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RESEARCH ARTICLE

POLYMORPHISMS OF GLUTATHIONE-S-TRANSFERASE M1, T1 AND THE RISK OF PROSTATE CANCER IN THE IRAQI PATIENTS

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ABSTRACT

Polymorphisms in *glutathione S-transferase* (GST) genes may influence response to oxidative stress and modify prostate cancer (PCA) susceptibility. These enzymes generallydetoxify endogenous and exogenous agents, but also participate in the activation and inactivation of oxidative metabolites that may contribute to PCA development. Several studies show some differences in association of glutathione S-transferase M1 and T1 genetic polymorphisms with the risk of prostate cancer in various populations. The current study was done with Iraqi patient to evaluate the association of the polymorphism of glutathione S-transferase subtypes (T, M) and the susceptibility of prostate cancer in Iraqi patients as compared to controls. Blood samples were collected from 35 prostate cancer patients and 25 health individuals as controls from Ghazi Al-hariri hospital, Baghdad, Iraq. The multiplex polymerase chain reaction PCRmethod was used to determine the polymorphism of the glutathione S-transferase theta (GSTT) 1 and glutathione S-transferase mμ (GSTM) 1 null allele. There was significant association in the GSTM1 gene polymorphism and prostate cancer risk ($P<0.05$). Moreover, significant relationship was found between the polymorphism of GSTT1 genes and higher risk of prostate cancer among Iraqi subjects ($P<0.05$). This study showed that polymorphism of GSTT1 and GSTM1 genes can be predisposing risk factors for prostate cancer among Iraqi subjects.

INTRODUCTION

Most common neoplasms of the male genital tract involve the prostate gland (Nicol *et al*, 1999). The prostate gland is a walnut size stricture which is located around the urethra at the base of the bladder (Andreoli *et al*., 1968). This gland in the male reproductive system that helps produce semen, the thick fluid that carries sperm cells (Hayes *et al*., 1999). Prostate cancer is the fifth common cancer in the world and the second in cancer mortality exceeded only by lung cancer (Bray *et al*., 1995). This disease is age related pathology and as such destined to be increasingly relevant in an ageing general population (Alberts and Blute, 2001). The identification of high frequency (>1%) genetic polymorphism in genes associated with carcinogens metabolism has explained the high degree of individual variability in cancer susceptibility that has been observed for example among smokers (Hein *et al*., 2000). Glutathione S-transferases (GSTs) are a family of cytosolic enzymes involved in the detoxification of various exogenous as well as endogenous reactive species (Ketterer, 1988). GSTs function as dimmers by catalyzing the conjugation of mutagenic electrophilicsubstrates to glutathione. In humans, 4 major subfamilies of GSTs can be distinguished and are designated as GSTa, GSTμ, GSTu, and GSTp (Mannervik,1992) Each of these subfamilies is composed of several members, some of which display genetic polymorphism. Within the GSTμ subfamily, the gene coding for GSTM1 exhibits a deletion polymorphism, which in case of homozygozity (GSTM1 null) leads to absence of phenotypic enzyme activity (Seidegard *et al*., 1988). A similar mechanism is described for GSTT1 within the GSTu subfamily. (Pemble *et al*., 1994). The increased evidences show that polymorphism of glutathione S-transferase genes can be associated with the risk of developing some types of cancer as these polymorphisms have been investigated in association with lung, bladder, colon (Ketterer *et al*., 1992), oral (Nair *et al*., 1992; Buch *et al*., 2002) and prostate cancers (Pemble *et al*., 1994; Steinhoff *et al*., 2000; Nakazato *et al*., 2003).

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MATERIALS AND METHODS

Sampling

Blood samples (2-3ml) were collected in EDTA tubes for DNA isolation (Molecular genetic studies) from (25) healthy controls and (35) patients all of them were males and diagnosed as prostate cancer attended the (Ghazi Al-Hariri) Hospital in Baghdad, The patients were interviewed and questioned according to special form. The inclusion criteria for patients were between 40 - 86 years old .suffering from prostate cancer based on diagnosis by physician. For the controls, over 40 years old men who were healthy.

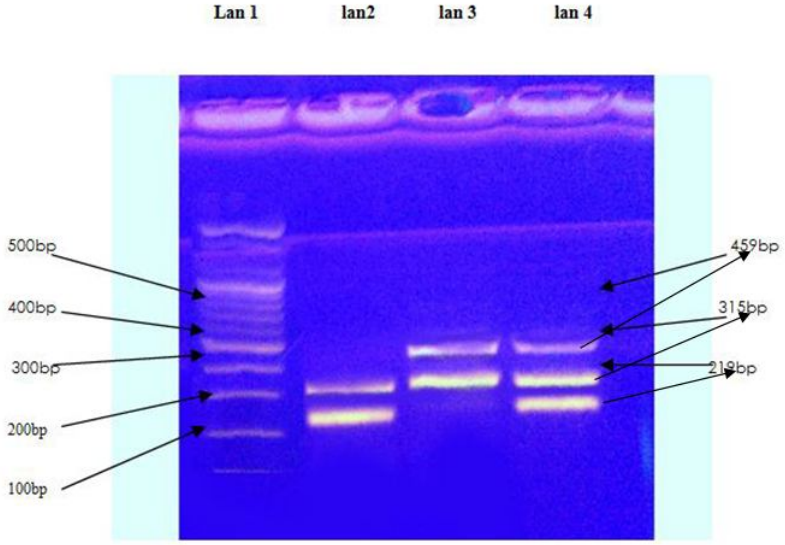
DNA extraction

Total genomic DNA isolated from the whole blood collected in EDTA anticoagulant tubes for molecular studies was applied using genomic DNA purification kits (geneaid) south Korea**.** The isolation of DNA was based on salting out methods (Sambrook *et al*., 1989). Multiplex Polymerase Chain Reaction (PCR) for GSTM1 and GSTT1genotypig was don using specific primer, primer was custom synthesized at Bioneer\south korea Company as a lyophilized product. Lyophilized primer was dissolved in a free DNase/RNase water to give a final concentration of (100 pmol/μl) (as stock solution), to prepare 20μM concentration as work primer resuspended 20 pmol/μl in 80 μl of deionized water to reach a final concentration 20μM. multiplex PCR was using primers like those presented in Table (1).

Table 1. Primer sequences used multiplex PCRamplification of GSTM1, GSTT1,CYTP1A1 genes

Primer	Primer sequences	TA	PCR Product size
CYP1A1 (used as control)	F 5-GAACTGCCACTTCAGCTGTCT -3	59°C	312bp
	R 5- CAGCTGCATTTGGAAGTGCTC -3	59°C	
GSTM1	F 5- GAA CTC CCT GAA AAG CTA AAGC -3	59°C	219bp
	R 5-GTTGGGCTCAAATATACGGTGG -3	59°C	
GSTT1	F 5- TTCCTTACTGGTCCTCACATCTC -3	59°C	459bp
	R 5- TCACCGGATCATGGCCAGCA -3	59°C	

TA= Temperature Annealing



- Note: (Middle band = CYP1A1gene)
- Figure 1:PCR product for GSTT1&GSTM1 polymorphisms on 2% agarose gel.Lane 1: DNA ladder.Lane 2: *GSTT1* deletion.Lane 3: *GSTM1* deletion.Lane 4: normal genotype.

Table 2. Number & percentage of prostate cancer patients and control according to type of gene

Group	No. & Percentage	Type of gene			
		GSTM1 gene deleted	*GSTM1* gene present	*GSTT1* gene deleted	*GSTT1* gene present
Patients	No.	19	16	5	30
(no. 35)	%	79.17	44.44	62.50	57.69
Control	No.	5	20	3	22
(no. 25)	%	20.83	55.56	37.50	42.31
Total No.	---	24	36	8	52
Chi-square–χ^2	---	11.59 *	4.03 *	10.34 *	4.89 *
		* (P<0.05)			

TA= Temperature Annealing

Multiplex PCR was performed in a 20µltotal volume, Primer forward1µl (20PM for each one), Primer reverse 1µl (20 PM for each one),Template DNA4 µl, (4- 6µg/ml) **.**A total of 35 PCR cycles with denaturation at 94°c for 1 minute, annealing at 59°c for 1 minute and extension at 72°C for 1 minute were conducted. An initial DNA denaturation at 95°c was carried out for 3 minutes and final extension at 72°c were carried out for 5 minutes each. The PCR product was then subjected to electrophoresis on a 2% agarose gel. The presence of bands of 459bp and 219bp was indicated of the *GSTT1* and *GSTM1* genotypes respectively, whereas the absence indicated the null genotype for that gene. CYP1A1indicated by a 312 bp product was used as an internal control Fig (1).

RESULTS AND DISCUSSION

The results showed that 23 samples of patients have deletion in one genes or both (Null genotype), whereas 12 samples only were normal. The chi-square test reflected significant association of the mutations and their combination, (deletion or presence), with the occurrence of prostate cancer as reflected from comparing the patients and control group Table (2). In the present study, we observe significantly different crude rates of the *GSTM1* and *GSTT1* null genotypes in the men diagnosed with prostate cancer and those in the control group. It is assumed that the presence of carcinogen-metabolizing enzymes in human prostate with a high inter-individual variability may be involved in the regulation of local levels of carcinogens and mutagens and may underlie interindividual differences in cancer susceptibility (Sreelekha *et al*., 2001). A similar study was done by Srivastava, *et al*., (2005) which showed an association between *GSTT1* and *GSTM1* with higher risk of prostate cancer. our observation matches to that reported in Scandinavian, German and Indian population (Autrup *et al*., 1999; Steinhoff *et al*., 2000; Mittal *et al*., 2004).Some studies have reported also a relationship between GST variants and risk of prostate cancer (Medeiros *et al* 2004; Kidd *et al*., 2003; Debes *et al*., 2004; Ntais *et al*., 2005). Monika *et al*., (2008) did not account for significant differences in the GST frequencies between healthy subjects and those diagnosed with prostate cancer. However, in Japanese, (Nakazato *et al*., 2003), United Kingdom,

(Kote-Jarai *et al.*, 2001) and in American (Kidd *et al., 2*003) studies reported non significant association also. These variations may be possibly attributed to the underlying geographic/ethnic factors. In the null genotype of *GSTT1* and *GSTM1* there are inactive forms of the GSTs enzymes, thus detoxification of activated carcinogen is reduced and this will be followed by progression to cancer (Guengerich *et al.*, 1995). Many studies have shown that GSTs are important for maintaining cellular genome (DNA) from damage andtheir absence may result in cancer susceptibility (Srivastava *et al.*, 2005). Our results provide the information that showed the null genotype of *GSTT1 and GSTM1* increase the risk of prostate cancer in Iraqi subjects. Mufeed *et al*. (2007) found that GSH concentration were found to significantly decrease in sera of patient with prostate cancer in comparison to the GSH level of serum in control group. So the depletion of GSH levels when compared with healthy control support the hypothesis those conceders GSH a protective factor against the development of prostate cancer. Prostate cancer is a multi-factorial disease influenced by complex genetic as well as environmental factors as noted by (Kolonel *et al.*, 2004).

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