RESEARCH ARTICLE

GSTP1 Gene Ile105Val Polymorphism Causes an Elevated Risk for Bladder Carcinogenesis in Smokers

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Abstract

Background: The glutathione S transferase (GST) family of enzymes plays a vital role in the phase II biotransformation of environmental carcinogens, pollutants, drugs and other xenobiotics. GSTs are polymorphic and polymorphisms in GST genes have been associated with cancer susceptibility and prognosis. *GSTP1* is associated with risk of various cancers including bladder cancer. A case control study was conducted to determine the genotype distribution of *GSTP1* A>G SNP, to elucidate the possible role of this SNP as a risk factor in urinary bladder cancer (UBC) development and to examine its correlation with clinico-pathologic variables inUBC cases. Materials and Methods: Using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, we tested the genotype distribution of 180 bladder cancer patients in comparison with 210 cancer-free controls from the same geographical region with matched frequency in age and gender. Results: We did not observe significant genotype differences between the control and bladder cancer patients overall with an odds ratio (OR)=1.23 (p>0.05). The rare allele (AG+GG) was found to be present more in cases (28.3%) than in controls (24%), though the association was not significant (p<0.05). However, a significant risk of more than 2-fold was found for the variant allele (AG+GG) with smokers in cases as compared to controls (p>0.05). Conclusions: Thus, it is evident from our study that *GSTP1* SNP is not implicated overall in bladder cancer, but that the rare, valine-related allele is connected with higher susceptibility to bladder cancer in smokers and also males.

Keywords: GST genes - SNP - polymorphic - bladder cancer - prognosis

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Introduction

Urinary bladder cancer ranks ninth in worldwide cancer incidence. It is the seventh most common malignancy in men and the 17th most common in women (Ploeg et al., 2009). Bladder cancer ranks as the seventh leading cancer and accounts for 5.90% of all prevalent cancers in a Kashmiri population (Arshad et al., 2012). Bladder cancer has been associated classically with exogenous and environmental risk factors, which primarily include smoking and occupational exposure (Clavel et al., 2007; Clayson et al., 2008).

Single nucleotide polymorphisms and inherited loss of both alleles are common in these gene superfamilies with varying frequencies among different populations (Senthilkumar and Thirumurugan, 2012; Sharma et al., 2012; Zhou et al., 2012). Genetic variations results in altered function or loss in activity of the corresponding enzymes with impaired cellular detoxification which makes the individual susceptible to cancer. Since activities of phase I and phase II enzymes are affected by genetic variations, the polymorphic enzyme variants in metabolic pathways are supposed to be responsible for the difference in cancer development risk between different individuals.

The glutathione S transferase (GST) family of enzymes play a vital role in the phase II biotransformation of environmental carcinogens, pollutants, drugs and other xenobiotics. GSTs are polymorphic and the polymorphisms in GST genes have been associated with cancer susceptibility and prognosis. Moreover, distinct ethnic differences have been observed in the type and frequency of GST gene polymorphisms. *GSTP1* have been associated with increased risk of various cancers including bladder cancer. The glutathione S-transferases (GST), a superfamily of phase II metabolic enzymes play an important role in the cellular mechanism of detoxification by conjugating reactive electrophilic compounds with soluble glutathione (Strange et al., 2000). GST enzymes are thus involved in the metabolism of xenobiotics that include environmental carcinogens, reactive oxygen species and chemotherapeutic agents (Hayes et al., 1995). A large number of structurally diverse xenobiotics are

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known to be substrates for the GSTs. Some substrates have relatively high specific activity with one or a few isoenzymes within a class and little or no activity within other classes (Whalen et al., 1998). In addition, these enzymes are also believed to play a crucial role in the protection of DNA from oxidative damage.

At least eight distinct classes of soluble GSTs that are highly expressed in the mammalian liver have been identified, which include alpha, mu, pi, sigma, theta, kappa, zeta and omega. In humans, hereditary differences in some GST enzyme activities are due to genetic polymorphisms. Polymorphism has been described in many genes in these families though to date, more attention has been focused on alleles in the mu, theta and pi families (Strange et al., 2000).

GSTP1 is a major enzyme involved in the inactivation of cigarette smoke carcinogens, such as benzo [a] pyrene diol epoxide, and other toxic constituents, such as acrolein (Hayes et al., 1995). *GSTP1* has a polymorphic site at codon 105 (exon 5), where an adenosine-to-guanosine (A-G) transition at nucleotide 313 results in an Ile-to-Val substitution (Ile105Val) in the substrate-binding site of *GSTP1*. The substitution of the less bulkier and more hydrophobic valine results in substrate-dependent alterations of *GSTP1* catalytic activity (Ali-Osmam et al., 1997). Since the polymorphisms of these metabolizing genes influence the detoxifying action, they have been suggested to play an important role in cancer susceptibility and prognosis. Allelic variants of *GSTM1*, *GSTT1*, and *GSTP1* have been associated with increased risk of various cancers like colorectal, lung, breast, prostate (Vijayalakshmi et al., 2005) and Bladder cancer. The allelic and genotypic variations have been observed in different populations and ethnic groups in various parts of the world.

Keeping in view the good frequency of bladder cancer and lack of any study regarding the polymorphism of *GSTP1* A>G, we designed a study to address the disease pathology associated with the bladder cancer in Kashmir Valley.

Materials and Methods

Subjects

The study population comprised of 180 bladder cancer patients and 210 healthy controls from Kashmir region and blood samples were collected at the Urology ward and clinics in Sher-I-Kashmir Institute of Medical Sciences. All the cases were histologically confirmed to be transitional cell carcinoma of the bladder, except for one case of adenocarcinoma. The pool of control subjects were recruited from the same hospital and belonged to the same geographic area, ethnic background, and approximately similar age group. The controls did not have a previous diagnosis of any type of cancer and were frequency-matched to the cases in terms of age and ethnicity. A written informed consent was obtained from each recruited subject, and the study was approved by the local Institutional Ethical Committee. Cases and controls went through a face-to-face interview during hospital admission using standard questionnaires. Of the 180 individuals, 36 (20%) were females and 144 (80%) were males. The patients group included, histological grading as 12 (6.66%) with G-I, 93 (51.66%) were with G-II and 75 (41.66%) as GIII/GIV of cancer. Moreover 57 (31.66%) were having stage pTa, 69 (38.33%) were pT1 stage and 54 (30%) were at pT2 stage or higher.

DNA extraction and genotyping

The *GSTP1* Ile/Val polymorphism was determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP) (Vijayalakshmi et al., 2005). Genomic DNA was isolated from whole blood by the salting out method (Miller et al., 1988). Genotypes of *GSTP1* were determined by PCR. PCR reaction was performed in a total 25μl reaction volume containing 50-100ng of genomic DNA, 1× PCR buffer (1.5mM $MgCl_2$,10 mM Tris (pH 9.0), 50mM KCl and 0.1% Triton X-100), 200μM dNTPs (Biotools, B & M Labs, Madrid, Spain), 50pM of each primer and 1 U of *Taq* DNA polymerase (Biotools, B & M Labs, Madrid, Spain). The primers used for amplification of *GSTP1* were forward 5´-ACCCCAGGGCTCTATGGGAA-3´ and reverse primer 5´-TGAG GGCACAAGAAGCCCCT-3´. The cycling condition were 95ºC for 5min of one cycle; 94º C for 30 sec, 55º C for 30 sec and 72º C for 30sec for 30 cycles and final elongation cycle of 72ºC for 5min. The PCR products were visualized by 2% agarose electrophoresis. The PCR amplicon of 176 bp was subjected to restriction digestion using *Alw26I* restriction enzyme (Fermentas Inc., MD) at 37˚C for 2-4 hour and the DNA bands were resolved by electrophoresis on a 3% agarose gel. The genotypes were determined based on the band pattern. The Ile allele was resistant to digestion by *Alw26I* and so the Ile/Ile genotype resulted in an undigested band of 176 bp, the Val allele with *Alw26I* site resulted in two fragments of 91bp and 85 bp. So an Ile/Val genotype was characterized by three fragments of 176bp, 91 bp and 85 bp. 20% of samples were repeated for reproducibility of results

Statistical analysis

The distribution of the allele and genotype frequencies of *GSTP1* was determined. Chi square test was applied to compare the allelic frequencies in different population using the SPSS (version 13) software programme for windows to determine p-Value which was considered significant as $p>0.05$.

Results

Overall 390 blood samples (180 cases and 210 controls) were successfully evaluated for *GSTP1* A>G polymorphism whose characteristics are given in Table 1. *GSTP1* gene Ile105Val genotype distributions of wild- and variant-type alleles among different clinico-pathologic characteristics are shown among the Kashmiri ethnic population for the cases (Table 2). The genotype frequencies observed in cases and controls were in Hardy-Weinberg equilibrium. In *GSTP1* A>G SNP, frequencies of AA (Ile/Ile), AG (Ile/Val), and GG (Val/Val) genotypes among controls were 76.0%, 22.0% , and 02.0 % while in

cases genotype frequencies were 71.6%, 25.0% and 3.4% respectively, with odds ratio (OR) 1.3 and 95% confidence interval (CI) 0.56-2.6, and this difference was statistically insignificant (p>0.05 Table 3). The rare allele (AG+GG) was found to be present more in cases (28.33%) than in controls(24%), though the association was not significant (p<0.05). We also found a significant risk of more than 2.5 fold of the variant allele (AG+GG) with smokers in cases as compared to controls (p<0.05). Stratified analysis of the *GSTP1* variation based on sex revealed that the male group showed a significant association with OR=5.09, CI (1.57-16.5) and p<0.05 (Table 2). The allele frequency for Ile (A) is 0.84 in cases versus 0.87 in controls and allele Val (G) is 0.16 versus 0.13 respectively indicating higher frequency of rare (G) alleles in cancer patients as compared to controls but the association was not significant (Table3).

For further classification into disease pathology, our study found a reverse relation in the distribution of variant genotype (AG+GG) with higher frequency in superficial tumor low stage (pTa) (36.8% versus 27.7%: Table 2) and high-grade (G-III/IV) (32% versus 25%:Table 2) in bladder cancer cases though the association was insignificant (p>0.05).

Discussion

Studies suggest that polymorphism in glutathione transferases are associated with cancer susceptibility and polymorphism at the *GSTP1* locus may be of particular importance, especially in view of the almost ubiquitous expression of this protein in a wide range of different cell types including lung (Ryberg et al., 1997), colon (Welfare et al., 1999), and breast cancer (Helzlsouer et al., 1999). In addition, this protein is often over-expressed in tumour cells made resistant to anticancer drugs. This raises the possibility that the polymorphism may also be important in the outcome of anticancer drug therapy.

The *GSTP1* A313G polymorphism is implicated in wide range of human cancers including bladder cancer (Katoch et al., 1999). Genetic polymorphisms are known to play a role on cancer susceptibility, and their role in bladder cancer is being largely evaluated. Since this polymorphism has been reported from this region bladder cancer, further evaluation was imperative, to elucidate the conformity of the results in the backdrop of different ethnic backgrounds. Thus, we conducted a case-control polymorphic study of *GSTP1* A>G to assess the role of these SNP in bladder cancer patients from the Kashmir region (North India).

The frequencies of the A/A, A/G, and G/G genotypes in the patient group were 71.66%, 25.0% and 3.33%, while as frequency in control group were 76.0%, 22.0%, and 2.0%, respectively. This distribution of the *GSTP1* A313G genotypes among two groups showed no statistical difference (p>0.05). Our report is in accordance with the study conducted in Germans (Steinhoff et al., 2000) and in Japanese population (Katoch et al., 1999) who observed no association for *GSTP1* A/G or G/G genotype with susceptibility to bladder cancer but in contrast to reports by Ryberg et al. (1997) from Caucasians and Coa et al. (2005).

Our study is in accordance to study conducted in south Indian population (Vettriselvi et al., 2006), the *GSTP1* allelic frequency was nearly similar for the A allele (0.78 versus 0.87 in our study) and 0.22 versus 0.13 (our study) for the G allele with similarity in the genotype frequency also.

Table 1. Distribution Analysis of Selected Demographic and Risk Factors in Bladder Cancer Cases and Controls

Characteristics		Cases	Controls	p value
Age	≤50	54 (30)	72 (35)	0.6
	>50	126 (70)	138 (65)	
Sex	Male	144 (80)	171 (81.4)	0.17
	Female	36 (20)	39 (18.9)	
Dwelling	Rural	138 (77)	138 (65)	0.17
	Urban	42 (23)	72 (35)	
Smoking status	Never	36 (20)	54 (25.7)	0
	Ever	144 (80)	156 (74.3)	
Mean age, years (±SD)		58.5 (12.3)	56.6 (11.6)	0.52
Mean no. of years smoking (±SD)		28.6 (14.0)	21.0 (14.7)	0.01

Table 3. Genotypic and Allelic Frequencies among Cases and Controls

Genotype	Controls N=210	Cases N=180	Odds Ratio	95% CI	p value
AA	159 (76.0%)	129 (71.6%)	1.00 (ref)		
AG	48 (22.0%)	45 (25.0%)	1.3	0.56-2.6	0.5
GG	03 (02.0%)	06 (3.4%)	2.6	0.22-28.47	0.2
A	366 (0.87)	303 (0.84)	1.00 (ref)		
G	54 (0.13)	57 (0.16)	1.28	0.58-2.8	0.2

Table 2. Genotypic Distribution between *GSTP1 Ile 105 Val SNP* and Clinic-pathologic Characteristics

Characteristics		Cases (%)	AA 71.66%	AG+GG 28.33%	Controls (%)	AA 76.00%	AG+GG 24.00%	OR (95%CI)	p value
Overall genotype		n=180	129	51	n=210	159	51	1.23 (0.56-2.68)	0.3
Age	≤50	54 (30)	42 (77.7)	12 (22.2)	72 (35)	60 (83.3)	12 (16.66)	1.43 (0.46-4.42)	0.6
	>50	126 (70)	87 (69.0)	39 (30.9)	138 (65)	117 (84.8)	21 (15.2)	2.50 (0.89-6.98)	0
Sex	Male	162 (90)	117 (72.2)	45 (27.7)	171 (81.1)	159 (92.9)	12 (7.01)	5.09 (1.57-16.5)	0
	Female	18 (10)	12 (66.6)	06 (33.3)	39 (18.9)	30 (76.9)	09 (23.1)	1.67 (0.2-14.05)	0.6
Dwelling	Rural	138 (77)	102 (73.9)	36 (26.0)	132 (65)	117 (84.8)	21 (15.21)	1.97 (0.07-5.52)	0.2
	Urban	42 (23)	27 (64.3)	15 (35.7)	72 (35)	60 (83.3)	12 (16.6)	2.78 (0.60-12.84)	0.1
Smoking status	Never	36 (20)	26 (72.3)	10 (27.7)	54 (25.7)	40 (74.1)	14 (25.9)	1.1 (0.5-2.6)	0.8
	Ever	144 (80)	105 (72.9)	39 (27.0)	156 (74.3)	135 (86.6)	21 (13.4)	2.5 (1.4-4.2)	0.003
Histological type	GI	12 (6.66)	09 (75)	03 (25)				Ref	
	GII	93 (51.6)	69 (74.2)	24 (25.8)				1.04 (0.09-11.44)	0.97
	GIII/IV	75 (41.6)	51 (68)	24 (32)				1.4 (0.13- 15.58)	0.77
Clinical stage	pTa	57 (31.6)	36 (63.1)	21 (36.84)				Ref	
	pT1	69 (38.3)	54 (78.3)	15 (21.73)				0.47 (0.012-1.81)	0.28
	pT2 or more	54 (30.0)	39 (72)	15 (27.77)				0.66 (0.16-2.64)	0.56

Aggregate variant genotype frequencies (AG+GG) found in cases was 28.33% as compared to 24% in controls which depicts variant allele frequency is implicated more in bladder cancer cases than in controls. This perhaps predisposes the patients to slightly increased risk of bladder cancer (OR=1.23, p>0.05). The heterozygote frequency shows inconsistency in the previous studies depicting it either as protective in bladder cancer (Kannika et al., 2009) or as a risk factor (Srivastava et al., 2005). The conflicts are possibly accounted to the difference of environmental pollutants, industrial chemicals, dietary, and smoking behavior.

This study shows that the risk factors for bladder carcinoma are smoking and older age. We found ever smokers with a 2.5 fold more risk of bladder cancer with twice higher heterozygote frequency in cases than in controls (27% versus 13.4%: p>0.05). Similar sort of results are shared by many studies confirming smoking as risk factor in *GSTP1* A>G polymorphism (Törüner 2001; Rama et al., 2005). Significant association of bladder carcinoma increased with patients >50 years of age with OR 2.50 (0.89-6.98), consistent with other studies (Brauers et al., 2000: Coa et al., 2005).

We also noticed that the variant allele (AG+GG) genotypes were frequently present in cases with rare genotypic ratio of 27.7% versus 7.01% in controls (p<0.05) showing a positive significance with bladder cancer and this may be possibly due to over exposure of males to smoking and various occupational exposures predisposing them to higher risk of bladder cancer. When disease pathology was analysed with genotypes of *GSTP1* A>G polymorphism, we could not find any association with either grade or stage of bladder cancer, but interestingly a reverse relation was observed in the distribution patterns of genotypes. Variant genotypes on one hand were implicated more in lower stage of the disease but on the other hand were observed highly in higher grades. The relation of the *GSTP1* A>G SNP with other clinic-pathological characteristics is shown in Table 2

In conclusion, our report suggests that *GSTP1* SNP is not implicated in bladder cancer patients in our population, but that the rare, valine-related allele is connected with higher susceptibility to bladder cancer in smokers and also males.

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