RESEARCH ARTICLE

Role of Household Exposure, Dietary Habits and Glutathione S-Transferases M1, T1 Polymorphisms in Susceptibility to Lung Cancer among Women in Mizoram India

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Abstract

Background: A case-control study was conducted to evaluate the effect of household exposure, dietary habits, smoking and Glutathione S-Transferases M1, T1 polymorphisms on lung cancer among women in Mizoram, India. Materials and Methods: We selected 230 newly diagnosed primary lung cases and 460 controls from women in Mizoram. Multivariate logistic regression analysis was performed to estimate adjusted odds ratio (OR). Results: Exposure of cooking oil fumes (p<0.003), wood as heating source for cooking (p=0.004), kitchen inside living room (p=0.001), improper ventilated house (p=0.003), roasting of soda in kitchen (p=0.001), current smokers of tobacco (p=0.043), intake of smoked fish (p=0.006), smoked meat (p=0.001), Soda (p<0.001) and GSTM1 null genotype (p=0.003) were significantly associated with increased risk of lung cancer among women in Mizoram. Significantly protective effect was observed for intake of bamboo shoots (p=<0.001) and egg (p<0.001). A clear increase in dose response gradient was observed for total cooking dish years. Risk for lung cancer tends to increase with collegial effect of indoor environmental sources (p=0.022). Significant correlation was also observed for interaction of GST polymorphisms with some of dietary habits. Conclusions: We confirmed the important role of exposure of cooking oil emission and wood smoke, intake of smoked meat, smoked fish and soda (an alkali preparation used as food additives in Mizoram) and tobacco consumption for increase risk of lung cancer among Women in Mizoram.

Keywords: Cooking oil fumes - lung cancer - Mizoram - women - bamboo shoots

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Introduction

Lung cancer is the leading cause of cancer death worldwide with an annual mortality of 18.2% cancer death (Ferlay et al., 2010). In India, it continues to be important (D'Souza et al., 2013) and the incidence of lung cancer is highest in Aizawl district of Mizoram among females with an age adjusted rate of 38.7 per 10^5 population (NCRP, 2010). Mizoram situated in between 92.15´ and 93.29´E longitude and 21.58´ to 24.35´N latitude; surrounded by Myanmar in east and Bangladesh in west. Mizoram have their ancestral origin in China; later which shares a similar mortality trend with United States according to China Health Statistical Yearbook, published by Ministry of health in 2010 (China's Health Statistics Yearbook, 2010).

A unique pattern of tobacco consumption and dietary habits with high incidence of lung cancer was observed in Mizoram (Chaturvedi et al., 1998; Phukan et al., 2006; Malakar et al., 2014). Smoking of tobacco is undoubtly one of predominant risk factor for lung cancer (Tredaniel et al., 1994; Pisani et al., 2006; Sellappa et al., 2009). Apart from smoking exposure, indoor smoke from household activities (Zhong et al., 1999; Reid et al., 2012; Yin et al., 2013) and dietary habits (Marchand et al., 2002; Shen et al., 2008) were also suspected to have casual relationship. Exposure to cooking oil fumes (COF) and wood smoke are known to contain considerable amount of carcinogens such as benzo[a]pyrene, 2, 4-decadienal, carbon monoxide, carbon dioxide and other polycyclic aromatic hydrocarbons (PAH) (Yang et al., 2000; Lissowska et al., 2005). Exposure to COF may potentially play a role in occurrence of lung cancer. Mizo cooking often involves stir-frying and deep-frying to a high temperature that produces ample amount of COF containing several harmful compounds (Straif et al., 2006). Incense burning, a traditional practice in Mizo household also produces a considerable amount of PAH, carbonyls and benzene (Koo et al., 1995).

Glutathione S-transferases (GST) are a family of phase II enzymes, metabolize carcinogenic compounds that are

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found in tobacco smoke and environmental carcinogens (Jourenkova-Mironova et al., 1998; Wenzlaff et al., 2005; Nosheen et al., 2010; Senthilkumar and Thirumurugan, 2012). Functional genetic polymorphisms of Phase II metabolic pathways genes GST also may account for differences in metabolic inactivation of dietary or other environmental carcinogens (Ye et al., 2006). Among these, the best known are deletion of GST mu 1 (GSTM1) or GST theta 1 (GSTT1) genes (null variant), which result in no functional activity (Kelsey et al., 1997).

The present study was a population based case control study design to investigate whether exposure from COF, house hold combustion and other indoor environmental sources plays a significant role in enhancing risk of lung cancer among women in Mizoram. We also wished to explore the join effect of these indoor environmental sources and whether the impact of these compounds and other dietary carcinogens is modified by GST polymorphisms.

Materials and Methods

The basic design of present study was an age and sex matched population based case-control study. The work was carried out at Regional Medical Research Centre (RMRC), Indian Council of Medical Research (ICMR), North East Region; India in collaboration with Civil Hospital, Aizawl, Mizoram. The study duration was from October 2009 to December 2012. All incident cases and controls subject willing to participate in the study were ethnic tribes of Mizo women. Histopathologically confirmed cases of no evidence of pulmonary inflammation or benign lung tumors and only lung as their primary site of cancer were included in the study. Cases too aged to be interviewed elaborately and who refused to be interviewed, were excluded from this study. Sources of data collection for the cases include Radiotherapy and Pathology Department of Civil hospital Aizawl, other private laboratory and nursing homes of Mizoram. Cancer free controls with age and sex matched were selected from healthy population of the state.

In order to reduce potential bias in our study none of the controls had consanguinity with the cases or had any non-communicable diseases. Information of dietary habits, socio-demographic characteristics and tobacco consumption were recorded in a structured pre designed questionnaire. Subjects who reported that they were regularly smoking during index year were defined as current smokers or current users, those who reported that they had stopped smoking the year before index year or before were defined as ex-smokers or ex-users, and those who reported that they never had smoked before or during index year were defined as never-smokers or never users (Phukan et al., 2005). A composite index, cooking disk years was constructed to quantify regular cooking habits based on frequency and duration of cooking, as previously described (Yu et al., 2006). One stir-frying dish year means cooking one stir fried dish with cooking oil daily for a year. The total cooking dish year was used to express amount of exposure to cooking oil emissions, which was also categorized into five groups with interval of 50 dish year. The time period for exposure of COF, heating sources for cooking, types of ventilation, position of kitchen in house, use of fume extractor and roasting of soda in kitchen was set for since last 25 years and all participants were asked to recall exposures for the above mention practices, 25 years before age of diagnosis for lung cancer cases as well as for controls group. In the dietary section, we calculated following items: smoked fish, dried fish, fresh fish, smoked meat, dried meat, fresh meat, intake of tea, soda (an alkali preparation), bamboo shoots, soybean, pickle, egg and fruits. Based on dietary style of Mizo population we divided consumption of these items into following groups: (i) Nil (ii) Occasionally (iii) Twice or more in a week. A total of 230 female lung cancer cases and 460 controls matched for age sex were enrolled in the study. Written informed consent was taken from all subjects for participation in the study in a protocol approved by institutional ethical committee of Regional Medical Research Centre, North East Region (Indian Council of Medical Research).

DNA extraction and genotyping of GSTM1 and GSTT1 genotyping: Four milliliter of blood was collected from all subjects in EDTA vials. DNA was isolated by using standard phenol chloroform method (Sambrook and Russel, 2001) and stored at -80º C till further analysis. Multiplex PCR for homozygous null polymorphisms of GSTM1 and GSTT1 genotypes were carried out based on previous reports using Albumin gene as an internal positive control with modifications. The primer sequences used were-GSTM1 F (5'GTA CCC TAC TTG ATT GAT GGG 3'), GSTM1 R (5'CTG GAT TGT AGC AGA TCA TGC 3') (Wang et al., 2002); GSTT1 F (5'TTC CTT ACT GGT CCT CAC ATC TC 3'), GSTT1 R (5'TCA CCG GAT TCA TGG CCA GCC 3') (Kiran et al., 2010); Albumin F (5' GCC CTC TGC TAA CAA GTC CTA C 3') and Albumin R (5' GCC CTA AAA AGA AAA TCG CCA ATC 3') (Cho et al., 2005). Final reaction conditions contained 10 pmol of each primer (Sigma make), 10mM of 1X PCR buffer (Roche), 10mM $MgCl_2$ (Roche), 10mM of deoxyribonucleotide triphosphate (Roche), 2.5U of Taq DNA polymerase (Roche); and 50-100ng of genomic DNA. Amplified PCR products (GSTM1-273 base pair (bp), GSTT1-480bp and albumin-350bp) were separated by 2% agarose gel electrophoresis and visualized in a gel documentation system (Cell Biosciences). GSTM1 homozygous null genotype was indicated by absence of a 480bp fragment. Amplification of 350bp albumin fragment, used as an internal control, confirmed successful PCR reaction.

Statistical analysis

Difference in consumption of dietary and cooking habits, distribution of socio-demographic characteristics and genotype frequencies between cases and controls were evaluated using the Chi Square ($\chi 2$) test. Estimates of lung cancer risk, imparted by genotypes, other covariates as tobacco smoking and other dietary factors were determined by deriving odds ratio (OR) and its corresponding 95% confidence interval (95% CI) using multivariable logistic regression after adjusting for age, education and occupation. For all the tests a two sided

Table 1. Socio-Demographic Information and Risk of Lung Cancer

Category		Control (460) n (%)	Case (230) n (%)	Crude OR (95% CI)	p value	Adjusted OR (95% CI)**	p value
Mean age		58.60±13.99	59.01±13.02	---	0.711	---	---
Educational status	College and above	102 (22.2)	44 (19.1)	Reference	0.194	Reference	0.349
	Illiterate	51 (11.0)	32 (14.0)	1.46 (0.83-2.56)	0.322	1.35 (0.72-2.54)	0.397
	Primary-middle	206 (44.8)	110 (47.8)	1.24 (0.81-1.89)	0.969	1.23 (0.77-1.97)	0.485
	Secondary	101 (22.0)	44(19.1)	1.01 (0.61-1.67)		1.21 (0.71-2.09)	
Occupational status	Office workers	83 (18.0)	28(12.1)	Reference	0.939	Reference	0.845
	Cultivators	88 (19.1)	29(12.6)	0.98 (0.54-1.78)	0.643	0.94 (0.49-1.78)	0.833
	Skilled workers	40 (8.7)	16 (7.0)	1.19 (0.58-2.44)	0.005	1.09 (0.49-2.42)	0.047*
	House wife	200 (43.5)	135 (58.7)	2.00 (1.24-3.24)	0.396	1.68 (1.01-2.80)	0.526
	Unskilled workers	49 (10.7)	22 (9.6)	1.33 (0.69-2.58)		1.26 (0.62-2.57)	
Fuel used	Liquefied Petroleum Gas	256 (55.7)	94(40.9)	Reference	0.68	Reference	0.824
	Coal	61 (13.3)	25(10.8)	1.12 (0.66-1.88)	<0.001	1.07 (0.58-1.97)	0.044*
	Wood	143 (31.0)	111 (48.3)	2.11 (1.50-2.98)		1.50 (1.01-2.22)	
Location of kitchen	In separated room	302 (65.7)	108 (47.0)	Reference	<0.001	Reference	0.001*
	In the living room	158 (34.3)	122 (53.0)	2.16 (1.56-2.98)		1.79 (1.25-2.55)	
Ventilation type	Ventilated	367 (79.8)	146 (63.5)	Reference	<0.001	Reference	0.003*
	Improper ventilated	93 (20.2)	84(36.5)	2.27 (1.60-3.23)		2.10 (1.36-3.22)	
Cooking oil fumes	No Exposure	244 (53.0)	87(37.8)	Reference	<0.001	Reference	0.003*
	Exposure	216 (47.0)	143 (62.2)	1.86 (1.34-2.57)		2.56 (1.39-4.74)	
Use of fume extractor	Yes	38 (8.3)	23(10.0)	Reference	0.449	Reference	0.621
	No	422 (91.7)	207 (90.0)	0.81 (0.47-1.40)		1.17 (0.63-2.14)	
Roasting of soda in kitche	No	108 (23.5)	31(13.5)	Reference	0.002	Reference	0.001*
	Yes	352 (76.5)	199 (86.5)	1.97 (1.27-3.05)		2.30 (1.38-3.80)	
Use of incense	No	194 (42.2)	83(36.1)	Reference	0.124	Reference	0.533
	Yes	266 (57.8)	147 (63.9)	1.29 (0.93-1.79)		0.80 (0.39-1.62)	
Meal per day	1/2	111 (24.1)	54(23.5)	Reference	0.85	Reference	0.292
	>2	349 (75.9)	176 (76.5)	1.04 (0.71-1.50)		0.74 (0.42-1.30)	

*Significant; **Adjusted OR were estimated by adjusting age in multiple logistic regression model

Table 2. Total Cooking Dish Years and Risk of Lung Cancer

Category		Control (460) n (%)[b]	Case (230) n (%)[b]	Crude OR (95% CI)	p value	Adjusted OR (95% CI)[a]	p value
Total dish-years	≤50	22 (4.8)	7 (3.0)	Reference		Reference	
	51-100	86 (18.7)	30 (13.0)	1.10 (0.43-2.83)	0.849	1.26 (0.47-3.33)	0.646
	101-150	80 (17.4)	58 (25.2)	2.28 (0.91-5.69)	0.078	2.76 (1.04-7.32)	0.041*
	151-200	67 (14.6)	56 (24.3)	2.63 (1.05-6.60)	0.04	3.02 (1.14-7.98)	0.026*
	>200	21 (4.6)	24 (10.4)	3.59(1.28-10.09)	0.015	4.56(1.51-13.78)	0.007*

*Significant; [a]Adjusted OR were estimated by adjusting age, education and occupational status in multiple logistic regression model; [b]Calculated only for those cases that use these peculiar cooking methods (stir frying and deep frying); hence column-wise percentage does not add up to 100%

p≤0.05 was considered statistically significant. All the statistical analysis were done using SPSS version 17.0.

Results

The distributions of socio-demographic characteristics of cases and controls subject were analyzed in Table 1. There was no statistically significant difference between cases and controls in terms of mean age (p=0.711) of study subjects. However, we observed a significant difference in terms of occupation (Housewife, p= 0.047). Furthermore, in terms of indoor air environmental sources; significant association was observed: exposure of cooking oil fumes (OR=2.56, CI=1.39-4.74), wood as heating source for cooking (OR=1.50, CI=1.01-2.22), kitchen inside living room (OR=1.79, CI=1.25-2.55), improper ventilated house (OR=2.10, CI=1.36-3.22) and roasting of soda in kitchen (OR=2.30, CI=1.38-3.80). Analysis of effects of cooking dish–years, both cases and controls were grouped by five dish year levels (with an interval of 50) (Table 2). A dose response gradient with total cooking dish years was displayed, with an OR >3 in second highest dish years and >4 in highest dish years.

We also observed that, with increasing level of sources for smoke inside the house, risk for lung cancer tend to increase (OR= 4.45, CI=1.38-14.39). An enhanced risk was observed for stir frying after smoke emission with these sources (OR=5.30, CI=1.40-20.12). Although indoor smoking was not significantly associated with increase of lung cancer, risk was even more with the collegial effect of indoor smoking with these sources (OR=6.42, CI=1.15-

Table 3. Indoor Environmental Sources and Risk of Lung Cancer

Levels	Indoor air pollution[a]	Control (460) n (%)[b]	Case (230) n (%)[b])	Univariate OR (95% CI)[a]	p value
1	Use of wood as burning fuel+emission of cooking oil fumes +roasting of soda in kitchen+improper ventilated house				
	No	11 (2.4)	6 (2.6)	Reference	
	Yes	14 (3.0)	34(14.8)	4.45 (1.38-14.39)	0.010*
2	Stir frying+level 1				
	No	9 (2.0)	4 (1.7)	Reference	
	Yes	14 (3.0)	33 (14.3)	5.30 (1.40-20.12)	0.010*
3	Indoor smoking+level 2				
	No	7 (1.5)	2 (0.9)	Reference	
	Yes	12 (2.6)	22 (9.6)	6.42 (1.15-35.90)	0.022*

*Significant; [a]Combustion of LPG for cooking, no emission of cooking oil fumes, no roasting of soda in kitchen, properly ventilated house, no stir frying and no indoor smoking were taken in reference group marked as "No", while Combustion of wood for cooking, emission of cooking oil fumes, roasting of soda in kitchen, improper ventilated house, stir frying and indoor smoking were taken in risk group marked as "Yes"; [b]Calculated only for those cases that were exposed to the above household scenario ([a]); hence column-wise percentage does not add up to 100%

Table 4. Risk Factors According to Tobacco Consumption Habits

Category		Control (460) n (%)	Case (230) n (%)	Crude OR (95% CI)	p value	Adjusted OR (95% CI)**	p value
Smoking status	Never smoker	132 (28.7)	38 (16.5)	Reference		Reference	
	Ex-smoker	175 (38.0)	66 (28.7)	1.31 (0.83-2.07)	0.248	0.91 (0.40-2.09)	0.825
	Current smoker	153 (33.3)	126 (54.8)	2.86 (1.86-4.40)	<0.001	2.19 (1.03-4.67)	0.043*
Smoking types	Never smoker	132 (28.7)	38 (16.5)	Reference		Reference	
	Meiziol	279 (60.7)	167 (72.6)	2.08 (1.38-3.13)	<0.001	2.25 (1.27-4.01)	0.006*
	Cigarette	49 (10.6)	25 (10.9)	1.77 (0.97-3.24)	0.062	1.68 (0.80-3.52)	0.171
Indoor smoking	No	155 (33.7)	59 (25.7)	Reference		Reference	
	Yes	305 (66.3)	171 (74.3)	1.47 (1.03-2.10)	0.031	0.70 (0.40-1.21)	0.200
Passive smoking	No	220 (47.8)	80 (34.8)	Reference		Reference	
	Yes	240 (52.2)	150 (65.2)	1.72 (1.24-2.38)	0.001	1.56 (1.02-2.39)	0.041*
Tuibur status	Never user	309 (67.2)	134 (58.3)	Reference		Reference	
	Ex-user	77 (16.7)	35 (15.2)	1.05 (0.67-1.64)	0.837	0.76 (0.47-1.23)	0.261
	Current user	74 (16.1)	61 (26.5)	1.90 (1.28-2.82)	0.001	1.59 (1.02-2.47)	0.039*

*Significant; **Adjusted OR were estimated by adjusting age education and occupational status in multiple logistic regression model

Table 5. Dietary Habits and Risk of Lung Cancer

Category		Control (460) n (%)	Case (230) n (%)	Crude OR (95% CI)	p value	Adjusted OR (95% CI)**	p value
Smoked fish	Nil	227 (49.3)	108 (47.0)	Reference		Reference	
	Occasionally	149 (32.4)	59 (25.7)	0.83 (0.57-1.22)	0.342	0.89 (0.50-1.60)	0.696
	Twice or more in a week	84 (18.3)	63 (27.3)	1.58 (1.06-2.35)	0.025	2.57 (1.31-5.08)	0.006*
Dried fish	Nil	250 (54.3)	123 (53.5)	Reference		Reference	
	Occasionally	126 (27.4)	46 (20.0)	0.74 (0.50-1.11)	0.144	1.10 (0.57-2.10)	0.785
	Twice or more in a week	84 (18.3)	61 (26.5)	1.48 (0.99-2.19)	0.053	1.34 (0.64-2.83)	0.437
Fresh fish	Nil	164 (35.7)	86 (37.4)	Reference		Reference	
	Occasionally	196 (42.6)	91 (39.6)	0.89 (0.62-1.27)	0.508	0.79 (0.43-1.48)	0.468
	Twice or more in a week	100 (21.7)	53 (23.0)	1.01 (0.66-1.54)	0.961	0.68 (0.31-1.50)	0.340
Smoked meat	Nil	255 (55.4)	111 (48.3)	Reference		Reference	
	Occasionally	113 (24.6)	41 (17.8)	0.83 (0.55-1.27)	0.397	1.08 (0.54-2.16)	0.821
	Twice or more in a week	92 (20.0)	78 (33.9)	1.95 (1.34-2.83)	<0.001	3.03 (1.60-5.74)	0.001*
Dried meat	Nil	239 (52.0)	108 (47.0)	Reference		Reference	
	Occasionally	131 (28.5)	50 (21.7)	0.85 (0.57-1.26)	0.405	1.71 (0.82-3.57)	0.152
	Twice or more in a week	90 (19.5)	72 (31.3)	1.77 (1.21-2.60)	0.004	1.98 (0.90-4.35)	0.091
Fresh meat	Nil	249 (54.1)	118 (51.3)	Reference		Reference	
	Occasionally	109 (23.7)	45 (19.6)	0.87 (0.58-1.31)	0.510	0.90 (0.45-1.80)	0.775
	Twice or more in a week	102 (22.2)	67 (29.1)	1.39 (0.95-2.02)	0.091	0.93 (0.43-2.01)	0.854
Intake of tea	Nil	65 (14.1)	34 (14.8)	Reference		Reference	
	Occasionally	79 (17.2)	40 (17.4)	0.99 (0.55-1.70)	0.910	1.80 (0.85-3.78)	0.124
	Twice or more in a week	316 (68.7)	156 (67.8)	0.94 (0.60-1.49)	0.804	1.29 (0.73-2.30)	0.378
Soda	Nil	200 (43.5)	74 (32.2)	Reference		Reference	
	Occasionally	102 (22.2)	42 (18.3)	1.11 (0.71-1.74)	0.639	1.86 (0.82-4.22)	0.136
	Twice or more in a week	158 (34.3)	114 (49.5)	1.95 (1.36-2.79)	<0.001	7.96 (4.09-15.48)	<0.001*
Bamboo shoots	Nil	172 (37.4)	119 (51.7)	Reference		Reference	
	Occasionally	93 (20.2)	55 (23.9)	0.86 (0.57-1.28)	0.450	0.91 (0.44-1.86)	0.786
	Twice or more in a week	195 (42.4)	56 (24.4)	0.42 (0.28-0.61)	<0.001	0.15 (0.07-0.30)	<0.001*
Soybean	Nil	187 (40.7)	92 (40.0)	Reference		Reference	
	Occasionally	119 (25.9)	59 (25.7)	1.01 (0.68-1.50)	0.970	1.14 (0.57-2.28)	0.721
	Twice or more in a week	154 (33.4)	79 (34.3)	1.04 (0.72-1.51)	0.824	0.83 (0.38-1.80)	0.638
Pickle	Nil	126 (27.4)	72 (31.3)	Reference		Reference	
	Occasionally	155 (33.7)	75 (32.6)	0.85 (0.57-1.26)	0.415	1.18 (0.60-2.32)	0.639
	Twice or more in a week	179 (38.9)	83 (36.1)	0.81 (0.55-1.20)	0.293	1.43 (0.73-2.81)	0.303
Egg	Nil	81 (17.6)	93 (40.4)	Reference		Reference	
	Occasionally	258 (56.1)	103 (44.8)	0.35 (0.24-0.51)	<0.001	0.09 (0.05-0.18)	<0.001*
	Twice or more in a week	121 (26.3)	34 (14.8)	0.25 (0.15-0.40)	<0.001	0.05 (0.02-0.11)	<0.001*
Fruits	Nil	156 (33.9)	76 (33.0)	Reference		Reference	
	Occasionally	41 (8.9)	21 (9.2)	1.05 (0.58-1.90)	0.869	2.11 (0.82-5.40)	0.121
	Twice or more in a week	263 (57.2)	133 (57.8)	1.04 (0.74-1.47)	0.832	1.41 (0.81-2.46)	0.228

*Significant; **Adjusted OR were estimated by adjusting age education and occupational status in multiple logistic regression model

Table 6. Risk Factors According to Distributions of GSTM1 & GSTT1 Genotype

Category		Control (460) n (%)	Case (230) n (%)	Crude OR (95% CI)	p value	Adjusted OR (95% CI)**	p value
GSTM1	Present	292 (63.5)	114 (49.6)	Reference		Reference	
	Null	168 (36.5)	116 (50.4)	1.77 (1.28-2.44)	<0.001	1.85 (1.24-2.76)	0.003*
GSTT1	Present	267 (58.0)	117 (50.9)	Reference		Reference	
	Null	193 (42.0)	113 (49.1)	1.34 (0.97-1.84)	0.074	0.96 (0.64-1.44)	0.855

*Significant; **Adjusted OR were estimated by adjusting age, education and occupational status in multiple logistic regression model

Table 7. Dietary Habits and Risk of Lung Cancer

Model	Interaction parameters [b]			Control (460) n (%)	Case (230) n (%)	Crude OR (95% CI)	p value	Adjusted OR (95% CI)[a]	p value
1	GSTM1 Smoked fish	Present	No	138 (30.0)	45 (19.6)	Reference		Reference	
		Present	Yes	154 (33.5)	69 (30.0)	1.37 (0.89-2.13)	0.157	1.38 (0.88-2.16)	0.163
		Null	No	89 (19.3)	63 (27.4)	2.17 (1.36-3.46)	0.001	2.09 (1.30-3.36)	0.002*
		Null	Yes	79 (17.2)	53 (23.0)	2.06 (1.27-3.34)	0.003	2.27 (1.37-3.76)	0.002*
2	GSTM1 Dried fish	Present	No	153 (33.3)	64 (27.8)	Reference		Reference	
		Present	Yes	139 (30.2)	50 (21.7)	0.86 (0.56-1.33)	0.497	0.87 (0.55-1.35)	0.527
		Null	No	97 (21.1)	59 (25.7)	1.45 (0.94-2.25)	0.092	1.50 (0.96-2.36)	0.076
		Null	Yes	71 (15.4)	57 (24.8)	1.92 (1.22-3.02)	0.005	1.95 (1.21-3.15)	0.006*
3	GSTM1 Fresh fish	Present	No	92 (20.0)	38 (16.5)	Reference		Reference	
		Present	Yes	200 (43.5)	76 (33.0)	0.92 (0.58-1.46)	0.723	0.82 (0.51-1.34)	0.426
		Null	No	72 (15.7)	48 (20.9)	1.61 (0.95-2.73)	0.074	1.62 (0.94-2.82)	0.085
		Null	Yes	96 (20.8)	68 (29.6)	1.72 (1.05-2.80)	0.031	1.53 (0.90-2.61)	0.115
4	GSTM1 Smoked meat	Present	No	160 (34.8)	57 (24.8)	Reference		Reference	
		Present	Yes	132 (28.7)	57 (24.8)	1.21 (0.79-1.87)	0.384	1.21 (0.78-1.88)	0.403
		Null	No	95 (20.6)	54 (23.4)	1.60 (1.02-2.50)	0.042	1.64 (1.03-2.60)	0.037*
		Null	Yes	73 (15.9)	62 (27.0)	2.38 (1.51-3.75)	<0.001	2.47 (1.53-3.98)	<0.001*
5	GSTM1 Dried meat	Present	No	156 (33.9)	60 (26.1)	Reference		Reference	
		Present	Yes	136 (29.6)	54 (23.4)	1.03 (0.67-1.59)	0.886	1.04 (0.67-1.63)	0.855
		Null	No	83 (18.0)	48 (20.9)	1.50 (0.95-2.39)	0.085	1.60 (0.99-2.58)	0.055
		Null	Yes	85 (18.5)	68 (29.6)	2.08 (1.35-3.22)	0.001	2.09 (1.32-3.33)	0.002*
6	GSTM1 Fresh meat	Present	No	158 (34.3)	63 (27.4)	Reference		Reference	
		Present	Yes	134 (29.2)	51 (22.2)	0.96 (0.62-1.48)	0.834	0.96 (0.61-1.52)	0.871
		Null	No	91 (19.8)	55 (23.9)	1.52 (0.97-2.36)	0.066	1.57 (0.99-2.50)	0.055
		Null	Yes	77 (16.7)	61 (26.5)	1.99 (1.27-3.10)	0.003	2.02 (1.26-3.26)	0.004*
7	GSTM1 Alkali (Soda)	Present	No	116 (25.1)	45 (19.6)	Reference		Reference	
		Present	Yes	176 (38.3)	69 (30.0)	1.01 (0.65-1.57)	0.963	0.98 (0.62-1.55)	0.934
		Null	No	84 (18.3)	29 (12.6)	0.89 (0.52-1.53)	0.675	0.89 (0.51-1.56)	0.690
		Null	Yes	84 (18.3)	87 (37.8)	2.67 (1.69-4.21)	<0.001	2.68 (1.65-4.34)	<0.001*
8	GSTT1 Smoked fish	Present	No	131 (28.5)	60 (26.1)	Reference		Reference	
		Present	Yes	136 (29.6)	57 (24.7)	0.92 (0.59-1.41)	0.689	0.96 (0.62-1.49)	0.845
		Null	No	96 (20.9)	48 (20.9)	1.09 (0.69-1.73)	0.710	1.08 (0.67-1.74)	0.743
		Null	Yes	97 (21.0)	65 (28.3)	1.46 (0.94-2.27)	0.089	1.59 (1.00-2.53)	0.052
9	GSTT1 Dried fish	Present	No	145 (31.5)	68 (29.6)	Reference		Reference	
		Present	Yes	122 (26.5)	49 (21.3)	0.86 (0.55-1.33)	0.489	0.83 (0.53-1.31)	0.425
		Null	No	105 (22.8)	55 (23.9)	1.12 (0.72-1.73)	0.618	1.11 (0.70-1.74)	0.664
		Null	Yes	88 (19.2)	58 (25.2)	1.41 (0.91-2.18)	0.129	1.41 (0.88-2.24)	0.149
10	GSTT1 Fresh fish	Present	No	82 (17.8)	44 (19.1)	Reference		Reference	
		Present	Yes	185 (40.2)	73 (31.7)	0.74 (0.47-1.16)	0.186	0.60 (0.37-0.97)	0.038*
		Null	No	82 (17.8)	42 (18.3)	0.96 (0.57-1.61)	0.861	0.88 (0.51-1.52)	0.636
		Null	Yes	111 (24.2)	71 (30.9)	1.19 (0.74-1.91)	0.466	1.01 (0.61-1.68)	0.962
11	GSTT1 Smoked meat	Present	No	151 (32.8)	58 (25.2)	Reference		Reference	
		Present	Yes	116 (25.2)	59 (25.7)	1.32 (0.86-2.05)	0.207	1.26 (0.81-1.97)	0.301
		Null	No	104 (22.6)	53 (23.0)	1.33 (0.85-2.08)	0.217	1.30 (0.82-2.06)	0.268
		Null	Yes	89 (19.4)	60 (26.1)	1.76 (1.12-2.74)	0.013	1.81 (1.12-2.91)	0.015*
12	GSTT1 Dried meat	Present	No	140 (30.4)	58 (25.2)	Reference		Reference	
		Present	Yes	127 (27.6)	59 (25.7)	1.12 (0.73-1.73)	0.606	1.13 (0.72-1.76)	0.601
		Null	No	99 (21.5)	50 (21.7)	1.22 (0.77-1.93)	0.396	1.28 (0.80-2.05)	0.310
		Null	Yes	94 (20.5)	63 (27.4)	1.62 (1.04-2.52)	0.033	1.61 (1.00-2.58)	0.050*
13	GSTT1 Fresh meat	Present	No	145 (31.5)	63 (27.4)	Reference		Reference	
		Present	Yes	122 (26.5)	54 (23.5)	1.02 (0.66-1.58)	0.933	1.01 (0.64-1.59)	0.965
		Null	No	104 (22.6)	55 (23.9)	1.22 (0.78-1.89)	0.382	1.24 (0.78-1.98)	0.357
		Null	Yes	89 (19.4)	58 (25.2)	1.50 (0.96-2.34)	0.073	1.49 (0.93-2.39)	0.100
14	GSTT1 Alkali (Soda)	Present	No	108 (23.5)	35 (15.2)	Reference		Reference	
		Present	Yes	159 (34.5)	82 (35.7)	1.59 (1.00-2.53)	0.050	1.50 (0.92-2.42)	0.102
		Null	No	92 (20.0)	39 (17.0)	1.31 (0.77-2.23)	0.325	1.27 (0.73-2.22)	0.404
		Null	Yes	101 (22.0)	74 (32.1)	2.26 (1.39-3.67)	0.001	2.16 (1.30-3.57)	0.003*

*Significant; [a]Adjusted OR were estimated by adjusting age education and occupational status in multiple logistic regression model; [b]'Yes' includes both occasionally and twice or more in a week

35.90) (Table 3).

The OR for current smokers (OR=2.19, CI=1.03-4.67) was found to be statistically significant as compared to never smokers. Significantly higher risk was observed for Meiziol users (OR=2.25, CI=1.27-4.01) as compared to cigarette users (OR=1.68, CI=0.80-3.52). Current users of Tuibur was also significantly associated than never users (OR=1.59, CI= 1.02-2.47) (Table 4).

Significantly increased risk was observed with intake of smoked fish (OR=2.57, CI=1.31-5.08), smoked meat (OR=3.03, CI=1.60-5.74) and Soda (OR=7.96, CI=4.09-15.48). An inverse correlation was observed for bamboo shoots (OR=0.15, CI=0.07-0.30) and egg (OR=0.05, CI=0.02-0.11) (Table 5).

Discussion

We examined the effect of cooking exposure and dietary habits on lung cancer risk among women in Mizoram, and their modification by indoor tobacco smoking and GST polymorphisms. In Mizo tradition, males always occupy a superior position in family, and their behavior is not restricted; while females are mostly housewife. Because of traditional culture of Mizo, responsibility of cooking lies mostly within women. Meanwhile, we observed a strong association between exposure to cooking oil fumes from frying and lung cancer risk. This was indicated by the result of total cooking dish years which depicts a significant contribution and there was a clear exposure response trend observed in the study subject. Mizo cooking often involves stir frying at high temperature, which produces ample amount of COF containing several carcinogens (Straif et al., 2006). Our study was consistent with the studies from China for significant association of COF and risk of lung cancer among women (Metayer et al., 2002). An experimental approach performed by Hung et al. (2007) reported that COF was capable of causing cellular destruction. Another studies from China also reported that women were at higher risk for lung cancer if they were exposed to COF emitted at high temperature, and risk were higher when fumes were not reduced by an extractor (Ko et al., 2000). Most household in Mizoram depends on combustion of biofuels, primarily wood for heating with no fume extractor, poor ventilation with roasting of soda inside houses. Soda, an alkaline preparation frequently used as food additives was significantly associated with increased risk of stomach cancer in Mizoram (Phukan et al., 2006). Perhaps roasting of soda inside houses, may enhance smokier environment forming complicated reaction with wood smoke and COF. These sources combined with foods being cooked with cooking oil and indoor tobacco smoke, often make quite smoky environment of several harmful carcinogens. Combustion of wood also contains many hazardous pollutant including benzo(a)pyrene, formaldehyde, benzene and other PAH (Lissowska et al., 2005).The association of wood use as burning fuel and lung cancer risk as observed in our study was important, because IARC (International Agency for Research on Cancer) classified biomass use (primarily wood) as a Group 2A carcinogen due to limited epidemiological evidence (IARC, 2010). Significantly, fewer studies of lung cancer in association with combustion of wood were reported. Association of combustion of wood and lung cancer in our study will support conclusion that indoor air pollution from wood combustion was a possible carcinogens (Group 2A).

We also observed a significant association for consumption of smoked fish and smoked meat with lung cancer among women in Mizoram. Increase risk of lung cancer with consumption of smoked meat and fish in present study may be due to PAH and other nitrosamines present in smoked fish (Stolyhwo and Sikorski, 2005; Viksna et al., 2008). Precise type of cooking also exert an influence, since heterocyclic amines are formed when meat was cooked at high temperatures, particularly when it is fried at high temperatures; PAH are formed during grilling leading to PAH -DNA adducts, which are known to cause mutation, ultimately cancer (Rithman et al., 1990). However lack of association of fresh and dried fish as well as meat with lung cancer in the present study warrants further studies. Present study also indicates that consumption of both egg and bamboo shoot are related to significant risk reduction for lung cancer among women in Mizoram. It is known fact that eggs are rich source of n-6 PUFA (Lewis et al., 2000) that contributes to increase risk of breast cancer (Murff et al., 2011). Thus, the same role of association of egg intake and protective effect against lung cancer has to be studied in depth to make a conclusive statement. The protective effect of bamboo shoot on lung cancer risk may be due to presence of physterol and dietary polyphenolic compounds in bamboo shoot (Gong et al., 2010; Chongtham et al., 2011). Polyphenolic compounds inhibit promotion of carcinogenesis by inhibiting oxygen radical-forming enzymes that contributes to DNA synthesis, inhibiting DNA topoisomerase II activity which further contributes to proliferative signal transduction (Galati and Brien, 2004).

As expected, our results confirmed well-established association between smoking and lung cancer. Although, result was not statistically significant for indoor smoking; risk tends to increase with collegial effect of indoor smoking with the sources of indoor environment. A distinctive pattern of tobacco consumption was observed in Mizoram. Unlike other smokeless tobacco product, a unique tobacco-infused water (locally known as Tuibur) and locally made cigarette (Meiziol) is predominant in Mizoram. Both Tuibur and Meiziol demonstrated a high risk for lung cancer development among women in Mizoram.

Present study is the first study to describe significant association of GSTM1 null genotype with lung cancer risk among women in Mizoram (Table 6). For studying the role of gene-diet interaction that might modify susceptibility of lung cancers, we also made an attempt at evaluating potential interactions of GST polymorphisms with known dietary risk factors (Table 7). Significant risk estimates were observed for interaction of GSTM1 null genotype with soda, smoked fish and smoked meat. Risk estimates were also observed for interaction of GSTT1 null genotype with soda, smoked and dried meat. However an inverse correlation was observed when GSTT1 homozygous genotype interacts with consumption of fresh fish. Present study indicates carcinogen-specific modulation of cancer risk by GST genotype and was contrary to the classical roles assumed by GST genes. Understanding the mechanism of GST polymorphisms and dietary fish meat interaction will require further studies; though we can speculate that individual with GSTM1 null genotype would suffer an increase level of toxic metabolites while an increase level of PAH were found in smoked fish and smoked meat (Stolyhwo and Sikorski, 2005; Miculis et al., 2011). To our knowledge, this is the first study that has analyzed interaction between dietary fish and meat consumption with GST polymorphisms in lung cancer risk among women. Our current knowledge of diet related carcinogenesis is still limited, so individual variability in potential relationship between dietary constituents and cancer risk markers merits further investigation.

Our study has several strengths and findings. It is a population based case control study with a high participation rates and was carried only among the ethnic tribes of Mizo women. Newly diagnosed cases were included in the present study. Newly diagnosed cases were less likely to change their habits and exposure. Intense care has been taken while collecting epidemiological data to minimize recall bias and observer bias. Major strength of our studies was that great efforts were made to quantify cooking emission exposure on the frequency and duration of cooking exposure as expressed in total cooking dish year. In addition to these, dietary and GST gene polymorphisms were also taken into account to find out for possible association with lung cancer. Our studies were the first to find out protective effect of bamboo shoot with lung cancer. Exposure to Mizo cooking style of cooking emission may pose serious health impact. Our results underscore the necessity and priority of formulating an effective strategy to modify and improve cooking practice in Mizo populations.

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