Preoperative serum glutathione S-transferase P1(GSTP1) hypermethylation predicts the risk of serum PSA recurrence in men following radical prostatectomy

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Abstract:

In our study,we evaluated the association of preoperative GSTP1 hypermethylation in men with clinically localized PCa who underwent radical prostatectomy with serum PSA recurrence, on a period of 24 months.We included in our study a number of seventy males with clinically localized PCa, who underwent radical prostatectomy at the Urology Clinic from County Emergency Hospital Timisoara.All serum samples were collected before prostatectomy .PSA recurrence was defined as a single postoperative PSA level ≥0.2ng/ml.Of the 70 males from our study, 39(55.71%) experienced biochemical serum PSA recurrence within the study period (median time to PSA recurrence 2 years),compared to the 31(44.29 %) nonrecurrent males who did not experienced PSA recurrence.Our study suggests that preoperative serum GSTP1 might be used as a predictive biomarker for men with clinically localized PCa following radical prostatectomy.

Key words: prostate cancer (PCa); methylation-specific polymerase chain reaction (MSP); prostate-specific antigen (PSA);glutathione S-transferase P1 (GSTP1)

1.Introduction:

Prostate cancer (PCa) represents one of the most common malignancy among men worldwide,with an estimated 193.000 new cases and 27.500 prostate cancer related deaths in the United States in 2009[1].Primary therapy in the form of radical prostatectomy is one of the treatment modalities available for men with clinically localized disease. Several clinicopathological scoring systems have been developed to identify the patients who present risk of recurrence after surgery, including the weighted risk of recurrence, determined by the lymph node status, seminal vesicle status,surgical margin status, and postoperative Gleason score and the Kattan nomograms [2].Biochemical (prostate-specific antigen) recurrence of prostate cancer after radical prostatectomy remains a major problem [3]. Men who experience early biochemical PSA recurrence, within the first 2-3 years after surgery, are likely to develop metastatic lesions and are likely to die of their prostate cancer[4].

Early occult dissemination of PCa cells from the primary tumor through the bloodstream into secondary organs is a critical step in tumor progression. Currently, repeated measurements of PSA blood serum levels are done after the primary treatment of PCa (e.g.surgery or radiotherapy). An increase in PSA levels (called biochemical recurrence) cannot distinguish between a local or metastatic relapse. Moreover, even if overt metastases are subsequently confirmed by current imaging technologies (e.g., bone scans) the patient has become already incurable.Thus, a biomarker indicating early spread of tumor cells as the potential seed for future metastases is highly desirable. It has been demonstrated that about two thirds of patients treated by radical prostatectomy, remain disease free following radical prostatectomy,but there is still one third of patients who will present serum PSA recurrence within the first 2-3 years after surgery, and they are likely to develop metastatic lesions and to die of prostate cancer[5]. Because of these, a minimally invasive, preoperative test, that might identify patients at risk for early serum PSA recurrence postsurgery, would be useful in the treatment management.

Epigenetic changes represent changes in gene expression, which are not caused by alterations in the primary sequence of the nucleotides that compose the gene. It has been demonstrated that, one of the most common epigenetic change and the most frequent molecular alterations in human cancers is DNA hypermethylation [6]. Gluthatione S-transferase P1(GSTP1) is the most common somatic genome abnormality in human prostate cancer [7]. The somatic GSTP1 hypermethylation has been observed in more than 90% of PCa cases, whereas it has not been observed in normal or benign prostate [8]. Although GSTP1 hypermethylation is thought to be an early event during prostate carcinogenesis, it has also been detected in patients with advanced prostate cancer and metastatic lesions.Analyses of body fluids from men with the diagnosis of PCa showed that DNA contains GSTP1 hypermethylation, thus suggesting a role of this epigenetic gene as a predictive biomarker in detection of PCa recurrence [9].

In our study, using the methylation-specific polymerase chain reaction (MSP) technique, we examined the methylation status of GSTP1 gene in the preoperative serum of men with localized PCa, to determine its association with serum PSA biochemical recurrence and found found that GSTP1 hypermethylation is the strongest predictor of early biochemical recurrence following surgical monotherapy for this malignancy[10].

2.Materials and methods:

In our study we recruited consecutive 70 patients with localized PCa undergoing radical prostatectomy. Serum samples were obtained preoperatively from the Department of Urology, County Clinical Emergency Hospital Timisoara, between 2008-2010. The clinico-pathological characteristics of the patients included in our study are presented in Table 1.The study was conducted in accordance with The World Medical Association Declaration of Helsinki statements and written informed consent was obtained from each patient. Routine serum PSA follow-up consisted of measurements 3 months postoperatively during the first year, biannually for the second year,and annually thereafter.

Table 1 Clinoco-pathological characteristics of the patients

We collected all clinical and pathologic data for all patients, including here: age, preoperative serum PSA, clinical and pathologic stage, Gleason grade, surgical margins status. All of the patients were followed for at least 2 years. Biochemical progression was defined as PSA values > 0.2 ng/ml and higher. None of the patients included in the study group received adjuvant or hormonal treatment at the time of biochemical recurrence.

2.1. Sample collection and DNA extraction:

Serum samples from all the patients included in the study were collected. Genomic DNA was extracted from serum using the QIAmp® DNA Mini Kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany).After extraction, the DNA level was measured using the PicoGreen® dsDNA Quantification Kit (Molecular Probes, Leiden,Netherlands).

2.2.Bisulfite treatment:

After denaturation with 3 M NaOH, DNA was treated over 3 hours with 100 µL of a solution of 2.5 M sodium bisulfite and 6.25 mM hydrochinone. For the purification of the bisulfite-treated DNA, we used the QIAquick® PCR Purification Kit Protocol (Qiagen,Germany), according to the manufacturer's instructions.

2.3. Methylation-specific polymerase chain reaction (MSP):

MSP was performed using primers and conditions selected to especially amplify bisulfite-treated promoter DNA for the gene of interest.The positive control was normal human DNA treated with Sss I methyl transferase before bisulfite modification. The negative control was distilled water without template. Positive and negative controls were added to all MSP reactions. MSP products were loaded on 3% agarose gels, prestained with ethidium bromide,separated electrophoretically, and visualized under ultraviolet illumination.

The primers used for amplification were:

Forward primer: 5'-TTCGGGGTGTAGCGGTCGTC-3' (methylated);

5'-GATGTTTGGGGTGTAGTGGTTGTT-3' (unmethylated)

Reverse primer: 5'- GCCCCAATACTAAATCACGACG-3' (methylated)

5'- CCACCCCAATACTAAATCACAACA-3' (unmethylated)

The PCR conditions were as follows: Hot start at 95ºC for 5 minutes (to fully denaturate the bisulfite-modified genomic DNA), 35 amplification cycles (94ºC for 30 seconds for denaturation,

58ºC for 30 seconds for primer annealing and 72ºC for 60 seconds for extension), and a final full extension at 75ºC for 3 minutes.

Fig 5 Electrophoresis of GSTP1 on 3% agarose gel

3.Statistical analysis:

For comparison of age distribution,serum PSA level and DNA methylation between the two groups Mann-Whitney U tests were performed. The correlations between GSTP1 methylation status and clinico-pathological parameters were analyzed using the chi-square test and Fisher exact test. All statistical analyses were performed with SPSS 16.0 (SPSS, Inc., Chicago, IL, 2008). P-values < 0.05 were considered as statistical significant.

The major statistical end point of our study was time to progression (TTP). Progression included PSA elevations of >0.2 ng/ml , metastasis and death. The event time distributions for this end point were estimated with the method of Kaplan and Meier

4.Results:

The maximum period of follow-up was 24 months .Using the MSP technique to analyse the methylation status of GSTP1 in the preoperative serum samples from the patients included in our study, we found that 31 (44.29%) of them presented hypermethylated GSTP1, compared to 39 (55.71%) who had unmethylated GSTP1.We also analyzed whether serum GSTP1 hypermethylation is associated with established clinicopathological parameters, such as: age, preoperative serum PSA, and Gleason score.

4.1.Correlation of age with preoperative GSTP1 hypermethylation:

The mean age of men with recurrencce was significantly higher compared to the mean age of nonrecurrent patients (Independent sample test; $p=0.009$;$\alpha=0.013$).

Fig 1 Comparison between the men ages for the two groups

Table 2 Descriptive statistics for age variable:

4.2.Correlation of preoperative serum PSA with GSTP1 hypermethylation:

A significant difference in the preoperative serum PSA was observed between the nonrecurrent and the recurrent group(Independent t-test; $p<0.001$; $\alpha=0.001$).

Fig 2 Comparison between the mean PSA values for the two groups of males

Table 3. Descriptive statistics for PSA variable

4.3. Correlation of recurrence with the clinicopathological factors:

We further analyzed the association between prostate cancer recurrence with the Gleason score. Gleason score, based on pathology was significantly higher in local recurrent cases compared with the nonrecurrent cases (Unequal-variance t-test; p=0.003).

Biochemical recurrence was observed in 31 patients(time to recurrence:17 months (mean), 12 months (median).

Fig 3 Percentage distribution for recurrence between the two groups

Kaplan-Meier analysis for the time to PSA progression following radical prostatectomy, showed a survival advantage for the patients without GSTP1 hypermethylation in the preoperatively serum samples (e.g, serum PSA recurrence in 44.29% vs.55.71% patients without GSTP1 hypermethylation). The recurrence probability after 12 months was of 50% in the study group.

Fig 4 Kaplan-Meier survival analysis regarding the serum PSA recurrence in the study group

5.Discussion:

The risk of recurrence and metastatic progression for prostate cancer patients who undergo radical prostatectomy can be estimated in relative accuracy based on the PSA doubling time.Predicting the probability of recurrence in males undergoing radical prostatectomy using the molecular techniques, will enable urologists to distinguish between patients who have an indolent cancer not requiring any form of treatment, and patients with biochemical relapse who may have a more aggressive course requiring early intervention.

In the present study, we have shown that men with clinically localized PCa and methylated preoperative serum GSTP1, experienced biochemical serum PSA recurrence within the first 2 years following radical prostatectomy. Chuang et al reported GSTP1 hypermethylation status in 36 patients with prostate cancer using a restriction enzyme based, real-time PCR approach.They found GSTP1 hypermethylation in 11 of 36 plasma samples of patients with prostate cancer, but did not report a correlation with diagnostic or prognostic variables[11].

In one of their studies,Goessl et al. [12] reported that hypermethylation of GSTP1 was present in serum samples of patients with advanced PCa.

These findings warrant further analysis of the genes in a large study cohort to explore the implications of a panel of genes for prediction of prostate cancer risk of recurrence[13].

Our study has several limitations,such as the sample size and the relatively short follow-up, which could have limited our ability to detect differences attributed to other variables.

6.Conclusion

The detection of DNA hypermethylation in bodily fluids (serum,plasma or urine)samples is technically possible, it may improve the current screening and detection methods for prostate cancer and its recurrence. In our study, we have demonstrated the ability of GSTP1 to predict the risk of early PSA recurrence, which may aid in the adjuvant treatment strategies within 2 to 3 years after radical prostectomy.

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Tables and Figures

Table 1

Table 1. Clinico-pathological characteristics of 70 men with clinically localized prostate cancer treated with radical prostatectomy

	Study cohort, n = 70
Age (y), mean (range)	68,9 (50 – 71)
Preoperative PSA (ng/mL), mean (range)	7,68 (0,9 – 38)
Pathologic Gleason score (%)	
5 – 6	19 (27)
7	37 (53)
8 - 10	14 (20)
Pathologic stage (%)	
Organ confined	31 (44)
Extraprostatic extension	27 (39)
Seminal vesicle invasion	8 (11)
Positive surgical margins	4 (6)

Table 2

No.	Mean for age	Std. Deviation	Std. Error Mean	Range
39 without recurrences	61.49	9.673	1.549	47-80
31 with recurrences	67.74	9.619	1.728	48-82

Table 3

No.	Mean for PSA	Std. Deviation	Std. Error Mean	Range (ng/mL)
39 without recurrences	16.46	2.32	0.371	12-23
31 with recurrences	20.90	3.91	0.702	14-28

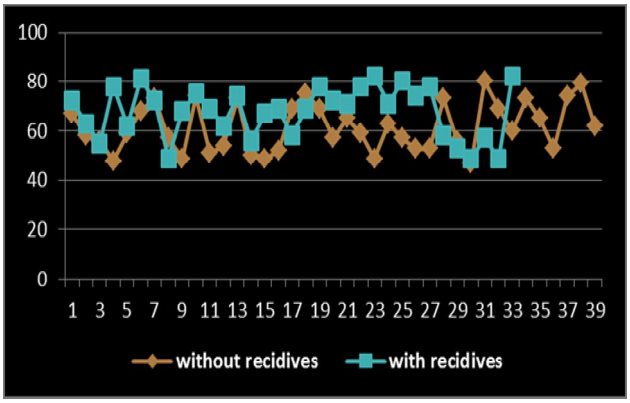

Figure 1. Comparison between the men ages for the two groups

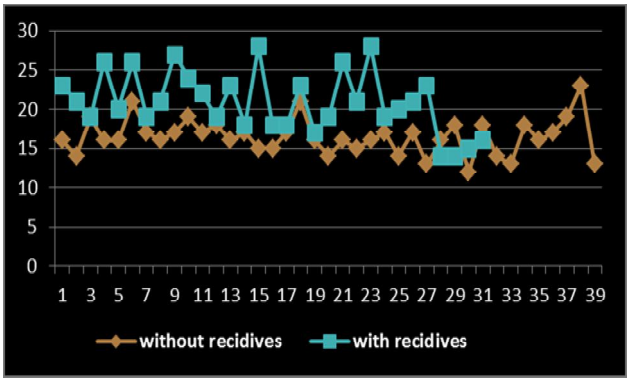

Figure 2. Comparison between the mean PSA values for the two groups of males

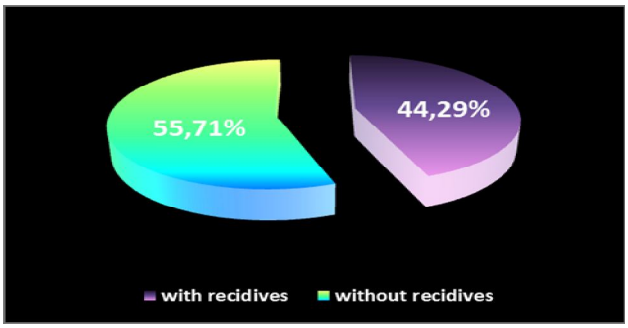

Figure 3.Percentage distribution for recurrence between the two groups

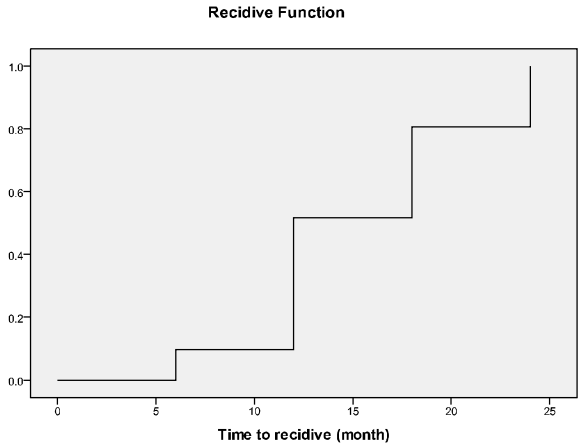

Figure 4. Kaplan-Meier survival analysis regarding the serum PSA recurrence in males from the study group

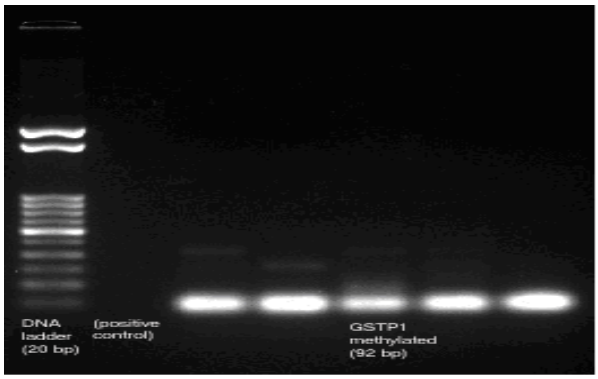

Figure 5. Electrophoresis of GSTP1 on 3% agarose gel