HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi: 10.12980/APJTB.4.2014APJTB-2014-0476

Genetic polymorphisms of *GSTM1*, *GSTP1* and *GSTT1* genes and lung cancer susceptibility in the Bangladeshi population

Mir Muhammad Nasir Uddin[1], Maizbha Uddin Ahmed[1], Mohammad Safiqul Islam[2], Mohammad Siddiqul Islam[1], Muhammad Shahdaat Bin Sayeed[1], Yearul Kabir[3], Abul Hasnat[1*]

[1]*Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Dhaka-1000, Bangladesh*

[2]*Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh*

[3]*Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh*

PEER REVIEW

Peer reviewer

Dr. Md. Kamrul Hossain, Professor and Chairman, Department of Pharmacy, University of Chittagong-4331, Chittagong, Bangladesh.
Tel: +880 31 2606001 4486, 621726
E-mail: mkhossain73@yahoo.com

Comments

This is an interesting study of the association of *GST* family genes and lung cancer in the Bangladeshi population as *GSTM1*, GSTTI and GSTP1 genotypes are well established risk factors for lung cancer. The interaction of GSTP1 gene and tobacco use on lung cancer risk is quite interesting in the current study. The finding about GSTP1 genotyping and it's correlation to lung cancer is also quite interesting.

Details on Page 987

ABSTRACT

Objective: To verify possible associations between polymorphisms of glutathione S-transferase Mu (*GSTM1*), glutathione S-transferase θ (*GSTT1*) and glutathione S-transferase Pi (GSTP1) genes and susceptibility to lung cancer.

Methods: A total of 106 lung cancer patients and 116 controls were enrolled in a case-control study. The *GSTM1* and *GSTT1* were analyzed using PCR while GSTP1 was analyzed using PCR-restriction fragment length polymorphism. Risk of lung cancer was estimated as odds ratio at 95% confidence interval using unconditional logistic regression models adjusting for age, sex, and tobacco use.

Results: *GSTM1* null and *GSTT1* null genotypes did not show a significant risk for developing lung cancer. A significantly elevated lung cancer risk was associated with GSTP1 heterozygous, mutant and combined heterozygous+mutant variants of rs1695. When classified by tobacco consumption status, no association with risk of lung cancer was found in case of tobacco smokers and nonsmokers carrying null and present genotypes of *GSTM1* and *GSTT1*. There is a three-fold (approximately) increase in the risk of lung cancer in case of both heterozygous (AG) and heterozygous+mutant homozygous (AG+GG) genotypes whereas there is an eight-fold increase in risk of lung cancer in cases of GG with respect to AA genotype in smokers.

Conclusions: Carrying the *GSTM1* and *GSTT1* null genotype is not a risk factor for lung cancer and GSTP1Ile105Val is associated with elevated risk of lung cancer.

KEYWORDS

Lung cancer, Glutathione S-transferase, Genetic polymorphism, Smoking

1. Introduction

Lung cancer is currently one of the most common malignant diseases and is responsible for the leading cause of cancer related deaths worldwide[1,2]. It is considered to be the leading cancer site in males and accounts for 17% of the total new cancer cases and 23% of the total cancer deaths. Among females, it was the fourth most commonly diagnosed cancer and the second leading cause of cancer death[3]. Overall five-year survival rates remain poor and are in the range of 5% to10%[4]. According to the World

*Corresponding author: Dr. Abul Hasnat, Professor, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
Tel: +88-02-9667850, Ext.8164
Fax: +880-2-8615583
E-mail: ahasnat@du.ac.bd

Foundation Project: Partially supported by the Ministry of National Science, Information and Communication Technology (NSICT) of People's Republic of Bangladesh (Grant No.: 39.012.002.01.03.018.2012-721).

Article history:
Received 29 Aug 2014
Received in revised form 9 Sep 2014
Accepted 8 Oct 2014
Available online 17 Oct 2014

Health Organization data published in April 2011, lung cancers account 1.89% of total deaths in Bangladesh. Total number of lung cancer patients aged 30 years was estimated to be 196 000 in Bangladesh[5,6]. In the USA, the lifetime chance of developing lung cancer is 1 in 13 (men) and 1 in 16 (women)[7]. Approximately, half of all newly diagnosed cases in the US screening offers former smokers[1]. Smoking is believed to be the primary cause of cancer, not only smokers but also many non-smokers including passive smokers develop lung cancer[8,9]. One of thirteen lifetime smokers develop lung cancers, implying that the differential risk for lung cancer may be explained by genetic susceptibility factors[10,11]. Polymorphism of human genes that encodes the enzymes involved in metabolic activation and detoxification of pulmonary carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines has been reported. Inter individual differences in the ability to activate and detoxify these pulmonary carcinogens are expected to affect the risk of developing lung cancer[12]. Glutathione S-transferases (GSTs) are phase II transformation enzymes involved in the detoxification of hazardous agents[13,14]. *GST* gene family encodes genes that are critical for certain life processes, as well as for detoxication and toxification mechanisms. The main role of GSTs is to detoxify xenobiotics by catalyzing the nucleophilic attack by glutathione synthetase on electrophilic carbon, sulfur, or nitrogen atoms and converts to nonpolar xenobiotic substrates, thereby preventing their interaction with crucial cellular proteins and nucleic acids[15]. Several studies performed in different populations that examined the role of genetic polymorphisms to lung cancer often showed contradictory results[16]. Glutathione S-transferase Mu (*GSTM1*), presents in human lung tissue is characterized by two active alleles *GSTM1*A, GSTM1*B* and a non-functional null allele which resulting from the entire *GSTM1* gene deletion mutation. Unlike *GSTM1*, Glutathione S-transferase θ (*GSTT1*) is polymorphic and characterized by a functional (wild) allele and a non-functional (null) allele. This null allele results from total or partial deletion of the gene. *GSTT1* gene may have a diminished ability to metabolically eliminate carcinogenic compounds. Individuals who are carriers of such genotypes may therefore be at increased cancer risk[12,17-19]. Glutathione S-transferase P1 (GSTP1) is the most predominant GSTs in lung tissue also considered to be most important in determining risk for lung cancer[20]. Four GSTP1 alleles have been recognized the wild type allele (GSTP1*A) differs by an A:G transition at nucleotide 313 (Val 105-Ala114) from GSTP1*B and from GSTP1*C by this transition and a C:T transition at 341 (Val 105 –Val 114). A GSTP1*D allele (Ile 105 –Val 114) has also been identified. Most frequently observed single nucleotide polymorphisms in GSTP1 are rs1695 (formerly rs947894 which is due to an A313G substitution resulting in an Ile105Val amino acid change) and rs1138272 (formerly rs1799811 which is due to a C341T substitution resulting in an Ala114Val amino acid change reduce catalytic activity of the enzyme[18,19,21]. It has the highest specific activity towards the active benzo(a)pyrenediol epoxide metabolite of cigarette, and is almost exclusively active towards the (+)-enantiomer of anti-benzo pyrenediol epoxide, thought to be the ultimate mutagenic form of benzo(a)pyrene[22]. Accumulation of these two to have a direct relation to lung cancer[23]. thus, individual carrying val variant expected to have lower detoxication potential and greater risk for cancer because it has generally lower activity towards polycyclic aromatic hydrocarbon diol epoxides, especially benzo pyrenediol epoxide[24]. We conducted a case-control study to investigate the association between the risk of lung cancer and *GSTM1*, *GSTT1* and GSTP1 polymorphisms for first time in Bangladesh. No study of *GSTM1*, GSTP1 and *GSTT1* as risk factors for lung cancer has been conducted on Bangladeshi lung cancer patients. A few case-control studies of *GSTM1* and *GSTT1* have been conducted in Indian subcontinent populations that make up around one-sixth of the world's population. The ancestry in Indian subcontinent is exceptional and the researchers showed that most Indian populations are genetic admixtures of two ancient, genetically divergent groups, which each contributed around 40%-60% of the DNA to most present-day populations. The researchers also found that Indian populations were much more highly subdivided than European populations but whereas European ancestry is mostly carved up by geography, Indian segregation was driven largely by caste[25]. Bangladesh which is situated in the south eastern region of Indian subcontinent supposed to have similar racial admixture and genetic diversity as populations in other part of Indian subcontinent still expected to have unique genetic characteristics.

2. Materials and methods

2.1. Study subjects and data collection

This study was conducted on 106 lung cancer patients and 116 healthy volunteers matched by age, sex and smoking status. Histologically confirmed lung cancer patients were recruited from three main cancer treatment based hospitals in Bangladesh (Ahsania Mission Cancer and General Hospital, Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University) between the period of January 2009 and December 2011. Controls were selected after physical examination by matching age, sex and smoking status to lung cancer patients. No lung cancer case had a history or evidence of any other severe diseases like cardiovascular disease, kidney disease, previous cancer, and metastasized cancer and if preset they were excluded from the study. Controls were not relatives to the patients and no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations or any acute or chronic diseases based on medical history, clinical examination and laboratory investigation (hematology, blood biochemistry and

urine analysis). Other information like smoking status, demographic characteristics, and lifestyle factors were collected through interviews by trained nurses in the presence of expert physicians. Former smokers quit for >1 year before the recruitment, current and ex-smokers were considered as ever smokers. The guidelines of International Association of Lung Cancer were followed and the patients were histologically diagnosed with lung cancer[26]. The study protocol was approved by the ethical committees of the respective hospitals and the study was conducted in accordance with the declaration of Helsinki and its subsequent revisions[27]. Patients group (n=106) and the healthy volunteers had participated in a preliminary genotyping study of Islam *et al*[5].

2.2. Genotyping

Genomic DNA was extracted from blood samples of all subjects. Three milliliters of venous blood was collected from all patients and control subjects in ethylene diamine tetraacetic acid-Na_2-containing sterile tubes and kept at 80 C until DNA extraction. Genomic DNA was extracted using Daly's chemical method[5,28]. Genotyping was performed by PCR for *GSTM1* and *GSTT1*[18,19] (Figures 1 and 2), whereas genotyping of GSTP1 was performed by PCR-restriction fragment length polymorphism (RFLP) and primers were designed from the published paper[18,19] (Figure 3). Briefly, 25 μL PCR mixture consisted of 1 μL genomic DNA samples (50–70 ng/μL), 2.5 μL of 10 standard Taq reaction buffer (with $MgCl_2$), 0.5 μL dNTPs (10 mmol/L), 0.5 μL of each primer (10 mmol/L), 0.13 μL Taq DNA polymerase (5 IU/μL) (New England Biolabs, Ipswich, MA) and 20 μL nuclease free water. PCR products of *GSTM1* and *GSTT1* were analyzed on a 2% agarose gel by staining with ethidium bromide whereas that of GSTP1 was analyzed with RFLP using the restriction enzyme *BsmAI*. The presence of one *GSTM1* allele [*GSTM1* (+)] and *GSTT1* allele [GSTT (+)] was identified by the presence of 273 bp and 459 bp PCR amplified product respectively. PCR amplified product of breast canncer 2 (BRCA2) has been used as an internal control (marker) for *GSTM1* and PCR amplified product of cytochrome 3A5*3 (CYP3A5*3) has been used as an internal control (marker) for *GSTT1*. The required primers, PCR

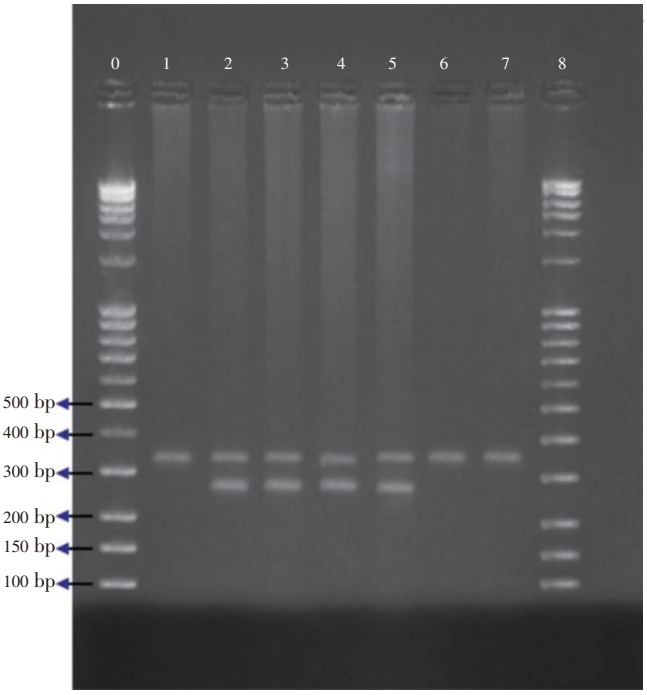

Figure 1. PCR assay for *GSTM1* gene polymorphism.
Lanes 2, 3, 4, 5: *GSTM1* positive genotype (273 bp); Lanes 1, 6, 7: *GSTM1* null genotype; Lanes 0, 8: Marker; *BRCA2* gene was used as an internal positive control (346 bp).

Table 1
Primers, PCR conditions, restriction enzymes and expected DNA fragments on digestion to genotype the selected polymorphisms.

Polymorphism	Primers	PCR condition	No. of cycles	Restriction endonuclease	Digestion condition	Digested fragments /Size of PCR products
GSTM1	FP: 5′-CTG CCC TAC TTG ATT GAT GGG-3′ RP: 5′-CTG GAT TGT AGC AGA TCA TGC-3′	94 C for 1 min 59 C for 1 min 72 C for 1 min	30	None	No digestion	273
GSTT1	FP: 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ RP: 5′-TCA CCG GAT CAT GGC CAG CA-3′	94 C for 1 min 59 C for 1 min 72 C for 1 min	35	None	No digestion	459
GSTP1	FP: 5′-ACC CCA GGG CTC TAT GGG AA-3′ RP: 5′-TGA GGG CAC AAG AAG CCC C*T*-3′	95 C for 30 seconds 56 C for 30 seconds 72 C for 1 min	35	*BsmAI*	Incubated over night with *BsmAI* at 55 C	NH (176) HE (176, 83, 93) MH (83, 93)
*CYP3A5*3*	FP: 5′-CCT GCC TTC AAT TTT TCA C*T*-3′ RP: 5′-GGT CCA AAC AGG GAA GAG G*T*-3′	94 C for 1 min 59 C for 30 seconds 72 C for 1 min	35	None	No digestion	196
BRCA2	FP: 5′-TGG AAT ACA GTG ATA CTG AC-3′ RP: 5′-TTG GAT TAC TCT TAG ATT TG-3′	94 C for 30 seconds 56 C for 30 seconds 72 C for 30 seconds	35	None	No digestion	346

NH: Normal homozygote; HE: Heterozygote; MH: Mutant homozygote.

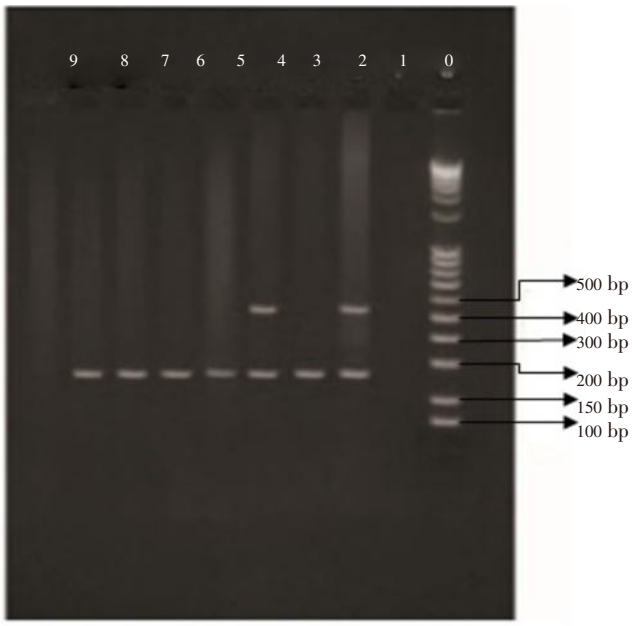

Figure 2. PCR assay for *GSTT1* gene polymorphism.
Lanes 2, 4: *GSTT1* positive genotype; Lanes 3, 5, 6, 7, 8: *GSTT1* null genotype; Lane 0: Marker; *CYP3A5*3* gene was used as an internal positive control.

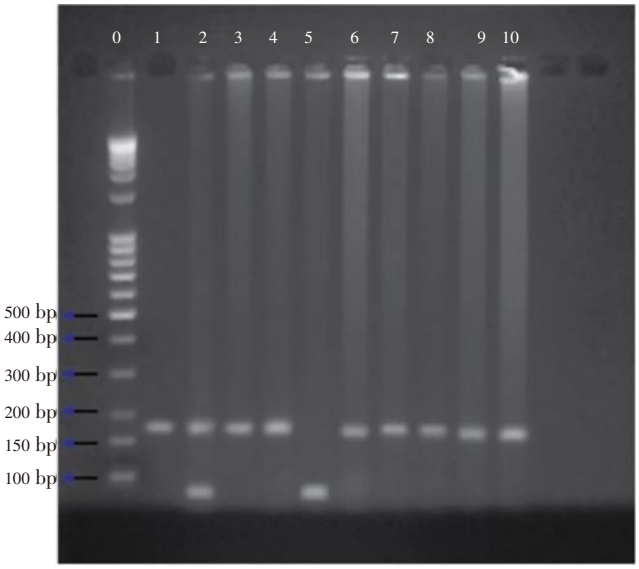

Figure 3. Gel electrophoresis of the digested PCR products showing individuals DNA for the *GSTP1* polymorphism.
Heterozygous polymorphism: Lane 2; ile/val heterozygous; Homozygous polymorphism: Lanes 1, 3, 4, 6, 7, 8, 9, 10; val/val; Mutant homozygous: Lane 5; Electrophoresis of the digested PCR products showed individuals homozygous (ile/ile) for the *GSTP1 BsmAI* polymorphism as one band of 176 bp. Heterozygous (ile/val, val) for the polymorphism resulted in three bands of 176, 91 and 85. Homozygotes mutant (val/val) showed two bands of 91 and 85 bp (which appear as one band due to close molecular size).

2.3. Statistical analysis

χ^2-tests and two-sided unpaired *t*-tests were used for comparing demographic variables, distribution of genotype between cases and controls. Unconditional logistic regression was used to estimate crude odds ratio (OR), adjusted OR and their 95% confidence intervals (*CI*s), with adjustment for age, sex and tobacco consumption status using the statistical software package SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Cases and controls characteristics

The distributions of demographic characteristics and clinical data among study subjects are summarized in Table 2. Briefly, there were no significant differences in gender (P=0.505), mean age (P=0.576) and smoking status (P=0.228) between the two groups. The histological subtypes of lung cancer were squamous cell carcinoma (43.39%), adenocarcinoma (34.91%), small cell carcinoma (18.87%), large cell carcinoma (1.89%) and adenosquamous cell carcinoma (0.94%). Current smokers had been smoking regularly and non-smokers had never smoked during his/her lifetime. Those smokers who quit for more than 1 year before the recruitment were considered as former smokers. Current and ex-smokers were considered as ever smokers. Among the ever smokers 26.42% and 18.10% were former smokers in cases and controls, respectively whereas the observed ever smoking rate was 91.51% in the cases and 89.65% in controls. No significant difference of ever smokers and never smokers (P=0.637) was found between cases and controls. The distributions of demographic characteristics, clinical data, histological subtype of lung cancer and smoking status among study subjects was also summarized in Islam *et al*[5].

Table 2
Distribution of demographic variables of the lung cancer patients and controls.

Variables		Cases (n=106)	Control (n=116)	P
Gender [n(%)]	Male	93 (87.74)	105 (90.52)	0.505[a]
	Female	13 (12.26)	11 (9.48)	
Age (years)	Mean age	57.87±10.12	58.14±9.77	0.576[b]
	Range	18-85	20-87	
Smoking status [n(%)]	Current smoker	69 (65.09)	83 (71.55)	0.637[a]
	Former smoker	28 (26.42)	21 (18.10)	
	Ever smoker	97 (91.51)	104 (89.65)	
	Never smoker	9 (8.49)	12 (10.35)	
Histological type [n(%)]	Adenocarcinoma	37 (34.91)		
	Squamous cell carcinoma	46 (43.39)		
	Small cell carcinoma	20 (18.87)		
	Large cell carcinoma	2 (1.89)		
	Adenosquamous cell carcinoma	1 (0.94)		

[a]: *Chi*-square test; [b]: Unpaired *t* test; Mean age is expressed as mean±SD.

3.2. GSTM1 and GSTT1 polymorphisms

The frequency of subjects carrying the *GSTM1* null genotype was slightly higher in the patient group (57.54%) compared with controls (56.03%). No significant difference was found between the

genotype frequency distribution of the two groups (P=0.934). Risk of lung cancer by *GSTM1* null genotype is not statistically significant (OR=1.06, 95% *CI*=0.62-1.81, P=0.820) (Table 3). Among the 106 cases 71.69% were carrying *GSTT1* null genotype, and 28.30% were *GSTT1* positive whereas among the 116 controls 75.86% were carrying *GSTT1* null genotype, and 24.13% were *GSTT1* positive. No significant difference was found between the genotype frequency distribution of the two groups (P=0.481). Risk of lung cancer by *GSTT1* null genotype is not statistically significant (Adjusted OR=0.84, 95% *CI*=0.46-1.55, P=0.573) (Table 3).

Table 3

GSTM1, *GSTP1* (rs1695), *GSTT1* genotypes among lung cancer patients and controls.

Genotypes		Cases [*n*(%)]	Control [*n*(%)]	Crude OR (95% *CI*)	*P*	Adjusted OR (95% *CI*)	*P*
GSTP1 (rs1695)	AA	67 (63.20)	101 (87.06)	1	-	-	-
	AG	31 (29.24)	13 (11.20)	3.59 (1.75-7.37)	0.001	3.57 (1.70-7.46)	0.001
	GG	8 (7.54)	2 (1.70)	6.03 (1.24-29.27)	0.019	6.58 (1.28 -33.81)	0.024
	AG+GG	39 (36.79)	15 (12.93)	3.92 (2.00-7.67)	0.000	3.96 (1.99-7.87)	0.000
GSTM1	*GSTM1*-positive	45 (42.45)	51 (43.97)	1	-	1	-
	Null genotype	61 (57.55)	65 (56.03)	1.06 (0.63-1.81)	0.820	1.02 (0.59-1.76)	0.934
GSTT1	*GSTT1*-positive	30 (28.30)	28 (24.14)	1	-	1	-
	Null genotype	76 (71.70)	88 (75.86)	0.81 (0.44-1.47)	0.481	0.84 (0.46-1.55)	0.573

NH: Normal homozygote; HE; Heterozygote; MH: Mutant homozygote; Lung cancer cases n=106; Control group n=116.

3.3. GSTP1 polymorphisms

The frequency distribution of patients obeys the Hardy-Weinberg equilibrium (χ^2=2.83, P=0.092), whereas that of controls deviates from the equilibrium (χ^2=4.29, P=0.038). The distribution of the GSTP1 genotypes was significantly different between the cases and controls (AA, AG and GG genotypes; 63.20%, 29.24% and 7.54% *vs* 87.06%, 11.20% and 1.7%, P=0.001). AG, GG and AG+GG genotypes increased the risk of lung cancer (Adjusted OR=3.56, 95% *CI*=1.70-7.46, P=0.001; adjusted OR=6.57, 95% *CI*=1.28-33.81, P=0.024; adjusted OR=3.95, 95% *CI*=1.98-7.87, P=0.0005, respectively) compared to the AA genotype.

3.4. Association between lung cancer risk and tobacco consumption

As tobacco consumption is the potential risk factors to lung cancer, we further calculated the modifying effect of *GSTM1*, *GSTT1* and GSTP1 genotypes on the association of tobacco consumption with lung cancer (Table 4). Current, ex-smokers and chewing tobacco constitutes tobacco user. Never smoker is considered as tobacco non-user. In tobacco user *GSTM1* null and *GSTT1* null genotypes do not increase the lung cancer risk significantly (OR=1.17, 95% *CI*=0.67-2.04, P=0.579; OR=0.73 95% *CI*=0.39-1.39, P=0.349) whereas AG, GG and AG+GG genotypes of GSTP1 increase the lung cancer risk significantly(OR=3.23, 95% *CI*=1.56 to 6.71, P=0.001; OR=8.71, 95% *CI*=1.02-74.14, P=0.048; OR=3.73, 95% *CI*=1.86-7.49, P=0.0005, respectively)

Table 4

Effect of *GSTM1, GSTP1* and *GSTT1* genotypes on the association of tobacco use with lung cancer.

Genotype		Patients [*n*(%)]	Control [*n*(%)]	OR (95% *CI*)	*P*
GSTP1	AA	62 (58.49)	90 (77.58)	1	
	AG	29 (27.35)	13 (11.20)	3.23 (1.56 -6.71)	0.0016
	GG	6 (5.66)	1 (0.86)	8.71 (1.02-74.14)	0.048
	AG+GG	35 (33.01)	14 (12.06)	3.73 (1.86-7.49)	0.0002
GSTM1	Present	41(38.67)	48 (41.37)	1	-
	Null	56 (52.83)	56 (48.27)	1.17 (0.67-2.04)	0.579
GSTT1	Present	28 (26.41)	24 (20.68)	1	-
	Null	69 (65.09)	80 (68.96)	0.73 (0.39-1.39)	0.349

Lung cancer cases n=106; Control group n=116; *OR and 95% CI were calculated by logistic regression and adjusted for age, gender and tobacco use.*

4. Discussion

Several molecular studies have proved few significant piece of information regarding the relationship of *GSTM1* and *GSTT1* with cancer susceptibility. First, the frequencies of homozygous *GSTM1* and *GSTT1* deletion carriers are very high (*i.e.*, 20%-50%) in most populations studied to date[18,19,29-32]. Second, *GSTM1* and possibly, *GSTT1* may be involved in the etiology of cancer at more than one site[33-36]. Third, the risk conferred to individuals who carry homozygous deletions in *GSTM1* or *GSTT1* appears to be small in magnitude (*e.g.*, OR<2). However, the magnitude of risk is larger (*e.g.*, OR=3-5) when interactions of *GSTM1* or *GSTT1* with other factors (*e.g.*, cigarette smoking) are considered[37]. In our current study we investigated three high risk genetic polymorphisms of *GSTM1*, *GSTT1* and GSTP1 as a genetic modifier of risk for individuals with lung cancer as susceptible genotypes. The mu and theta classes of *GST* isozymes (*GSTM1* and *GSTT1* respectively) have a common and broad range of substrate specificities, and they detoxify the reactive metabolites of benzo-a-pyrene and other polycyclic aromatic hydrocarbons[19,38-40]. Carriers of homozygous deletion in *GSTM1* and *GSTT1* genes have an absence of GS*T*-m and GS*T*-q enzyme activity respectively[19, 41]. These deletion variants are very useful in epidemiological studies of cancer because they divide individuals in two well-defined susceptibility classes: those who are and those who are not able to detoxify potential carcinogens by the metabolic pathways regulated by *GSTM1* and *GSTT1* genes. GSTP1 is the most abundant isoform in the lung and is also involved in response to oxidative stress[41,42]. A number of studies have tried to establish links between polymorphic expression of different GSTs and lung cancer risk in different ethnic populations and the results have been conflicting[16]. Among studies investigating *GSTM1* null genotype and risk of developing lung cancer in different populations, some of them found significantly increased risk[43-45]. In few studies it was also observed that *GSTM1* null genotypes appeared to play a protective role for cancer[46]. A meta-analysis of 11 studies found an OR of 1.6 (95% *CI*=1.26–2.04) for an association between the *GSTM1* null genotype and lung cancer risk. In another meta-analysis

study, it was reported that there was no statistically significant relationship between the individuals carrying *GSTM1* null genotype and susceptibility to lung cancer but the number of patients carrying this genotype was greater in the lung cancer group. *GSTM1* null allele in the present study is 57.54% in patient group, which is not similar to the frequencies reported in Indian subcontinent. Additionally, the rate of *GSTM1* null genotype was higher in the control group with compare to other control group in different Indian ethnic population[31,32,35,47], but results are consistent with studies conducted in Indian subcontinent which did not show any significant association between *GSTM1* null genotype and lung cancer risk. The incidence of the *GSTT1* null allele differs among global populations[31,48]. Significant differences in *GSTT1* null allele frequencies were observed between Caucasian, Asian, African and African American populations[30,31,48]. The prevalence of *GSTT1* null allele in the present study in cases is 71.69%, which is not similar to the frequencies reported in Indian subcontinent[30-32,35,47,48]even our result is not consistent with studies conducted in Indian subcontinent[29,30]. Among world population, Korean population showed higher frequency of *GSTT1* null allele compared with the white Americans, African Americans, Mexican-Americans and Turkish populations[18,30-31,48]. Although, some researchers reported a significant increase on lung cancer risk with *GSTT1* null genotype in various populations[29]. In our study, we found no significant relation between *GSTM1* and GTTT1 null genotype and susceptibility to lung cancer. Our results are in sequence with previous case-control studies and a meta-analysis study which observed no significant relationship between *GSTT1* deletion and lung cancer for Caucasians[18,49]. Earlier studies carried out in different populations which analyzed GSTP1 exon 5 polymorphism and lung cancer risk did not expose any significant association[49]. These apparently indicate that lack of involvement between GSTP1 exon 5 genotypes and the risk of developing lung cancer might be steady. Our findings suggest that GSTP1 exon 5 polymorphism (Ile105Val) is associated with increase risk in developing overall lung cancer and statistical analysis also found significance to support such finding. Thus, our results are in line up with the study of Wang *et al.* which found a significant increase in lung cancer risk with the GSTP1 exon 5 polymorphism. GSTP1 exon 5 polymorphism (Ile105Val) results inactive proteins with different enzyme activity. GSTP1 enzymes with 105Val allele showed decreased *GST* detoxification capacity resulting in an increased concentration of carcinogens in their lung tissue[19]. Individuals with the 105Val allele have a higher risk of developing lung cancer than Individuals with the 105Ile allele.

The present work thus provides probably the first study of this nature from Bangladesh. We believe that further investigation of *GSTM1*, *GSTT1* and GSTP1 allelic variants in Bangladesh should provide useful information for identification of founder mutations and ethnic predisposition alleles that results various cancerous disease phenotypes.

Our observations showed that carrying the *GSTM1* and *GSTT1* null genotype is not a risk factor alone for lung cancer. Our findings also suggest that GSTP1 exon 5 polymorphism (Ile105Val) is associated with high risk of lung cancer and especially in tobacco users. As other *GST* polymorphisms play important overlapping roles in detoxifying tobacco carcinogens and because risk might be associated with these polymorphisms, further larger populations studies of risk associated with multiple polymorphisms are needed to fully understand the genetic interactions underlying risk susceptibility. Large scale multicenter studies are necessary to obtain more reliable and correct results.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The research project was partially supported by the Ministry of National Science, Information and Communication Technology of People's Republic of Bangladesh (Grant No.: 39.012.002.01.03.018.2012-721). The authors have no other relevant affiliations or financial involvement with any organization. The authors thank physicians and nurses of Ahsania Mission Cancer Hospital, Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh for their technical assistance during blood collection and patient counseling.

Comments

Background

Lung cancer is a major cause of cancer-related death in the developed countries and the overall survival rate has still an extremely poor. The study of genetic polymorphisms has touched every aspect of pulmonary and critical care medicine. The very nature of such studies promises to help in defining pathophysiologic mechanisms, to identify individuals who are at risk for developing disease and to suggest novel targets for drug treatment.

Research frontiers

The present research work depicts role of *GSTM1*, *GSTT1* and GSTP1 polymorphisms as susceptible genotypes for lung cancer especially in relation to tobacco use. The *GSTM1* and *GSTT1* were analyzed using PCR while GSTP1 was analyzed by using PCR and also by RFLP.

Related reports

The relationship between *GSTM1* polymorphism and lung cancer has already been observed in two large studies from Japan (Hayashi *et al.*, 1992; Kiyohara *et al.*, 2002b) and two from China. The prevalence of *GSTT1* null allele in the present study is 72%, which is also not similar to the frequencies reported in Indian subcontinent (Konwar *et al.*, 2010). Genetic polymorphism of GSTP1 has been observed in studies of Anttila *et al.*, 1993 and Hayes *et al.*, 1995.

Innovations and breakthroughs

A few case-control studies have been conducted in Indian sub-continent but the results are quite conflicting. In the present study the prevalence of genetic polymorphism in GSTP1, *GSTM1* and *GSTT1* genes and their association with risk to lung cancer has been

observed. This is the first of its kind in Bangladesh.

Applications

It is possible that genetic susceptibility to lung cancer may in part be determined by the genetic factors associated with inter-individual variations in carcinogen metabolizing enzymes. This article points out criteria that should be applied to design large scale multicenter studies.

Peer review

This is an interesting study of the association of *GST* family genes and lung cancer in the Bangladeshi population as *GSTM1*, GSTTI and GSTP1 genotypes are well established risk factors for lung cancer. The interaction of GSTP1 gene and tobacco use on lung cancer risk is quite interesting in the current study. The finding about GSTP1 genotyping and it's correlation to lung cancer is also quite interesting.

References

[1] Tota JE, Ramanakumar AV, Franco EL. Lung cancer screening: review and performance comparison under different risk scenarios. *Lung* 2014; **192**(1): 55-63.

[2] Haws L Jr, Haws BT. Aerodigestive cancers: lung cancer. *FP Essent* 2014; **424**: 32-47.

[3] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**(2): 69-90.

[4] Van Meerbeeck JP, Fennell DA, De Ruysscher DK. Small-cell lung cancer. *Lancet* 2011; **378**(9804): 1741-1755.

[5] Islam MS, Ahmed MU, Sayeed MS, Maruf AA, Mostofa AG, Hussain SM, *et al*. Lung cancer risk in relation to nicotinic acetylcholine receptor, *CYP2A6* and *CYP1A1* genotypes in the Bangladeshi population. *Clin Chim Acta* 2013; **416**: 11-19.

[6] Palipudi KM, Sinha DN, Choudhury S, Zaman MM, Asma S, Andes L, *et al*. Predictors of tobacco smoking and smokeless tobacco use among adults in Bangladesh. *Indian J Cancer* 2012; **49**(4): 387-392.

[7] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**(5): 277-300.

[8] Lam TK, Ruczinski I, Helzlsouer KJ, Shugart YY, Caulfield LE, Alberg AJ. Cruciferous vegetable intake and lung cancer risk: a nested case-control study matched on cigarette smoking. *Cancer Epidemiol Biomarkers Prev* 2010; **19**(10): 2534-2540.

[9] Tang L, Zirpoli GR, Jayaprakash V, Reid ME, McCann SE, Nwogu CE, *et al*. Cruciferous vegetable intake is inversely associated with lung cancer risk among smokers: a case-control study. *BMC Cancer* 2010; **10**: 162.

[10] DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, *et al*. Cancer treatment and survivorship statistics, 2014. *CA Cancer J Clin* 2014; **64**(4): 252-271.

[11] Risch A, Plass C. Lung cancer epigenetics and genetics. *Int J Cancer* 2008; **123**(1): 1-7.

[12] López-Cima MF, Alvarez-Avellón SM, Pascual T, Fernández-Somoano A, Tardón A. Genetic polymorphisms in *CYP1A1*, *GSTM1*, *GSTP1* and *GSTT1* metabolic genes and risk of lung cancer in Asturias. *BMC Cancer* 2012; **12**: 433.

[13] Shukla RK, Kant S, Mittal B, Bhattacharya S. Polymorphism of cytochrome p450, glutathione-s-transferase and N-acetyltransferases: influence on lung cancer susceptibility. *Niger J Med* 2010; **19**(3): 257-263.

[14] Dekant W. The role of biotransformation and bioactivation in toxicity. *EXS* 2009; **99**: 57-86.

[15] Josephy PD. Genetic variations in human glutathione transferase enzymes: significance for pharmacology and toxicology. *Hum Genomics Proteomics* 2010; doi: 10.4061/2010/876940.

[16] Altinisik J, Balta ZB, Aydin G, Ulutin T, Buyru N. Investigation of glutathione S-transferase M1 and T1 deletions in lung cancer. *Mol Biol Rep* 2010; **37**(1): 263-267.

[17] Dzian A, Halasova E, Matakova T, Kavcova E, Smolar M, Dobrota D, *et al*. Lung adenocarcinoma and squamous cell carcinoma in association with genetic polymorphisms of GSTs in Slovak population. *Neoplasma* 2012; **59**(2): 160-167.

[18] Ada AO, Kunak SC, Hancer F, Soydas E, Alpar S, Gulhan M, *et al.* Association between *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and lung cancer risk in a Turkish population. *Mol Biol Rep* 2012; **39**(5): 5985-5993.

[19] Ramzy MM, Solliman MEDM, Abdel-Hafiz HA, Salah R. Genetic polymorphism of *GSTM1* and *GSTP1* in lung cancer in Egypt. *Int J Collab Res Intern Med Public Health* 2011; **3**: 41-51.

[20] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005; **45**: 51-88.

[21] Harris MJ, Coggan M, Langton L, Wilson SR, Board PG. Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. *Pharmacogenetics* 1998; **8**(1): 27-31.

[22] Robertson IG, Guthenberg C, Mannervik B, Jernström B. Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7 beta,8 alpha-dihydroxy-9 alpha,10 alpha-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. *Cancer Res* 1986; **46**(5): 2220-2224.

[23] Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science* 1996; **274**(5286): 430-432.

[24] Coles B, Yang M, Lang NP, Kadlubar FF. Expression of *hGSTP1* alleles in human lung and catalytic activity of the native protein variants towards 1-chloro-2,4-dinitrobenzene, 4-vinylpyridine and (+)-anti benzo[a]pyrene-7,8-diol-9,10-oxide. *Cancer Lett* 2000; **156**(2): 167-175.

[25] Reich D, Thangaraj K, Patterson N, Price AL, Singh L. Reconstructing Indian population history. *Nature* 2009; **461**(7263): 489-494.

[26] Travis WD. Pathology of lung cancer. *Clin Chest Med* 2011; **32**(4): 669-692.

[27] [The Helsinki Declaration of the World Medical Association (WMA). Ethical principles of medical research involving human subjects]. *Pol Merkur Lekarski* 2014; **36**(215): 298-301. Polish.

[28] Daly AK, Monkman SC, Smart J, Steward A, Cholerton S. Analysis of cytochrome P450 polymorphisms. *Methods Mol Biol* 1998; **107**: 405-422.

[29] Yang X, Qiu MT, Hu JW, Wang XX, Jiang F, Yin R, *et al. GSTT1* null genotype contributes to lung cancer risk in Asian populations: a meta-analysis of 23 studies. *PLoS One* 2013; **8**(4): e62181.

[30] Sharma A, Pandey A, Sardana S, Sehgal A, Sharma JK. Genetic polymorphisms of *GSTM1* and *GSTT1* genes in Delhi and comparison with other Indian and global populations. *Asian Pac J Cancer Prev* 2012; **13**(11): 5647-5652.

[31] Kumar A, Yadav A, Giri SK, Dev K, Gulati S, Gautam SK, *et al.* Allelic variation of *GSTM1* and *GSTT1* genes in Haryana population. *Genomic Med Biomark Health Sci 2012*; **4**(3): 98-102.

[32] Senthilkumar KP, Thirumurugan R. *GSTM1* and *GSTT1* allele frequencies among various Indian and non-Indian ethnic groups. *Asian Pac J Cancer Prev* 2012; **13**(12): 6263-6267.

[33] Zhang BL, Sun T, Zhang BN, Zheng S, Lü N, Xu BH, *et al.* Polymorphisms of *GSTP1* is associated with differences of chemotherapy response and toxicity in breast cancer. *Chin Med J* 2011; **124**(2): 199-204.

[34] Safarinejad MR, Shafiei N, Safarinejad SH. Glutathione S-transferase gene polymorphisms (*GSTM1*, *GSTT1*, *GSTP1*) and prostate cancer: a case-control study in Tehran, Iran. *Prostate Cancer Prostatic Dis* 2011; **14**(2): 105-113.

[35] Konwar R, Manchanda PK, Chaudhary P, Nayak VL, Singh V, Bid HK. Glutathione S-transferase (*GST*) gene variants and risk of benign prostatic hyperplasia: a report in a North Indian population. *Asian Pac J Cancer Prev* 2010; **11**: 1067-1072.

[36] Sailaja K, Surekha D, Rao DN, Rao DR, Vishnupriya S. Association of the *GSTP1* gene (Ile105Val) polymorphism with chronic myeloid leukemia. *Asian Pac J Cancer Prev* 2010; **11**(2): 461-464.

[37] Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1997; **6**(9): 733-743.

[38] Fedets' OM. [Structure and functions of glutathione transferases]. *Ukr Biokhim Zh* 2014; 86(3): 23-32. Ukrainian.

[39] Board PG, Menon D. Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim Biophys Acta* 2013; **1830**(5): 3267-3288.

[40] Wu B, Dong D. Human cytosolic glutathione transferases: structure, function, and drug discovery. *Trends Pharmacol Sci* 2012; **33**(12): 656-668.

[41] Zimniak P, Nanduri B, Pikula S, Bandorowicz-Pikula J, Singhal SS, Srivastava SK, *et al.* Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *Eur J Biochem* 1994; **224**(3): 893-899.

[42] Manevich Y, Hutchens S, Tew KD, Townsend DM. Allelic variants of glutathione S-transferase P1-1 differentially mediate the peroxidase function of peroxiredoxin VI and alter membrane lipid peroxidation. *Free Radic Biol Med* 2013; **54**: 62-70.

[43] Liu D, Wang F, Wang Q, Guo X, Xu H, Wang W, *et al.* Association of glutathione S-transferase M1 polymorphisms and lung cancer risk in a Chinese population. *Clin Chim Acta* 2012; **414**: 188-190.

[44] Liu X, Li Z, Zhang Z, Zhang W, Li W, Xiao Z, *et al.* Meta-analysis of *GSTM1* null genotype and lung cancer risk in Asians. *Med Sci Monit* 2014; **20**: 1239-1245.

[45] Langevin SM, Ioannidis JP, Vineis P, Taioli E, Genetic Susceptibility to Environmental Carcinogens group (GSEC). Assessment of cumulative evidence for the association between glutathione S-transferase polymorphisms and lung cancer: application of the Venice interim guidelines. *Pharmacogenet Genomics* 2010; **20**(10): 586-597.

[46] Yadav DS, Devi TR, Ihsan R, Mishra AK, Kaushal M, Chauhan PS, *et al.* Polymorphisms of glutathione-S-transferase genes and the risk of aerodigestive tract cancers in the Northeast Indian population. *Genet Test Mol Biomarkers* 2010; **14**(5): 715-723.

[47] Vettriselvi V, Vijayalakshmi K, Solomon FDP, Venkatachalam P. Genetic variation of *GSTM1*, *GSTT1* and *GSTP1* genes in a South Indian population. *Asian Pac J Cancer Prev* 2006; **7**(2): 325-328.

[48] Piacentini S, Polimanti R, Porreca F, Martínez-Labarga C, De Stefano GF, Fuciarelli M. *GSTT1* and *GSTM1* gene polymorphisms in European and African populations. *Mol Biol Rep* 2011; **38**(2): 1225-1230.

[49] Honma HN, De Capitani EM, Perroud MW Jr, Barbeiro AS, Toro IF, Costa DB, *et al.* Influence of p53 codon 72 exon 4, *GSTM1*, *GSTT1* and *GSTP1*B* polymorphisms in lung cancer risk in a Brazilian population. *Lung Cancer* 2008; **61**(2): 152-162.