RESEARCH COMMUNICATION

Evaluating Polymorphic Status of Glutathione-S-Transferase Genes in Blood and Tissue Samples of Prostate Cancer Patients

Rama Devi Mittal*, Dhruva K. Mishra, Anil Mandhani

Abstract

Prostate cancer is the most common urologic malignancy, involving multiple factors. There is evidence that suggests that detoxification enzymes and growth factors may play a role in its development . The glutathione S-transferase (GST) enzymes detoxify several carcinogens and genetic polymorphisms in GSTM1, T1, and P1 (Ile105Val) have been reported to be associated with prostate cancer, mainly from blood samples. As expression studies suggest differential expression of different genes in tissues, we hypothesize that polymorphic status may be differently expressed for GSTM1, GSTT1 and GSTP1 gene in blood and tissues of prostate cancer patients and BPH controls, impacting on the development of prostate cancer. To study this, we extracted DNA from blood and tissue samples of patients undergoing biopsy procedures or transurethral resection of prostate tissue. Genotyping for GSTM1 and T1 was conducted by multiplex PCR and for GSTP1 by the PCR-RFLP method. Our results suggested no significant differences in frequency distribution of M1, T1 and P1 between blood and tissue samples of patients and controls, but in a few patients differences in polymorphic status were observed. However, they were not significant. Furthermore, we observed a significant risk of prostate cancer with null allele of GSTT1 and GSTM1 and Val allele of GSTP1, supporting our previous findings. A study with large sample size using radical prostectomy tissue now needs to be performed to attain a specific conclusion.

Key Words: GSTM1 - GSTT1 - GSTP1 - prostate cancer - North Indian population

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Introduction

Prostate cancer remains one of the most common cancers afflicting men today. It is the third most common cancer in the world and the most frequently diagnosed male cancer in western countries (Ferlay et al, 2001). In Asia, however, the incidence of prostate cancer is significantly lower and it often plays second fiddle to lung, stomach and colon cancer. In India, it is the sixth most common cancer among men (Sinha et al, 2003).

The molecular events involved in neoplastic initiation and progression is poorly understood. Various studies suggested the role of metabolic genes in prostate cancer development, especially from blood sample. The genes for glutathione S-transferases, which are involved in the metabolism of carcinogens and the defense against reactive oxygen species, may link exposure to genome-damaging stress to increased genomic instability during prostatic carcinogenesis. Many studies have been conducted evaluating association of genetic variants of GSTP1, GSTM1, and GSTT1 with prostate cancer risk. The 313A>G germline genetic variant of GSTP1 gene, which results in an amino acid substitution that alters the function of the enzyme, has been linked to an increased risk (Srivastava et al, 2005). Individuals with homozygous deletions of GSTM1 or GSTT1 lack glutathione S-transferase and therefore may be unable to eliminate electrophilic carcinogens as efficiently, that may increase the risk of somatic mutations leading to tumor formation. Combinations of various unfavorable deletion genotypes theoretically confer an even higher risk to the patients with prostate cancer.

Prostate carcinogenesis is a complex process involving genetic as well as environmental factors. Expression and functional studies suggested a differential expression and activity of various genes in different tissues. The variation in expression and activity may be due to various epigenetic and genetic events such as mutations. The accuracy of CaP detection through biopsy depends mainly upon the site from where core is taken. If the cancerous lesion during biopsy is missed, cancer would not be detected. So, in this study, we hypothesized that there may be differences in polymorphic status of these genes in blood and tissue within the same individual and awareness of this may substantiate and improve the detection process. We also attempted to investigate the risk of CaP with GSTM1, GSTT1 and GSTP1 variants.

Department of Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road,Lucknow-226014, India
**Correspondence: Phone 091-522-2668004-8, Ext. 2116 Fax: 091-522-2668 017 E-mail: rmittal@sgpgi.ac.in, ramamittal@yahoo.com*

Materials and Methods

Study Subjects

The retrospective study included consecutive North Indian patients (n=54) with histologically confirmed prostate cancer (CaP) during the time frame of January 2003 and November 2005 in the Department of Urology of the Institute. The control group consisted of 105 age matched, of similar ethnicity and unrelated healthy men with BPH (Benign prostatic hyperplasia). The mean age of patients and controls were 66.2 ± 8.7 and 64.1 ± 9.5 respectively. Clinical details of each individual were taken according to a detailed questionnaire set up in our department. Most of the patients visiting our hospital were in advanced stage because of lack of any structured screening programme. PSA was measured in both patients as well as in healthy controls. Informed consent from the patient as well as healthy volunteers was taken. The local ethical committee of our institute approved the protocol and the study

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Laboratory Methods

Genomic DNA was extracted from peripheral blood leukocytes by salting out procedure (Miller et al, 1988) and Quiagen DNA kit was used to extract DNA from tissues.

GSTM1 and GSTT1 Genotyping

Multiplex PCR was performed as described by Abdel-Rahman et al (1998) to determine the presence or absence of GSTM1 and GSTT1 genes. CYP1A1 was co-amplified and used as an internal standard. 50ng of DNA was amplified in a total volume of 25ml reaction mixture containing 10pmol of each primer as described (Mishra et al., 2004), 200mmol of dNTPs and 1.5U Taq polymerase (Gibco-BRL).

PCR was performed with initial denaturation at 94˚C for 5 min, followed by 35 cycles of 94˚C for 1min, 59˚C for 1min, 72∞C for 1min and a final extension at 72˚C for 10 min. Presence of M1 and T1 genotypes yielded bands of 215bp and 480bp respectively with internal control of 312bp.

GSTP1 Genotyping

GSTP1 was identified by digestion of the PCR products using PCR restriction fragment polymorphism method. (Harries et al., 1997). PCR was done in a total volume of 25 ml using 10pmol each of forward and reverse primers (Mishra et al, 2004). Initial denaturation was carried out at 95˚C for 5min followed by 30 cycles at 95˚C for 2min, 55˚C for 30sec, 72˚C for 30sec and final extension at 72˚C for 5min. 10ml of 176bp PCR product was digested for 2 hrs at 55˚C with 5U of Alw261 (NEB, UK) and separated on 3.5% Ethidium bromide stained agarose gel. As a result of A to G transition Alw261 cleaved to yield two fragments of 91bp and 85bp.

Statistical Analysis

Statistical analysis was conducted with SPSS software 11.5. Binary Logistic Regression Model assessed difference in genotype prevalence and association between case and control group. Univariate and multivariate analysis, correlation coefficient, odds ratio (OR) and its 95% confidence interval (CI) were used to describe the strength of association. P-value <0.05 was considered as statistically significant.

Table 1. Frequency Distribution of GSTM1, GSTT1 and GSTP1 Genes in Blood and Tissue Samples of CaP Patients (n=54) and Controls (n=105)

Gene	Genotype	C	P	OR (95% CI)	p-value
Tissue					
GSTM1	Presence	69	24	1.00	
	Absence	36	30	2.34 (1.22-4.69)	0.01
GSTT1	Presence	77	29	1.00	
	Absence	28	25	2.37 (1.19-4.72)	0.014
GSTP1	Ile/Ile	55	16	1.00	
	Ile/Val	44	28	2.18 (1.05-4.54)	0.036
	Val/Val	6	10	5.73 (1.81-18.18)	0.003
Blood					
GSTM1	Presence	67	23	1.00	
	Absence	38	31	2.38 (1.22-4.65)	0.011
GSTT1	Presence	75	30	1.00	
	Absence	30	24	2.00 (1.01-3.96)	0.047
GSTP1	Ile/Ile	58	17	1.00	
	Ile/Val	42	28	2.27 (1.11-4.68)	0.026
	Val/Val	5	9	6.14 (1.81-20.79)	0.004

Results

We have analyzed 54 CaP patients and 105 BPH controls to evaluate the difference in tissue and blood samples of same patients for GSTM1, GSTT1 and GSTP1 gene polymorphism. Genotype distributions in controls were in agreement with Hardy –Weinberg Equilibrium for each gene. No significant difference was observed in GSTM1, GSTT1 and GSTP1 polymorphism in blood and tissue samples (Figure 1). However, in a few tissue samples deviation from blood genotype was apparent.

A significant association were observed for null genotypes of the GSTM1 (OR=2.3, 95% CI= 1.22-4.65, P= 0.01) and GSTT1 (OR=2.00, 95% CI= 1.01-3.96, P= 0.047); as well as the heterozygous genotypes (Ile/ Val) and mutant

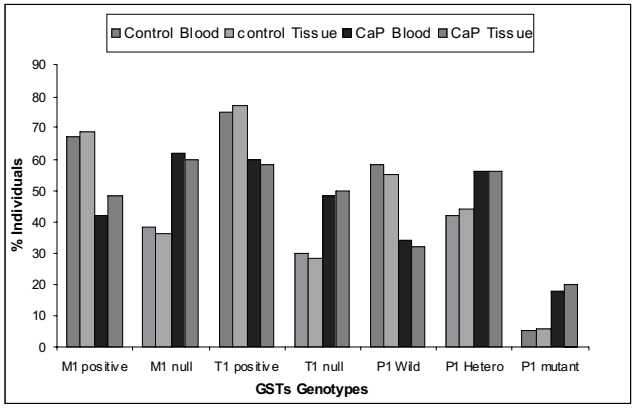

Figure 1. Comparative Distribution of GST Genotypes in Blood and Tissue Samples of Patients and Controls

(Val/Val) of the GSTP1 gene to prostate cancer risk (OR = 2.27, 95% CI= 1.11-4.68, P= 0.026; OR = 6.14, 95% CI= 1.81-20.79, P= 0.004) (Table 1).

Discussion

Prostate cancer is multifactorial and polygenic in origin. Its development and progression is a very complex process. We have hypothesized in this study that there may be difference in the polymorphic status of GSTM1, GSTT1 and GSTP1 genes in blood and prostate tissue of the same individual which lead to the initiation of tumorogenesis in prostate cells as the mutant variants makes it prone for the cancer progression. We did not observe significant difference in polymorphic status of any of these genes when the frequency distribution was compared in DNA obtained from blood and tissue from the same patient. However, a significant association with risk of prostate cancer was observed with GSTM1 and GSTT1 null and val allele of GSTP1 gene in blood, which supported the previous study from, our laboratory (Srivastava et al., 2005). The observed non-significant difference in polymorphic status of theses genes between prostate tissues of patients or controls may be due to constant genetic make up of the body. The difference may arise only in those cells, which become timorous. As in few cases for GSTM1 and GSTT1, the positive genotype in blood was later identified as null genotype in tissues while for GSTP1, in few cases wild type (Ile/Ile) genotype in blood sample was genotyped to be heterozygous in tissue and in two cases heterozygous conditions were identified to be mutant.

Our study had limitations with regard to the method of collection of prostate tissue samples through biopsy. It was rather not viable to dissect out the core tissue containing only the cancerous cells as the pieces collected were very small in size. Therefore the whole piece of biopsy tissue was taken assuming to be cancerous, after being histopathologically proved. It was likely that there was contamination of normal cells in the tissue samples as well. In few samples we observed that wild type genotype of GSTP1 was turned to heterozygous and heterozygous condition was turned to mutant one.

To the best of our knowledge, till date there is no such study reported in any population in the world. A more precise study using laser dissection technique and high thorough put technology identifying the cancerous lesions is required with greater sample size to reach any conclusive decision. If any significant difference can be obtained for these gene frequency distributions at expression level it might improve the diagnostic measure for the early detection of prostate cancer.

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