Original Article

Association of genetic polymorphisms at the glutathione S-transferase Pi locus with prostate cancer

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ABSTRACT

Objective: The present report evaluates a possible association of the GST-pi Ile105Val polymorphism with sporadic prostate cancer.

Methodology: Tissue samples from 35 prostate adenocarcinomas and 26 geographically and age matched benign prostatic hyperplasia (BPH) patients as control subjects were tested for GST-pi polymorphism by using restriction fragment length polymorphism (RFLP) method for the polymerase chain reaction (PCR) product.

Results: Among prostate carcinoma patients, 60% were homozygous for the wild type allele (Ile/Ile), 22% for heterozygous (Ile/Val) and 18% homozygous for mutant allele (Val/Val). While, the control group showed 64% of the subjects were homozygous for wild type allele, 28% were heterozygous and 8% homozygous for mutant allele.

Conclusion: Val/Val genotype of GST-pi was significantly connected with prostate cancer risk, hence, GST-pi polymorphism may take part in developing prostate cancer.

KEY WORDS: Prostate carcinoma; Glutathione S-transferase; GST-pi polymorphism.

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INTRODUCTION

Prostate cancer (PCa) is a common malignancy and a leading cause of cancer death among men worldwide, accounting for 13.6% of the total men cancers.[1] In 2009, it ranked 6th among cancers in males and accounted (7.9%) of Jordanian male cancers.[2] Most of the cases (75%) occur after 65 years of age. The median age at diagnosis is 70 years and ranges from 40 years to 87 years. The vast majority of PCa morphology is adenocarcinomas (93.3%).[2] Several environmental and genetic factors are implicated in PCa, however, the interaction between these factors is still uncertain.[3,4]

A strong genetic predisposition to PCa, especially in young adult, has been reported by several studies, which pointed to both high and low penetrance genes.[5,6] Susceptibility to PCa might be due to alteration in the enzymes activity, which are involved in the detoxification process of carcinogens as well as DNA repair. The competence of these functions of such enzymes is genetically influenced.[7] Hence, polymorphism in such genes, which code for detoxifying enzymes may be involved in PCa susceptibility. The glutathione S-transferases (GSTs) comprise a supergene family of phase-2 enzymes that catalyze the detoxification of cytotoxic drugs and carcinogens by glutathione conjugation, the process that guards DNA from harm and adduct formation.[8] GSTs detoxify the resultants of oxidizing reaction, completed by phase 1 enzymes.[9] Glutathione S-transferase-pi is a major

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GST, which is expressed in normal tissue of prostate gland and prostatic adenocarcinoma.[10,11] GST-pi gene is located on chromosome 11q. Polymorphisms in exon 5 (*Ile*105*Val*) and exon 6 (*Ala*114*Val*) of the GST-pi gene have been identified.[12,13] The affected regions lie adjacent to the hydrophobic binding site of GST-pi. Moreover, polymorphism is known to alter the function of the enzyme.[9] Few studies have demonstrated lower or higher specific activity and affinity of the 105*Val* variant than that of 105*Ile* depending on the substrate.[14,15]

The possibility of GST-pi polymorphism involvement in the pathogenesis of PCa has received great attention. Some epidemiological studies on the potential association between genetic polymorphisms of GST-pi and PCa have produced inconsistent results.[16-20] Some of these studies consistently failed to detect association between PCa and GST-pi polymorphism, and the results and conclusions have been highly variable. This contradiction might be partly due to differences in population, and their exposures to factors of PCa development. The evidence regarding a possible association of GST-pi polymorphism with PCa is thus controversial and, in view of the high incidence of this tumor, requires clarification. Based on these considerations, we evaluated this issue for the first time in North Jordan population.

The present study investigates the relationship between the I105V GST-pi polymorphism and the possibility for developing prostate cancer.

METHODOLOGY

This study includes 35 samples of paraffin embedded tissue blocks of individuals previously diagnosed with prostatic adenocarcinoma and were treated with radical prostatectomy, and 26 samples of paraffin embedded BPH tissue as a control population, in addition to normal nearby tissue at surgical margins from prostate cancer patients. All control cases were geographically and age matched. All samples and clinical data including age group, TNM stage and Gleason score were collected from the medical records and the Pathology Department at Jordan University of Science and Technology (Table-I). The median age of the patients and control group was 71 and 70 years, respectively (ranges from 40 to 85 years).

DNA Extraction: Sections of 10µm were cut from paraffin blocks of prostatic adenocarcinoma, which were resected by radical prostatectomy, and BPH (Transurethral Resection of the Prostate tissue [TURP]). Later, DNA from microdissected sections of paraffin embedded tissue blocks belonging to all cases (cancer and controls) were extracted with an Extraffin kit (Nanogen Advanced Diagnostics S.r.L., Buttigliera Alta, Italy) according to the manufacturer's instructions. Five 10 µm sections placed in a 0.5 ml extraction microtube, followed by 100 µl of Extraffin reagent. The mixture was incubated in a heater for 10 minutes at +95°C. Later, the heater was set to a temperature of +55°C. Ten µl of protease was transferred into the tube and was mixed vigorously. The mixture was incubated for 3 hours at +55°C with shaking every 30 minutes. Later, the heater was set to a temperature of +95°C. Resin was shake vigorously and 50 µl of the resuspended resin was transferred into the microtube and were mixed and incubated for 10 minutes at +95°C, then was centrifuged at ambient temperature for 10 minutes at 12,000-14,000 RPM. Later, extraction product was drawn into the tip through the surface layer of solidified paraffin. The extraction product was stored at -20°C.

Genotyping: The polymorphism (Ile 105 → Val) in exon 5 coding region of GST-pi gene was identified by using Restriction Fragment Length Polymorphism (RFLP) procedure. Standard GST-pi primers (Table-II) bought from Alpha DNA in Canada were used for the amplification reactions and restriction enzyme corresponding to RFLP (Fermentas, Canada) for the digestion reactions.

The initial denaturation step of PCR reaction were carried out for five minutes at 95°C in a 30 mL volume containing 5 µL of genomic DNA template, 200 ng of each primer and PCR master mix, which is bought from Promega (USA) and contains 200 mM each dNTP, 1.5 mM $MgCl_2$, 1x PCR buffer and 2 unit Taq DNA polymerase. Then the samples were processed through 35 temperature cycles of 30 s at 94°C, 1 minute at 59°C, and 1 minute at 72°C. Later, final round of 72°C for 10 minutes were completed. Successful amplification of PCR product was confirmed by electrophoreses on 2% agarose gel.

5 units of BsmA1 restriction enzyme was added to 10 ml of PCR products for digestion overnight at 55°C. Finally, different alleles were detected by horizontal 4% agarose gel electrophoresis labeled with ethidium bromide, along with a 100-bp DNA ladder. Genotypes were determined as homozygous for the wild type allele (Ile/Ile; 176 bp), heterozygous (Ile/ Val; 176, 91, 85 bp) or homozygous for mutant allele (Val/Val; 91, 85 bp).

Statistical Analyses: All statistical analyses were performed using EpiInfo version 6. Mid-P exact test (The mid *p*–value falls between the conservative

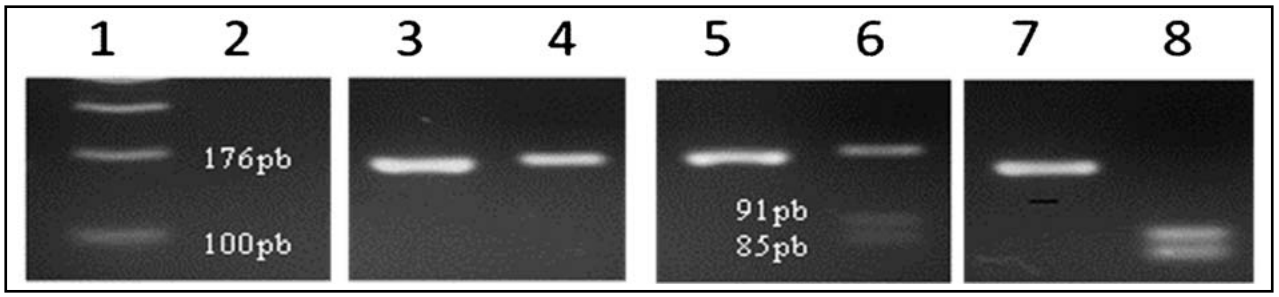

Fig.1: Agarose gel electrophoresis (4%) of the GSTP1 exon 5, codon 105 polymorphism.
Lane 1 represent ladder. Lane 2 represent negative control. Lanes 3, 5, & 7 represent untreated samples. Lanes 4, 6, & 8 treated samples with BsmA1 enzyme. Sample in lane 4 represent homozygous normal allele. Sample in lane 6 represent heterozygous allele. Sample in lane 8 represent mutant allele.

Fisher's *p*–value and the "radical" normal *p*–value or chi–squared *p*–value) was used to find out if there is any significant difference in the incidence of polymorphism in the cancer cases in contrast with control population. Statistics were calculated using 95% confidence intervals (p< 0:05 significant).

RESULTS

PCR based genotyping assay was used to investigate a GSTP1 polymorphism in PCa susceptibility. The genotypic results of GSTP1 are presented in Table-II and Fig.1. Among prostate cancer patients, 60% were homozygous for the wild type allele (Ile/ Ile), 22% for heterozygous (Ile/Val) and 18% homozygous for mutant allele (Val/Val). While, in the control group, 64% of the subjects were homozygous for the GSTP1 wild type allele, 28% were heterozygous and 8% homozygous for mutant allele (Table-III). There is some evidence of an increasing association of prostate cancer risk with GSTP1 homozygous genotype of mutant allele Val/Val (*P* = 0.03768).

Table-I: Pathologic and clinical data of patient with prostate cancer.

Demographics	*Cancer cases*
• Age 40-49	1
• Age 50-59	4
• Age 60-69	6
• Age > 70	24
Gleason score	
• Low (2-4)	3
• Moderate (5-7)	13
• High (8-10)	19
Tumor stage	
• Localized (I, II and III)	24
• Metastatic (IV)	11

There were no remarkable differences in the distribution of the GSTP1 wild type and mutant alleles between cases or controls. The observed percentage of GSTP1 wild type and GSTP1 mutant alleles were 78.8% and 21.2%, respectively in the control group. In the 35 prostate cancer cases, the corresponding frequencies of GSTP1 alleles were 71.4% and 28.6% for wild type and mutant alleles, respectively (Table-III). No relationship was found between GSTP1 genotype and pathological parameters (grade or stage) in cancer patients.

DISCUSSION

There is a growing evidence signifying relationship between *GSTP1* polymorphism and susceptibility to bladder, testicle, breast, and lung carcinomas.[9,16,21] The effects of these polymorphism on prostate cancer development, however, remains controversial.[16,22-24] Although the sample size, in the present study, is small, the results of GSTP1

Table-II: Standard GST-pi primers.

Forward	5¢– ACC CCA GGG CTC TAT GGG AA–3¢
Reverse	5¢–TGA GGG CAC AAG AAG CCC CT–3¢

Table-III: Genotype and allele percentage of the GSTP1 polymorphism in PCa patients.

	Patients (n = 35)		*Control; BPH tissues (n = 26)*		*Mid-P exact test P value*
Genotype	*No.*	*%*	*No.*	*%*	
Ile/Ile	21	60%	16	64%	P = 0.4543
Ile/Val	7	22%	9	28%	P = 0.1095
Val/Val	7	18%	1	8%	P = 0.03768

Allele	*No. of alleles in 35 cancer cases*	*Percentage of alleles in 35 cancer cases*	*No. of alleles in 26 controls*	*Percentage of alleles in 26 controls*	*Mid-P exact test P value*
Ile	49	71,4%	41	78.8%	P = 0.1409
Val	21	28.6%	11	21.2%	P = 0.1409

genotypes showed some influence of the Ile105Val GSTP1 polymorphism on prostate cancer risk in Jordanian population. These findings indicate a linear relationship between the risk of developing prostate cancer and the homozygous 105 Val genotypes (*P* = 0.03768), which may be a sign of low levels of enzyme activity. These findings are in agreement with many studies, where the mutant allele genotypes were allied with a considerable growth in the risk of prostate cancer in Italian[25], Japanese[13], North Indian[18] and Kashmiri populations.[26]

The results of the present study, based on the equal distribution of Ile/Ile genotype between cancer patients and control group, contradict the findings of Harries et al. who tested 36 prostate cancer patients and found that Ile/Ile genotype has a protective effects against the risk of prostate cancer.[16] In the same study, the frequencies of GST-pi 105 Ile homozygotes and heterozygotes (Ile/Val) were significantly decreased and increase respectively amongst prostate cancer patients.[16] Harries et al also reported a significant relationship between the GST-pi mutant allele and bladder and testicular cancers (OR 3.6, CI 1.4±9.2 and OR 3.3, CI 1.5±7.7, respectively).[16] On the other hand, in another study that tested 178 cases of Scandinavian male population, there was no association between the GST-pi genotypes and the risk of prostate cancer.[23]

It is presumably clear that the GST-pi catalytic activity (detoxifying properties) fluctuate largely in association with its genotype i.e. the amino acid at 105 position, which possibly facilitates recognizing the binding site of substrate, in addition to the nature of the electrophilic substrate.[12,13,27,28] Furthermore, hypermethylation of CpG islands of the regulatory region of the gene abolishes GSTP1 expression in the majority of prostate tumours.[29] This evidence further supports our findings that GST-pi enzyme function could be an important element in the etiology of prostate cancer. In other words, alteration in carcinogen metabolizing activity due to one or two 105 Val alleles, in addition to the silencing or down regulation of the GST-pi gene may predispose an individual to the development of prostate cancer.

In conclusion, the results of this study show that the Val/Val genotype of GSTP1 may contribute to the risk of prostate cancer.

Recommendation: Because of limitations of this study due to the small sample size, more significant results could be obtained investigating larger sample sizes.

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