM1T1 Null Genotypes among Women With Normal, Precancerous and Cervical Cancer Lesions

Cervical cytology	N	GSTM1 null	GSTM1 non null	p	OR	CI (95%)
Normal [a] (N)	457	160	297			Reference
%		35.01	64.99			
Precancerous [b] (N)	25	10	15	0.67	1.24	0.54-2.8
%		40.0	60.0			
Cervical cancer[c] (N)	135	79	56	<0.0001*	2.62	1.77-3.88
%		58.5	41.5			
Cervical cytology	N	GSTT1 null	GSTT1 non null	p	OR	CI (95%)
Normal [a] (N)	457	65	392			Reference
%		14.2	85.8			
Precancerous [b] (N)	25	4	21	0.77	1.15	0.38-3.45
%		16.0	84.0			
Cervical cancer[c] (N)	135	26	109	0.16	1.44	0.87-2.38
%		19.3	80.7			
Cervical cytology	N	GST M1T1-	GST M1T1+	p	OR	CI (95%)
Normal [a] (N)	457	53	404			Reference
%		11.6	88.4			
Precancerous [b] (N)	25	2	23	0.75	0.66	0.15-2.89
%		8.0	92.0			
Cervical cancer[c] (N)	135	23	112	0.11	1.56	0.92-2.67
%		17.0	83.0			

**Significance <0.05, Frequencies of [a] compared to [b] and [c]

cases, 74 (54.8%) women were in ≤35 years of age group where as 61 (45.2%) were in >35 years age group.

The number of smokers in women with normal, precancerous and cancerous lesions was 39(8.5%), 4 (16.0%), and 22 (16.3%) respectively. HPV infection was observed in 91/457 (19.9%) women with normal cytology, 6/25 (24.0%) in precancerous and 89/135 (65.9%) in cervical cancer cases. Of 91 women with HPV infection in the control group, 15 (16.5%) were smokers and 76 (83.5%) were non-smokers. 3 (50.0%) women in precancerous lesions group and 17 (19.1%) in the cervical cancer cases were smokers as well as HPV infected. The

Table 3. Association of GSTM1, GSTT1, GSTM1T1 Homozygous Null Genotypes According to Smoking Status among Women with Normal, Precancerous and Cervical Cancer Lesions

Cervical cytology	N		N	GSTM1 null	GSTM1non null	p	OR	CI (95%)
Normal (N)	457	Smokers	39	15	24	0.64	1.18	0.59-2.31
%				38.5	61.5			
(N)		Non-Smokers	418	145	273			
%				34.7	65.3			
Precancerous (N)	25	Smokers	4	2	2	0.66	1.62	0.19-13.93
%				50.0	50.0			
(N)		Non-Smokers	21	8	13			
%				38.1	61.9			
Cervical cancer (N)	135	Smokers	22	13	9	0.95	1.03	0.40-2.60
%				59.1	40.9			
(N)		Non-Smokers	113	66	47			
%				58.4	41.6			
Cervical cytology	**N**		**N**	**GSTT1null**	**GSTT1 non null**	**p**	**OR**	**CI (95%)**
Normal (N)	457	Smokers	39	9	30	0.10	1.94	0.87-4.30
%				23.1	76.9			
(N)		Non-Smokers	418	56	362			
%				13.4	86.6			
Precancerous (N)	25	Smokers	4	1	3	0.59	2.0	0.15-26.18
%				25.0	75.0			
(N)		Non-Smokers	21	3	18			
%				14.3	85.7			
Cervical cancer (N)	135	Smokers	22	8	14	0.03*	3.01	1.10-8.23
%				36.4	63.6			
(N)		Non-Smokers	113	18	95			
%				15.9	84.1			
Cervical cytology	**N**		**N**	**GST M1T1_**	**GST M1T1+**	**p**	**OR**	**CI (95%)**
Normal (N)	457	Smokers	39	6	33	0.44	1.43	0.57-3.60
%				15.4	84.6			
(N)		Non-Smokers	418	47	371			
%				11.2	88.8			
Precancerous (N)	25	Smokers	4	1	3	0.22	6.67	0.32-137.41
%				25.0	75.0			
(N)		Non-Smokers	21	1	20			
%				4.8	95.2			
Cervical cancer (N)	135	Smokers	22	5	17	0.44	1.55	0.51-4.74
%				22.7	77.3			
(N)		Non-Smokers	113	18	95			
%				15.9	84.1			

*Significance <0.05

risk of cervical cancer was found to be twice in smokers and almost eight times in HPV positive individuals (OR-2.09, 95%CI, 1.19-3.67; p=0.01 and OR-7.78, 95%CI, 5.09-11.89; p<0.0001) respectively (Table 1).

Risk estimation between GST null genotypes and cervical cancer

Table 2 shows the genotype distribution of *GSTM1* and *GSTT1* and *GSTM1T1* in women with normal,

Table 4. Association of GSTM1 and GSTT1 Homozygous Null Genotypes in HPV Positive Smoker Women with Normal, Precancerous and Cervical Cancer Lesions

Cervical cytology	Total No	HPV +Smoking status		N	GSTM1 null(%)	GSTM1 non null(%)	p	OR	CI(95%)
Normal	457	HPV Positive + smokers	(No)	15	6	9	0.89	1.08	0.35-3.35
			%	3.28	40.0	60.0			
		HPV positive + Non smokers	(No)	76	29	47			
			%	16.6	38.2	61.8			
Precancerous	25	HPV Positive + smokers	(No)	3	1	2	1.0	1.0	0.03-29.81
			%	12.0	33.3	66.7			
		HPV positive + Non smokers	(No)	3	1	2			
			%	12.0	33.3	66.7			
Cervical cancer	135	HPV Positive + smokers	(No)	17	11	6	0.86	1.1	0.36-3.31
			%	12.6	64.7	35.3			
		HPV positive + Non smokers	(No)	72	45	27			
			%	53.3	62.5	37.5			
Cervical cytology	Total No	HPV +Smoking status		N	GSTT1 null(%)	GSTT1 non-null(%)	p	OR	CI(95%)
Normal	457	HPV Positive + smokers	(No)	15	2	13	0.81	0.82	0.16-4.11
			%	3.28	13.3	86.7			
		HPV positive + Non smokers	(No)	76	12	64			
			%	16.6	15.8	84.2			
Precancerous	25	HPV Positive + smokers	(No)	3	1	2	0.43	4.2	0.12-151.98
			%	12.0	33.3	66.7			
		HPV positive + Non smokers	(No)	3	0	3			
			%	12.0	0	100			
Cervical cancer	135	HPV Positive + smokers	(No)	17	6	11	0.02*	4.36	1.27-15.03
			%	12.6	35.3	64.7			
		HPV positive + Non smokers	(No)	72	8	64			
			%	53.3	11.1	88.9			
Cervical cytology	Total No	HPV +Smoking status		N	GST M1T1_	GST M1T1+	p	OR	CI(95%)
Normal	457	HPV Positive + smokers	(No)	15	2	13	0.75	1.31	0.25-6.87
			%	3.28	13.3	86.7			
		HPV positive + Non smokers	(No)	76	8	68			
			%	16.6	10.5	89.5			
Precancerous	25	HPV Positive + smokers	(No)	3	1	2	0.43	4.2	0.12-151.98
			%	12.0	33.3	66.7			
		HPV positive + Non smokers	(No)	3	0	3			
			%	12.0	0	100			
Cervical cancer	135	HPV Positive + smokers	(No)	17	5	12	0.04*	3.87	1.05-14.23
			%	12.6	29.4	70.6			
		HPV positive + Non smokers	(No)	72	7	65			
			%	53.3	9.7	90.3			

*Significance <0.05

precancerous lesions and cervical cancer. The frequency of *GSTM1* homozygous null genotype was 79 (58.5%) in cervical cancer cases, 10 (40.0%) in women with precancerous lesions as compared to 160 (35.01%) in women with normal cytology. We observed that there was a significant difference (p<0.0001) in *GSTM1* homozygous null genotype among the cervical cancer cases with an odds ratio of 2.62 (95% CI, 1.77-3.88) but not in women with precancerous lesions (OR-1.24, 95% CI, 0.54-2.8; p=0.67) as compared with normal cytology. No significant association of *GSTT1* and *GSTM1T1* homozygous null genotypes was observed among women with cervical cancer (p=0.16; 0.11) and precancerous lesions (p=0.77; 0.75) respectively as compared to normal cytology (Table 2).

Comparison of *GSTM1*, *GSTT1* and *GSTM1T1* homozygous null genotypes was done in ≤35 years and >35 years of age group in the women with normal cytology, precancerous lesions and cervical cancer cases. No association of *GSTM1*, *GSTT1* or *GSTM1T1* homozygous null genotypes with age was observed in the above stated groups (Table not shown).

The distribution of *GSTM1*, *GSTT1* and *GSTM1T1* homozygous null genotypes in all the three groups has been presented according to smoking habits in Table 3. Considering smoking habits, significant association (p=0.03) was observed only in *GSTT1* homozygous null cervical cancer smokers with a prevalence of 36.4% (8/22) as compared to non-smokers with 15.9% (18/113). A threefold increased risk was observed in smoker women with *GSTT1* genotype (OR-3.01, 95% CI, 1.10-8.23), but not with *GSTM1* and *GSTM1T1* homozygous null genotypes (Table 3). In other two groups smokers with *GSTM1*, *GSTT1*, or *GSTM1T1* homozygous null genotypes were not found to be at increased risk in women with normal (p=0.64; p=0.10; p=0.44 respectively) and precancerous lesions (p=0.66; p=0.59; p=0.22 respectively) as compared to non-smokers (Table 3).

HPV positive women with *GSTM1*, *GSTT1* or *GSTM1T1* homozygous null genotypes were not found to

Table 5. Frequency of GSTM1 Homozygous Null Genotypes in Cervical Cancer Cases Worldwide

Name	Year	Country/Population	No. of Cases/ Controls	No. of GSTM1 null Cases/ Controls	OR, CI (95%)	P value
Agorastose et al	2007	Greece /Caucasian	176/114	33/60	0.21 (0.12-0.35)	<0.0001*
Agodi et al	2010	Italy/Caucasian	27/162	15/17	10.66 (4.29-26.49)	<0.0001*
Palma et al	2010	Italy/Caucasian	25/111	15/58	1.37 (0.57-3.31)	0.48
Kiran B et al	2010	Turkey/Caucasian	46/52	25/30	0.87 (0.39-1.94)	0.74
De Carvalho et al	2008	Brazil/latino	43/86	28/49	1.41 (0.66-3.01)	0.37
Warwick et al	1994	Caucasian	77/190	40/94	1.10 (0.65-1.87)	0.71
Chen et al	1999	USA/Caucasian	190/206	101/118	0.85 (0.57-1.26)	0.41
Goodman et al	2001	America	131/180	74/98	1.09 (0.69-1.71)	0.72
Sierra-Torres et al	2003	America	69/72	35/29	1.53 (0.78-2.97)	0.21
Sierra-Torres et al	2006	America	91/92	36/38	0.93 (0.51-1.68)	0.81
Ivana Stosic et al	2014	Serbia	32/50	22/28	1.73 (0.68-4.39)	0.25
Huang et al	2006	China/Asian	47/78	30/32	2.54 (1.20-5.35)	0.01*
Song et al	2006	China/Asian	130/130	77/57	1.86 (1.14-3.04)	0.01*
Zhou et al	2006	China/Asian	125/125	73/54	1.84 (1.12-3.05)	0.02*
Liu et al	2009	China/Asian	62/45	40/13	4.48 (1.95-10.25)	0.0004*
Ma CL et al	2009	China/Asian	43/45	29/15	4.14 (1.70-10.08)	0.002*
Kim et al	2000	Korea/Asian	181/181	95/96	0.98 (0.65-1.48)	0.92
Lee SA et al	2004	Korea/Asian	81/86	42/42	1.13 (0.61-2.07)	0.7
Niwa et al	2005	Japan/Asian	131/320	70/184	0.85 (0.56-1.28)	0.43
Nishino et al	2008	Japan/Asian	124/125	77/59	1.83 (1.11-3.04)	0.02*
Ueda et al [38]	2008	Japan/Asian	144/54	75/28	1.01 (0.54-1.89)	0.98
Setheetham-Ishida et al	2009	Thailand/Asian	90/94	54/56	1.02 (0.56-1.84)	0.39
Sharma et al	2004	India/Asian	142/96	81/33	2.53 (1.48-4.33)	0.0007*
Joseph et al	2006	India/Asian	147/165	79/54	2.39 (1.51-3.78)	0.0002*
Sobti et al	2006	India/Asian	103/103	42/38	1.18 (0.67-2.06)	0.57
Singh et al	2008	India/Asian	150/168	64/46	1.97 (1.23-3.15)	0.004*

*Significant

Table 6. Frequency of GSTT1 Homozygous Null Genotypes in Cervical Cancer Cases Worldwide

Name	Year	Country/Population	No. of Cases/ Controls	No. of GSTT1 Null Cases/ Controls	OR, CI (95%)	P value
Warwick et al	1994	Caucasian	70/168	9/27	0.77(0.34-1.74)	0.53
Palma et al	2010	Italy/Caucasian	25/111	8/22	1.90(0.73-4.98)	0.19
Kiran B et al	2010	Turkey/Caucasian	46/52	15/16	1.09(0.46-2.55)	0.84
De Carvalho et al	2008	Brazil/latino	43/86	22/16	4.58(2.04-10.28)	0.0002*
Ivana Stosic et al	2014	Serbia	32/50	12/20	0.90(0.36-2.24)	0.82
Zhou et al	2006	China/Asian	125/125	67/55	1.47(0.89-2.42)	0.13
Kim et al	2000	Korea/Asian	181/181	120/92	1.90(1.24-2.91)	0.003*
Lee SA et al	2004	Korea/Asian	81/86	38/54	0.52(0.28-0.97)	0.04*
Niwa et al	2005	Japan/Asian	131/320	63/145	1.12(0.74-1.68)	0.59
Setheetham-Ishida et al	2009	Thailand/Asian	90/94	42/38	1.29(0.72-2.31)	0.39
Sharma et al	2004	India/Asian	142/96	28/12	1.72(0.83-3.58)	0.15
Joseph et al	2006	India/Asian	147/165	24/16	1.82(0.92-3.57)	0.08
Sobti et al	2006	India/Asian	103/103	16/26	0.54(0.27-1.09)	0.09
Singh et al	2008	India/Asian	150/168	40/18	3.03(1.65-5.57)	0.0004*

*Significant

Table 7. Frequency of *GSTM1T1* null genotypes in cervical cancer cases worldwide

Name	Year	Country/Population	No. of Cases/ Controls	No. of GSTM1T1 Null Cases/Controls	OR, CI (95%)	P value
Ivana Stosic et al	2014	Serbia	32/50	11/9	1.49(0.43-5.19)	0.53
Zhou et al	2006	China/Asian	125/125	39/27	1.65(0.93-2.91)	0.87
Kim et al	2000	Korea/Asian	181/181	62/48	1.44(0.92-2.27)	0.11
Setheetham-Ishida et al	2009	Thailand/Asian	90/94	26/18	1.72(0.86-3.41)	0.12
Sharma et al	2004	India/Asian	142/96	27/11	1.81(0.85-3.86)	0.12
Sobti et al	2006	India/Asian	103/103	8/9	0.88(0.32-2.38)	0.8
Singh et al	2008	India/Asian	150/168	23/2	15.03(3.48-64.94)	0.0003*

*Significant

be at increased risk in normal, precancerous and cervical cancer cases (Table not shown). However, a significant association in cervical cancer cases was observed in HPV positive smokers with *GSTT1* (p=0.02) and *GSTM1T1* (p=0.04) null genotypes when compared with HPV positive non-smokers. These results showed almost four fold increased risk in HPV positive smokers with *GSTT1* (OR-4.36, 95% CI, 1.27-15.03) and *GSTM1T1* null genotypes (OR-3.87, 95% CI, 1.05-14.23) (Table 4).

Discussion

The present study was planned to generate data on prevalence of GST polymorphism in routine cervical scrapes, and cervical cancer biopsies. The study was further extended to investigate whether GST polymorphisms could influence the risk to develop cervical cancer, individually or in combination with smoking habit and/or HPV infection in these women.

A significant association of *GSTM1* homozygous null genotype with cervical cancer cases as compared to those with normal cervical cytology was observed in the present study. We have observed that individuals carrying *GSTM1* homozygous null genotype were approximately thrice at risk of having cervical cancer as compared to *GSTM1* non-null individuals (Table 2). When these results were compared with studies reported worldwide it was observed that *GSTM1* homozygous null genotype is associated with the development of cervical cancer, especially in Chinese (Huang et al., 2006; Song et al., 2006; Zhou et al., 2006; Liu et al., 2009; Ma et al., 2009) and Indian population (Sharma et al., 2004; Joseph et al., 2006; Singh et al., 2008), whereas only two studies from Caucasian population (Agorastos et al., 2007; Agodi et al., 2010) reported an association of *GSTM1* homozygous null genotype with cervical cancer (Table 5). No relationship between *GSTM1* homozygous null genotype and cervical cancer risk was reported in studies of the Hawaiian population (Au, 2004), Caucasian populations (Warwick et al., 1994; Chen et al., 1999; Goodman et al., 2001; Sierra-

Torres et al., 2003; Sierra-Torres et al., 2006; de Carvalho et al., 2008; Beray et al., 2010; Palma et al., 2010; Stosic et al., 2014) and in Asian populations (Kim et al., 2000; Lee et al., 2004; Niwa et al., 2005; Sobti et al., 2006; Ueda et al., 2008; Settheetham-Ishida et al., 2009) (Table 5). Meta-analyses performed recently, have also indicated a significant association of *GSTM1* null genotypes with cervical cancer (Economopoulos et al., 2010; Gao et al., 2011; Wang et al., 2011; Zhang et al., 2012).

In the present study, no risk of cervical cancer was observed in *GSTT1* homozygous null genotype (Table 2). Similar type of observations were also reported in other studies (Warwick et al., 1994; Sharma et al., 2004; Joseph et al., 2006; Niwa et al., 2005; Sobti et al., 2006; Zhou et al., 2006; Beray et al., 2010; Palma et al., 2010; Stosic et al., 2014) (Table 6), along with two meta analyses (Economopoulos et al., 2010; Wang et al., 2011). Whereas a few studies have reported a significant correlation between *GSTT1* null genotypes and cervical cancer (Kim et al., 2000; Lee et al., 2004; de Carvalho et al., 2008; Singh et al., 2008) (Table 6) and in a meta-analysis study (Gao et al., 2011).

An interaction between the complete deletion of both *GSTM1* and *GSTT1* homozygous null genotypes and cervical cancer has been reported only in a few studies. Significant association with cervical cancer was reported in a study from India (Singh et al., 2008) and in two meta analyses (Gao et al., 2011; Wang et al., 2011), whereas other studies failed to find such correlation (Kim et al., 2000; Sharma et al., 2004; Joseph et al., 2006; Sobti et al., 2006; Zhou et al., 2006; Settheetham-Ishida et al., 2009; Stosic et al., 2014) (Table 7). Like other studies, in the present study, individuals with null homozygous genotypes of *GSTM1T1* were not found to be at risk of cervical cancer. Cseh et al. (2011) have reported an increased risk for cervical precancerous lesions in *GSTM1*, *GSTT1*, and *GSTM1T1* homozygous null genotypes.

A few studies have been done to know the association of age with *GSTM1* and *GSTT1* homozygous null genotypes in cervical cancer. A significant difference was found by Sharma et al. (2004) between the cervical cancer cases and controls in the distribution of the null genotype of *GSTM1* in individuals aged above 45 years (p=0.04) but this difference was not significant in individuals aged under 45 years (p=0.06). Stosic et al (Stosic et al., 2014) also reported that the risk of cervical lesions might be significantly related to the *GSTM1* homozygous null genotype, especially in women aged above 45 yrs. The numbers of individuals in >50 years of age group were less, so in the present study the comparison was done in ≤ 35 and >35 years of age. We have not observed any association between *GSTM1*, *GSTT1* or *GSTM1T1* homozygous null genotypes and ≤35years or >35years of age groups in any group.

Known risk factors for the development of cervical cancer are smoking and HPV infection. Cigarette smoking has been found to be an independent risk factor of cervical cancer (Giuliano et al., 2002). The tobacco smoke constituents are modified by metabolizing enzymes and may promote malignant cellular growth (Prokopczyk et al., 1997). Some of the tobacco related carcinogens are substrates for GSTs; therefore, it is important to examine the tobacco/gene interaction as a modulating factor. It was hypothesized that the lack of GST activities by an inherited deletion of the GST and smoking status may synergistically influence the cancer development. GST null genotypes have been reported to have an increased risk of several tobacco-related cancers (Kietthubthew et al., 2001; Spurdle et al., 2001; Lee et al., 2002; Sweeney et al., 2003; van der et al., 2003). A significant increase in the risk of developing high grade squamous intraepithelial lesions in smokers was reported by Ueda et al. (2005), whereas others reported a significant association between cervical cancer and passive smoking but not active smoking (Trimble et al., 2005). Abbas et al. (2013) also reported that passive smokers with *GSTM1* null and *GSTP1* (G/G) genotypes have an increased risk for cervical cancer.

In the present study, the number of smokers was very less. *GSTT1* homozygous null genotypes among smokers showed a significant increase in the risk of cervical cancer as compared to non-smokers, but in remaining two groups, smoking does not seem to play any role in individuals with *GSTM1*, *GSTT1* or *GSTM1T1* homozygous null genotypes (Table 4). A few studies have reported elevated risk of cervical cancer development in smoker women with *GSTM1* null, *GSTT1* homozygous null and *GSTP1* (Ile 105Val) genotype (Jee et al., 2002; Sobti et al., 2006). In two Meta analysis studies, Zhang et al. (2012) and Zhen et al. (2013) have reported that *GSTM1* homozygous null genotype is associated with cervical cancer and stated further that smoking and HPV infection may modify the association between the *GSTM1* homozygous null genotypes and cervical cancer. The majority of studies cited in literature, however, did not find any interaction between smoking habits and the polymorphisms of *GSTM1* or *GSTT1* genes (Goodman et al., 2001; Agorastos et al., 2007; Nishino et al., 2008; Singh et al., 2008; Settheetham-Ishida et al., 2009; Palma et al., 2010; Ben Salah et al., 2012) and *GSTP1* (Beray et al., 2010). These variations found in different populations may be due to different lifestyles, diet, environmental factors and other genetic factors (LiuXu, 2012), as well as more efficient metabolic and detoxifying activity of tobacco compounds in some individuals than in others (Palma et al., 2010).

Though HPV infection has been documented as a major risk factor for cervical cancer development worldwide, we have not observed any risk of cervical cancer in HPV positive women with *GSTM1*, *GSTT1* or *GSTM1T1* homozygous null genotypes. However, we have observed that *GSTT1* & *GSTM1T1* homozygous null genotypes have almost four fold increased risk of cervical cancer among HPV positive smoker women. Chen & Nirunsuksiri, (Chen and Nirunsuksiri 1999) also found no correlation between *GSTM1*, *GSTT1* or *GSTM1T1* null genotypes and risk of HPV infection, although *GSTM1* seems to be down regulated in HPV-transfected cells. Evidence from other studies suggests that inherited susceptibility in the form of GST genotype may modulate the risk of developing HPV related cancer as evidenced by *GSTM1* homozygous null genotype, which, in addition to HPV infection and smoking, has been

found to increase the risk of developing cervical cancer (Sierra-Torres et al., 2003). Moreover, in the patients with cervical cancers having a positive association with GST homozygous null genotype, as studied by Kim et al. (2000), HPV 16or 18 infections were confirmed in all. The *GSTM1* null genotype was found to be significantly associated with an increased risk of cervical cancer in Korean women with HR-HPV infection (Lee et al., 2004) and in HPV infected smokers (Au et al., 2003; Sierra-Torres et al., 2003). Kim et al. (2000) reported that the risk of developing cervical cancer before 40 is high ($p<0.05$) among patients who carried HPV. A significant increased risk for developing early stage cervical lesion associated with *GSTM1* homozygous null genotype but not with *GSTT1* homozygous null genotypes was also observed in Chinese and Indian women (Joseph et al., 2006; LiuXu 2012; Zhang et al., 2012) but no risk in Japanese, European and American populations. These differences showed variations in cancer susceptibility by ethnicities.

Conclusion: The results demonstrate that the *GSTM1* null genotype is associated with the development of cervical cancer, but smoking modified the association between *GSTT1* null genotype and cervical cancer.

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