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Association between Asn142Asp Genetic Polymorphism of GSTO2 and Susceptibility to Bladder Cancer

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Abstract

Background The glutathione-S-transferases (GSTs) comprise a class of enzymes that detoxify carcinogenic compounds by conjugating glutathione to facilitate their removal. Polymorphisms in glutathione S-transferase Omega 1,2 (GSTO1, GSTO2), and GSTP1 genes have been related to risk for bladder cancer.

Objective To assess a comprehensive picture of the relationship between smoking and GSTO2 gene Asn142Asp variant (rs156697) with bladder cancer

Methods A case control study was conducted at Chemistry and Biochemistry Department, College of Medicine and DNA Research and Training Center, Al-Nahrain University from February 2014 to September 2014. Forty one bladder cancer patients and 41 age matched apparently healthy controls were participated in this study. Genotyping of the GSTO2 Asn142Asp polymorphism was evaluated using a polymerase chain reaction fragment length polymorphism (PCR-RFLP) method. The odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of the combined effect of cigarette smoking and the GSTO2 Asn142Asp polymorphism on bladder cancer risk.

Result It was found that subject with the GSTO2 Asp/Asp genotype have significantly increased bladder cancer risk (OR 4.92; 95% CI =1.32 - 18.30). A statistically highly significant increased the bladder cancer risk was also found in ever smoker of the GSTO2 (Asn/Asn) (OR =11.8; 95% CI=2.43 - 57.84) and (Asn/Asp +Asp/Asp) (OR =12.8; 95% CI=3.23 - 51.41) compared with never smoker Ala/Ala genotype.

Conclusion The study suggests that smokers having GSTO2 Asn/142Asp polymorphism could play an important role as risk factor for the development with bladder cancer.

Keywords Bladder cancer, single nucleotide polymorphism, glutathione S-transferase, GSTO2, Asn142Asp, smoking, rs156697.

List of Abbreviation: GST = Glutathione transferases, GSH = glutathione, GSTO2 = glutathione S-transferase Omega 2, ROS = reactive oxygen species.

Introduction

Glutathione transferases (GST) are detoxification enzymes that play a role in the conjugation of endogenous or exogenous xenobiotic toxins to glutathione (GSH); however several GSTs function as GSH peroxidases [(1)].

Human cytosolic GST super family contains at least 16 genes subdivided into eight distinct classes designated as: Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta [(2,3)].

GSTs catalyze the conjugation of GSH to a wide variety of endogenous and exogenous electrophilic compound [(4)].

Unlike other GSTs, glutathione S-transferase Omega (GSTO) has an active site cysteine that is able to form a disulfide bond with GSH and

exhibits glutathione dependent thiol-transferase and dehydroascorbate reductase activities, reminiscent of thioredoxin and glutaredoxin enzymes [5].

Human Omega class GST contains two expressed gene hGSTO1 and hGSTO2 [6]. The hGSTO1 and hGSTO2 are 12.5 and 24.5 kb, respectively, and lie 7.5 kb apart on chromosome 10q24.3, between the markers D10S603 and D10S597.

Three polymorphisms in hGSTO genes: hGSTO1*Ala140Asp, hGSTO1 and hGSTO2*Asn142Asp have been identified in ethnic groups [7] but their relationship with bladder cancer are not yet fully understood.

Recently studies reported that carcinogens in the cigarettes like hydrocarbons, polycyclic aromatic, aromatic amines and N-nitroso compounds could be one of the major causes of bladder cancer [8].

Tobacco smoking has been identified as a major lifestyle risk factor for developing transitional cell carcinoma (TCC). It has been estimated that around 50% of all TCC cases can be attributed to tobacco smoking, with considerable variation in groups of former and current smokers [9,10].

Chemicals and carcinogens contained in cigarettes require the detoxification by phase II enzymes like Glutathione-S-transferases, this leads to formation of less toxic and more hydrophilic derivatives, which is more readily excreted.

However, the deficiency in detoxification-related enzymes generates oxidized products including reactive oxygen species (ROS) which can cause DNA damage and the accumulation of genetic mutations [11].

hGSTO2*Asn142Asp is a single nucleotide polymorphism of GSTO2 gene causing variations in enzyme activity and may influence individual susceptibility to bladder cancer [12].

This study was aimed to investigate the joint effect of smoking on GSTO2 Asn142Asp polymorphism susceptibility in patient with bladder cancer.

Methods

This case control study was approved by the Ethical Committee of College of Medicine Al-Nahrain University, Baghdad, Iraq. The study was carried out during the period from (February 2014 to September 2014). It included 82 subjects, 41 subjects (30 males, 11 females) with bladder cancer mean age ± SD (60.95 ± 10.74) and 41 subjects, (23 males and 18 female) healthy volunteers (control) mean age ± SD (59.35 ± 9.914).

This study was conducted at Chemistry and Biochemistry Department, College of Medicine Al-Nahrain University and Forensic DNA Research and Training Center, Al-Nahrain University, Baghdad.

All patient were first diagnosed with bladder cancer and investigated by urologist and underwent cystoscopy examination for transurethral resection of bladder tumor or underwent cystoscopy with biopsy of bladder lesion for histopathological examination.

Urine cytology was requested for all patients to detect the presence of bladder cancer in all patients

The patients were recruited at Gazi Al-Harery Hospital for Specialized Surgery. The main exclusion criteria were as follows: subjects with history of urinary tract infection, bladder stones, benign bladder tumor and prostate cancer.

All participants provided informed consents and then were interviewed by a well-trained interviewer using a structured questionnaire to collect information including a history of cigarette smoking.

Study subjects who had smoked more than 100 cigarettes during their lifetime were regarded as ever smokers, while those who had smoked less than 100 cigarettes were defined as never smokers.

Genomic DNA was isolated from the whole fresh blood sample using the Geneid Genomic DNA Mini Kit Vogelstein [13]. The DNA was made into aliquot and store at -60^{o}C for future use.

Genotyping was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, GSTO2 Asn142Asp polymorphism was determined by PCRRFLP according to Marahatta et al. [14].

Primer sequence ->3')

Forward primer: (5'AGG CAG AAC AGG AAC TGG AA 3')

Reverse primer: (5'GAG GGA CCC CTT TTT GTA CC3')

The PCR conditions were obtained by making an optimization using multiple samples and multiple annealing temperatures.

PCR conditions were: one cycle at 95 ^{o}C for 5 min; 35 cycles of 95 ^{o}C for 30 sec, 58 ^{o}C for 30 sec and 72 ^{o}C for 45 sec, and a final extension at 72 ^{o}C for 10 min.

The amplified PCR product was 181 bp and visualized by electrophoresis in a 2% agarose gel as shown in fig. 1.

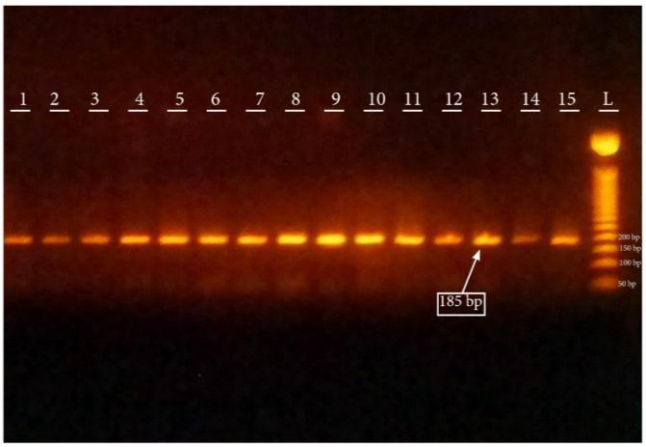

Fig. 1. PCR product for the of GSTO2 rs156697 polymorphism using Promega master mix on 1% agarose, 70V, and for 60 minute (7 µl of DNA loaded in each well). Lane L: 50 bp Ladder, Lane 1-15: PCR product

After the complete digestion with the restriction enzyme MobI at 37 ^{o}C for 18 h, the resulting DNA fragments which represented the Ala142Asp polymorphism of GSTO1 gene were analyzed by electrophoresis in a 3% agarose gels as shown in fig. 2.

To ensure quality, a random 10% of the samples were genotyped repeatedly.

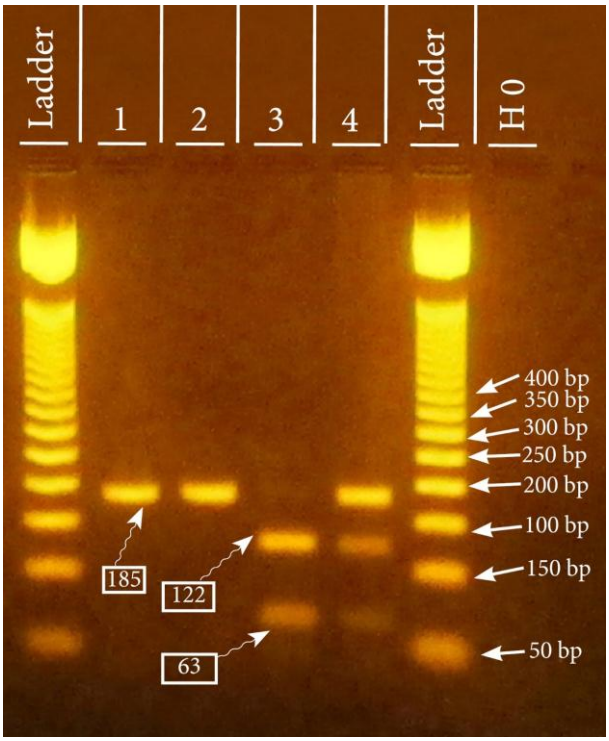

Fig. 2. Restriction digestion of PCR products of GSTO2 rs156697 polymorphism demonstrated the patterns of digestion in different genotypes of GSTO1 rs4925 polymorphism in 3% agarose, 70V, and for hour (7 µl of DNA loaded in each well). Lane L: 50 bp Ladder, Lane 1 and 2 show 1 band (185); Asn/Asn Genotype, Lane 3 show 2 bands (63 and 122); Asp/Asp Genotype, Lane 4 show 3 bands (68,122 and 185); Asn/Asp Genotype

Statistical Analysis

The data of the study were stored in Microsoft excel spread sheet and analyzed on the computer using (IBM SPSS Advanced Statistics 20.0 software) and Microsoft excel program (2013). Numeric variables were expressed as mean ± SD. Students t-test was used for comparison of mean between two groups. Chi-square test was used to compare frequency. Chi- square test was performed to determine if the control samples demonstrated Hardy-

Weinberg equilibrium for GSTO2 polymorphism. Logistic regression was used to calculate ORs and 95% CI for bladder cancer risk associated with the genetic polymorphisms of GSTs as well as the joint effects of cigarette smoking and the GSTO2 Asn142Asp polymorphisms on bladder cancer risk.

Results

The Basic characteristics for bladder cancer patients and controls are shown in table 1. There were no significant differences between age, weight, height and body mass index ($P > 0.05$) between bladder cancer patient and control.

Table 1. Basic characteristics of study groups

Feature	Patient group N = 41	Control group N = 41	*P* value
Age (year)	60.95 ± 10.74	61.0 ± 9.91	0.47
Weight (Kg)	74.42 ± 12.0	75.0 ± 9.9	0.113
Height (Cm)	169.5 ± 7.1	169.0 ± 6.9	0.06
BMI (Kg/m^2)	26.27 ± 4.46	26.0 ±3.7	0.12

Table 2 shows that there were no significant differences ($P > 0.05$) in the distribution of age between bladder cancer patients and control.

On the other hand a highly significant increased ($P < 0.001$) bladder cancer risk was notice in ever smokers subjects (OR=7.44; 95%CI =2.79- 19.75).

Table 2. Demographic characteristics of the patients and controls

Variable		Patient group (n=41)	Control group (n=41)	OR	95% CI	*P* value
Age	< 55	9 (21.95)	14 (34.14)	1		
	55-65	20 (48.78)	17 (41.46)	1.83	0.63-5.27	0.26
	> 65	12 (29.26)	10 (24.39)	1.03	0.57-6.10	0.30
Gender	Female	11 (26.82)	18 (43.90)	1		
	Male	30 (73.1)	23 (56.09)	1.6	0.84-5.3	0.1086
Cigarette smoking	Never	11 (26.82)	30 (73.17)	1		
	Ever	30 (73.1)	11 (26.82)	7.44	2.79- 19.75	0.0001

GSTO2 Asn142Asp polymorphism distribution of the observed genotype frequencies among control group was consistent with Hardy–Weinberg equilibrium (HWE), ($P = 0.4$). The gene frequency of Asn142 in control group was 0.73 and was 0.27 for allele Asp142, in bladder cancer patients gene frequency of Asn142 was 0.52 and for Asp142 was 0.47.

Table 3. GSTO2 rs156697 polymorphism Genotypes and Allele frequency among patients and control groups

GSTO2	Genotype, n (%)		Gene frequency				*P* value
	No.	Asn142/Asn142	Asn142/Asp142	Asp142/Asp142	Asn142	Asp142	
Control	41	23 (56.09)	14 (34.14)	4 (9.75)	0.73	0.27	0.4
Patients	41	14 (34.14)	15 (36.58)	12 (29.26)	0.52	0.47	0.08

As shown in table 4, study subjects who carried the Asp/Asp genotypes of GSTO2 gene had a significantly higher BC risk of 4.92 (95% CI = 1.32 - 18.30) comparing to individuals who carried the Asn/Asn genotype (P = 0.01), While a non-significant higher BC risk 1.7 (95% CI = 0.65 – 4.71) was found in subject carried the Asn/Asp comparing to individuals who carried the Asn/Asn genotype. Furthermore there was a significantly higher BC risk of 2.46 (95% CI = 1.0 - 6.0) in subjects who carried the combination of Asp/Asp and Asn/Asp genotypes of GSTO2 gene when compared to individuals who carried the Asn/Asn genotype (*P* = 0.04).

Table 4. Distribution of GSTO2 Asn142Asp polymorphism in patients and control groups

GSTO2 genotype	Patient group No. (%)	Control group No. (%)	OR	95% CI	P value
Asn/Asn	14 (34.14)	23 (56.09)			
Asn/Asp	15 (36.58)	14 (34.14)	1.76	0.65- 4.71	0.26
Asp/Asp	12 (29.26)	4 (9.75)	4.92	1.32 - 18.30	0.01
Asn/Asn	14 (34.14)	23 (56.09)			
Asn/Asp+Asp/Asp	27 (56.85)	18 (43.90)	2.46	1.0 to 6.0	0.04

To Find the Joint effect of cigarette smoking and the Asn142Asp polymorphism of GSTO2 gene on the development of bladder cancer

As shown in table 5, comparing with never smoking who carried Asn/Asn genotype of the GSTO1 gene as a reference group, it was found a non-significant higher BC risk (OR = 3.4; 95% CI = 0.84 - 14.16) in never smokers who carried Asp/Asp and Asn/Asp genotypes of the GSTO1 gene; a very high significant bladder cancer risk in ever smoking group carried Asp/Asp and Asn/Asp genotypes of 12.8 (95% CI = 3.23 – 51.41) (*P* = 0.0003); and a very high significant bladder cancer risk in ever smoking group carried Asn/Asn genotypes of 11.8 (95% CI = 3.23 – 51.41) (*P* = 0.0022).

Table 5. Combined effect of GSTO2 Asn142Asp polymorphism and cigarette smoking on bladder cancer risk

Smoking	GSTO2 genotype	Patient group	Control group	OR	95% CI	P value
Never	AsnAsn	4 (9.75)	19 (46.34)	1		
	AsnAsp+AspAsp	8 (19.51)	11 (26.82)	3.4	0.84 - 14.16	0.0851
Ever	AsnAsn	10 (24.39)	4 (9.75)	11.8	2.43 - 57.84	0.0022
	AsnAsp+AspAsp	19 (46.34)	7 (17.07)	12.8	3.23 - 51.41	0.0003

Discussion

In the present study we have shown that mutant heterozygous GSTO2 Asn142Asp or homozygous mutant GSTO2 Asp142Asp genotypes are associated with development bladder cancer. GSTO2 as genetic markers may have a prognostic or pharmacogenomics role in patients with muscle invasive bladder cancer [15]. Recently, many studies have investigated the association between single nucleotide polymorphisms on glutathione S-transferases and susceptibility to bladder cancer [19]. previous studies described that cigarette smoking can induce Single-nucleotide polymorphisms in the detoxification enzymes like GSTs, which may cause a substitution in amino acid leading to a changing in the activity

of these enzymes and affecting the biological metabolism [7,12].

The combined effect of polymorphisms in GSTO2/rs156697, gene on the risk of bladder cancer was studied as well as the joint effect of smoking and polymorphisms on bladder cancer risk.

In this study, it was found a highly significant increased (P < 0.001) bladder cancer risk in ever smokers cancer patients (OR=7.44; 95%CI =2.79- 19.75) (as shown in Table 2) these results were in consistent with previous studies that showed that ever smokers had a significantly increased risk of bladder cancer [10,16].

The GSTO2 enzymes are encoded by the omega class glutathione S-transferase (GST). GSTO2 widely expressed in all tissues, it has been seen in liver, kidney, skeletal, muscle with lower expression in the heart and high levels in testis [17].

GSTs are involved in the metabolism of xenobiotics and carcinogens, in human; the GSTO2 is polymorphic with an Asn142Asp substitution in the coding region [18].

Previous study reported that the GSTO2 Asn142Asp polymorphism may have an effect on individual susceptibility to many multifactorial diseases, however Marahatta reported that there is no association between GSTO2 Asn142Asp polymorphism and the risk of the hepatocellular carcinoma, cholangiocarcinoma, colorectal cancer and breast cancer as well [14].

Recently, a study demonstrated that GSTO2 polymorphism may significantly increase cancer risk in Caucasian population and is associated with elevated risk of breast cancer [12]; other study shows that GSTO2 could use as genetic markers may have a prognostic or pharmacogenomics role in patients with muscle invasive bladder cancer [19].

In this study, the gene frequency of GSTO2 Asp142 in healthy control was 0.27 as shown in table 3. The gene frequency of GSTO2 ASP142 has been reported, 0.31 among European Australians in Canberra, 0.86 among Bantu Africans in Durban, and 0.27 among Chinese from Hong Kong [19].

The present study revealed that the control group exhibited GSTO2 ASP142 gene frequency, which was similar to Chinese, reflecting resemblance in Asian population; however the allele frequency of Asp142 in total bladder cancer was 0.47 and was higher than the control group.

Moreover, there was higher significant bladder cancer risk of 4.92 (95% CI = 1.32 - 18.30) for subjects who carried the Asp/Asp genotypes of GSTO2 comparing to individuals who carried the Asn/Asn genotype (P = 0.01).

Furthermore, there is a significantly higher BC risk of 2.46 (95% CI = 1.0 - 6.0) in the subjects who carried the combination of Asp/Asp and Asn/Asp genotypes of GSTO2 gene when compared to individuals who carried the Asn/Asn genotype (P = 0.04).

GSTO2 Asn142Asp creates a non-conservative amino acid change from uncharged polar to acidic amino acid.

It is unfortunate that the insolubility of GSTO2 has prevented its characterization and comparison with GSTO1 Pervious study reported that the GSTO2 142Asp (142Asp) variant allozyme showed 20% reduction in level of expression compared with the level of the GSTO2 wild type (142Asn) allozyme [20].

The effect of tobacco smoking with formation of the Asn142Asp genotype GSTO2 polymorphism, we find A very high significant bladder cancer risk in ever smoking subjects carring Asp/Asp and Ala/Asp genotypes of 12.8, (P = 0.0003) and a very high significant bladder cancer risk of 11.8 in ever smoking subject who carring Ala/Ala genotypes, (P = 0.0022).

In conclusion, GSTO2 rs156697, polymorphism is associated with bladder cancer risk but the gene-environmental factor (GSTO2-smoking) may increase the bladder cancer risk.

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Author contribution

Literatures survey, manuscript preparation by Mr. Mahmood; manuscript writing and editing by Dr. Abdul-Rasheed; sample choosing and collection by Dr. Al-Nasiri; and genetic studies by Dr. Al-Awadi and Al-Zubaidi.

Declaration of interest

The author declare no conflict of interest.

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