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Could GSTP1, PTEN and NKX3.1 gene expression be novel markers in the relationship between prostate cancer and epigenetics?

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Abstract

Background Although numerous treatment modalities exist for Prostate Cancer (PCa) with a complex pathophysiology, a significant worldwide health concern for males, early identification remains the paramount factor influencing disease prognosis. Proposed new biomarkers, including GSTP1, PTEN, and NKX3.1, may provide predictive and prognostic functions, hence enhancing the efficacy of PSA in diagnosing prostate cancer. Epigenetic variables associated with nutrition influence the blood gene expression levels of the new biomarkers GSTP1, PTEN, and NKX3.1. This study aimed to elucidate the correlation between nutrition-related epigenetic variables and the expression of the tumor suppressor genes GSTP1, PTEN, and NKX3.1 in benign prostate and prostate cancer patients.

Methods This study was conducted with 41 newly diagnosed prostate cancer and 42 benign prostate patients, all aged 40 years and older. Participants had specific anthropometric measurements taken, and seven-day food records were kept. Serum levels of novel biochemical markers GSTP1, PTEN, and NKX3.1 were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) method. Expression analyses of tumor-protective GSTP1, PTEN, and NKX3.1 genes were performed on prostate tissues obtained from fresh prostate biopsies taken from the groups and were associated with nutritional status.

Results A significant relationship was found between the serum levels of GSTP1, PTEN, and NKX3.1, which are important in the formation and treatment of prostate cancer, and Gleason grade, tumor stage, and surgical margin status ($p < 0.05$). However, significant relationships were found between many macro and micronutrients obtained from the dietary pattern and effective in epigenetic pathways and GSTP1, PTEN, and NKX3.1 gene expressions ($p < 0.05$).

Conclusions The findings of this investigation corroborated the notion that prospective novel markers, including GSTP1, PTEN, and NKX3.1, may serve predictive and prognostic functions in prostate cancer, hence enhancing the efficacy of PSA in its diagnosis. Dietary trends may influence the optimization of many epigenetic processes. Targeted

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therapeutic nutritional interventions designed to enhance gene expression will facilitate the formulation of prostate cancer prevention methods.

Keywords Prostate cancer, Novel biomarker, GSTP1, PTEN and NKX3.1 gene expression, Epigenetic, Diet

Introduction

Prostate Cancer (PCa) ranks as the second most prevalent malignancy among men globally and is the fourth primary cause of cancer-related mortality worldwide [1, 2]. The number of people diagnosed with PCa worldwide is approximately 1.5 million in 2022 [2–4]. The five-year prevalence of prostate cancer among males in Türkiye is reported to be 21% [5].

Multi-step differentiations resulting from the accumulation of various genetic changes play a role in the process from normal prostate epithelial tissue to the formation of PCa tissue [6]. The carcinogenic defense mechanism in prostate cancer cells includes essential components that modulate tumor suppressor (GSTP1) and growth factor signaling pathways (NK3 Homeobox 1 (NKX3.1), Phosphatase and tensin homolog (PTEN), p27, and AR). In normal prostate tissue, NKX3.1, PTEN, and p27 govern the proliferation and viability of prostate cells. Insufficient amounts of PTEN, NKX3.1, and p27, or a reduction in their levels, result in enhanced proliferation and reduced apoptosis, thereby promoting the proliferation of prostate cancer cells [7].

The Glutathione S-Transferase P1 (GSTP1) gene governs various physiological processes, including metabolism, detoxification, and the elimination of potentially genotoxic foreign complexes, thereby detoxifying metabolites and safeguarding DNA from damage caused by oxidants and metabolites [8]. The weakening of GSTP1 expression or activity, which acts as a cellular “keeper”, through genetic (deletion or mutation) or epigenetic alterations, reduces cellular detoxification capacity [9]. Phosphatase and tensin homolog is one of the tumor suppressor genes most significantly impacted in the development of PCa [10]. Insufficient PTEN levels or a reduction in its levels results in enhanced proliferation and reduced apoptosis, thereby promoting the proliferation of PCa cells. The loss of PTEN function (silencing) is prevalent (50–80%) among individuals with PCa [11]. The prostate-specific gene NKX3.1 plays a role in normal prostate development [12]. The prostate-specific gene NKX3.1 is involved in normal prostate development. While NKX3.1 expression in prostate tissue is elevated in adults, it is diminished in PCa [13].

Dietary components can modulate the gene expression of prostate cancer-related pathways via epigenetic mechanisms [14]. Epigenetic variables associated with diet influence the expression levels of the GSTP1, NKX3.1, and PTEN genes [15–17]. Moreover, diet correlates with prostate cancer through its micro and macronutrient

composition and its impact on inflammatory responses and health indices [18]. Growing data should indicate that elements within dietary patterns may influence prostate cancer development by altering DNA methylation [19].

Although many studies have shown that nutrition is individually associated with the prognosis of various cancers, the relationships and interactions between PCa prognosis remain unclear. This study aimed to evaluate the expression levels of GSTP1, PTEN, and NKX3.1 genes, biochemical parameters, and dietary patterns together for the first time in malignant PCa patients and individuals with benign prostate tissue.

Materials and methods

Study design, setting, and participants

This research was designed and executed as a case-control study involving 83 male participants, comprising 41 with prostate cancer (malignant prostate) and 42 without prostate cancer (benign prostate), all over the age of 40. Diagnoses were based on pathology results validated by the attending pathologist, with participants seeking treatment at the Urology Polyclinic of Gazi University Faculty of Medicine Hospital from December 2021 to September 2022. Participants were included in the study based on their voluntary participation.

The sample size was determined using the G*Power 3.1 software based on analogous study outcomes [20]; 38 participants were identified for 95% power and 35 for 80% power, resulting in an effect size of 0.48 and an alpha error of 0.05. In light of the attrition of participants at the study's onset, the sample size was established to be a minimum of 40 for each group.

The study's exclusion criteria consisted of the following: a Body mass index (BMI) < 18.5 or ≥ 40 kg/m², existence of endocrine or metabolic disorders, autoimmune diseases, liver diseases, cardiovascular diseases, kidney diseases, inflammatory conditions (including inflammatory bowel disease), a diagnosis of any cancer aside from prostate cancer, previous chemotherapy and/or radiotherapy, adherence to a specific diet in the preceding six months, and consistent use of vitamin, mineral, or herbal supplements.

Ethics Committee Approval for the study was obtained from the Gazi University Hospital Clinical Research Ethics Committee, under the Republic of Türkiye Ministry of Health, on 15 November 2021, with the reference number 103. The study's research budget was funded by the

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Informed consent was obtained from all participants involved in the study. The study design is illustrated in Fig. 1.

Obtaining biopsy samples

Biopsy specimens were acquired via a fusion biopsy technique by a specialized urologist from patients who were not on Prostate Specific Antigen (PSA) -lowering medications, exhibited no urinary tract infection, had a PSA level exceeding 4 ng/mL, and presented with a Prostate Imaging Reporting and Data System (PIRADS) lesion rated above 4 on MRI. Patients slated for fusion biopsy underwent standard clinical evaluations to detect any disorders that could contraindicate the surgery. Prostates of patients having fusion biopsy were examined using a correctly positioned transrectal ultrasonography instrument, and periprostatic anesthesia was administered with a combination of 5 mg lidocaine and 5 mg bupivacaine. Tissue samples from the highest-grade PIRADS lesions seen on MRI were acquired using fusion biopsy utilizing a BK Medical Focus 500 Biplanar 7.5 MHz transrectal ultrasound probe. A sample of paired specimens from the target lesion, acquired by fusion biopsy, was dispatched to the pathology laboratory for examination, while additional fresh prostate tissues were preserved at -80°C by the investigator in a sterile Eppendorf tube. Fusion biopsy

specimens from individuals were categorized into malignant or benign tumor categories according to pathology results. The samples were subsequently preserved at -80°C for the analysis of GSTP1, PTEN, and NKX3.1 gene expression.

Biochemical analyses

The biochemical values of the study participants of PSA (ng/mL), free PSA (ng/mL), total testosterone (ng/dL), fasting blood glucose (mg/dL), hemoglobin (mcg/mL), albumin (mcg/mL), CRP (mg/L), total protein (mcg/mL), triglycerides (mg/dL), cholesterol (mg/dL), total bilirubin (mg/dL), direct and indirect bilirubin (mg/dL), creatinine (mg/dL), sodium (mg/dL), potassium (mg/dL), calcium (mg/dL), phosphorus (mg/dL), chloride (mg/dL), Blood Urea Nitrogen (BUN) (mg/dL), Alanine Aminotransferase (ALT) (IU/L), Aspartate Aminotransferase (AST) (IU/L), Gamma-Glutamyltransferase (GGT) (U/L), and uric acid (mg/dL) were sourced from the hospital system of Gazi University Faculty of Medicine Hospital. Blood samples were collected from all participants in the morning after an overnight fast of at least 8 h. These samples were subsequently analyzed to determine the levels of MDA (mg/dL), GSTP1 ($\mu\text{g/L}$), NKX3.1 (ng/mL) ve PTEN (ng/mL), TAS (mmol troloxEqv/L), TOS ($\mu\text{mol H}_2\text{O}_2$ Eq/L) and OSI. For the analysis of MDA, GSTP1, NKX3.1 and PTEN TAS, TOS, and OSI, serum samples were centrifuged according to standard protocols and

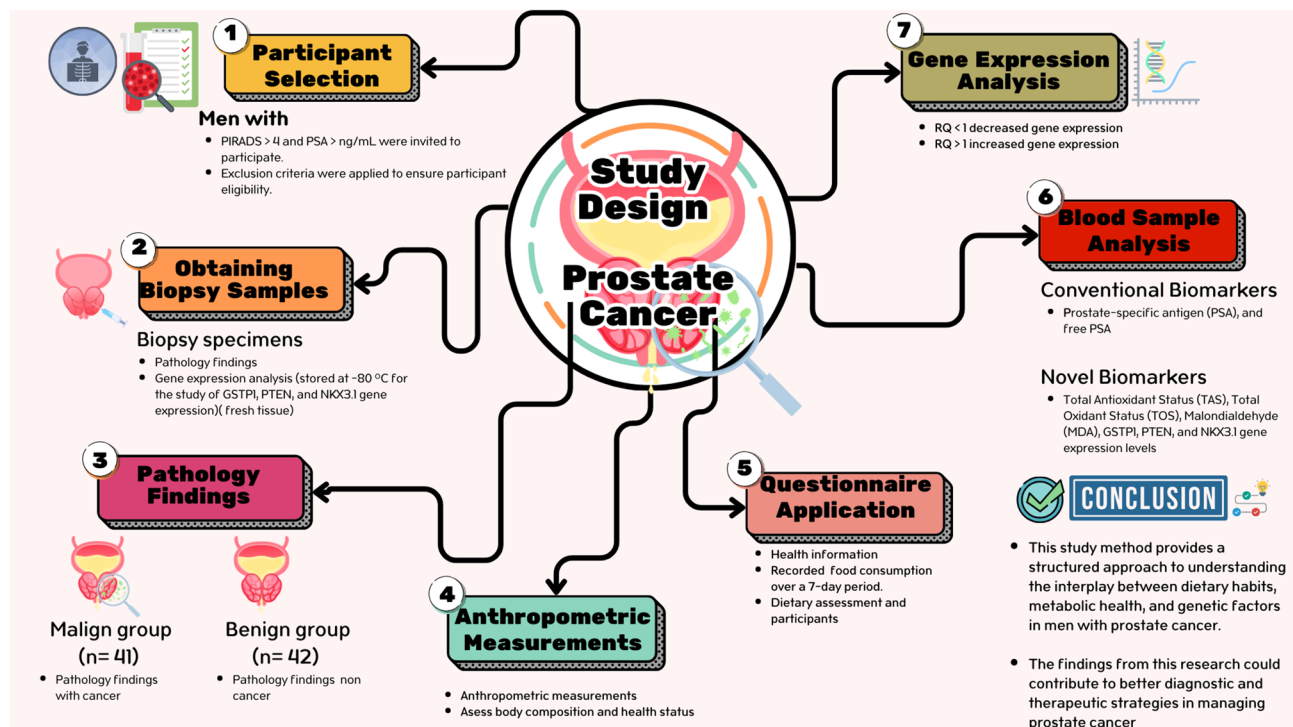


Fig. 1 Study design. Relative Quantity (RQ), Glutathion S-Transferase P1 (GSTP1), phosphatase and tensin homolog (PTEN), NK3 Homeobox 1 (NKX3.1) and Prostate Imaging Reporting and Data System (PIRADS)

stored at -80°C until the conclusion of the study. Malondialdehyde, GSTP1, NKX3.1 and PTEN TAS, TOS, OSI levels were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kits, specifically the Human Malondialdehyde ELISA Kit, Human GSTP1 ELISA Kit, Human NKX3.1 ELISA Kit, Human PTEN ELISA Kit, Human TAS ELISA Kit, and Human TOS ELISA Kit, respectively.

mRNA gene expression analyses of prostate tissues

Total RNA extraction article protocol

Total RNA was extracted from prostate tissues using the Trizol method (15596026, Invitrogen). Briefly, 50–100 mg of tissue was weighed and homogenized in Trizol reagent and total RNA was recovered with 50 μl of nuclease-free dH_2O after following the steps according to the manufacturer's protocol. The concentration and quality of extracted RNA was determined by NanoDrop (Colibri Titertek Berthold).

CDNA article protocol

The cDNA processing of the quantified RNA samples was performed with SOLIScript[®] RT cDNA synthesis KIT (06-35-00050, SolisBIODYNE, Estonia) following the manufacturer's instructions. Briefly, 2 μl 10x RT Reaction Buffer, 1 μl reverse transcription enzyme, 1 μl Oligo (dT) primer (or stem-loop primer), 0.5 μl dNTP MIX, 0.1 μl RNase Inhibitor and variable amounts of distilled water were used for a total volume of 20 μl . The PCR protocol was reverse transcription at 50°C for 5 min and finally enzyme inactivation at 85°C for 5 min.

Realtime PCR article protocol

SolisFAST[®] SolisGreen[®] qPCR Mix (no ROX), 5X (28-46-00001, SolisBIODYNE, Estonia) was used to determine gene expression levels. The total volume for the 1X PCR reaction was 20 μl , consisting of 4 μl master mix, 5 μl mixB (containing 0.3 μM forward and reverse primers), 6 μl dH_2O and 5 μl cDNA. The reaction was performed on a realtime PCR instrument (BioRAD CFX-96, Germany) with initial denaturation 95°C 5 min, 40 repeats 95°C 5 s, 55°C (GSTP1)/ 59°C (ActinBeta)/ 61°C (PTEN, and NKX3.1) 30 s (read). Relative mRNA expression levels obtained for specific genes through the device were determined using the $2^{-\Delta\Delta\text{Ct}}$ method [21], with the R package (qpcrtools 1.0.1, ggpubr 0.6.0, dplyr 1.1.4, tidyverse 2.0.0, car 3.1-2). The housekeeping gene ActinBeta was used as a normalizer in the study. The normal distribution of the data was checked with the “shapiro_test” parameter in the R packages and the homogeneity of the variances was checked with the “leveneTest” and the significance between the groups was evaluated with the Kruskal Wallis test. At the end of the test, the significance between the groups was determined using “dunnTest” and “bonferroni” correction. A shift of 1 log signifies a

tenfold variation. In the first sample, if the Target/ref ratio exhibited a Log10-fold increase post-exercise, the Target/ref ratio in the 41 sample for the malign prostate group and the Target/ref ratio in the 42 sample for the benign prostate group were deemed $\text{RQ} = 1$ in the cells, while $\text{RQ} = 0$ was assigned in the absence of a Log10 expression alteration. Despite minor modifications in Cdna, this proportional difference was recognized as a sensitive predictor of alterations in physiological expression. In the future, the relative expression ratio, an indicator of mRNA differentiation, is anticipated to serve as an optimal and straightforward tool, replacing complex and time-consuming calibration curve quantification models. RQ is a method employed to assess variations in gene expression within a specific sample relative to a reference sample, such as an untreated control. Tissues with an RQ score exceeding 1 in cells tested for gene expression are regarded as having positive gene expression. Tissues with an RQ value less than 1 are classified as having negative gene expression. Tissues exhibiting a relative RQ value greater than 1 in gene expression analysis are classified as having elevated gene expression. Tissues exhibiting an RQ value less than 1 are classified as having low gene expression.

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in the R packages and the homogeneity of the variances was checked with the “leveneTest” and the significance between the groups was evaluated with the Kruskal Wallis test. At the end of the test, the significance between the groups was determined using “dunnTest” and “bonferroni” correction. A shift of 1 log signifies a tenfold variation. In the first sample, if the Target/ref ratio exhibited a Log10-fold increase post-exercise, the Target/ref ratio in the 41 sample for the malignant prostate group and the Target/ref ratio in the 42 sample for the benign prostate group were deemed RQ = 1 in the cells, while RQ = 0 was assigned in the absence of a Log10 expression alteration. Despite minor modifications in Cdna, this proportional difference was recognized as a sensitive predictor of alterations in physiological expression. In the future, the relative expression ratio, an indicator of mRNA differentiation, is anticipated to serve as an optimal and straightforward tool, replacing complex and time-consuming calibration curve quantification models. RQ is a method employed to assess variations in gene expression within a specific sample relative to a reference sample, such as an untreated control. Tissues with an RQ score exceeding 1 in cells tested for gene expression are regarded as having positive gene expression. Tissues with an RQ value less than 1 are classified as having negative gene expression. Tissues exhibiting a relative RQ value greater than 1 in gene expression analysis are classified as having elevated gene expression. Tissues exhibiting an RQ value less than 1 are classified as having low gene expression.

Primer sequences

Gene Region	5'>3' Primer sequence	Tm
PTEN_hsa_F	CATGTACTTTGAGTTCCT	61 °C
PTEN_hsa_R	TCCTGGTATGAAGAATGTATT	
NKX3-1_hsa_F	CAAGAACCTCAAGCTCAC	61 °C
NKX3-1_hsa_R	TGCTTTCGCTTAGTCTTATAG	
GSTP1_hsa_F	TACACCAACTATGAGGC	55 °C
GSTP1_hsa_R	TAGTCAGCGAAGGAGAT	
ACTB-hsa-F	TGAAGATCAAGATCATTGCT	59 °C
ACTB-hsa-R	ATACTCCTGCTTGCTGAT	

Dietary assessment

A seven-day food record (comprising five weekdays and two weekend days) was maintained by participants to assess their daily dietary energy and nutrient intakes. Due to the heightened vulnerability of seven-day food records in elderly male participants to recall bias and underreporting, individuals in our study, who provided informed consent (either for themselves or through a caregiver), underwent training on accurately documenting food intake. The training involved gathering food intake using The “Meal and Food Photograph Catalog” [22], utilizing a three-step methodology: step 1 (quick list), step

2 (detailing), and step 3 (assessment). Training lasting around 15 min was conducted on documenting food consumption utilizing portion sizes, measurement utensils (such as cups, teaspoons, etc.), models, and images. Potential food intake records were validated during daily teleconferences. The “Meal and Food Photograph Catalog” [22] and “Standard Recipes for Caterings” [23] were utilised to determine the portion sizes and quantities of food consumed during each meal. The collected data were analyzed using a computer-assisted nutritional program, Nutrition Information Systems (BeBiS) [24].

Anthropometric measurements and body composition

The researcher, a specialised dietitian, assessed the participants’ anthropometric measurements and body composition, including body fat percentage, muscle mass (kg), and body water percentage, using a Tanita BC 601 bioelectrical impedance device in the early morning after a minimum of 8 h of overnight fasting. Body height was measured without footwear using a stadiometer after the participant inhaled deeply, ensuring their head was aligned in the Frankfort plane and their heels, back, and shoulders were positioned upright. The Body Mass Index (BMI) was determined using the formula: body weight divided by height squared (kg/m²) [25]. The waist circumference was measured with the individual standing, arms at their sides, legs together, and the midpoint between the lowest rib and the cristae marked using a non-stretchable tape measure, ensuring no tissue compression and that the tape was parallel to the ground. Waist circumference measurements were categorised as normal if < 80 cm for women, risky if between 80 and 88 cm, and high risk if ≥ 88 cm, in relation to chronic disease risk according to WHO categorisation [26].

Statistical analysis

Relationships between serum biomarkers, dietary variables, and gene expression were examined statistically in groups with benign and malignant prostate cancer. Qualitative data obtained from the participants were used to calculate number (n) and percentage values (%). The relationship between categorical variables was examined by means of a chi-square. Kruskal-Wallis tests were utilised to identify broad differences between the groups prior to using Mann-Whitney U-tests for pairwise comparisons (benign and malignant). Using the same technique, the statistical analysis was performed between histopathological groups, including Gleason grade, tumour stage, and surgical margin status. With additional markers at $p < 0.01$ and $p < 0.001$ for greater confidence levels, significance thresholds were set at $p < 0.05$. Pearson correlation coefficients (r values) were computed to evaluate the direction and the degree of linear relationship between neopterin and other biochemical and nutritional data.

Seaborn, a Python-based visualisation package for creating box plots showing the distribution of gene expression levels, serum biomarker levels, and dietary components, were used [27]. Individual data points, enabling a visual depiction of variance within groups, were superimposed on each box plot. To highlight important differences, the statistical significance of group comparisons is indicated inside the plots with the symbols * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

Results

The relationship between traditional and novel serum biomarkers and epigenetic factors in individuals with malignant and benign prostate cancer was thoroughly investigated, and the related relationships were highlighted in the results section. Serum levels of essential biomarkers such as GSTP1, PTEN, NKX3.1, and MDA, which may be novel serum biomarkers, were investigated. In addition, both total and free PSA levels were investigated as diagnostic markers for prostate cancer. The relationships between serum biomarkers, gene expressions, epigenetic factors, and histopathological features of prostate cancer, such as Gleason score, tumor stage, and surgical margin status, were investigated. Dietary macro and micronutrients were analysed, and statistical comparisons identified variables associated with GSTP1, PTEN, and NKX3.1 gene expression, showing significant differences between groups.

The mean age, BMI, and waist circumference for the benign prostate group were 64.5 ± 5.94 years, 28.50 ± 4.44 , and 99.90 ± 10.55 , respectively, while for the malignant prostate group, they were 66.0 ± 6.64 years, 28.30 ± 3.99 , and 100.60 ± 8.97 ($p > 0.05$). Moreover, there was no statistically significant difference between the study groups for the number of snacks and main meals ($p > 0.05$). In both groups, a statistically significant difference was found in terms of education status, smoking status, alcohol consumption, and family history of cancer ($p < 0.05$). Due to the analogous selection of individuals, body weight (kg), body fat percentage (%), muscle mass (kg), and body water percentage (%) exhibited no significant differences between the benign and malignant prostate groups (Table 1) ($p > 0.05$).

In the light of serum biomarkers and GSTP1, PTEN, and NKX3.1 gene expression analyses performed for individuals in the benign and malignant prostate groups, significant differences between the groups are presented in Fig. 2. The PSA value, a crucial parameter with its sensitivity and specificity in the diagnosis of prostate cancer, was significantly higher in the malignant group than in the benign group, confirming the literature information ($p < 0.05$). Serum hemoglobin, which correlates with disease prognosis with serum PSA levels, was found at elevated levels in the malignant prostate group, and both

were demonstrated to be closely associated with survival outcomes ($p < 0.05$). Serum levels of GSTP1, which regulates various pathways associated with cell proliferation, differentiation, and apoptosis, are markedly elevated in the benign prostate group compared to the malignant prostate group ($p < 0.05$). PTEN, which leads to enhanced prostate cell proliferation and reduced apoptosis when its levels are inadequate, is markedly lower in the malignant group compared to the benign group ($p < 0.05$). Moreover, the serum concentration of NKX3.1, integral to proper prostate growth, was considerably diminished in the malignant group compared to the benign group, akin to the serum PTEN level ($p < 0.05$). Concurrently with the serum levels of GSTP1, PTEN, and NKX3.1, which corroborate the validity of our investigation, the gene expression levels of GSTP1, PTEN, and NKX3.1 were markedly diminished in the malignant group relative to the benign group ($p < 0.05$). The results, along with the PSA value, which holds significant diagnostic importance for prostate cancer, indicate that the serum levels of GSTP1, PTEN, and NKX3.1, as well as the gene expression levels

Table 1 General characteristics of benign and malignant participants

	Benign Group (n:42)	Malign Group (n:41)	P value
Age (years)	64.50 ± 5.94	66.00 ± 6.64	0.281 ^b
Marital status			
Married	42(100)	39(95.1)	0.000 ^a
Single	0(0)	2(4.9)	
Education level			
≤ High school	32(79.6)	37(65.9)	0.026 ^a
>High school	9(21.4)	14(34.1)	
Smoking status			
Yes	8(19.0)	4(9.8)	0.000 ^a
No	34(81.0)	37(90.2)	
Alcohol consumption			
Yes	3(7.1)	4(9.8)	0.000 ^a
No	39(92.9)	37(90.2)	
Family history of cancer			
Yes	7(16.6)	24(58.6)	0.000 ^a
No	35(84.3)	17(41.4)	
Number of main meals	2.45 ± 0.50	2.26 ± 0.44	0.082 ^b
Number of snacks	1.04 ± 0.49	1.19 ± 0.67	0.262 ^b
Anthropometric Measurements			
Body weight (kg)	80.50 ± 12.72	82.00 ± 12.53	0.593 ^b
BMI (kg/m ²)	28.50 ± 4.44	28.30 ± 3.99	0.830 ^b
Waist circumference (cm)	99.90 ± 10.55	100.60 ± 8.97	0.744 ^b
Body fat percentage (%)	26.70 ± 6.27	26.80 ± 4.97	0.853 ^b
Body muscle mass (kg)	55.60 ± 6.80	56.80 ± 6.67	0.412 ^b
Body water percent- age (%)	52.70 ± 4.58	51.60 ± 5.87	0.360 ^b

Abbreviations BMI body mass index, n number, SD standard deviation

* $p < 0.05$, Data are presented as number and percentage (Chi-Square Test) and mean ± SD (Independent Sample-t test and Mann-Whitney U), respectively denoted by a, b, and c

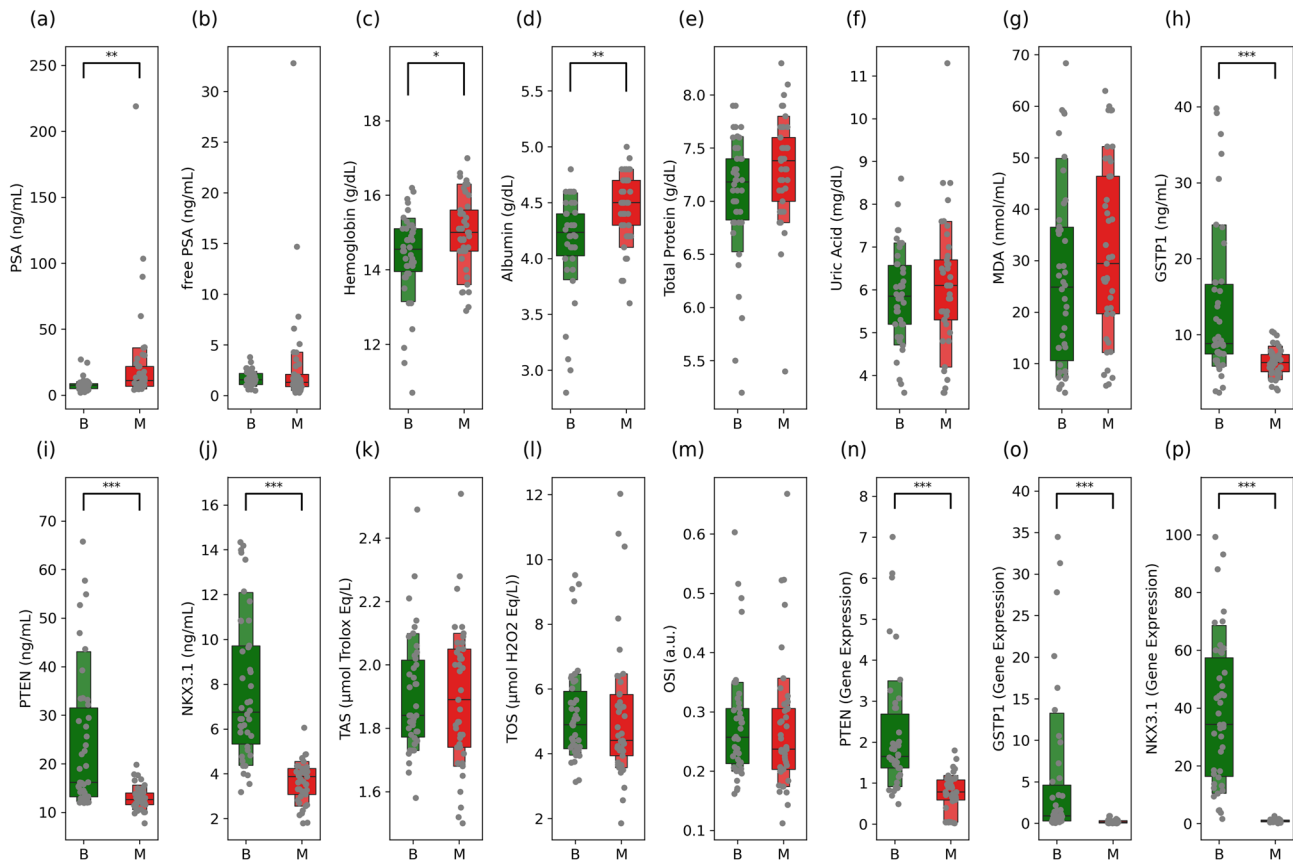


Fig. 2 Comparison of Various Biochemical Parameters and Gene Expression in the Benign and Malignant Prostate Groups. Multiple scatter plots (a–p) showing the relationships between biochemical parameters and gene expression across two study groups, and Tensin Homolog (PTEN), NK3 Homeobox 1 (NKX3.1), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) across benign and malignant prostate cancer groups. Data represent the mean $\pm$ SD for benign group (green), and malignant cases (red), with individual data points shown as grey dots for each group. Statistical significance is indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The Kruskal–Wallis test followed by a post-hoc Mann–Whitney U-test for independent samples was used for analysis

of GSTP1, PTEN, and NKX3.1, were significantly different between the groups, suggesting their potential in diagnostic applications.

Figure 3 presents a thorough overview of several traditional and novel biomarkers relevant to the diagnosis and treatment of prostate cancer. These traditional and novel biomarkers offer significant new insights into the diagnosis and management of the disease. According to the statistical study, significant differences were found between different histological groups, such as gleason grade, tumor stage, surgical margin status, and variables of traditional prostate cancer biomarkers. In addition, a significant difference was found between TOS and OSI, which are promising novel biomarkers and indicators of the oxidant system, and the tumor stage variable. One of the most important findings is that a significant relationship was found between the expression of tumor suppressor genes and serum levels of GSTP1, PTEN, and NKX3.1, which are vital in the formation and treatment of prostate cancer, tumor stage, and surgical margin status ($p < 0.05$).

Figure 4 illustrates the correlation between the expression levels of the GSTP1, PTEN, and NKX3.1 genes in the research participants and a total of 16 variables, which include gene expression, various biochemical markers, and signs of prostate tumors. The most striking finding in the malignant group is the negative correlation between the serum uric acid group ($r = -0.35$, $p < 0.05$) and PTEN gene expression level. In the benign prostate group, high correlations were identified between the expression levels of GSTP1, PTEN, and NKX3.1 genes and five parameters. The most notable finding in the benign prostate cohort is the correlation between serum GSTP1 ($r = 0.52$, $p < 0.05$) and PTEN ($r = 0.70$, $p < 0.05$) levels, alongside the oxidant parameters TOS ($r = 0.51$, $p < 0.05$) and OSI ($r = 0.52$, $p < 0.05$) levels, and GSTP1 gene expression levels. This indicates that elevated levels of PTEN, TOS, and OSI correlate with increased GSTP1 gene expression. The expression level of the PTEN gene expression showed a substantial association with all blood anti-prostate indicators (GSTP1 ($r = 0.52$, $p < 0.05$), PTEN ($r = 0.70$, $p < 0.05$), and NKX3.1 ($r = 0.52$, $p < 0.05$)).

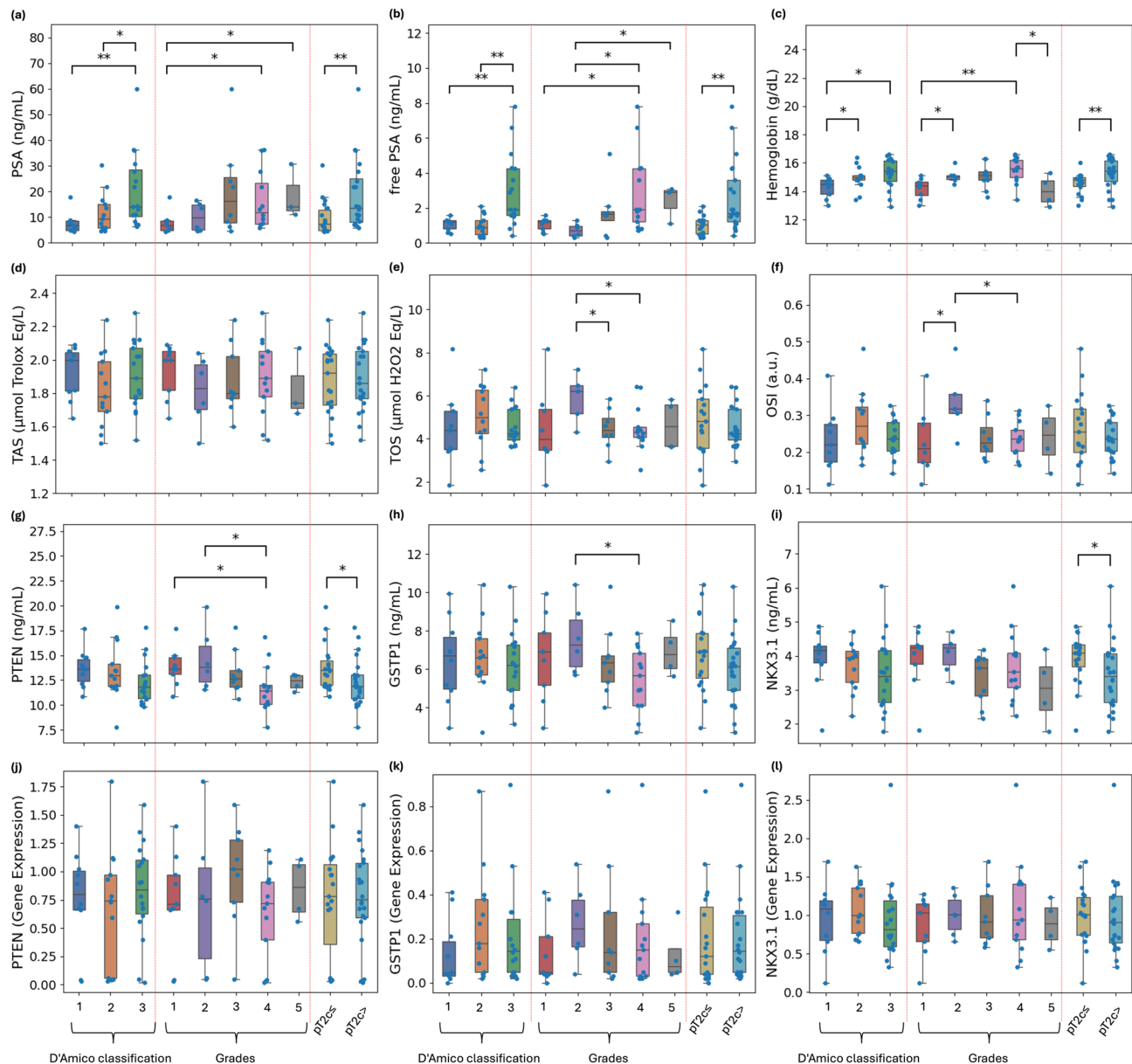


Fig. 3 Various traditional and new biomarkers, in the diagnosis and treatment of prostate cancer, level comparisons between different tumour classifications and grades. Comparison of prostat cancer patient's serum levels of prostate-specific antigen (PSA), and free PSA, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), glutatyon S-Transferaz P1 (GSTP1, phosphatase and tensin homolog (PTEN), NK3 Homeobox 1 (NKX3.1) and gene expression level with histopathological characteristics of biopsy specimens (patients with malignant tumour; $n=41$). The "Levene p-value" values were calculated for each supergroup and then, if significant, the "Wilcoxon p-value" values of the subgroups were calculated. Data illustrate mean $\pm$ SD. * $p_{\text{value_wilcox}} < 0.05$; ** $p_{\text{value_wilcox}} < 0.01$; *** $p_{\text{value_wilcox}} < 0.001$. Grade, combination analysis of Gleason score 1 and 2 (1); pT2c, pathological tumor stage; R0, (negative surgical margin); R1, (positive surgical margin). Note: upper groups are those between the red dashed lines, lower groups are those between the boxes

The relationship between the GSTP1, PTEN, and NKX3.1 gene expression levels of the participating individuals and the daily dietary energy and nutrient intake is presented in Table 2. While a positive significant relationship was observed between the GSTP1 gene expression level of the volunteers in the malignant prostate group and dietary protein (%) ($r=0.372$; $p<0.05$), omega-3 (g) ($r=0.421$; $p<0.05$), vitamin A (mcg) ($r=0.338$; $p<0.05$),

niacin (mg) ($r=0.530$; $p<0.05$), potassium (mg) ($r=0.317$; $p<0.05$), iron (mg) ($r=0.512$; $p<0.05$) intake, a negative significant relationship was observed between the dietary cholesterol (mg) ($r=-0.404$; $p<0.05$) intake. A positive significant relationship was found between the NKX3.1 gene expression level of the malignant prostate group individuals and dietary vitamin E (mg) ($r=0.310$; $p<0.05$), vitamin B1 (mg) ($r=0.345$; $p<0.05$), vitamin

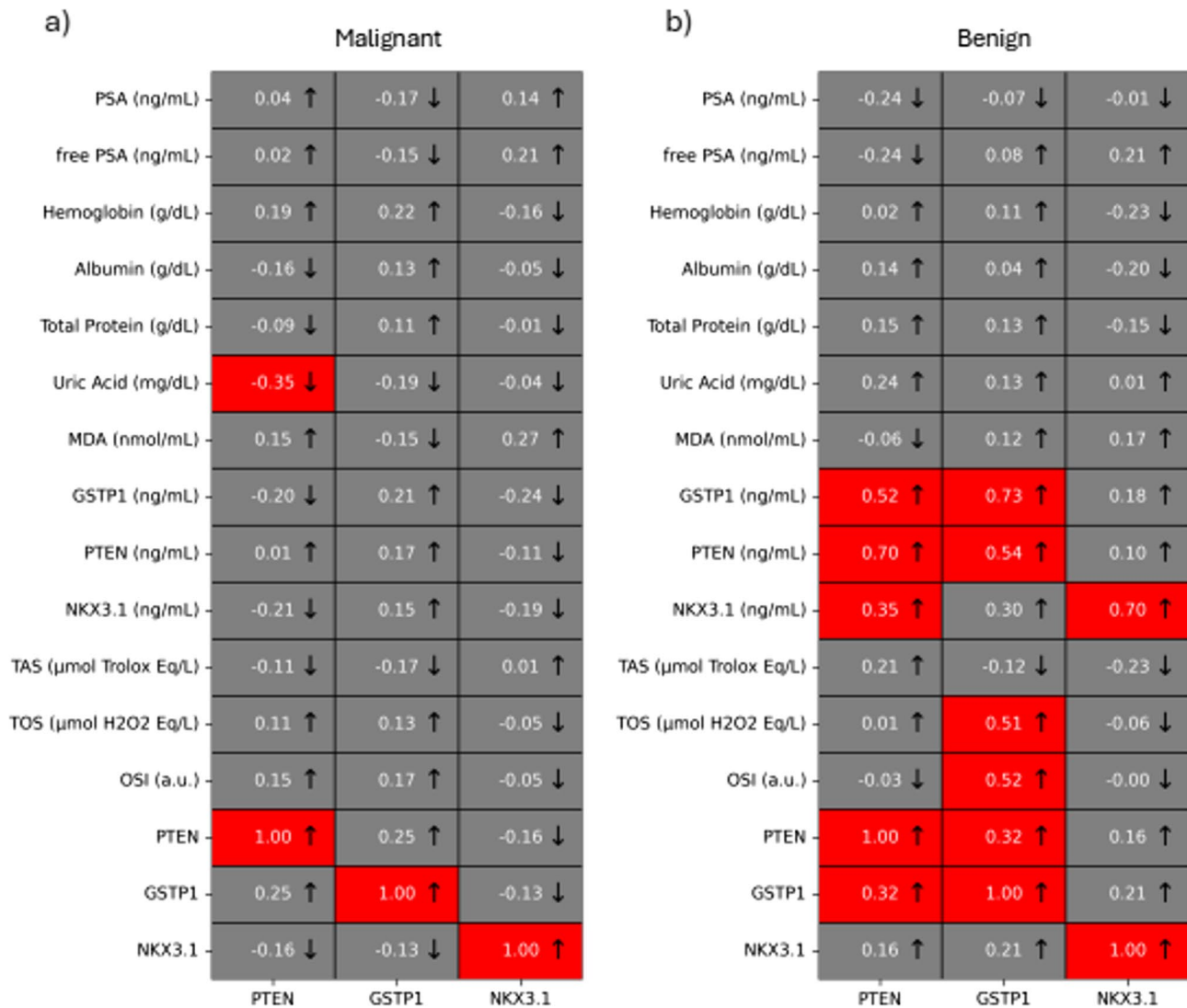


Fig. 4 Gene expression and various traditional and novel biochemical markers correlations with gene expression levels (GSTP1, PTEN and NKX3.1) in malignant (a) and benign prostate (b) individuals. Arrows indicate the direction of correlation in GSTP1, PTEN and NKX3.1 gene expression levels and traditional and novel biochemical markers among groups, while colored cells indicate p-value ranges for statistical significance (red for $p < 0.05$, and gray for $p > 0.05$). To determine correlation coefficients (r) and p-values, Pearson correlation analysis was used. The threshold for statistical significance was set at $p < 0.05$. Python (version 3.9.13) and the SciPy statistics package (version 1.13.0) were used for the analysis. Heatmaps created with the Seaborn library (version 0.13.0) were used to display statistically significant correlations, making it easy to discern between positive and negative correlations

B2 (mg) ($r=0.369$; $p<0.05$), folic acid (mcg) ($r=0.323$; $p<0.05$), potassium (mg) ($r=0.315$; $p<0.05$), magnesium (mg) ($r=0.318$; $p<0.05$) and zinc (mg) ($r=0.369$; $p<0.05$) intake, while a negative significant relationship was found between diet and omega-6 (g) ($r=-0.320$; $p<0.05$) and phosphorus (mg) ($r=-0.320$; $p<0.05$) intake. In the malignant prostate group, no significant relationship was observed between the PTEN gene expression levels of the groups and daily dietary energy and nutrient intake ($p>0.05$) (Table 2). While a positive significant relationship was found between the GSTP1 gene expression levels of the benign prostate group volunteers and dietary PUFA (%) ($r=0.331$; $p<0.05$), vitamin E (mg) ($r=0.307$;

$p<0.05$) and DTAK (mmol/day) ($r=0.360$; $p<0.05$) intake, a negative significant relationship was found between dietary omega-6 (g) ($r=-0.309$; $p<0.05$) intake. In the benign prostate group, no significant relationship was observed between the PTEN and NKX3.1 gene expression levels of the groups and daily dietary energy and nutrient intake ($p>0.05$) (Table 2).

Discussion

Prostate cancer is a significant malignancy that poses a global threat to men's health, and its pathogenesis is notably intricate [28]. Alterations in GSTP1, PTEN, and NKX3.1 are critical determinants of tumor phenotype,

Table 2 Correlation between GSTP1, PTEN, and NKX3.1 gene expressions in individuals and their daily dietary energy and food consumption

Gene Expression	Malign Prostate Group (n = 41)				Benign Prostate Group (n = 42)			
	GSTP1	PTEN	NKX3.1	PTEN	GSTP1	PTEN	NKX3.1	PTEN
Energy and nutrients								
Energy (kcal)	0.035	0.829	0.643	0.075	0.169	0.193	0.953	0.630
Energy (kcal/kg)	-0.034	0.832	0.418	0.130	0.886	0.402	0.593	0.461
Protein (g)	0.142	0.375	0.380	-0.141	0.068	0.468	0.952	0.310
Protein (g/kg)	0.089	0.581	0.905	-0.019	0.344	0.496	0.659	0.674
Protein (%)	0.372	0.017	0.350	-0.150	0.176	0.827	0.232	0.138
Vegetable protein (g)	-0.066	0.680	0.699	-0.062	0.187	0.604	0.882	0.957
Animal protein (g)	0.089	0.582	0.309	-0.163	0.208	0.678	0.167	0.291
Carbohydrate (g)	-0.103	0.523	0.276	-0.174	0.638	0.228	-0.210	0.183
Carbohydrate (%)	-0.033	0.837	0.387	-0.139	0.686	0.061	-0.239	0.127
Fat (g)	-0.071	0.661	0.322	0.159	0.531	0.143	0.742	0.638
Fat (%)	-0.059	0.714	0.198	0.205	0.978	0.404	0.368	0.678
Fiber (g)	-0.015	0.924	0.614	-0.081	0.878	0.831	0.102	0.522
SFA (%)	-0.067	0.678	0.407	0.133	0.402	0.996	0.047	0.767
MUFA (%)	0.460	0.002	0.666	0.070	0.675	0.728	0.211	0.179
PUFA (%)	0.132	0.410	0.206	0.202	0.235	0.032	-0.015	0.925
Cholesterol (mg)	-0.404	0.009	0.663	-0.070	0.209	0.268	0.050	0.755
Omega-3 (g)	0.421	0.006	0.537	0.101	0.261	0.238	0.051	0.749
Omega-6 (g)	0.100	0.533	0.453	0.121	-0.320	0.041	-0.062	0.698
Omega 6/Omega 3	0.040	0.803	0.985	0.003	-0.096	0.369	-0.163	0.302
Vitamin A (mcg)	0.338	0.031	0.984	0.003	0.106	0.266	-0.020	0.899
Vitamin E (mg)	0.215	0.178	0.368	0.144	-0.310	0.050	-0.049	0.758
Vitamin B ₁ (mg)	0.021	0.896	0.381	-0.141	-0.345	0.202	0.060	0.706
Vitamin B ₂ (mg)	0.021	0.896	0.860	0.028	-0.369	0.278	0.033	0.837
Niacin (mg)	0.530	≤0.001	0.529	-0.101	-0.263	0.091	0.161	0.307
Vitamin B ₆ (mg)	0.125	0.437	0.780	0.045	-0.210	0.286	-0.004	0.979
Vitamin B ₁₂ (mcg)	0.212	0.184	0.149	0.229	-0.225	0.307	-0.023	0.886
Folic acid (mcg)	-0.015	0.928	0.677	0.067	-0.323	0.912	-0.050	0.755
Vitamin C (mg)	0.106	0.508	0.752	0.051	-0.082	0.349	-0.114	0.470
Calcium (mg)	-0.109	0.498	0.711	0.060	-0.273	0.507	0.018	0.908
Potassium (mg)	0.317	0.043	0.684	0.066	-0.315	0.104	0.079	0.621
Magnesium (mg)	0.062	0.702	0.977	-0.005	-0.318	0.439	≤0.001	0.996
Phosphorus (mg)	0.096	0.551	0.992	-0.002	-0.316	0.925	0.116	0.465
Iron (mg)	0.512	≤0.001	0.957	-0.009	-0.198	0.822	0.047	0.766
Zinc (mg)	0.174	0.276	0.250	0.184	-0.369	0.793	0.033	0.833
Copper (mg)	0.041	0.797	0.697	-0.063	-0.194	0.437	-0.021	0.893

Table 2 (continued)

	Malign Prostate Group (n = 41)			Benign Prostate Group (n = 42)		
Selenium (mcg)	-0,240	0,130	0,024	0,879	-0,159	0,321
DTAK (mmol/gün)	0,154	0,336	0,085	0,596	-0,037	0,818
<i>PTEN</i> Phosphatase and tensin homolog					0,206	0,191
<i>SFA</i> Saturated Fatty Acid					0,019	0,454
<i>MUFA</i> Monounsaturated Fatty Acid					-0,014	0,321
<i>PUFA</i> Polyunsaturated Fatty Acid						0,932

influencing growth control, detoxification, and immune regulation. Alongside PSA, which remains indispensable but limited in specificity, it is crucial to establish novel molecular profiles to improve diagnosis and prognosis [29–31]. GSTP1, PTEN, and NKX3.1 are among the novel factors studied for their role in prostate carcinogenesis, with evidence suggesting dietary modulation via epigenetic pathways [32, 33]. This study was conducted to determine the relationship between nutrition-related epigenetic factors and the expression of tumor suppressor novel potential factors GSTP1, PTEN, and NKX3.1 genes in prostate cancer patients.

Advanced age is one of the most significant unmodifiable risk factors for prostate cancer, with risk rising sharply after age 50 [34]. A meta-analysis reported higher risk of high-grade PCa among single men, potentially linked to lifestyle and socioeconomic differences [35]. Education, another sociodemographic factor, also influences PCa risk [36]. The general features of people with malignant and benign prostate conditions participating in the study are detailed in Table 1.

In patients with prostate cancer, the monitoring of specific biochemical and genetic indicators is crucial [37]. Catalona et al. (2020) reported that free and total PSA are reliable for detecting PCa and avoiding unnecessary biopsies [38]. In our study, PSA levels were significantly higher in the malignant group ($p < 0.01$) (Fig. 2). We also observed higher hemoglobin and albumin in the malignant group, consistent with previous reports [39, 40]. A wide variety of research indicates that oxidative stress (OS) contributes to both aging and prostate cancer progression [41]. In individuals with PCa, oxidative stress markers such as TAC, TOS, MDA, and OSI are useful indicators of antioxidant status [42, 43]. PTEN and NKX3.1 are involved in disease initiation, while GSTP1 has diagnostic potential in high-risk cases [32]. Monitoring serum GSTP1 shown higher specificity than PSA in follow-up and may serve as a complementary marker [44]. Since the combination of PSA with NKX3.1 improves sensitivity for detecting PCa and metastasis, it may be clinically valuable [45]. Furthermore PTEN and serum miR-214 have been suggested as non-invasive biomarkers [46]. Serum GSTP1 has also been linked to survival in advanced PCa [47], though genomic alterations in GSTP1 are less consistent [48]. Our study revealed that serum levels of the novel biomarkers GSTP1, PTEN, and NKX3.1 were elevated in the malignant prostate cohort compared to the benign prostate cohort, which correlated with increased GSTP1, PTEN, and NKX3.1 gene expression levels. This data is significant for the prospective application of these markers in the diagnosis and treatment of prostate cancer ($p < 0.001$) (Fig. 2). Our research validates the significant importance of PSA in the surveillance of prostate cancer. The recognized

detrimental impacts of inflammation on health must not be overlooked to safeguard overall well-being, including prostate cancer, and to maintain optimal metabolic function. While the novel biomarkers serum GSTP1, PTEN, and NKX3.1 play a significant role in safeguarding against prostate cancer, additional research is required to elucidate the correlation between the levels of GSTP1, PTEN, and NKX3.1 and the risk of developing prostate cancer. Nevertheless, for serum GSTP1, PTEN, and NKX3.1 levels to be utilised as standard innovative biochemical parameters in assessing the risk of developing prostate cancer in the future, it is anticipated that the prices of measurement should be diminished through the development of new methodologies.

Positive surgical margin (PSM), ISUP grade, and extra-prostatic extension have been identified as independent predictors of biochemical recurrence in PCa [49]. The Gleason score on biopsy is associated with pathological stage, tumor volume, and molecular biomarkers such as serum PSA levels [50]. A study by Shukla et al. (2020) reported oxidative damage and antioxidant status in high-risk prostate cancer patients, revealing that oxidative damage significantly contributes to prostate carcinogenesis, particularly during its development and early stages. Oxidative Stress Index (OSI) may therefore serve as a valuable prognostic marker [51]. Reduction of GSTP1 activity through genetic or epigenetic mechanisms has been observed in more than 70% of prostate carcinomas and PIN lesions [52]. Similarly, alterations in miRNAs regulating *PTEN* impair the PI3K/AKT/mTOR pathway, driving proliferation and apoptosis dysregulation [53]. Loss of *NKX3.1* expression correlates with high Gleason scores in PCa and PIN lesions [54], while DNA methylation-mediated suppression of *GSTP1* has been associated with aggressive disease [55]. Loss of *PTEN* expression is also linked to poor prognosis and elevated Gleason scores [56]. Our findings confirm correlations between PSA, hemoglobin, and oxidative stress indices with Gleason grade and tumor stage, supporting their role in PCa progression ($p < 0.05$) (Fig. 3). We also observed significant reductions in GSTP1, PTEN, and NKX3.1 in advanced disease, correlating with Gleason grade and surgical margin positivity ($p < 0.05$), underscoring their prognostic significance (Fig. 3).

The prostate-specific homeobox gene *NKX3.1* inhibits PSA gene expression by DNA binding. The diminished expression of the *NKX3.1* gene accounts for the elevated PSA expression levels in prostate cancer [57]. Additional studies have shown regulatory interactions among these genes, such as PTEN stabilizing *NKX3.1*, PTEN–GSTP1 connections via miR-153, and inverse GSTP1–PTEN expression patterns [58–62]. The role of serum uric acid (SUA) remains controversial, with studies showing both protective and risk-enhancing associations with

PCa [63–66]. The expression levels of GSTP1, *NKX3.1*, and *PTEN* genes, which modulate carcinogenic defense mechanisms and growth factor signaling pathways in prostate cancer cells, were assessed collectively in our study as precursors. These were analyzed alongside various traditional and novel biochemical markers, revealing a significant association between *PTEN* gene expression and uric acid levels in the malignant group ($p < 0.05$) (Fig. 4). In benign cases, serum and tissue levels of GSTP1, PTEN, and *NKX3.1* showed consistent correlations, further validating their relevance ($p < 0.05$) (Fig. 4) [67].

Evidence suggests that diets low in animal fats and dairy but rich in fruits and vegetables may mitigate disease risk [68], while omega-6-rich, high-trans-fat diets promote tumor progression [69, 70]. Cholesterol accumulation has been linked to reduced PTEN and enhanced tumor growth in PCa cells [71]. Micronutrients such as retinol, folate, magnesium, zinc, and iron have demonstrated gene-regulatory effects [72–75].

Diet may regulate epigenetic mechanisms such as DNA methylation and histone modification [76]. Evidence suggests that diets low in animal fats, protein and dairy but rich in fruits and vegetables may mitigate disease risk [77], while omega-6-rich, high-trans-fat diets promote tumor progression [67, 68]. Cholesterol accumulation has been linked to reduced PTEN and enhanced tumor growth in PCa cells [69]. Micronutrients such as retinol, folate, magnesium, zinc, and iron have demonstrated gene-regulatory effects [70–73]. Our data showed positive associations between protective gene expression and nutrients such as omega-3, vitamins (A, E, B-complex, folate), and minerals (zinc, magnesium, potassium, iron), while higher cholesterol and omega-6 intake correlated negatively ($p < 0.05$) (Table 2). Consistent with these findings, optimal diet and appropriate levels of energy and minerals may confer preventive effects against prostate cancer by enhancing the expression of the protective genes GSTP1, PTEN, and *NKX3.1*.

Existing literature may inadequately assess the GSTP1, PTEN, and *NKX3.1* gene expression levels in malignant PCa and benign prostate cases alongside nutrition-related epigenetic pathways. To our knowledge, this study is a pioneering study evaluating simultaneous changes in PTEN and *NKX3.1* gene expression in clinical PCa samples.

Conclusion

While the significance of multiple variables in the etiology of prostate neoplasms is recognised, the underlying reasons for these factors influencing prostate tumor growth remain unclear at present. Due to the heterogeneity of prostate diseases and the fact that PSA levels can be affected by different factors, it is not sufficient alone

as a biomarker for the diagnosis and prognosis of prostate cancer. Our research indicates that novel biomarkers, including serum GSTP1, PTEN, and NKX3.1, may serve predictive and prognostic functions in diagnosis and therapy, thereby enhancing the efficacy of PSA in prostate cancer diagnosis. The study we conducted shows that dietary habits influence the risk of prostate cancer development, and this influence is associated with lifestyle modifications. This indicates that for individuals with prostate cancer or at risk for PCa, personalized and balanced dietary adjustments may be a crucial strategy for disease prevention and therapy. To our knowledge, this study is the pioneer study assessing concurrent alterations in GSTP1, PTEN, and NKX3.1 gene expression levels in clinical prostate cancer samples. Various variables influence the reduced expression levels of the GSTP1, PTEN, and NKX3.1 genes in prostate cancer. This study is anticipated to serve as a foundational reference for future research by examining the expression levels of GSTP1, PTEN, and NKX3.1 genes alongside nutritional and lifestyle modifications in clinical prostate cancer samples. Significant correlations were noted between daily dietary intake of nutrients, including protein, MUFA, PUFA, cholesterol, omega-3 and omega-6 fatty acids, vitamin A, vitamin E, vitamin B1, vitamin B2, niacin, folic acid, potassium, magnesium, phosphorus, and iron, and the expression levels of the GSTP1, PTEN, and NKX3.1 genes. Targeted therapeutic nutritional therapies and lifestyle modifications designed to enhance diminished gene expressions may prove advantageous in formulating prostate cancer preventive strategies.

Limitations of study

This research was designed and executed on Turkish participants. Firstly, the cross-sectional design precludes the establishment of causal links. This strategy indicates that all biomarkers were assessed at a singular time point.

Prostate cancer is a complex disease, and the study groups were not controlled for all potential causative factors. A further constraint is the impossibility of achieving optimal genetic matching. The study's focus on a single breed restricts the generalizability of its findings.

The dietary assessment, derived from seven consecutive daily average food records (comprising five weekdays and two weekend days), may not correctly represent habitual dietary consumption and is susceptible to potential bias.

The data for this study were gathered during the global Coronavirus disease (COVID-19) pandemic. COVID-19 infection experienced during this era may influence biochemical markers. Moreover, the quarantines instituted during the epidemic altered the nutrition and lifestyle practices of individuals.

Strengths of study

Our study involves the analysis of the expressions of tumor-protective genes GSTP1, PTEN, and NKX3.1 in tissues acquired from fresh prostate biopsies from various groups and linking these expressions with dietary and lifestyle factors.

This work is the pioneer investigation assessing the impact of epigenetic mechanisms associated with nutrition and lifestyle modifications on the expression levels of the GSTP1, NKX3.1, and PTEN genes, enriching the existing literature in this domain.

In our investigation, mRNA expressions of the GSTP1, PTEN, and NKX3.1 genes were analyzed in tissues derived from fresh prostate biopsies rather than in serum, whereas blood serum levels of GSTP1, PTEN, and NKX3.1 were examined concurrently.

Our study examined the mRNA expression levels of multiple genes (GSTP1, PTEN, and NKX3.1) rather than a single gene.

Based on the findings of this study, it is believed that implementing healthy lifestyle protocols, alongside tailored nutritional interventions and medical or surgical treatments for prostate cancer, may enhance the quality of life for individuals with the condition in the future.

Abbreviations

PCa	Prostate cancer
GSTP1	Basal metabolic rate the glutathione S-transferase P1
NKX3.1	NK3 Homeobox 1
PTEN	Phosphatase and tensin homolog
PIRADS	Prostate imaging reporting and data system
PSA	Prostate specific antigen
TAC	Total antioxidant capacity
TOS	Total oxidant Score
MDA	Malondialdehyt
OSI	Oxidative stress indexes
BMI	Body mass index
FFM	Fat free mass
REE	Resting energy expenditure
SD	Strandard deviation

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Authors' contributions

Tevfik Koçak: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, and Conceptualization. Nilüfer Acar-Tek: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, and Conceptualization. Suleyman Yesil: Writing – review & editing, Supervision, Methodology, Investigation, and Conceptualization. Mehmet Ali ERGUN: Writing – review & editing, Supervision, Methodology, Investigation, and Conceptualization. Yusuf Icer: Formal analysis.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations**Ethics approval and consent to participate**

Ethics Committee Approval for the study was obtained from the Gazi University Hospital Clinical Research Ethics Committee, under the Republic of Türkiye Ministry of Health, on 15 November 2021, with the reference number 103. Clear explanations were provided for the parents with regard to the purpose of the study, after which written informed consent was obtained from all the parents in accordance with the Declaration of Helsinki (World Medical Association).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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