The Egyptian Journal of Medical Human Genetics (2017) 18, 41–45

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Ain Shams University

The Egyptian Journal of Medical Human Genetics

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ORIGINAL ARTICLE

Genotypes of GSTM1 and GSTT1: Useful determinants for clinical outcome of bladder cancer in Pakistani population

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Received 10 February 2016; accepted 6 March 2016
Available online 28 April 2016

KEYWORDS

GSTM1;
GSTT1;
Bladder cancer;
PCR;
Polymorphism

Abstract *Background:* Incidence of bladder cancer has increased rapidly worldwide in the past few years. Environmental as well as genetic factors are involved in the etiology of bladder cancer. Glutathione S transferase mu 1 (GSTM1) and glutathione S transferase theta 1 (GSTT1) genes are two xenobiotic metabolizing genes in phase II of detoxification process.

Aim: The current study was aimed to find out the association of different environmental factors and *GSTM1* and *GSTT1* gene polymorphisms with susceptibility to bladder cancer in Pakistani population.

Method: Bladder cancer cases (236) and control blood samples (270) were screened using phenol chloroform method of DNA extraction followed by multiplex PCR.

Results: With respect to age; bladder cancer was more prevalent in age >60 years and low grade tumors were more frequent than high grade tumors. Smokers had a significantly higher incidence rate of cancer; also family history of cancer was found to be strongly associated ($P < 0.05$) with bladder cancer. Commonly reported symptoms by the patients of bladder cancer were hematuria, lower urinary tract symptoms (LUTS) and flank pain. A larger number of patients had undergone surgery, chemotherapy and radiotherapy. Similarly *GSTM1* (OR 2.24; CI 1.5–3.2; $P = 0.0001$) and *GSTT1* (OR 2.9; CI 1.4–6.1; $P = 0.002$) gene deletion showed a highly significant association with bladder cancer. Simultaneous deletions of both *GSTM1* and *GSTT1* genes also showed highly significant association (OR 5.3; CI 2.1–13.1; $P = 0.0001$) with cancer risk. No association was found when both of the two genes deletion was compared with bladder cancer among smokers.

Conclusion: This study suggests that *GSTM1* and *GSTT1* gene polymorphisms may be associated with increased susceptibility toward bladder cancer in Pakistani population.

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Abbreviations: GSTs, glutathione S transferases; GSTM1, glutathione S transferase mu 1; GSTT1, glutathione S transferase theta 1; BC, bladder cancer; LUTS, lower urinary tract symptoms; PCR, polymerase chain reaction; IRB & EC, Institutional Review Board and Ethics Committee; OR, odds ratio; CI, confidence interval; NORI, National Oncology and Radiotherapy Institute; EDTA, ethylenediaminetetraacetic acid

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2016.03.001
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1. Introduction

The incidence rate of bladder cancer is higher in men as compared to women and increases with age [1]. Smoking is the major environmental factor involved in the development of urinary bladder cancer. Cigarette smokers have four times greater risk of onset of bladder cancer as compared to non-smokers. More than 50% cases of bladder cancer have a history of smoking. In Pakistan smoking, fried items (fried chicken, fried potatoes, samosay, pakoray, cutlets, cheese fritters, roasted beef etc.) and sedentary life style up-regulated bladder cancer development while consumption of fruits and vegetables together with increased uptake of fluids down-regulated urinary bladder carcinoma [2]. Regarding symptoms of bladder cancer, about 85% patients have painless hematuria as a major problem. Other commonly reported problems are vesical irritability, frequency urgency and dysuria as well as three p's (PPP) i.e., Profuse, Periodic and Painless hematuria [3]. Bladder cancer can be muscle invasive or noninvasive and classified as high grade or low grade tumor respectively [4].

Bladder cancer development involves a complex interplay of chemical, environmental and genetic factors. Different metabolic enzymes have been encoded by glutathione S transferases super family and play an important role in the mechanism of cellular detoxification. They catalyze different reactions with bladder cancer carcinogens like amino biphenyls and polycyclic aromatic hydrocarbons [5]. A number of studies have been conducted to find out the association of GSTs with bladder cancer. Both *GSTM1* and *GSTT1* genes exhibit homozygous deletion polymorphisms which results in null genotypes and the enzymatic activity is completely lost. The frequency of null genotypes of these two genes is greater in Asians as compared to Europeans. Positive association of GSTM1 and GSTT1 deletion polymorphism and bladder carcinoma was found among Chinese, Indian, American and Turkish populations [6–8]. *GSTM1* and *GSTT1* genes are also risk factors for gastric, prostate, lung and head and neck cancer due to their hereditary loss and lack of enzymatic activity [9–12]. However, data related to genetic status of *GSTM1* and *GSTT1* and their association to bladder cancer is not available in Pakistani population. Therefore, current study is designed to evaluate the association of *GSTM1* and GSST1 gene polymorphisms with bladder cancer risk in Pakistani population. It also sheds light on the role of different environmental risk factors in the onset and development of bladder carcinoma.

2. Subjects and methods

The work has been carried out in accordance with The Code of the World Medical Association (Declaration of Helsinki) for experiments in humans. After approval from institutional ethics committee (Reference: IRB & EC # 308-157-2013) 270 controls and 236 urinary bladder cancer patients were selected from Shifa International Hospital Islamabad (Urology department) and National Oncology and Radiotherapy Institute Islamabad (NORI) in the period of one year and 3 months. Informed consent was signed from controls and patients. Histopathologically confirmed bladder cancer patients by oncologists were included in this study. Clinical and demographic data were collected through a detailed questionnaire by interview. All patients and controls were of Pakistani origin.

Blood was collected in 5 ml EDTA containing vacutainer and stored in refrigerator at 4 °C for DNA extraction. DNA was isolated by phenol–chloroform extraction with the help of different chemicals obtained from Merck, Germany. Screening of *GSTM1* and *GSTT1* was done by multiplex PCR (UNIEQUIP Cat# CMG96G; Germany). Following primers were used for amplification of these two genes. *GSTM1* 5′-TCTGGGGAGGTTTGTTTTCA-3′, 5′-TGGACACAGAACATCATGGAA-3′; *GSTT1* 5′-GGCGAGAGAGCAAGACTCAG-3′, 5′-GGCAGCATAAGCAGGACTTC-3′. *CYPIA1* were used as internal control gene. Master Mix was prepared using dNTPs, magnesium chloride, buffer, Taq polymerase and PCR water obtained from Solis biodyne. PCR was performed at 95 °C for 5 min followed by 30 cycles at 94 °C for 45 s, annealing temperature of 59 °C for 45 s, 72 °C for 1 min and final extension was done at 72 °C for 10 min. Then, electrophoresis was performed by running all PCR products on 2% agarose gel and bands were visualized under UV light in gel documentation system (Wealtec Dolphin-DOC plus Cat#1141004 USA). Product size for GSTM1 and GSTT1 was 270 bp and 215 bp respectively obtained by comparing with the DNA ladder [13].

The genotypic distribution of *GSTM1* and *GSTT1* genes were analyzed by chi square test among control and bladder cancer patients. The association of genetic polymorphisms, bladder cancer, age, sex, smoking, residential area and family history of cancer was estimated from logistic regression analysis using odds ratio (OR) with 95% confidence interval (CI). Statistical analysis was performed using SPSS 17.0 version and significance level was observed at $P < 0.05$ (see Fig. 1).

3. Results

The mean age of bladder cancer patients and controls was 59.5 (±15.76) years and 56.5(±15.67) years respectively. More than 60% were >60 years of age. Different demographic features were presented in Table 1. It was found that smoking was highly significantly associated (cigarette $P = 0.03$ and huqqa $P = 0.002$) with bladder cancer risk among Pakistani population. Residential area (open or congested) of bladder cancer patients showed non-significant association with bladder cancer. A major finding in this study group was that family history of cancer played a major role in the onset of bladder cancer. It was found to be significantly ($P = 0.001$) associated with the incidence of bladder cancer. All the patients were having histological type transitional cell carcinoma (TCC) showing that it is very common. We observed that hematuria (94.8%), lower urinary tract symptoms (LUTS) (72.4%) and flank pain (67.2%) are major symptoms of urinary bladder cancer. LUTS refers to a group of symptoms including storage or irritative symptoms (increased frequency and urgency of urination, painful urination and excessive urination at night) and voiding or obstructive symptoms (poor stream, hesitancy, terminal dribbling, incomplete voiding and overflow incontinence). Low grade tumors (58%) and non-invasive muscle bladder cancer (55%) were more frequent among patients. Most of the bladder cancer patients (68%) had undergone surgery. A

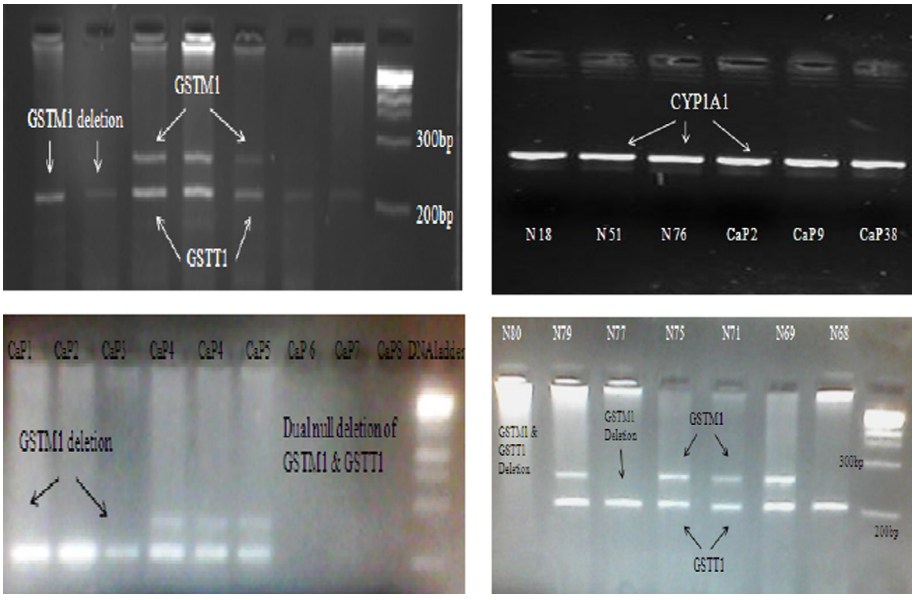

Figure 1 2% agarose gel showing *GSTM1* and *GSTT1* bands and null genotypes in controls (N) and bladder cancer patients (CaP). CYP1A1 is used as an internal control.

Table 1 Demographic features of normal healthy controls and bladder cancer (BC) patients.

Parameters	Control group (%) *n*	BC patients (%) *n*	OR (95% CI)	Chi square	*P* value
Age (years)					
Mean ± SD	56.5(±15.67)	59.5(±15.76)			
26–46	(21.5)	(22.4)			
46–66	(65.5)	(65.3)			
66–86	(9.4)	(7.14)			
>86	(3.4)	(5.10)			
Gender					
Women	(17.03) 46	(17.34) 41	0.9 (0.6–1.5)	0.01	0.9
Men	(82.96) 224	(82.65) 195			
Residential area					
Open	(84.4) 228	(86.8) 205	0.87 (0.4–1.3)	0.59	0.43
Congested	(15.5) 42	(13.13) 31			
Smoking					
Cigarette Yes	*(14.4) 39*	*(51.6) 122*	*5.0 (3.3–7.6)*	*80.5*	*0.03*
No	*(85.5) 231*	*(48.3) 114*			
Huqqa Yes	*(0.7) 2*	*(5.08) 12*	*7.7 (1.5–32.4)*	*8.83*	*0.002*
No	*(99.2) 268*	*(94.9) 224*			
Cigarettes per day					
<15	(23.0) 9	(36.8) 45	0.5 (0.2–1.1)	2.52	0.11
>15	(76.9) 30	(63.1) 77			
Mean years					
Smoking	24.5	15.6	–	–	–
Naswar chewing	0	1	–	1.9	0.15
Family history of cancer	0	(10.5) 25	–	27.1	0.001

Significant factors are italicized.
Huqqa is a kind of smoking.

large number of bladder cancer patients were at chemotherapy (87%) and radiotherapy (73%). It showed that one kind of treatment is not enough to cure the disease.

GSTM1 (OR 2.24; CI 1.5–3.2; $P = 0.0001$) and GSTT1 (OR 2.9; CI 1.4–6.1; $P = 0.002$) gene deletions were strongly associated with the increased risk of bladder cancer as

Table 2 *GSTM1* and *GSTT1* genotype distributions among controls and bladder cancer patients.

Genotypes	BC patients (*n*) 236	Control (*n*) 270	OR (95% CI)	*P* value
GSTM1				
Present	*131 (55.5)*	*199 (73.7)*		
Null	*105 (44.5)*	*71 (26.3)*	*2.24 (1.5–3.2)*	*0.0001*
GSTT1				
Present	*226 (95.8)*	*239 (88.5)*		
Null	*10 (4.2)*	*31 (11.5)*	*2.9 (1.4–6.1)*	*0.002*
Both GSTM1 & GSTT1				
Present	*230 (97.5)*	*239 (88.5)*		
Null	*6 (2.5)*	*31 (11.5)*	*5.3 (2.1–13.1)*	*0.0001*
GSTM1 and smoking				
Smokers in study	122 (51.7)	39 (14.4)	0.8 (0.4–1.7)	0.63
Present	51 (41.8)	18 (46.2)		
Null	71 (5)	21 (53.8)		
GSTT1 and smoking				
Smokers in study	122 (51.7)	39 (14.4)	0.6 (0.1–3.2)	0.62
Present	113 (92.6)	37 (94.9)		
Null	9 (7.4)	2 (5.1)		

n = number of controls and bladder cancer patients.
BC = bladder cancer.
Significant factors are italicized.

illustrated in Table 2. Deletion of both GSTM1 and GSTT1 genes was also associated (OR 5.3; CI 2.1–13.1; $P = 0.0001$) with greater risk toward bladder cancer. GSTM1 ($P = 0.63$) and GSTT1 ($P = 0.62$) gene deletions and smoking were found not to be associated with bladder cancer risk in Pakistani population. However, percentage of gene deletions among smokers was greater than non-smokers but we are unable to find any significance.

4. Discussion

In this study mean age of patients was 59.5($\pm$15.76) years and approximately 60% of patients were above the age of 60 years. Approximately 90% of bladder cancer cases were of transitional cell carcinoma (TCC). Similar results were observed in different studies [8,14–15].

Cigarette smoking is an important factor in the development of bladder cancer. In this study, cigarette and huqqa smoking ($P = 0.03$ and $P = 0.002$) proved to be a significant risk factor for bladder cancer. These results were also presented in a number of studies among Indian, Chinese, Turkish and Serbian populations (6, 8, 14 and 16]. It was noted that occurrence of low grade tumors was a little higher than high grade tumors and similar results were observed for muscle invasive and non-invasive tumor stages similar to the studies in Turkish, Korean and Iranian populations [7,8–15].

GSTM1 and *GSTT1* gene polymorphisms have been associated with a wide variety of cancers including bladder cancer [16]. In our study it was found that *GSTM1* null polymorphism was associated ($P = 0.0001$) with risk of bladder cancer. Similar results have been reported in other studies that *GSTM1* deletion was a risk factor for the development of bladder cancer [17,18]. Two different review papers also depicted association between *GSTM1* null deletion and bladder cancer risk [19,20]. We tried to find the association between smoking, *GSTM1* null deletion and bladder cancer risk however, no significant association was found. Similar results had been shown by an Iranian, an Indian and two German studies that *GSTM1* deletion does not show any association with smoking and bladder cancer [8,21–23]. We also investigated the association of *GSTT1* and bladder cancer. It was found that *GSTT1* was strongly associated ($P = 0.002$) with urinary bladder cancer. Our results were similar to a number of other studies previously conducted in Turkey, India, Brazil and Korea [8,23–26]. It was found that role of *GSTT1* gene deletions in bladder cancer was not dependent on smoking status as among Indians, Germans, Iranians and Spanish populations [8,18,21–23]. Both genes *GSTM1* and *GSTT1* have been evaluated in combination with each other. It was depicted that dual null deletion of *GSTM1* and *GSTT1* genes was also associated with increased susceptibility to bladder cancer.

5. Conclusion

It was concluded that smoking was an important risk factor for urinary bladder carcinoma. *GSTM1*, *GSTT1* and combination of the two gene polymorphisms were strongly associated with bladder cancer risk in Pakistani population. No association was found between genetic deletions of *GSTM1* and *GSTT1* and bladder carcinoma among smokers.

Ethical approval

All procedures performed in this study (involving human participants) were in accordance with the ethical standards of the institutional research committees.

Conflict of interest

There is no conflict of interest with study.

Contributors

All the authors participate in the sampling, lab work and article writing.

Acknowledgements

The authors acknowledge all the controls, patients, their family members and the hospital staff as well. My prayers goes to all of them. I would also like to thank Dr. Saeed Akhtar (Shifa International Hospital Islamabad, Department of Urology) for his help and guidance. No particular grant is available from any funding body for this research.

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