Role of glutathione-S-transferase gene P1 in the diagnosis of prostate cancer in patients with 'grey level' prostate-specific antigen values

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Abstract. Prostate cancer (PC) represents the second most frequent cancer diagnosis in men and, at the same time, is one of the top six causes of death worldwide. The aim of the present study was to evaluate the diagnostic value of glutathione-S-transferase gene P1 (GST-P1) in patients that fall within the 'grey area' of the prostate-specific antigen (PSA) values. A retrospective observational study on 80 patients with prostate abnormal volumes and PSA values in the range 4-10 ng/ml was performed. The prostate gland was extracted following transrectal ultrasonography, and GST-P1 gene expression was analysed. A histopathological examination was considered the gold standard for PC diagnosis. Among the 53 patients diagnosed with PC, 69.8% (n=37) were GST-P1-positive, whereas, among the 27 patients diagnosed with benign prostatic hyperplasia, 18.5% (n=5) were GST-P1-positive. The sensitivity for diagnosing PC in patients with PSA values between 4 and 10 ng/ml was 69.81%, and the specificity was 81.48%. The positive predictive value was 88.1% [95% confidence interval (CI), 74.37-96.02%] and the negative predictive value was 57.89% (95% CI, 40.82-73.69%). Collectively, these results show the potential of using GST-P1 gene expression in patients who are suspected of having PC, but where the PSA values are inconclusive.

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Abbreviations: PC, prostate cancer; BPH, benign prostatic hyperplasia; GST-P1, glutathione-S-transferase gene P1; PSA, prostate-specific antigen; DRE, digital rectal examination

Key words: prostate cancer, diagnosis, glutathione-S-transferase gene P1, prostate-specific antigen, genetic markers

Introduction

Prostate cancer (PC) is one of the most commonly and frequently diagnosed malignant solid tumours in men. It is the second most diagnosed cancer worldwide, representing one of the major causes of death among men in both industrialized countries and developing countries according to recently published data, with increases in cases of urinary tract carcinomas, such as penile carcinoma, having been identified among the developing countries of Africa, Asia and South America (1,2). The progression of PC worldwide is expected to grow to almost 2.3 million new cases, and 740,000 deaths, by 2040 (1). In Romania, PC is the second most common diagnosed malignancy, with high incidence numbers compared with other neoplastic diseases (3), and the second most common cause of death by cancer in men.

During the course of PC diagnosis, several laboratory and clinical tests are routinely performed. Screening tests are frequently used, including the test for prostate-specific antigen (PSA). Despite its low sensitivity, this screening test is widely used (4,5) in detecting PC when a 4 ng/ml cut-off point is used. Furthermore, if the PSA value of the patients falls within 4-10 ng/ml, also known as the 'borderline' or 'grey-level', this poses serious concerns in terms of making the correct diagnosis (6). Therefore, a combination of several other diagnostic tests are recommended, such as digital rectal examination (DRE), prostate health index, the 4k score, IsoPSA™ (Cleveland Diagnostics) and imaging testing (7). Considering all these tests, expanding the pool of biomarkers

that contribute to the early and accurate detection of PC would be of great interest for researchers, medical staff, and people at risk (8).

In the present study, the possibility of using glutathione-S-transferase gene P1 (GST-P1), a genetic marker involved in carcinogen detoxification, antineoplastic product activation and metabolism of chemotherapeutic agents (9), in patients that are in the 'grey area' of the PSA values was evaluated.

Materials and methods

Patient study. This observational, retrospective study was conducted on consecutive patients that presented either for control examination or due to lower urinary tract symptoms (LUTS) at the Urology Clinic of County Hospital of Constanta between January 2018 and January 2020. A total of 80 patients that met the inclusion criteria of having a PSA value between 4 and 10 ng/ml were recruited.

Ultrasound control was conducted in all patients, with the prostatic volume measured by ~~DRE~~ Afiniti 30-Philips Ultrasound Machine with a C9-4v transducer probe. For all patients with abnormal prostate volumes, the PSA level was evaluated using the electrochemiluminescent immunoassay method (Cobas INTEGRA® 411 Analyzer). Transrectal ultrasonography with prostate biopsy was also performed. On the extracted tissue, GST-P1 gene expression was analysed, and histopathological examination was performed to confirm the diagnosis. The histopathological examination (hematoxylin-eosin staining) was considered as being the golden standard for PC diagnosis.

Isolation of genomic DNA from harvested tissue was performed with the aid of a QIAamp DNA mini kit from Qiagen GmbH, which combines the selective property of links on a silicon membrane with a flexible elution volume of 20-100 μl. Isolation of genomic DNA was performed from small amounts of tumour tissue biopsies (<10 mg), which were transferred immediately after harvesting to cryotubes with DNA/RNA shield solution (Zymo Research Corp.) to preserve the integrity of the genetic material. Sodium bisulfite conversion of genomic DNA was performed using an EpiTect Bisulfite Kit (Qiagen GmbH), and subsequently, methylation-specific PCR was performed using a CpG WIZ GST-P1 Amplification Kit (Merck KGaA; see below for further details).

According to the results of the histopathological examination, patients were divided into two groups: Patients with PC and patients with benign tumours, or benign prostatic hyperplasia (BPH; control group). The results from the two groups were compared to identify possible differences in age, prostate volume, PSA value, environment, LUTS and GST-P1 methylation status. The diagnostic accuracy of GST-P1 methylation status in these particular patients for whom the PSA values were inconclusive was evaluated.

The index test (GST-P1 methylation status). The index test (GST-P1 methylation status) can be methylated or unmethylated. Methylation-specific PCR for GST-P1 was performed using a CpG WIZ GSTpi Amplification Kit (Merck KGaA), according to the manufacturer's instructions. Concerning the protocol, the U Primer Set was defined as that which annealed to unmethylated DNA that has undergone a chemical modification, the M Primer Set was that which annealed to methylated DNA, and the W Primer Set was that which served as a control for efficiency of chemical modification. The primer sequence was not provided by the manufacturer, which only specified that the amplified region is defined as the sequence between the 3'-nucleotide of the sense primer and the complement of the 3'-nucleotide of the anti-sense primer for each gene promoter. The nucleotide numbering system was the one used in the GenBank submission, identified as AY324387 for GSTpi. For each experiment, the controls provided by the test were used, namely U control DNA and M control DNA, which were amplified with their corresponding primer set and served as the controls for unmethylated and methylated DNA, respectively, and untreated W genomic control DNA, which was amplified with the W primer set and served as a control for the efficiency of chemical modification. The PCR products were electrophoresed on a 2% agarose gel and visualized with ethidium bromide. Finally, a negative PCR control (i.e., no DNA) was performed for each set of primers (Figs. S1 and S2).

The specificity and sensitivity of the test were determined, to yield positive and negative predictive values of the test. 95% confidence intervals (CI) were calculated to quantify the statistical precision of the measurements (10). For comparing continuous variables, the mean and the standard deviation (mean ± SD) are presented, and comparisons were made using Student's t-test for independent variables. For comparing proportions, in the case of dichotomous variables, the χ^2 test was used. The summary data for these variables are presented as proportions. To determine the relationship between PSA values and the GST-P1 methylation status, a point-biserial correlation was used. This method represents a special case of Pearson's product moment correlation applied to a dichotomous and a continuous variable, as described in IBM documentation for SPSS (v.19.0). $P<0.05$ was considered to indicate a statistically significant difference.

The study received the ethical committee approval (no. 446/30.03.2018) of the Ethical Committee for Clinical Studies of the Emergency County Hospital Constanta. Procedures at all stages of the study were carried out in compliance with the principles of the Declaration of Helsinki. Informed consent forms were received from all participants prior to their enrolment in the study group.

Results

The total number of patients was 80. As the present study was a retrospective study, tests were performed on all of the patients, with no dropouts. The main characteristic of the sample group of patients was that all the participants had PSA values between 4 and 10 ng/ml. The results of the test are detailed in Fig. 1.

Subsequently, the characteristics of patients with PC and those with a benign tumour, or BPH, were analysed (Table I). Patients diagnosed with PC tended to be older (70.02 years; SD=8.7) compared with patients with BPH (64.07 years; SD=8.9), and these patients also came predominantly from urban areas, i.e., a higher percentage of patients from urban areas were diagnosed with PC. All other measured parameters, including prostate volume, LUTS and PSA values, were found not to have statistically significant differences (all P-values ≥0.5). DRE raised the suspicion of PC in 69.8% of the

Table I. Descriptive statistics of the sample (n=80).

Variable	Prostate cancer (n=53)	Benign tumour (n=27)	P-value
Mean age ± SD (years)	70.02±8.70	64.07±8.90	0.005[a,b]
Mean prostate volume ± SD	46.579±13.025	42.226±13.029	0.162[b]
PSA value (ng/ml)	7.08±1.81	7.13±1.87	0.91[b]
Environment (urban/rural)	31/22	8/19	0.015[a,c]
LUTS (present/absent)	22/31	16/11	0.133[c]
Suspicion at digital rectal exam (yes/no)	37/16	8/19	0.001[a,c]
GST-P1 expression (positive/negative)	37/16	5/22	0.001[a,c]

[a]Statistically significant (P<0.05), as highlighted in bold; [b]ANOVA test; [c]Fisher's exact test. LUTS, lower urinary tract symptoms; PSA, prostate-specific antigen; GSTp1, glutathione S-transferase gene P1.

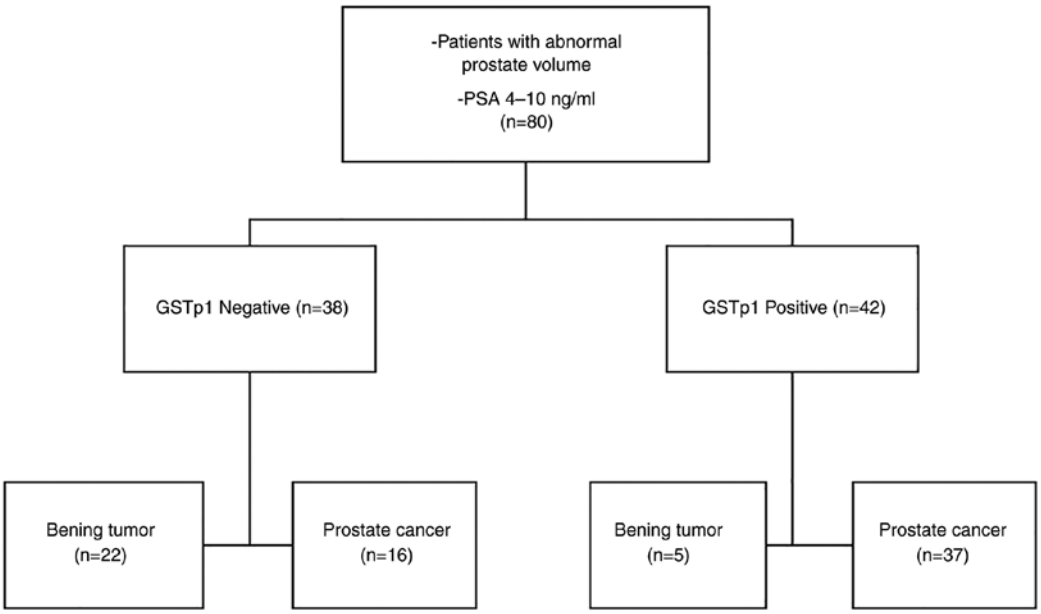

Figure 1. Patient flow chart. PSA, prostate-specific antigen; GSTp1, glutathione S-transferase gene P1.

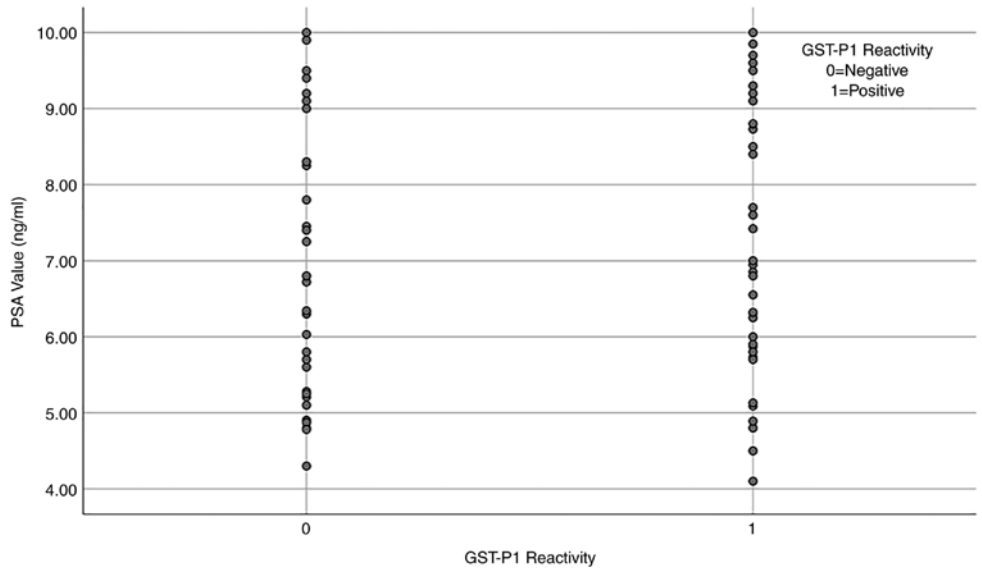

Figure 2. Simple scatter of PSA values by GST-P1 methylation status. GST-P1/PSA correlation curve is shown. PSA, prostate-specific antigen; GST-P1, glutathione S-transferase gene P1.

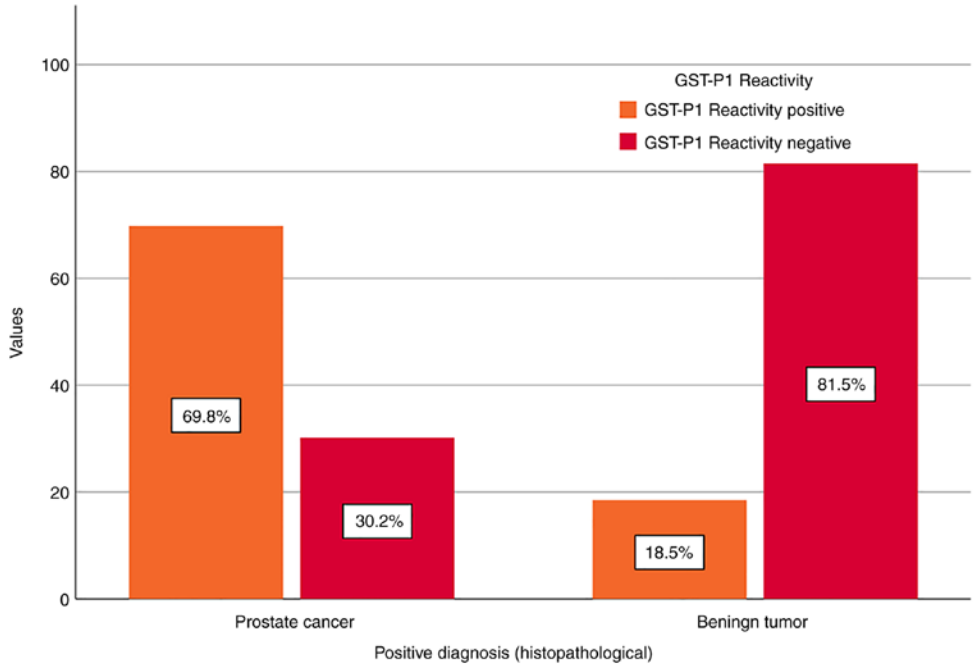

Figure 3. Patients' distribution-GST-P1 reactivity and diagnosis. GST-P1, glutathione S-transferase gene P1.

Table II. Screening test results.

Variable	Value	95% CI
Sensitivity	69.81%	55.66-81.66%
Specificity	81.48%	61.92-93.70%
AUC	0.76	0.65-0.85
Positive likelihood ratio	3.77	1.68-8.48
Negative likelihood ratio	0.37	0.24-0.58
Disease prevalence	66.25%	54.81-76.45%
Positive predictive value	88.10%	74.37-96.02%
Negative predictive value	57.89%	40.82-73.69%

AUC, area under the receiver operating characteristic curve.

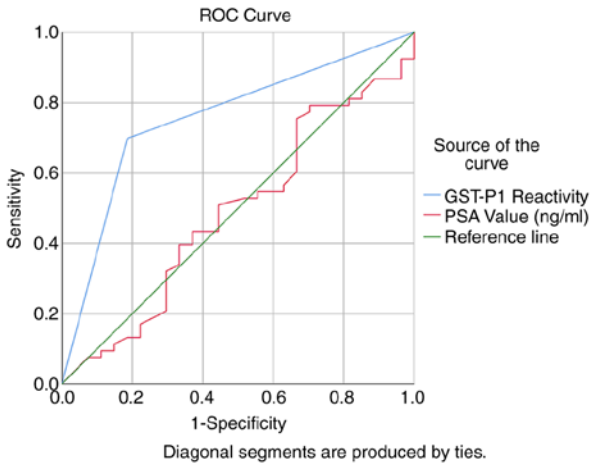

Figure 4. ROC curves for GST-P1 and PSA for diagnosing Prostate Cancer. PSA, prostate-specific antigen; GST-P1, glutathione S-transferase gene P1; ROC, receiver operating characteristic curve.

patients diagnosed with PC, but also raised the suspicion of malign tumour in 29.6% of the patients with a BPH.

A point-biserial correlation analysis was performed to determine the relationship between PSA values and GST-P1 methylation status. A positive correlation was identified, although this was not found to be statistically significant (r_{pb}=0.081; n=80; p=0.473) (Fig. 2).

Furthermore, more detailed attention was paid to the results for GST-P1 reactivity in patients within the grey area of PSA values. Among the 53 patients diagnosed with PC, 69.8% (n=37) were GST-P1-positive, whereas, among the 27 patients diagnosed with BPH, 18.5% (n=5) were GST-P1-positive. The calculated accuracy of the test was 73.75%, as it correctly identified 37 patients with PC and 22 patients with BPH (Fig. 3).

The calculated sensitivity for diagnosing PC in patients with PSA values between 4 and 10 ng/ml was 69.81% (95% CI, 55.66-81.66%), and the specificity was 81.48% (95% CI, 61.92-93.70%) (Table II). At the same time, based on the prevalence given by the study population, the positive predictive value was determined to be 88.1% (95% CI, 74.37-96.02%), and the negative predictive value had a lower value of 57.89% (95% CI, 40.82-73.69%). The receiver operating characteristic (ROC) curve was subsequently drawn for GST-P1 and PSA for the diagnosis of PC (Fig. 4).

Discussion

The present study aimed to evaluate the potential of using the GST-P1 gene as a biomarker for the diagnosis of PC in patients for which the PSA value is inconclusive, i.e., within the 'grey area', defined as values between 4 and 10 ng/ml. The results of the analysis indicate that GST-P1 has good potential to discriminate between patients with PC or BPH.

The calculated sensitivity was 69.81%, whereas the specificity of the test was 81.48%, with a positive predictive value of 88.1% and a negative predictive value of 57.89%. These results suggest that the evaluation of GST-P1 in patients for which the PSA is inconclusive may prove to be useful for diagnosing the presence or absence of PC, allowing for a faster detection time and treatment initiation.

Methylation of the GST-P1 gene represents the most common genetic alteration that is reported in PC (11,12), being observed in >90% of cases of PC, whereas it is seldom observed in benign prostate tissue (13). A recently published systematic review and meta-analysis (14) estimated that the incidence of GST-P1 methylation was higher in patients with PC than in those without, with an odds ratio (OR) of 18.58 (95% CI, 9.6-35.35; P<0.001). The detection of GST-P1 was considered in several studies as a non-invasive diagnostic tool for early detection of PC (15,16), being evaluated within meta-analysis (17). The results tend to vary a lot, and, as determined by Wu *et al* (17), the pooled specificity of GST-P1 was found to be excellent (89%; 95% CI, 80-95%) with a lower sensitivity, of 63% (95% CI, 50-75%). Another meta-analysis that analysed >35 studies which focused on the usefulness of GST-P1 in PC diagnosis (18) concluded that the sensitivity for GST-P1 (on biopsies) was 81.7±8.3%, and the specificity was 95.8±0.6%.

Another recent study suggested that GST-P1 may be involved in the development and progression of various types of cancers, fincluding lung cancer, colorectal cancer, gastric cancer, and even metabolic diseases, with these roles being evaluated in recent works (19).

Although, in general, research conducted previously has been carried out on participants that were evaluated for the presence of PC (and thus the characteristics of the test were applicable to the general population), the particularity of our study was the fact that it was focused solely on patients for which the PSA is inconclusive (within the range of 4-10 ng/ml). This might explain the lower value of the specificity when compared with other studies, and also could account for the higher value of the sensitivity.

Another major difference, which, in the context of screening purposes may be a limitation of our study, refers to the method of measuring the methylation status of GST-P1, which was executed by DNA genomic isolation from the harvested tissue. Previously published studies (16,20-22) have indicated that there is a correlation between the detection of GST-P1 from tissue samples and the methylation status examined from urine samples, within various limits. Other studies showed significant differences in the sensitivity and specificity of GST-P1 for PC, depending on the testing method (23); therefore, new research on the potential of GST-P1 usage as a screening test in patients within the 'grey-area' of PSA values could bring valuable new information for the development of novel methods of identifying patients with PC. Another possible limitation of the present study was the absence of other methods for determining the level of GST-P1 expression (i.e., immunohistochemistry).

The usage of genetic markers for the diagnosis of oncological conditions is increasing, as their potential to serve this purpose is very promising. In the present study, the potential of GST-P1 marker usage was evaluated in the diagnosis of PC in patients for which the PSA values were uncertain (within the 'grey area'). The results indicated a good sensitivity of 69.8% and a good specificity of 81.48%, when compared with the golden standard of diagnosis-histopathological examination. These results have the potential of sustaining the use of this diagnosis method in patients for which the suspicion of PC exists, but the PSA values are inconclusive.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MS, VB, DOC, AIS, CT and FV contributed to the conception and design of this study. MS, APS, LM, AM, CB and DS were responsible for the data collection and analysis. MS, AIS, APS, CT and DOC oversaw drafting the manuscript. LM, DS, VB, AM, CT, CB and FV revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript. MS and FV confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Committee of the Clinical County Emergency Hospital 'St. Andrew' Constanta (approval no. 446, approval date: 30.03.2018). The written informed consent was obtained from all subjects. The research was carried out respecting all the international and national regulations and in agreement with the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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