# Diagnostic Value of GSTP1, RASSF1, and RASSF2 Methylation in Serum of Prostate Cancer Patients

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**Purpose:** Considering the inadequacy of PSA measurement in the diagnosis of prostate cancer, it is aimed to establish a potential liquid biopsy diagnostic panel.

**Materials and Methods:** 39 patients who underwent TRUS-biopsy and 15 healthy volunteers were included. Approximately 15 ml of venous blood samples taken from healthy volunteers and patients before biopsy were separated as plasma. Hypermethylation status of GSTP1 and RASSF1:RASSF2 genes was revealed in cfDNA materials collected from plasma samples. Correlation of this epigenetic change detected in PCa, BPH and healthy volunteer groups with pathology results was examined.

**Results:** Pathology reports of 39 patients included were 13 PCa, 3 ASAP, 3 HGPIN, and 20 BPH. In total, 3 of the patients with PCa had positive GSTP1, 4 had RASSF1 and 9 had positive RASSF2 methylation. It was seen that RASSF2 had the highest sensitivity (69%), specificity (39%) and NPV (80%), while RASSF1 had the highest PPV (30%). When the binary combinations of genes were examined it was observed that the GSTP1:RASSF1 combination had the highest sensitivity (46%), specificity (76%) and NPV (82%). When the methylation of all three genes was examined, it was observed that the sensitivity was quite low (8%), but the specificity (83%) increased significantly.

**Conclusion:** Although we observed that the GSTP1 and RASSF1 methylation positivity rates that we examined in our study were higher in patients without prostate cancer, we found that the RASSF2 methylation rate was higher in patients with prostate cancer. randomized controlled studies are needed.

**Keywords :** GSTP1; RASSF1; RASSF2; Prostate Cancer

## INTRODUCTION

Prostate cancer (PCa) is the most common type of cancer in men. PCa ranks second in cancer-related deaths in men.[1] Although prostate specific antigen (PSA) measurement is widely used in PCa screening, it has important disadvantages. While the false positive rate of PSA measurement is approximately 60%, the false negative rate is around 15%, and 2% of these are aggressive prostate cancers.[2] For this reason, the search for new biomarkers for the diagnosis of PCa has been continuing for many years.

Liquid biopsy was first described in 1869 by pathologist Thomas Ashworth, who demonstrated the presence of free- CTCs in the blood of a patient with metastatic cancer.[3]

CTCs are rare cells originating from primary and metastatic tumors that circulate throughout the body to form metastatic foci in other tissues.[4,5] Nucleic acids present in CTCs, proteins expressed on or within the surface of CTCs, and the number of these CTCs are potential cancer biomarkers, allowing monitoring of epigenetic and genetic changes.[6]

Cell free nucleic acids are circulating cell-free DNA(cf-DNA) or RNA(cf-RNA) fragments released after destruction of apoptotic or necrotic cells.[7,8] cf-DNA in the blood can be detected at higher levels in PCa patients, with 80% sensitivity and 82% specificity compared to control individuals.[9] In addition, more than 50% of blood samples and more than 70% of urine samples taken from PCa patients showed cf-DNA changes that can be used as PCa biomarkers.[10]

Recent studies on DNA methylation in prostate tumors have revealed a gene panel that is frequently hypermethylated in PCa.[11] The most common epigenetic alteration in prostate carcinogenesis is hypermethylation in the promoter region of the glutathione-S-transferase P1 (GSTP1) gene. GSTP1 promoter methylation is present in up to 90% of prostate cancer tissues and two-thirds of intraepithelial neoplasia tissues, but is rarely present in BPH tissue.[12] Other genes commonly found to be methylated in prostate cancer include RASSF1, RARB, APC, MDR1, PTGS2, TIMP3, and CDH1.[11]

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**Table 1.** Primer design [16]

| | | |
|---|---|---|
| GSTP1 | Non-methylated targets | 5′- GATGTTTGGGGTGTAGTGGTTGTT-3′,<br>5′- CCACCCCAATACTAAATCACAACA-3′ |
| | Methylated targets | 5′-TTCGGGGTGTAGCGCTCGTC-3′ (sense),<br>5′-GCCCCAATACTAAATCACGACG-3′ |
| RASSF1 | Non-methylated targets | 5′-ATCGTGGTTTATTTTTTAGTTCGA-3′<br>3′-ATAAAAAAATTCGAATCTCTCCGA-5′ |
| | Methylated targets | 5′-TATTGTGGTTTATTTTTTAGTTTGA-3′,<br>3′-ATAAAAAAATTCAAATCTCTCCAAA-5′.<br>ACTB5′-TGGTGATGGAGGAGGTTTAGTAAGT-3′,<br>5′-AACCAATAAAACCTACTCCTCCCTTAA-3′ |
| RASSF2 | Promoter region | CTAAAACCTCAACCTAAC<br>GATTTAGAGTTGAATGTAAAGTAA |
| b-actin | Normalization factor | TGGTGATGGAGGAGGTTTAGTAAGT<br>AACCAATAAAACCTACTCCTCCCT-TAA |

While most studies of epigenetic changes in PCa have focused primarily on the evaluation of prostate tissue [13], only a limited number of studies[14-15] have analyzed a panel of DNA methylation biomarkers in serum samples from PCa. In this context, our study aimed to examine the methylation of GSTP1, RASSF1:RASSF2 genes in liquid biopsy materials that are aimed to be detected in blood samples taken at the diagnostic stage in patients with suspected PCa, and to demonstrate that this methylation state can be used as an alternative diagnostic tool to PSA in the pre-biopsy period in the diagnosis of PCa.

## MATERIALS AND METHODS

Study group: 39 patients and 15 healthy volunteers who were scheduled for TRUS-prostate biopsy in our clinic were included in our study. After obtaining approval from the ethics committee of our hospital (Approval Number: 2325), patients who applied to our clinic from February 2020 until March 2021, and who had prostate biopsy indications according to PSA measurement and digital rectal examination(DRE) findings, were evaluated prospectively. At this stage, the PSA cut-off value was accepted as 4 ng/dl, and patients with a PSA value above 4 ng/dl or with a normal PSA value but with suspicious findings in the prostate on rectal examination were included in the study. However, patients who had undergone an invasive procedure to their prostate (TUR-P, TVP, TRUS-Biopsy) or had another cancer disease were not included in the study.

Among the prostate tissue samples taken from 39 patients, plasma samples of 13 patients evaluated as prostate cancer, 3 patients evaluated as atypical small acinar proliferation (ASAP), 3 patients evaluated as high grade prostatic intraepithelial neoplasia (HGPIN) and 20 patients evaluated as BPH were studied comparatively. In addition, the study was carried out comparatively with plasma samples taken from 15 healthy men who did not have any urological complaints and did not have any other cancer history as the control group.

cf DNA isolation: Approximately 15 ml of venous blood samples were taken from the healthy volunteers and patients before the biopsy. These blood samples were centrifuged at 4500 rpm for 15 minutes and the

**Table 2.** Demographic data

| | PCa(n) | ASAP(n) | HGPIN(n) | BPH(n) | Healthy (n) |
|---|---|---|---|---|---|
| Study group (n) | 13 | 3 | 3 | 20 | 15 |
| Age (Average) | 66 | 64 | 58 | 63 | 51 |
| Family story (Yes: 1, No:0)) | 0 | 0 | 0 | 1 | 0 |
| DRE | | | | | |
| Abnormal | 9 | 1 | 1 | 5 | 0 |
| Normal 4 | 2 | 2 | 15 | 15 | |
| PSA ng/ml (Average) | 19,86 (2,6-65) | 10,06 (7,1-15,85) | 17,25 (5,9-38,6) | 8,94 (2,36-15,47) | 1,37 ( 0,28-2,51) |
| high >10 | 6 | 1 | 1 | 4 | 0 |
| low <10 | 7 | 2 | 2 | 16 | 15 |
| fPSA µg/l | 2,26 (0,48-3,28) | 1,14 (1,11-1,17) | 2,85 (0,23-5,47) | 2,01 (0,3-4,41) | 0,36 (0,03-1,01) |
| fPSA/tPSA | 0,13 (0,01-0,28) | 0,08 (0,01-0,16) | 0,06 (0,01-0,14) | 0,18 (0,01-0,55) | 0,28 (0,11-0,50) |
| PSA density | 0,4 (0,03-1,08) | 0,18 (0,08-0,34) | 0,37 (0,24-0,54) | 0,19 (0,05-0,58) | 0,04 (0,01-0,1) |
| PCA % | 57,5 (10-100) | - | - | - | - |
| ISUP grade | | | | | |
| 1 | 4 | - | - | - | - |
| 2 | 3 | - | - | - | - |
| 3 | 2 | - | - | - | |
| 4 | 0 | - | - | - | - |
| 5 | 4 | - | - | - | - |
| D'amico risk classiffication | | | | | |
| Low | 3 | - | - | - | |
| Medium | 6 | - | - | - | |
| High | 4 | - | - | - | |
| GSTP1 methylation positive | 3 | 2 | 2 | 13 | 11 |
| RASSF1 methylation positive | 4 | 1 | 3 | 13 | 12 |
| RASSF2 methylation positive | 9 | 3 | 1 | 10 | 11 |

**Abbreviations:** PCa , Prostate cancer ; ASAP, atypical small acinar proliferation ; HGPIN, high grade prostatic intraepithelial neoplasia; BPH , benign prostatic hyperplasia; GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

**Table 3.** Genetic methylation positivity according to patient subgroups

| | PCa D'amico risk classiffication | | | HGPIN(n:3) | ASAP(n:3) | BPH(n:20) |
|---|---|---|---|---|---|---|
| | Low (n:3) | Medium (n:6) | High (n:4) | | | |
| GSTP1 | 1 | 1 | 1 | 2 | 2 | 13 |
| RASSF1 | - | 3 | 1 | 3 | 1 | 13 |
| RASSF2 | 2 | 4 | 3 | 1 | 3 | 10 |

**Abbreviations:** PCa , Prostate cancer ; ASAP, atypical small acinar proliferation ; HGPIN, high grade prostatic intraepithelial neoplasia; BPH , benign prostatic hyperplasia; GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

plasma part was separated as approximately 7 ml. Then, for cfDNA isolation, it was stored at -80 C to be used in DNA isolation processes. After the genomic DNA and debris were separated from the plasma according to the manufacturer's kit recommendations, cfDNA (Maxwell Promega) was separated by robotic application. cfDNA quantity and quality were checked with nanodrop spectrophotometer (Thermo).

PCR analysis: Potential epigenetic changes in the samples were examined by real-time PCR with the PCR primers[16] in Table 1 . Although APP and ACTB were selected as control targets in the samples, bisulfite treatment was examined in two separate parts (with and without bisulfite treatment) of the cfDNA obtained in each sample before PCR. Positive methylated DNA included in the kit was used as positive or negative control. PCR success rate was accepted as 80% efficiency value.

Statistical data analysis: The obtained data were coded and accumulated in MS Office Excel database. The datasets obtained here were collected for comparative analysis at GraphPad Prism version 8.0.0, GraphPad Software, San Diego, California USA, www.graphpad.com. The temperature graph and the distribution graphs of the data were prepared with the same software. Shapiro-Wilk normality analysis was used in the research. Pearson correlation analysis, CI values were prepared for both PKa and control groups. G-Power 3.1 program was used to determine the sample size of the study. Considering the effect size as 0.33, the $\alpha$ margin of error as 5%, and the power of the study as 80% compared to the group averages in similar studies , a total of 52 people should be included in the study. A total of 54 people were included in our study.

## RESULTS

As a result of the pathological evaluation, 13 patients were evaluated with prostate cancer, 3 patients ASAP, 3 patients HGPIN, and 20 patients BPH out of 39 patients. When the prostate cancer patients were classified according to the D'amico risk classification, 3 patients were in the low-risk group, 6 patients were in the medium-risk group, and 4 patients were in the high-risk group. (**Table 2**).

Evaluation of GSTP1, RASSF1:RASSF2 results with cfDNA obtained from serum in control and patient groups:

GSTP1 was evaluated before and after bisulfite-mediated methylation from cfDNA samples isolated from serum. Figure 1 shows a total of 54 cases evaluated in both control and patient groups (BPH, ASAP, HGPIN and prostate cancer) according to Ct numbers. In this context, although there are methylated GSTP1 targets in the control group, high variation is observed in the patient group.Similarly, a similar distribution was observed for RASSF1 in **Figure 1**. RASFF2 differed from GSTP1 and RASSF1 (**Figure 1**).

GSTP1 methylation was observed in 1 of 3 patients subgrouped as PCa in the low risk group, while 2 cases methylated for RASSF1 were detected. Again, 1 of the same group is methylated for RASSF2. In only 1 of 3 cases, the RASSF1:RASSF2 target was seen together (**Table 3**). In this context, the rate of GSTP1 methylation tends to increase in the low-risk group PCa group. The number of cases subgrouped as intermediate risk PCa was 6 in our study, and the number of methylated cases was determined as 1 for GSTP1, 3 for RASSF1, and 4 for RASSF2. The number of cases seen together with RASSF1 and GSTP1 is 3, and the number of cases with methylation with RASSF2:GSTP1 is 1. In the high-risk PCa group, the number of methylated cases for GSTP1 was determined as 2, 1 for RASSF1, and 3 for RASSF2. In these cases, RASFF1:GSTP1 co-methylation was 3, and RASSF1:RASSF2:GSTP1 co-methylation was found to be 1.

There are 3 people in the HGPIN group and 3 people in the ASAP group. In these cases, cases with GSTP1 methylation were defined as 2 cases. In HGPIN cases, RASSF1 methylation was detected in 3 individuals and RASSF2 methylation was detected in 1 person. In the HGPIN group, 1 person with GSTP1: RASSF1: RASSF2 methylation was detected. On the other hand, RASSF1 methylation was determined as 1 and RASSF2 methylation was determined as 3 in the ASAP group. Co-methylation of GSTP1: RASSF1 and RASSF1: RASSF2 was observed in 1 of these cases. The cases

**Table 4.** Genetic combination analysis by patient subgroups

| | GSTP1:RASSF1 (n) | RASSF1:RASSF2 (n) | GSTP1:RASSF1:RASSF2 (n) | RASSF2:GSTP1 (n) |
|---|---|---|---|---|
| Low PCa | - | 1 | | |
| Medium PCa | 3 | - | - | 1 |
| High PCa | 3 | 1 | 1 | 1 |
| HGPIN | 1 | 1 | 1 | 1 |
| ASAP | 1 | 2 | - | 1 |
| BPH | - | 6 | - | 7 |
| Healthy | 8 | 9 | 6 | 8 |

**Abbreviations:** PCa , Prostate cancer ; ASAP, atypical small acinar proliferation ; HGPIN, high grade prostatic intraepithelial neoplasia; BPH , benign prostatic hyperplasia; GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

**Table 5.**Diagnostic value of genetic methylations in prostate cancer diagnosis

| | PCA+ (n:13) | PCA- (n:41) | Sensitivity (%) | Specificity (%) | NPV(%) | PPV(%) |
|---|---|---|---|---|---|---|
| GSTP1 | 3 | 28 | 23 (95% Cl 5% - 54%) | 32 (95% Cl 18% - 48%) | 57 (95% Cl 43% - 69%) | 10 (95% Cl 4% - 23%) |
| RASSF1 | 4 | 29 | 31 (95% Cl 6% - 61%) | 29 (95% Cl 16% - 46%) | 57 (95% Cl 42% - 71%) | 30 12 (95% Cl 6% - 24%) |
| RASSF2 | 9 | 25 | 69 (95% Cl 39% - 91%) | 39 (95% Cl 24% - 55%) | 80 (95% Cl 62% - 91%) | 26 (95% Cl 19% - 36%) |
| GSTP1:RASSF1 | 6 | 10 | 46 (95% Cl 19% - 75%) | 76 (95% Cl 60% - 88%) | 82 (95% Cl 72% - 88%) | 11 37 (95% Cl 21% - 57%) |
| GSTP1:RASSF2 | 2 | 17 | 15 (95% Cl 2% - 45%) | 59 (95% Cl 42% - 74%) | 69 (95% Cl 61% - 76%) | 1815 (95% Cl 3% - 31%) |
| RASSF1:RASSF2 | 2 | 18 | 15 (95% Cl 2% - 45%) | 56 (95% Cl 40% - 72%) | 68 (95% Cl 59% - 75%) | 1910 (95% Cl 3% - 29%) |
| GSTP1:RASSF1: RASSF2 | 1 | 7 | 8 (95% Cl 0,2%-36%) | 83 (95% Cl 68% - 93%) | 74 (95% Cl 70% - 78% | 812) (95% Cl 2% - 51%) |

**Abbreviations:** PCa , Prostate cancer ; NPV, negative predictive value; PPV, positive predictive value ;GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

with BPH are 20. While GSTP1 and RASSF1 methylation were observed in 13 cases, RASSF2 methylation was observed in 10 cases. RASSF1:RASSF2 methylation was detected together in 6 individuals. RASSF2:GSTP1 methylation was observed in 7 people. GSTP1:RASSF1:RASSF2 co-methylation was observed in only 2 individuals in the entire group. On the other hand, in the control group, GSTP1 and 11 RASSF2 methylation were observed in 11 out of 15 cases, and RASSF1 methylation was observed in 12. Co-methylation of GSTP1:RASSF1:RASSF2 was observed in 6 individuals. RASSF1:RASSF2 co-methylation is seen in 9 people. RASSF2:GSTP1 methylation was observed in 8 individuals (**Table 4**).

When the genetic methylations studied together with these findings are examined one by one, RASSF2 has the highest sensitivity (69% ,95% Cl 39% - 91%), specificity (39% ,95% Cl 24% - 55%) ,negative predictive value (NPV) (80% , 95% Cl 62% - 91, and positive predictive value (PPV) (26% , 95% Cl 19% - 36%) . When the binary combinations of genes were examined, it was seen that the GSTP1:RASSF1 combination had the highest sensitivity (46% , 95% Cl 19% - 75%), specificity (76% , 95% Cl 60% - 88%) ,NPV (82% , 95% Cl 72% - 88%) and PPV(37% , 95% Cl 21% - 57%). When the methylation of all three genes was examined, it was observed that the sensitivity was quite low (8%, 95% Cl 0,2% - 36% ), but the specificity (83%, 95% Cl 68% - 93% ) increased significantly. (**Table 5**)

When the studied gene methylations are evaluated in general, there is a general trend of increasing specificity with decreasing sensitivity as the number of genes combined increases.

The correlations between the three genes we examined in patients with and without prostate cancer were analyzed. There was a statistically significant negative correlation between GSTP1 methylation and RASSF1 methylation in patients without prostate cancer (r:-0.365; 95% CI:-0.6049, -0.06482; $p$ = 0,02). In other examinations, no statistically significant correlation was observed in terms of genetic methylation in both groups ($p$ > 0.05). (**Table 6-9**).

## DISCUSSION

For the first time, in 1948, Mandel and Metais described the extracellular nucleic acids and cfDNAs they detected in the blood of healthy individuals.[17]Following this discovery, it was demonstrated that circulating tumor DNA (ctDNA) fragments released into the bloodstream after active secretion or cell death in various cancer patients could be detected.[18,19]

Depending on different causes, such as tumoral cell load, number of metastases, and cell proliferation, ctDNAs can make up 0.01% to 90% of all ccfDNA detected in the blood of cancer patients.[19] Since ctDNAs have tumor-specific genetic properties of all tumoral subtypes, ccfDNAs are the most suitable candidates for liquid biopsies that allow for cancer diagnosis and prognostic prediction.[20]

DNA methylations are the first detected and most studied epigenetic modifications in cancers.[21,22] Since these changes can be evaluated in samples taken from different body fluids[23], liquid biopsy evaluations based on DNA methylation are promising not only in the detection of early stage/premalignant cancers, but also in terms of providing prognostic information.In addition, since some gene domains seem to exhibit tissue-specific DNA methylation, it may be possible to differentiate the primary tumor between different types of cancer in liquid biopsies or metastatic tumors.[23]

Because epigenetic changes are often multiple and do not need to overlap, multiple gene panels are crucial to enhance the lower susceptibility of individual genes. In this context Ellinger et al. showed that when a multiple gene panel containing GSTP1, PTGS2, RPRM and TIG1 was used in preoperative serum samples, the diagnostic coverage of PCa increased from 42% (GSPT1 alone) to 47% (panel) and retained 93% specificity. (24) In our study, on the contrary, it was observed that the sensitivity for GSTP1 alone, which was 23%, decreased to 8% with the combined gene panel.

In addition, Sunami et al. They reported that RASSF1A,GSTP1, and RARB 2 were hypermethylated in 24%, 13%, and 12%, respectively, of serum samples from patients diagnosed with CaP, while the tri-gene panel increased the diagnostic sensitivity rate to 29% and the specificity to 100% .[25] Addition of FOXA to

**Table 6.** Control patients according to Pearson correlation and CI values

| | mRASSF1 | mRASSF2 | mGSTP1 |
|---|---|---|---|
| mRASSF1 | 1 | 0,207 %95 CI (-0.1075, 0.4839) | -0,365 %95 CI (-0.6049 , -0.06482) |
| mRASSF2 | 0,207 %95 CI (-0.1075 , 0.4839) | 1 | -0,270 %95 CI (-0.5330 to 0.04150) |
| mGSTP1 | -0,365 %95 CI (-0.6049 , -0.06482) *$p$ = 0,019 | -0,270 %95 CI (-0.5330 , 0.04150) | 1 |

**Abbreviations:** GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

**Table 7.** PCa patients according to Pearson correlation and CI values

| | mRASSF1 | mRASSF2 | mGSTP1 |
|---|---|---|---|
| mRASSF1 | 1 | 0,083 (-0,4108-0,5397) | -0,365 (-0,7177-0,1365) |
| mRASSF2 | 0,083 | 1 | -0,426 %95 CI (-0,7510-0,06505) |
| mGSTP1 | -0,365 %95 CI (-0,7177-0,1365) | -0,426 %95 CI (0,7510-0,06505) | 1 |

**Abbreviations:** GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

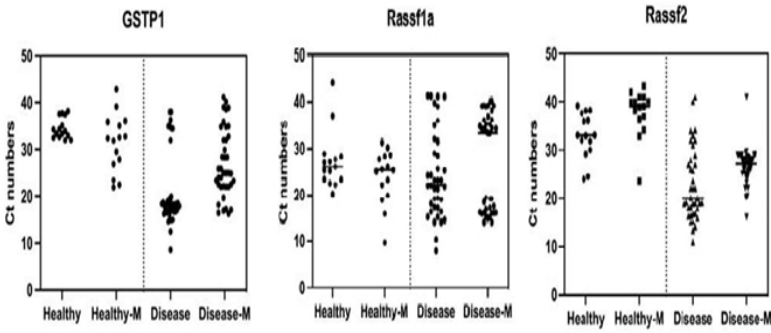


**Figure 1.** Detection of methylated and non-methylated targets in patient and control cases according to Ct numbers before and after bisulfite application. Ct > 40 was evaluated as the target could not be amplified by PCR. For each sample, at least 4 Ct differences between the b-actin ratio and the methylated and non-methylated targets were considered as the methylation parameter.

this genetic panel has been shown to increase sensitivity to 72%, despite lower specificity (72%).[26] Similarly, in our study, a general increase in specificity (83%) was observed with the combined gene panel, while sensitivity tended to decrease.

More recently, other genetic panels that do not contain GSTP1 have also been tested. Brait et al. In his study, the MCAM, ERα me and ERβ panel revealed 75% sensitivity and 70% specificity for early PCa detection.[27] Similarly, in serum, CCDC181me, ZNF660me, HAPLN3me and ST6GALNAC3me showed 26%, 22%,44% and31% sensitivity and 100% specificity for PKa, respectively. The best multıpl gene panel (CCDC181me, ST6GALNAC3me, and HAPLN3me) maintained 100% specificity, increasing the sensitivity to 67%.[28] In our study, it was observed that there was no noticeable change in PPV and NPV with the combined evaluation of genes. This shows that genes can also be used in combination for diagnostic purposes. Sanchez et al. A panel of GSTP1 and RASSF1A in patients with biopsy-confirmed PCa has been shown to have 73% PPV and 59.6% NPV in PCa, and these values are 81% and 66% when PSA is taken into account or increased.[29] Similarly, Reis et al. In another study, the addition of GAD45 methylation to PSA increased the sensitivity from 34% to 94%, although the specificity decreased from 98% to 88% compared to PSA alone.[30]

There are certain limitations of our study. The foremost of these is the low number of patients included in the study. When our genetic analysis results are compared with the results of other studies in the literature, we think that the lower sensitivity is due to our small number of patients.

## CONCLUSIONS

According to our study results , although we observed that GSTP1 and RASSF1 methylation positivity rates, which we examined in our study, were higher in patients without prostate cancer, we observed that RASSF2 methylation rate was higher in patients with prostate cancer.

## ACKNOWLEDGEMENT

None.

## CONFLICT OF INTEREST

Authors declared that there is no conflict of interest related to this article.

**Table 8.**Control group according to Pearson correlation and CI values

| | RASSF1 | RASSF2 | GSTP1 |
|---|---|---|---|
| mRASSF1 | r = 0,1024 %95 CI (-0,4325-0,5841) ns: $p$ = 0,71 | | |
| mRASSF2 | | r = 0,4331 %95 CI (-0,1017--0,7737) ns: $p$ = 0,11 | |
| mGSTP1 | | | r = -0,6028 %95 CI (-0,7716--0,3548) **** $p$ < 0.001 |

**Abbreviations:** GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family

**Table 9.** PCa patients according to Pearson correlation and CI values

| | RASSF1 | RASSF2 | GSTP1 |
|---|---|---|---|
| mRASSF1 | r = -0,4463 %95 CI (-0,6678--0,1522) ** p = 0,0044 $p$ = 0,004 | | |
| mRASSF2 | | r = -0,03315 %95 CI (-0,3451-0,2854) ns: $p$ = 0,8412 | |
| mGSTP1 | | | r = 0,6840 %95 CI (0,2643-0,8858) ** $p$ = 0,005 |

**Abbreviations: GSTP1, glutathione-S-transferase P1; RASSF ,ras association domain family**

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