RESEARCH COMMUNICATION

Genetic Polymorphism of Drug Metabolizing Enzymes (CYP2E1, GSTP1) and Susceptibility to Bladder Cancer in North India

Rama D Mittal, Daya Shankar L Srivastava, Mandhani A, Mittal B

Abstract

Glutathione-S-transferases (GSTs) are active in the detoxification of wide variety of endogenous or exogenous carcinogens and genetic polymorphisms of CYP2E1 and GSTP1 genes have been studied extensively to evaluate the relative risk of various cancers. In the present study, we examined associations with CYP2E1 and GSTP1 gene polymorphisms in sporadic bladder cancers from North Indian patients. The subjects were 106 bladder cancer (Ca-B) cases and 162 age-matched controls. The GSTP1 313 A/G polymorphism was determined by the PCR/RFLP method using peripheral blood DNA. Binary Logistic Regression Model was used for assessing differences in genotype prevalence and their associations between patient and the control group. We observed a non-significant association in Pst1 polymorphism of the CYP2E1 gene; though the A/G genotype (OR = 2.69, 95% CI=1.57- 4.59, P= 0.000) and G/G genotype (OR = 7.68, 95% CI=2.77- 21.26, P= 0.000) of the GSTP1 gene polymorphism alone or in combination with tobacco users were highly significant (OR=24.06; 95% CI: 4.80- 120.42; P =0.000) when compared to the controls. The results of our study demonstrated that the GSTP1 313 G/G polymorphism is a strong predisposing risk factor for bladder cancer in the North Indian population.

Key Words: GSTP1 313 A/G - CYP2E1 Pst1 - bladder cancers - environmental carcinogens - restriction fragment length polymorphisms (RFLPs).

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Introduction

The great majority of chemical carcinogens are incapable of producing DNA damage without activation by enzymes (such as Cytochrome P450) in order to become active for tumor initiation (Perera et al.,1997). Conversely phase II metabolizing enzymes, such as glutathione S-transferases (GSTs), epoxide hydroxylase and sulfotransferases are involved in detoxification of chemical carcinogen and subsequently their role is expected to be protective (Weber et al.,1997). Exposure to tobacco smoke and other environmental and occupational chemicals has been described as a risk factor for bladder cancer, with 2-napthylamine, benzidine and 4-aminobiphenyl now established as bladder carcinogens (Silverman et al., 1999). Genetic differences in the metabolism of these chemicals have been recently suggested to modify individual susceptibility to environmentally induced bladder cancer.

Cytochrome P4502E1 (CYP2E1) is a member of the cytochrome P-450 super family which is involved in the metabolic activation of many low molecular weight compounds, such as N-nitrosamines, aniline, vinyl chloride and urethane (Bartsch and Montsano, 1984; Guengerichi et al., 1991). N-nitrosamines present in tobacco and the diet are well recognized as etiological agents involved in cancer development in various sites. Functional CYP2E1 gene polymorphisms might therefore have an impact on susceptibility to cancer induction. The gene encoding the human CYP2E1 isoenzyme has been localized to chromosome 10. A number of polymorphisms have been detected in the gene, the best-studied being the Pst1/ Rsa1 polymorphism in the 5'flanking region and the Dra1 polymorphism in intron 6 . The restriction fragment length polymorphisms for Pst1 ($_{-1259}$ G change to A) and Rsa1 ($_{1019}$C change to T) appear to change the transcriptional activation of the gene (Hayashi et al. 1991).

Cytosolic GSTs are a family of related isozymes that catalyze the conjugation of reduced glutathione to a wide

Department of Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow-226014, Uttar Pradesh, India
Corresponding Author: Dr. Rama Devi Mittal, Additional Professor (Biochemistry), Department of Urology, SGPGIMS, Raebareli Road, Lucknow-226014, India Phone No: 091-522-2668004-8, Ext. 2116 Fax No: 091-522-2668 017 E-mail: rmittal@sgpgi.ac.in ramamittal@yahoo.com*

range of electrophilic substrates (Mannervik et al.1985). Among its polymorphisms, most extensively studied are the GSTM1 null, the GSTP1 313 A/G substitution and the GSTT1 null genotypes. The GSTP1 313 A/G polymorphism at the nucleotide level leads to an amino acid substitution of isoleucine / valine at codon 105 in the protein. Valine results in decreased enzyme activity (Ali-Osman et al.1997). Increase in GSTP1 313A/G or G/G genotype by 1.75 fold in a Turkish group of bladder cancer ptients has been reported by Toruner et al (2001).

Whether genetic variants can help explain part of the large differences in bladder cancer risk between populations needs clarification. In the present study, we therefore determined the genotypic frequencies of the CYP2E1 Pst1 and GSTP1 313 A/G gene polymorphisms in bladder cancer patients and controls in a North Indian population.

Materials and Methods

Subjects

The study group consisted of 106 bladder cancer patients mean age (62.5±10.2); all had transitional cell carcinomas; 32% were grade I tumors, 20 % were grade II and 48 % were grade III tumors); 162 healthy individuals mean age (54.5±13.9) were selected as controls. The ethnic origin of the cases and controls were same. Criteria for the patient selection were based on a questionnaire covering medical, pathological, and histo-pathological records from the outpatient department of Sanjay Gandhi postgraduate Institute of Medical Science, Lucknow from December 2001- December 2003. The inclusion criteria for the controls included absence of prior history of cancer or pre-cancerous lesions. The consumption of tobacco in any form (cigarette/ bidi smoking, chewing tobacco in betel leaf or gutka etc.) in both groups was noted through a detailed questionnaire.

DNA Extraction and Genotyping

Five ml of blood was collected in EDTA vials from cases and controls. DNA was extracted from blood lymphocytes using the proteinase K and phenol chloroform extraction procedure (Sambrook et al.1989). The 5‘- transcription regulatory region of CYP2E1 that includes the Pst1 restriction site of the gene was analyzed by a previously described method (Hayashi et al. 1991) with the primers:
Forward 5‘CCAGTCGAGTCTACATTGTCA 3’
Reverse 5‘ TTCATTCTGTCTTCTAACTGG 3’

The PCR product of 412 bp was digested with Pst1 overnight at 37 ˚C and subjected to 1.5% agarose gel electrophoresis. Presence of a restriction site resulted in only two fragments (122 bp and 290 bp) indicating a mutant allele and in the case of heterozygotes three fragments of 412bp, 122 bp and 290 bp (see Figure 1).

The A313G polymorphism of GSTP1 was analyzed as described by Harries et al (1997). Briefly, amplification was performed using the primers:
Forward, 5’-ACCCCAGGGCTCTATGGGAA-3’
Reverse, 5’-TGAGGGCACAA GAAGCCCCT-3’.

The 176-bp amplified product was digested with Alw261 and eletrophoresed on 3% agarose gels. Presence of a restriction site resulting in only two fragments (91bp and 85bp) indicated mutant alleles (G/G), and if a A/G polymorphism then three fragments of 176bp, 91bp and 85bp (see Figure 2).

Statistical Analysis

Statistical analysis was conducted with SPSS software 10.00. The Binary Logistic Regression Model (BLRM) was applied to assess differences in genotype prevalence and associations between case and control groups. The Chi square and Fisher exact tests were employed for habit strata (Tobacco users/ non-users). Correlation coefficients, odds ratios (ORs) and 95% confidence intervals (CIs) were used to describe the strengths of associations. A P-value <0.05 (two-tailed) was considered as statistically significant for the study.

Results

The genotype distributions in controls were in agreement with Hardy-Weinberg Equilibrium. GSTP1 genotypes in bladder cancer patients and the healthy controls are presented in Table1. In the control samples, frequency of GSTP1 A genotype present in homozygous state (A/A) was (57.6%), while the G genotype homozygous condition (G/G) was observed in 3.5%; the remaining 38.9% were heterozygous (A/G). We observed a significant association with the A/G

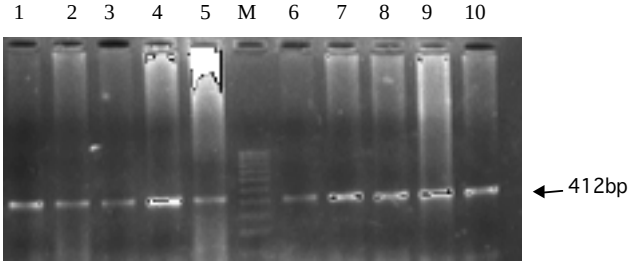

Figure 1. PCR Products from Amplification of CYP2E1 Gene Analyzed on a 1.5% Agarose Gel
Lane M- 100 bp ladder; Lanes1-10 CYP2E1 undigested BC

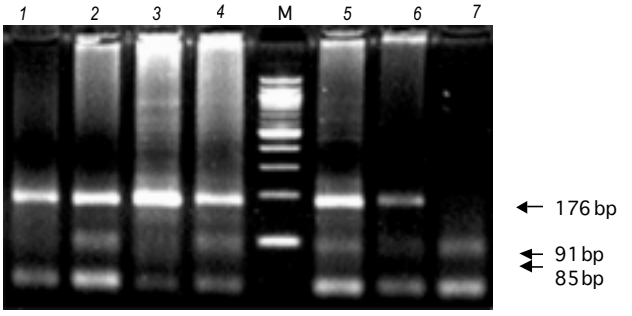

Figure 2. PCR Products from Amplification of the GSTP1 Gene Visualized on a 3.5% Agarose Gel
Lane M- 100 bp ladder, Lane-1, and 3 shows Ile/Ile genotype (176bp); lanes-2, 4, 5 and 6 Ile/Val genotype and lane-7 the Val/ Val genotype (91bp and 85bp).

Table 1. Distribution of CYP2E1 and GSTP1 Genotypes Among Bladder Cancer Patients and Controls

Genotype	Controls	Patients (Ca-B)	P-value	OR (95% CI)
CYP2E1	n=50	n= 50		
Wild	50 (100 %)	50 (100%)		1.0
Heterozygotes	0 (0 %)	0 (0%)	NS	NA
Mutant	0 (0 %)	0 (0%)	NS	NA
GSTP1	n= 162	n= 106		
Wild	95 (58.64 %)	33 (31.13%)		1.0
Heterozygotes	61 (37.65 %)	57 (53.77%)	0.000	2.69 (1.57- 4.59)
Mutant	6 (3.71 %)	16(15.10%)	0.000	7.68 (2.77- 21.26)

Table 2. Association of Tobacco Habit with GSTP1 Genotypes Among Bladder Cancer Patients and Controls

Habit	Genotype	Patients (Ca-B) N= 106	Controls N= 162	P-value	OR (95% CI)
Non-users	GSTP1				
	Wild	16 (25.40%)	77 (64.17%)	1.0	
	Heterozygotes	41 (65.08%)	39 (32.50 %)	0.001	5.06 (1.51- 4.08)
	Mutant	6 (9.52 %)	4 (3.33%)	0.005	1.83 (0.37- 5.64)
Tobacco-users	GSTP1				
	Wild	17 (39.53 %)	18 (42.86%)	0.001	4.55 (1.94- 10.68)
	Heterozygotes	16 (37.21 %)	22 (52.38 %)	0.003	3.5 (1.51- 8.10)
	Mutant	10 (23.26 %)	2 (4.76%)	0.000	24.06 (4.80- 120.42)

genotype (OR = 2.69, 95% CI=1.57- 4.59, P= 0.000) and G/G genotype (OR = 7.68, 95% CI=2.77- 21.26, P= 0.000) of the GSTP1 gene as compared to the controls. However, no association was observed with Pst1 polymorphism in CYP2E1 gene in north Indian population (Table 1).

An attempt was made to evaluate the association of GSTP1 genotypes with the risk of bladder cancer in tobacco users (Table 2). Taking the A/A genotype of GSTP1 of non-users as the reference we observed a significant association for the G allele of the GSTP1 genotype with tobacco users (OR=24.06; 95% CI: 4.80- 120.42; P =0.000) as compared to the controls. Since no effect of the polymorphism was observed with CYP2E1, any gene association with tobacco users was not determined.

Discussion

The polymorphic site of CYP2E1 gene was analyzed using the restriction enzyme Pst1 and the frequency distribution was the same in both cases and controls so that no association could be established with susceptibility to bladder cancer. Some investigators (Kato et al.1992) reported Pst1/Rsa1 polymorphism at a higher frequency in Japanese (25%) and another study in Chinese population where (19%) as compared to the Caucasians (African) and Americans (1- 4%). In a Japanese study, individuals homozygous for the variant DraI allele of CYP2E1 were reported to be decreased specially among the group with a high cumulative smoking dose (Uematsu et al. 1994). However, homozygosity for the RsaI allele was suggested to pose an increased risk of lung cancer in a Swedish study (Persson et al. 1997), while in Taiwanese this allele was associated with increased risk of nasopharyngeal carcinoma (Hildesheim et al. 1997).

We observed an increased risk for bladder cancer associated with the GSTP1 A/G or G/G polymorphism, in accordance with findings in Turkish (Tourner et al.2001) and British patients (Harries et al.1997), where significant increase in the risk of bladder cancer was observed for GSTP1 A/G or G/G, although Steinhoff et al (2000) in Germany and Katoh et al (1999) in Japan found no association. The present study indicates that polymorphic GSTP1 locus may be an important factor for genetic susceptibility to bladder cancer, persons having the G allele having lower potential for detoxification of carcinogenic compounds.

In the present study, we also evaluated the GSTP1 genotype polymorphism in smokers and non-smokers and observed a significant association with the G allele in tobacco users (p= >0.05) in our population. Tourner et al. 2001, in their Turkish population, reported a trend for association with G allele of GSTP1 genotypes with smoking status, and a significant association (>2 fold) emerged when combination effect of the null allele of GSTM1 and G allele of GSTP1 genotypes was assessed. Bladder cancer is a malignancy in which, in addition to genetic status of the individuals, gene environment interactions are thought to play an important role. Smoking is one of the important environmental risk factors (Lang and Pelkonnen et al 1999, Autrup et al, 2000).

In addtion to their impact on detoxification, GSTs also may modulate the induction of other enzymes and proteins important for cellular functions such as DNA repair. Hence, GSTP1 is important for maintaining cellular genomic integrity and as a result play an important role in cancer susceptibility (Pelkonnen et al 1999). They are known to be

involved in the deactivation of oxidative metabolites of exogenous or endogenous carcinogenic agents (industrial chemicals, dietary compound, tobacco products, drugs and environmental carcinogens etc.) that are probably associated with bladder cancer risk (Silverman et al.1999.).

To the best of our knowledge, this is the first genetic study on the association of GSTP1 with bladder cancer in the North Indian population. Our study provided evidence that the G allele of GSTP1 is a strong predisposing risk factor alone or in combination with tobacco use.

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