RESEARCH ARTICLE

Genetic Polymorphism of Glutathione S-transferases M1 and T1, Tobacco Habits and Risk of Stomach Cancer in Mizoram, India

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Abstract

Aim: The incidence of stomach cancer in Mizoram is highest in India. We have conducted a population based matched case-control study to identify environmental and genetic risk factors in this geographical area. Methods: A total of 102 histologically confirmed stomach cancer cases and 204 matched healthy population controls were recruited. *GSTM1* and *GSTT1* genotypes were determined by PCR and *H. pylori* infections were determined by ELISA. Results: Tobacco-smoking was found to be an important risk factor for high incidence of stomach cancer in Mizoram. Meiziol (local cigarette) smoking was a more important risk factor than other tobacco related habits. Cigarette, tuibur (tobacco smoke infused water) and betel nut consumption synergistically increased the risk of stomach cancer. Polymorphisms of *GSTM1* and *GSTT1* genes were not found to be directly associated with stomach cancer in Mizoram. However, they appeared to be effect modifiers. Persons habituated with tobacco smoking and/or tuibur habit had increased risk of stomach cancer if they carried the *GSTM1* null genotype and *GSTT1* non-null genotype. Conclusion: Tobacco smoking, especially meiziol is the important risk factor for stomach cancer in Mizoram. *GSTM1* and *GSTT1* genes modify the effect of tobacco habits. This study is a first step in understanding the epidemiology of stomach cancer in Mizoram, India.

Keywords: Stomach cancer - genetic polymorphism - *GSTM1 & GSTT1* - tobacco habits - Mizoram - India

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Introduction

Stomach cancer is the second leading cause of cancer death and forth most common malignancy in the world which accounts for 9.7% of total cancer deaths (Ferlay et al., 2010). In India, the incidence of stomach cancer is highest in the Aizawl district of the state of Mizoram with an age-adjusted rate (AAR) of 55.4 in male and 24.4 in female per 10^5 populations (NCRP, 2010). The age-adjusted rate (AAR) of stomach cancer for the entire state of Mizoram are 42.9 and 20.5 per 10^5 populations in male and female, respectively (NCRP, 2010). This incidence is quite high as compared to other regions of India where the incidence of stomach cancer per 10^5 varies from 1.0-12.3 in males and 0.6-11.0 in females.

There are considerable regional differences in the incidence of stomach cancer worldwide and the highest incidence among men is reported from Changle, China (AAR=145.0) and among women in Yamagata, Japan (AAR=38.9) per 10^5 populations (Parkin et al., 2002). Numerous studies have shown that consumption of alcohol, tobacco and different food habits are important risk factors of stomach cancer in addition to *Helicobacter pylori* infections (Correa, 1992; Russo et al., 2001; García-G et al., 2012). Earlier studies have shown that tobacco related habits are important risk factors for stomach cancer in Mizoram (Mahanta et al., 1998; Phukan et al., 2006). It was also shown that intake of tobacco in various traditional forms increases the risk of stomach cancer in Mizoram (Phukan et al., 2005).

Glutathione-s-transferases (GSTs), a supergene family of detoxification enzymes, provide protection against genotoxic and carcinogenic effects of numerous substances of both xenobiotic and endogenous origins (Pemble et al., 1994). The *GSTM1* gene is classified into the *mu* class and the *GSTT1* gene belongs to the *theta* class. Genes coding for *GSTM1* and *GSTT1* proteins are polymorphic in humans and *GSTM1* is absent in 35-60% of individuals (Bell et al., 1993). Similarly, *GSTT1* is absent in 10-65% of human populations (Chenevix-T et al., 1995). The phenotypic absence of *GSTM1* and *GSTT1* activity is due to homozygocity for an inherited deletion of these genes, termed the null genotype (Pemble et al., 1994). Individuals with the homozygous *GSTM1* null genotype

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express no protein and expected to have reduced abilities to detoxification of hazardous compounds, particularly epoxides (Cai et al., 2001). Many epidemiological studies have reported differently about the association of *GSTM1* and *GSTT1* gene polymorphism with stomach cancer (Katoh et al., 1996; Setiawan et al., 2000; Cai et al., 2001; Tripathi et al., 2011; Zang et al., 2011; García-G et al., 2012). The present study was undertaken to examine the association and interaction of *GSTM1* and *GSTT1* gene polymorphism and tobacco consumption with stomach cancer for the first time in this high risk state of Mizoram in the north eastern part of India.

Materials and Methods

This study was a population based matched case-control study carried out from March, 2009-2012. All cases and controls were ethnic Mizos of the Mizoram state. All cases (n=102) were newly diagnosed and histopathologically confirmed stomach cancer patients from the Aizawl civil hospital and other private clinics of Aizawl, Mizoram. The patients with severe clinical symptoms, patients with recurrent cancer or too old to be interviewed elaborately and who refused to be interviewed were excluded from this study. Two age (±5 years) and sex-matched population based healthy neighborhood controls (n=204) were selected for each case. Information on socio-demographic and other risk factors like consumption pattern of tobacco intake, alcohol drinking, betel nut chewing was collected from cases and controls by face-to-face interviews and information was recorded in a pre-designed questionnaire.

5 to 10 ml of peripheral whole blood was collected from each of the study subject in EDTA-containing vials and stored at -80^{0}C until analyzed.

The institutional ethical committee of the Regional Medical Research Centre, Dibrugarh approved this study. All participants were given an explanation of the nature of the study and informed and written consent were obtained from all cases and controls.

DNA extraction and PCR

Extraction and purification of high-molecular-weight genomic DNA was carried out with Quiagen DNeasy$^{(R)}$ Blood and Tissue Kit. The PCR reactions were performed by using two previously described methods (Wiencke et al., 1995; Cai et al., 2001) with slight modifications. A reaction mixture of 50 μL volume was prepared containing Promega 2X master mix (GoTaq DNA polymerase, 2X GoTaq Reaction Buffer, pH 8.5, 400 μmol.L^{-1} of each dNTP and 3 mM $MgCl_2$), 0.14 μmol.L^{-1} of each primer, 200 ng of template DNA and 0.1 mgml^{-1} of BSA. Primer sequences for *GSTM1* were 5'-GCTTCACGTGTTATGGAGGTTC-3' and 5'-GAGATGAAGTCCTCCAGATTT-3' which produced a 157 base-pair band. For *GSTT1* the primers used were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3' which produced a 480 base-pair band. β-globin gene was used as a positive internal control in all the PCR reactions. The primers used for β-globin gene amplification were 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'. The PCR amplification was carried out in Applied Biosystems Thermal Cycler. The PCR cycles for *GSTM1* and β-globin were 94°C for 8 min, followed by 35 cycles of 94°C for 30s, 60°C for 40s and 72°C for 1 min with a final extension at 72°C for 10 min. PCR cycles for *GSTT1* were 94°C for 8 min, followed by 35 cycles of 94°C for 30s, 62°C for 40s and 72°C for 1 min with a final extension at 72°C for 10 min. The PCR products were run in 2-2.5% of agarose gel, stained in ethidium bromide and documented in Gel-Doc.

Detection of Helicobacter pylori infection

Indirect *IgG* ELISA (BIO-RAD) was used to detect antibodies against *Helicobacter pylori* in serum of both cases and controls.

Statistical analysis

Univariate and multiple logistic regressions were used for data analysis. The conditional maximum likelihood method (Breslow et al., 1980) was used to estimate the parameters of the regression model because of the matched study design and significance was taken at $p \leq 0.05$ (two tailed). Initially a univariate analysis was carried out. The crude measure of association between single putative risk factor and stomach cancer was expressed as odds ratio (OR) and its 95% confidence interval (95%CI) was calculated from the standard error (SE) of the regression

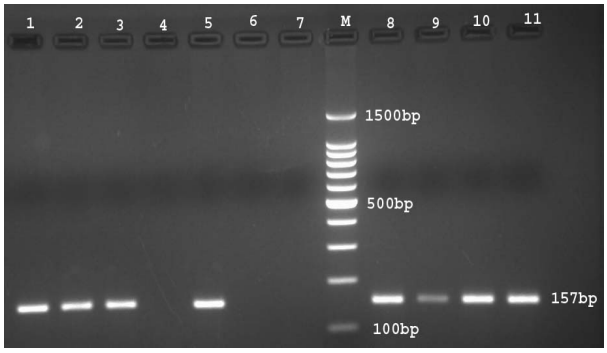

Figure 1. Agarose Gel Stained with Ethidium Bromide Showing *GSTM1* Polymorphism. Lane M=100 bp DNA ladder; Lane 4,6,7=*GSTM1* null genotype; Lane 1,2,3,5,8,9,10,11=*GSTM1* non-null genotype with 157 bp band.

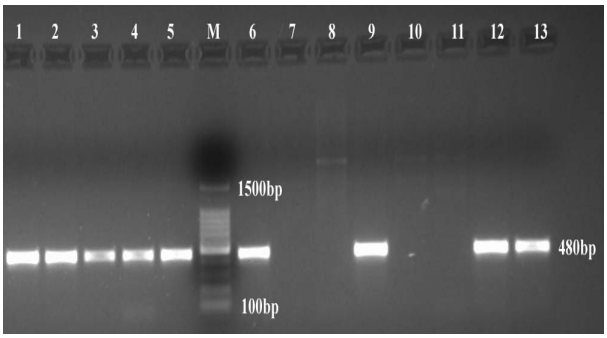

Figure 2. Agarose Gel Stained with Ethidium Bromide Showing *GSTT1* Polymorphism. Lane M=100 bp DNA ladder; Lane 7,8,10,11=*GSTT1* null genotype; Lane 1,2,3,4,5,6,9,12,13=*GSTT1* non-null genotype with 480 bp band.

co-efficient. To control the confounding variable and other co-variates such as smoking, alcohol drinking, level of education taken, occupation, income etc, the data were analyzed by conditional multiple logistic regression to evaluate the extent of risk association. The statistical package used for the analysis were SPSS version 17.0 and Epi-Info version 3.5.4.

Results

The main characteristics of the cases and controls. The mean age of the cases and controls were 59.3 (SD±13.4) and 59.4 years (SD±13.8), respectively. The distribution of sex and age among cases and controls were not statistically significant (p>0.05) which suggest that the age and sex matching was effective. Majorities (52.9%) of the stomach cancer cases were in the age group of 31-60 years at the time of diagnosis and 76.5% were males. To control for confounding, level of education, income groups and occupation were also included in the analysis.

The data in Table 1 indicates the risk of stomach cancer associated with tobacco habits. Odds ratios (OR) were calculated using non-smokers as reference group. The matched univariate OR for both ex-smokers and current smokers was 3.32 (95%CI, 1.66-6.64) and 2.55 (95%CI, 1.38-4.73), respectively. Higher risk of stomach cancer among past and current smokers was seen even after controlling for confounding factors which indicates its independent effect on the development of stomach cancer. High risk (OR, 23.02; 95% CI, 5.02-105.63) was observed in individuals smoking both cigarettes and *meiziol* (prepared from locally available tobacco). However, among individuals smoking only one type of tobacco, higher risk was observed in *meiziol* smokers (OR, 4.42; 95%CI, 1.94-10.07) in the multivariate model in contrast to cigarette only smokers. A significant higher risk was observed among smokers who smoked more than 10 times per day (OR, 5.38; 95%CI, 2.30-12.55). Longer duration of smoking increased the risk of stomach cancer significantly. Risk of stomach cancer also tended to decline significantly among ex-smokers who have quit smoking more than 10 years ago.

Tuibur (tobacco smoke infused water used in Mizoram) consumption and the risk of stomach cancer was also checked (Table 1) which revealed higher risk in individuals who takes *tuibur* for more than 15 years (OR, 2.04; 95%CI, 1.00-4.17) in the univariate analysis. In stratified analysis among tobacco smokers, *tuibur* user showed three times more risk (OR, 3.32; 95%CI, 1.13-9.74) of stomach cancer than *tuibur* non-users (Table 1).

Table 1. Tobacco use and Risk of Stomach Cancer

Habits		Cases n (%)	Controls n (%)	Univariate*, OR (95% CI)	p-value	Multivariate** adjusted OR (95% CI)	p-value
Smoking habit:	No	21 (20.6)	82 (40.2)	1.0 (reference)		1.0 (reference)	
	Yes	81 (79.5)	122 (59.8)	2.67 (1.49-4.79)	0	3.47 (1.64-7.33)	0
Smoking status: Ex-smokers		33 (32.4)	43 (21.1)	3.32 (1.66-6.64)	0	3.70 (1.56-8.76)	0
Current smokers		48 (47.1)	79 (38.7)	2.55 (1.38-4.73)	0	3.39 (1.54-7.50)	0
Smoking types: Cigarette smokers		9 (8.8)	50 (24.5)	0.60 (0.23-1.56)	0.29	0.98 (0.33-2.89)	0.97
Meiziol smokers		54 (52.9)	67 (32.8)	3.58 (1.89-6.77)	0	4.42 (1.94-10.07)	0
Cigarette+*Meiziol* smokers		18 (17.6)	05 (2.5)	19.26 (5.69-65.2)	0	23.02 (5.02-105.6)	0
Smoking frequency/day:	≤10	16 (15.7)	51 (25.0)	1.31 (0.62-2.73)	0.47	1.89 (0.77-4.64)	0.16
	>10	65 (63.7)	71 (34.8)	4.22 (2.17-8.20)	0	5.38 (2.30-12.6)	0
Smoking age started (in years):	≤15	27 (26.5)	28 (13.7)	4.01 (1.90-8.47)	0	4.24 (1.68-10.7)	0
	>15	54 (52.9)	94 (46.1)	2.45 (1.34-4.50)	0	3.28 (1.51-7.11)	0
Smoking duration (in years):	<15	4 (3.9)	17 (8.3)	0.97 (0.27-3.48)	0.96	1.65 (0.38-7.16)	0.5
	15-30	38 (37.3)	50 (24.5)	2.97 (1.48-5.98)	0	4.55 (1.91-10.8)	0
	>30	39 (38.2)	55 (27.0)	2.85 (1.49-5.46)	0	3.05 (1.31-7.10)	0.01
Years since stopped smoking:	<10	65 (63.7)	80 (39.2)	3.62 (1.94-6.78)	0	4.64 (2.07-10.4)	0
	≥10	16 (15.7)	42 (20.6)	1.51 (0.70-3.27)	0.29	1.81 (0.72-4.57)	0.21
Tuibur taking duration (in years):	Non-user	78 (76.5)	171 (83.8)	1.0 (reference)		1.0 (reference)	
	≤15	6 (5.9)	13 (6.4)	0.81 (0.25-2.60)	0.72	0.83 (0.22-3.16)	0.77
	>15	18 (17.6)	20 (9.8)	2.04 (1.00-4.17)	0.05	2.50 (0.95-6.60)	0.06
Among smokers, *tuibur* taking habit:	No	60 (74.1)	106 (86.9)	1.0 (reference)		1.0 (reference)	
	Yes	21 (25.9)	16 (13.1)	3.40 (1.29-8.98)	0.01	3.32 (1.13-9.74)	0.02

Table 2. Different Tobacco and Betel Nut Related Behavior and Risk of Stomach Cancer

Habits	Cases, n (%)	Controls, n (%)	Univariate*, OR (95% CI)	p-value	Multivariate**, adjusted OR (95% CI)	p-value
Meiziol only	71	74	4.08 (2.35-7.08)	0	3.72 (1.98-6.98)	0
Tuibur only	24	33	1.58 (0.86-2.91)	0.14	1.72 (0.79-3.77)	0.17
Tuibur+Cigarette	5	5	2.62 (0.70-9.77)	0.15	2.06 (0.41-10.2)	0.37
Tuibur+Meiziol	20	11	4.61 (1.92-11.1)	0	3.84 (1.36-10.9)	0.01
Betel nut+*Tuibur*	7	10	1.33 (0.48-3.69)	0.58	0.94 (0.28-3.17)	0.91
Betel nut+Cigarette	10	23	0.83 (0.35-1.96)	0.66	0.70 (0.27-1.85)	0.47
Betel nut+*Meiziol*	24	20	3.16 (1.54-6.49)	0	3.28 (1.39-7.75)	0.01

*Matched (cases and controls were matched for age, sex and ethnicity) univariate odds ratio estimated by conditional logistic regression analysis, **Adjusted odds ratios (adjusted for alcohol drinking, betel nut chewing, level of education, occupation and income group) obtained by matched conditional multiple logistic regression analysis using maximum likelihood approach.

Table 3. Association of *GSTM1* and *GSTT1* and Stomach Cancer

Genotype		Cases n (%)	Controls n (%)	Matched* OR (95% CI)
GSTM1				
Non-null		45 (44.1)	107 (52.5)	1.0 (reference)
Null		57 (55.9)	97 (47.5)	1.48 (0.90-2.43)
GSTT1				
Non-null		65 (63.7)	111 (54.4)	1.0 (reference)
Null		37 (36.3)	93 (45.6)	0.69 (0.42-1.13)
GSTM1	*GSTT1*			
Non-null	Non-null	30 (29.4)	61 (29.9)	1.0 (reference)
Non-null	Null	15 (14.7)	46 (22.5)	0.67 (0.32-1.40)
Null	Null	22 (21.6)	47 (23.0)	1.01 (0.51-2.01)
Null	Non-null	35 (34.3)	50 (24.5)	1.50 (0.78-2.86)

*Matched (cases and controls were matched for age, sex and ethnicity) odds ratio estimated by conditional logistic regression analysis.

The data in Table 2 indicates the risk involved with different tobacco related habits either in single or in combination. Significant high risk was observed in persons who practice *meiziol* and *tuibur* together (OR, 3.84; 95%CI, 1.36-10.86) followed by individuals who take *meiziol* and betel nut together (OR, 3.28; 95%CI, 1.39-7.75).

GSTM1 and GSTT1 polymorphism

The frequency distribution of homozygous *GSTM1* and *GSTT1* null and non-null genotype in stomach cancer cases and healthy controls were shown in Table 3. The data reveals that *GSTM1* and *GSTT1* null genotype are distributed unevenly between stomach cancer cases and controls. The *GSTM1* null genotype frequency was increased in stomach cancer cases (55.9%) as compared to controls (47.5%). Individuals with *GSTM1* null genotype showed higher risk of stomach cancer (OR, 1.48; 95%CI, 0.90-2.43) than *GSTM1* non-null genotype carrying people, while persons carrying *GSTT1* null genotype demonstrated decreased risk of stomach cancer (OR, 0.69; 95%CI, 0.42-1.13) than the non-null genotype carriers of *GSTT1*, although these associations failed to attain statistical significance. Among patients of stomach cancer, 36.3% (37/102) were *GSTT1* homozygous null genotype as compared to 45.6% (93/204) of controls.

The odds ratio of stomach cancer associated with combined genotypes of the polymorphisms of *GSTM1* and *GSTT1* genes are also shown in Table 3. No significant association was found between combined genotypes of *GSTM1* and *GSTT1* genotypes and stomach cancer. However, persons who carry the *GSTM1* null and *GSTT1* non-null genotype were found to have more risk involved (OR, 1.50; 95%CI, 0.78-2.86) than other combinations of *GSTM1* and *GSTT1* genotypes, though it could not attain statistical significance.

GSTM1 polymorphism, smoking, tuibur and alcohol habit

Data of Table 4 reveals that among non-smokers, persons carrying *GSTM1* null genotype had significantly higher risk of stomach cancer than non-smokers carrying *GSTM1* non-null genotype. Both ex-smokers as well as current smokers whether carrying *GSTM1* non-null or null genotype had significantly higher risk of stomach cancer than non-smokers non-null genotype carriers. Persons who smoked more than 10 times per day had significantly higher risk of stomach cancer irrespective of their genotypic status (*GSTM1* non-null or null genotype) as compared to non-smokers non-null *GSTM1* genotype carriers. All persons who smoked for more than 15 years had a higher risk of stomach cancer irrespective of whether they belong to *GSTM1* null or non-null genotype. Reduction in the risk of stomach cancer was seen in persons who left smoking for more than 10 years in both *GSTM1* non-null and null genotyped groups. The effect of the type of smoking and risk of stomach cancer was also similar in both *GSTM1* null and non-null genotype carriers. *Meiziol* smokers had significantly higher risk as compared to persons who smoked cigarette only.

The effect of *GSTM1* polymorphism on the risk of stomach cancer in *tuibur* users revealed that persons having *GSTM1* null genotypes using *tuibur* had 2.4 times (OR, 2.40; 95%CI, 1.03-5.59) higher risk of stomach cancer as compared to non-consumers of *tuibur* having non-null genotype (Table 4). Among alcohol drinkers, no significant effect of *GSTM1* polymorphism on the risk of stomach cancer was found (data not shown).

GSTT1 polymorphism, smoking, tuibur and alcohol habit

Table 4 reveals that among non-smokers, there was no effect of *GSTT1* polymorphism and risk of stomach cancer. Ex-smokers as well as current smokers had significantly higher risk of stomach cancer, if they carries *GSTT1* non-null genotype than non-smokers carrying *GSTT1* non-null genotype. Similarly, *GSTT1* non-null *meiziol* smokers had significantly higher risk of stomach cancer (OR, 4.05; 95%CI, 1.74-9.43) than *GSTT1* non-null non-smokers. Persons who smoked more than 10 times per day had significantly higher risk of stomach cancer than *GSTT1* non-null carriers of non-smokers. However, this risk was higher among persons who belong to *GSTT1* non-null genotype (OR, 5.76; 95%CI, 2.41-13.77) than persons belonging to *GSTT1* null genotype. Similarly, persons with *GSTT1* non-null genotype and who smoked for more than 30 years had significantly higher risk of stomach cancer (OR, 4.04; 95%CI, 1.66-9.86) as compared to non-smokers non-null genotype carriers. Persons who stopped smoking for last 10 years or more had a decreased risk of stomach cancer as compared to persons who stopped smoking less than 10 years ago. This decrease in risk of stomach cancer was similar in both *GSTT1* null or non-null genotypes.

Among *tuibur* consumers and alcohol drinkers, the effect of *GSTT1* polymorphism on the risk of stomach cancer was also examined, however, no significant effect was observed (data not shown).

Association of Helicobacter pylori with stomach cancer

The frequency of *Helicobacter pylori* in matched healthy population control was found to be 69.4% (n=193) based on the detection of antibodies against *H. pylori* using *IgG* indirect ELISA. Among stomach cancer patients, *H. pylori* infection was slightly higher (73.7%, n=99) than

Table 4. Association of *GSTM1 and GSTT1* Genotypes with Tobacco use and Risk of Stomach Cancer

Variables		Genotype	Cases n (%)	Controls n (%)	Matched*, OR (95% CI)	p-value
Tobacco consumption and risk of Stomach Cancer		GSTM1				
Smoking status	Non-smokers	Non-null	7 (6.9)	52 (25.5)	1.0 (reference)	
		Null	14 (13.7)	30 (14.7)	3.18 (1.18-8.56)	0.02
	Ex-smokers	Non-null	14 (13.7)	23 (11.3)	4.31 (1.44-12.8)	0.01
		Null	19 (18.6)	20 (9.8)	7.46 (2.62-21.2)	0
	Current smokers	Non-null	24 (23.5)	32 (15.7)	5.45 (2.03-14.6)	0
		Null	24 (23.5)	47 (23.0)	3.91 (1.51-10.1)	0.01
Smoking types	Cigarette smokers	Non-null	3 (2.9)	16 (7.8)	1.61 (0.36-7.24)	0.53
		Null	7 (6.9)	33 (16.2)	1.32 (0.38-4.66)	0.66
	Meiziol smokers	Non-null	26 (25.5)	37 (18.1)	5.26 (1.90-14.5)	0
		Null	27 (26.5)	30 (14.7)	8.03 (2.92-22.1)	0
	Cigarette+*Meiziol* smokers	Non-null	9 (8.8)	2 (1.0)	40.62 (6.83-241.6)	0
		Null	9 (8.8)	4 (2.0)	22.83 (4.70-110.8)	0
Smoking frequency/day	≤10	Non-null	6 (5.9)	18 (8.8)	2.47 (0.68-8.88)	0.16
		Null	10 (9.8)	33 (16.2)	2.47 (0.85-7.19)	0.09
	>10	Non-null	32 (31.4)	37 (18.1)	7.74 (2.82-21.2)	0
		Null	33 (32.4)	34 (16.7)	10.21 (3.68-28.3)	0
Smoking age started (in years)	≤15	Non-null	13 (12.7)	14 (6.9)	6.69 (2.14-20.9)	0
		Null	14 (13.7)	14 (6.9)	7.34 (2.43-22.2)	0
	>15	Non-null	25 (24.5)	41 (20.1)	4.49 (1.71-11.8)	0
		Null	29 (28.4)	53 (26.0)	4.30 (1.69-11.0)	0
Smoking duration (in years)	<15	Non-null	2 (2.0)	11 (5.4)	1.61 (0.28-9.39)	0.59
		Null	3 (2.9)	6 (2.9)	4.28 (0.80-23.0)	0.09
	15-30	Non-null	17 (16.7)	22 (10.8)	5.62 (1.88-16.8)	0
		Null	20 (19.6)	28 (13.7)	5.59 (1.96-15.9)	0
	>30	Non-null	19 (18.6)	22 (10.8)	5.76 (2.06-16.1)	0
		Null	20 (19.6)	33 (16.2)	4.27 (1.62-11.3)	0
Years since stopped smoking	<10	Non-null	30 (29.4)	32 (15.7)	7.25 (2.68-19.7)	0
		Null	35 (34.3)	48 (23.5)	5.70 (2.25-14.4)	0
	≥10	Non-null	8 (7.8)	23 (11.3)	2.51 (0.79-8.0)	0.11
		Null	8 (7.8)	19 (9.3)	2.96 (0.91-9.6)	0.07
Tuibur consumption	No	Non-null	34 (33.3)	88 (43.1)	1.0 (reference)	
		Null	44 (43.1)	83 (40.7)	1.40 (0.80-2.43)	0.23
	Yes	Non-null	11 (10.8)	19 (9.3)	1.36 (0.55-3.35)	0.5
		Null	13 (12.7)	14 (6.9)	2.40 (1.03-5.59)	0.04
Smoking habit and risk of Stomach Cancer		GSTT1				
Smoking status	Non-smokers	Non-null	11 (10.8)	45 (22.1)	1.0 (reference)	
		Null	10 (9.8)	37 (18.1)	1.04 (0.39-2.71)	0.96
	Ex-smokers	Non-null	20 (19.6)	21 (10.3)	4.32 (1.64-11.4)	0
		Null	13 (12.7)	22 (10.8)	2.54 (0.97-6.67)	0.05
	Current smokers	Non-null	34 (33.3)	45 (22.1)	3.35 (1.49-7.53)	0
		Null	14 (13.7)	34 (16.7)	1.57 (0.62-3.98)	0.34
Smoking types	Cigarette smokers	Non-null	7 (6.9)	29 (14.2)	0.82 (0.26-2.62)	0.73
		Null	3 (2.9)	20 (9.8)	0.65 (0.16-2.70)	0.55
	Meiziol smokers	Non-null	37 (36.3)	34 (16.7)	4.05 (1.74-9.43)	0
		Null	16 (15.7)	33 (16.2)	2.01 (0.83-4.87)	0.12
	Cigarette+*Meiziol* smokers	Non-null	10 (9.8)	3 (1.5)	13.01 (2.99-56.6)	0
		Null	8 (7.8)	3 (1.5)	13.83 (2.53-76.0)	0
Smoking frequency/day	≤10	Non-null	12 (11.8)	29 (14.2)	1.90 (0.73-4.95)	0.18
		Null	4 (3.9)	22 (10.8)	0.77 (0.23-2.64)	0.68
	>10	Non-null	42 (41.2)	37 (18.1)	5.76 (2.41-13.8)	0
		Null	23 (22.5)	34 (16.7)	3.22 (1.33-7.84)	0.01
Smoking age started (in years)	≤15	Non-null	16 (15.7)	15 (7.4)	5.27 (1.85-15.0)	0
		Null	11 (10.8)	13 (6.4)	3.13 (1.11-8.82)	0.03
	>15	Non-null	38 (37.3)	51 (25.0)	3.27 (1.45-7.35)	0
		Null	16 (15.7)	43 (21.1)	1.54 (0.63-3.72)	0.34
Smoking duration (in years)	≤30	Non-null	29 (28.4)	40 (19.6)	3.17 (1.30-7.75)	0.01
		Null	13 (12.7)	27 (13.2)	2.17 (0.83-5.74)	0.11
	>30	Non-null	25 (24.5)	26 (12.7)	4.04 (1.66-9.86)	0
		Null	14 (13.7)	29 (14.2)	1.70 (0.66-4.38)	0.26
Years since stopped smoking	<10	Non-null	44 (43.1)	44 (21.6)	4.43 (1.96-10.0)	0
		Null	21 (20.6)	36 (17.6)	2.45 (1.02-5.88)	0.04
	≥10	Non-null	10 (9.8)	22 (10.8)	1.87 (0.68-5.20)	0.22
		Null	6 (5.9)	20 (9.8)	1.22 (0.40-3.68)	0.73

*Matched (cases and controls were matched for age, sex and ethnicity) univariate odds ratio estimated by conditional logistic regression analysis.

the population controls, although this difference was not statistically significant [(univariate OR, 1.16; 95%CI, 0.66-2.04; p-value, 0.617), (multivariate adjusted OR, 0.71; 95%CI, 0.33-1.53; p-value, 0.375)].

Discussion

The state of Mizoram is a high risk region of stomach cancer in India (Rao et al., 1998; Phukan et al., 2004). Few studies have been carried out earlier to detect the risk factors for stomach cancer in India. Our study is the first population based matched case-control study of stomach cancer in Mizoram state of India which also considers *GSTM1* and *GSTT1* genetic polymorphism.

Tobacco smoking was found to be a significant risk factor of stomach cancer in our study which is consistent with the work of other investigators and also our earlier study (Phukan et al., 2005). Both current and ex-smokers showed significantly higher risk in comparison to the non-smokers. The *meiziol* smokers demonstrated high risk than the non-smokers and cigarette only smokers. Although cigarette smoking alone showed no significant association with stomach cancer, however synergistic interaction was found between cigarette and *meiziol* smokers, as persons who are exposed to the habits of cigarette smoking along with *meiziol*, showed nearly five times higher risk than those who smokes only *meiziol*. IARC monoghaph (IARC, 2002) and several cohort and case-control studies (Hansson et al., 1994; Tredaniel et al., 1997; Russo et al., 2001; García-G et al., 2012) reveal that tobacco smoking is a risk factor for stomach cancer. However, in Indian context, many studies reported variably about the involvement of the risk of tobacco-smoking for stomach cancer. Rao et al. (2002) and Yadav et al. (2010) did not get any relationship between tobacco use and stomach cancer. However, Gajalakshmi et al. (1996) and in one of our previous study (Phukan et al., 2005), we have reported tobacco-smoking as a significant risk factor for stomach cancer, which is consistent with this study also.

In our earlier study (Phukan et al., 2005), we have reported *tuibur* as a risk factor for stomach cancer in Mizoram which is consistent with this current study. No significant association between alcohol drinking and stomach cancer was seen like most of the previous studies (Hansson et al., 1994; Gajalakshmi et al., 1996; You et al., 2000; Rao et al., 2002); except a few studies (Boeing et al., 1991; Sumathi et al., 2009) reported alcohol drinking as risk factor for stomach cancer.

The present study is the first study to describe the *GSTM1* and *GSTT1* genetic polymorphism of stomach cancer in Mizoram. The *GSTM1* null genotype was found to be high in stomach cancer cases (55.9%) as compared to healthy neighborhood controls (47.5%), although this difference was not statistically significant. Another three studies carried out in China (Cai et al., 2001; Jing et al., 2012) and Japan (Katoh et al., 1996) also reported high frequency of *GSTM1* null genotype among stomach cancer cases than in controls. *GSTT1* homozygous null genotype was 36.3% in cases and 45.6% in controls which difference was also not significant. No significant association of *GSTM1* and *GSTT1* gene polymorphism and stomach cancer was found.

In this study, relationship between risk of gastric cancer, *GSTM1* polymorphism and smoking habit revealed that persons carrying *GSTM1* null genotype among non-smokers had significantly higher risk of stomach cancer than non-smokers carrying *GSTM1* non-null genotype. Among *tuibur* users, people who carry *GSTM1* null genotype have more than two fold higher risk of stomach cancer than *tuibur* non-users who carry *GSTM1* non-null genotype. Earlier two studies reported association of *GSTM1* null genotype with stomach cancer (Katoh et al., 1996; Saadat et al., 2001). The *GSTT1* non-null genotype carriers among ex-smokers as well as current smokers, *meiziol* smokers, persons who smoked more than 10 times per day showed higher risk of stomach cancer than non-smokers belonging to *GSTT1* non-null genotype carriers. Another study in Taiwan reported protective role of *GSTT1* null genotype for stomach cancer (Wang et al., 1998). A non significant increase of stomach cancer risk was observed among those persons, who carry *GSTM1* null and *GSTT1* non null genotype. One earlier study (Cai et al., 2001) showed similar increased risk among *GSTM1* null and *GSTT1* non null genotype carrying persons. Another study from India revealed protective role of *GSTT1* null genotype in oral cancer (Anantharaman et al., 2007).

In this study, no association of *H. pylori* infection and stomach cancer in Mizoram was found. Although several meta-analyses revealed a strong relationship between *H. pylori* and gastric cancer (Eslick et al., 1999; Xue et al., 2001), it is still controversial in different Asian countries (Miwa et al., 2002; Jing et al., 2012). Various studies from India also failed to show an association between *H. pylori* infection and gastric cancer (Kate et al., 2000; Khanna et al., 2002; Singh et al., 2006). These disparate observations have created an enigmatic situation in Indian context and needs serious approach to solve this problem.

In conclusion, this study reveals that tobacco-smoking either in single or in combined mode of habit is an important risk factor for stomach cancer in Mizoram state of India. *Meiziol* is the important risk factor among different tobacco habits. Cigarette, *tuibur* and betel nut modulates the risk when taken with *meiziol*. Among tobacco smokers, *tuibur* users have three times more risk of stomach cancer than *tuibur* non-users. Frequency of *GSTM1* null and *GSTT1* null genotype in the control population of Mizoram were 47.5% and 45.6% respectively, whereas among cases they were 55.9% and 36.3% respectively. Polymorphism of *GSTM1* and *GSTT1* gene are not found to be directly associated with stomach cancer in Mizoram. However, *GSTM1* and *GSTT1* genes modify the effect of tobacco habits. Persons who smoke tobacco and / or take *tuibur* had increased risk of stomach cancer if they carry *GSTM1* null genotype and *GSTT1* non-null genotype. *Tuibur* users carrying *GSTM1* null genotypes had 2.4 times higher risk of stomach cancer than non-consumers of *tuibur* having *GSTM1* non-null genotype. No association of *H. pylori* infection and stomach cancer in Mizoram was found. Similar types of studies with large population in different geographical areas may through important light and will help to understand and to confirm these preliminary findings in coming period.

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