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ORIGINAL PAPER

Expression and diagnostic potential of circulating miR-107, miR-134-5p, miR-149-5p, miR-370-3p, and miR-221 in prostate cancer and benign prostatic hyperplasia: A preliminary study

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Summary *Background: MicroRNAs (miRNAs) have shown promise as diagnostic biomarkers for prostate cancer (PCa). This study aimed to evaluate the expression of miR-107, miR-134-5p, miR-149-5p, miR-370-3p, and miR-221 in whole blood to distinguish PCa from benign prostatic hyperplasia (BPH) and potentially reduce unnecessary biopsies.*
Methods: Whole blood samples were collected from 20 PCa patients, 17 histologically-confirmed BPH patients (all with PSA > 4 ng/mL), and 20 healthy controls over 60 years without symptoms suggesting prostatic disease and PSA < 4 ng/mL. miRNA levels were quantified using qRT-PCR. Diagnostic potential was assessed via correlation analyses with clinical parameters and ROC curve evaluation. Statistical significance was set at $p < 0.05$.
Results: miR-107, miR-134-5p, miR-149-5p, and miR-370-3p were significantly overexpressed in PCa patients compared to BPH ($p<0.0001$). ROC analysis identified miR-134-5p (AUC: 0.94) and miR-149-5p (AUC: 0.93) as strong predictors of PCa. Additionally, miR-149-5p showed a positive correlation with PSA levels ($r = 0.2627$, $p < 0.05$).
Conclusions: This preliminary study demonstrated that miR-107, miR-134-5p, miR-149-5p, and miR-370-3p were significantly overexpressed in PCa patients compared to the BPH group. ROC analysis highlighted their diagnostic potential in distinguishing BPH from PCa. Despite the limited sample size, these findings provide early evidence to guide future research on the diagnostic value of miRNAs in prostate cancer.

KEY WORDS: *Prostate cancer; Benign prostatic hyperplasia; miRNA; PSA; Biomarker.*

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INTRODUCTION

Prostate cancer (PCa) is one of the most common malignancies in men and accounts for a significant proportion of cancer-related mortality worldwide. Typically diagnosed at mid-to-late stages in elderly men, PCa is highly likely to recur even after comprehensive treatment regimens (1-3). PCa is a complex and insidious disease that arises from hyperplasia, inflammation and a series of genetic mutations in the prostate gland, driven by factors such as aging, obesity, genetic predisposition, environmental influences and various comorbidities (4).

Prostate-specific antigen (PSA), a glycoprotein secreted by prostatic epithelial cells, remains the most widely used biomarker for the diagnosis and screening of PCa. Generally, serum PSA levels above 4.0 ng/mL, although age-adjusted thresholds are often applied, are associated with an increased risk of PCa (5,6). However, as a prostate-specific rather than a cancer-specific marker, PSA levels may also rise in benign conditions such as *benign prostatic hyperplasia* (BPH), prostatitis, or with advancing age, limiting its diagnostic specificity (6-9). Currently, the diagnosis of PCa relies on serum PSA measurements, *digital rectal examination* (DRE), *multiparametric magnetic resonance imaging* (mpMRI), and *transrectal ultrasound* (TRUS) -guided prostate biopsies (1, 4, 8). While histopathological evaluation of biopsy specimens remains the gold standard for confirming prostate cancer, the procedure is invasive and associated with potential complications, including bleeding, infection, anxiety, and other adverse outcomes. Therefore, there is a growing need for more specific, sensitive, and non-invasive biomarkers to improve diagnostic accuracy and minimize biopsy-related risks (6-9).

Endogenous short non-coding miRNAs, which play a crucial role in regulating gene expression, have been implicated in the pathogenesis of various cancers, including PCa. These small RNA molecules (20-22 nucleotides) are involved in key biological processes such as cell proliferation, differentiation, apoptosis and signal transduction. When dysregulated, they can modulate the expression of numerous genes, potentially involved in oncogenic pathways and contributing to cancer progression (1, 10). Importantly, miRNAs have recently been proposed as significant biomarkers in diagnostic and prognostic processes of various diseases due to their ease of detection in body fluids, such as blood, serum and urine, as well as their stable structures (11).

Numerous studies have identified deregulated miRNA

expression patterns in PCa tissues and circulating biofluids such as serum, plasma, and urine. Circulating miRNAs are of particular interest due to their stability, ease of detection, and potential utility as non-invasive biomarkers for cancer diagnosis and prognosis (1, 9, 12, 13). However, inconsistent findings, sample heterogeneity, and methodological variations have hindered their clinical translation. According to our literature review, among the miRNAs previously associated with PCa, conflicting results have been reported for miR-107 (14), miR-134-5p (13, 15), miR-149-5p (16, 17), miR-221 (18), and miR-370-3p (19). These miRNAs are known to participate in oncogenic or tumor suppressive pathways and may influence key cellular mechanisms such as apoptosis, cell survival, and migration (18, 19). Despite increasing interest, their diagnostic relevance and biological significance in PCa remain inadequately understood, highlighting the need for further investigation.

Despite accumulating evidence suggesting their potential diagnostic relevance, the circulating levels of these miRNAs and their combined diagnostic performance have not been extensively validated, particularly in distinguishing PCa from BPH. Therefore, this preliminary study aimed to investigate the circulating levels of miR-107, miR-134-5p, miR-149-5p, miR-370-3p, and miR-221-5p in patients with PCa, BPH, and healthy controls, and to assess their diagnostic value in differentiating PCa from non-cancerous conditions.

Materials and methods

Study design and patients

This prospective study included twenty patients with PCa, seventeen patients with BPH, and twenty healthy controls who visited the urology clinic between April 2020 and July 2022. The study was approved by the *Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University Faculty of Medicine* (Approval No. 13.02.2020/16-24), and written informed consent was obtained from all participants prior to enrollment.

The study population consisted of patients aged 40-70 years who underwent prostate biopsy due to either positive DRE findings or serum PSA levels greater than 4 ng/mL, and whose histopathological results confirmed prostate adenocarcinoma (PCa group); patients in the same age range with negative DRE findings but PSA levels above 4 ng/mL who underwent biopsy and were histopathologically diagnosed with benign prostatic hyperplasia (BPH group); and a control group of healthy volunteers aged over 60 years who attended routine urological check-ups, had normal DRE findings, serum PSA levels below 4 ng/mL, and no history or clinical suspicion of prostate disease.

Patients who had received medical treatment or undergone surgery for prostate diseases, had chronic inflammatory or infectious conditions, had been hospitalized within the past year due to a chronic illness, or had any other known malignancy were excluded from the study. Clinical evaluations and pathological assessments were performed at *Alanya Alaaddin Keykubat University Training and Research Hospital*.

For all participants, demographic and clinical parameters such as age, serum PSA level, and prostate volume (measured by pelvic ultrasound) were recorded. In addition, serum samples collected prior to biopsy were used to analyse miRNA expression profiles. Serum PSA levels were determined by *enzyme-linked immunosorbent assay* (ELISA). miRNA extraction was performed from whole blood, and expression levels were quantified by *quantitative real-time polymerase chain reaction* (qRT-PCR). Candidate miRNAs associated with PCa were identified using miRCancer (*http://mircancer.ecu.edu*) and miRBase (*https://www.mirbase.org*) databases and further supported by a comprehensive literature review.

Blood samples and PSA levels

Peripheral venous blood samples (2 mL) were collected from all study participants into EDTA tubes. Samples were stored at +4°C and processed for miRNA isolation within 24 hours. Serum PSA levels were measured using an automated immunoassay analyzer (*ADVIA Centaur XPT, Siemens, Germany*) as part of the clinical examination.

Pathological diagnosis of prostate cancer

Twelve-quadrant prostate needle biopsies were obtained from patients with PSA value > 4 ng/mL included in the study and preserved in a 10% buffered formaldehyde solution. After routine processing and paraffin embedding, 5-µm tissue sections were stained with *hematoxylin and eosin* (H&E) and examined under a light microscope (*Olympus CX41, Japan*). Immunohistochemical evaluation of tumor-suspected blocks was conducted using the BenchMark ULTRA IHC/ISH System (*Ventana Medical Systems, USA*). The following primary antibodies were used: VENTANA anti-p63 (4A4) mouse monoclonal antibody, anti-keratin (34βE12, HMWK) mouse monoclonal antibody, and anti-p504s (α methylacyl-CoA racemase = AMACR, SP116) rabbit monoclonal antibody (*Ventana Medical Systems, USA*). Nuclear staining of basal cells with p63 and HMWK confirmed benign histology; the absence of such staining supported a malignant diagnosis.

Gleason score and *International Society of Urological Pathology grade* (ISUP) were used for tumor grading, and patients were stratified using the D'Amico risk classification system based on PSA levels, Gleason scores, and clinical stage (20-22). Following histopathological confirmation of the tumors, Gleason scoring was performed. Tumor size, tumor percentage, lymphovascular invasion, and perineural invasion were assessed following the TNM classification system. In BPH, the glandular morphology resembled normal prostate tissue both histologically and immunohistochemically. Based on Gleason scores, patients were stratified into three groups: ≤ 6, 7, and > 7. Prognostic risk stratification was performed using the D'Amico classification, based on clinical TNM stage, Gleason score, and PSA level:

- Low risk: Clinical stage T1c-T2a, PSA ≤ 10 ng/mL, and Gleason score ≤ 6.
- Intermediate risk: Clinical stage T2b or PSA 10.1-20 ng/mL or Gleason score = 7.
- High risk: Clinical stage T2c or PSA > 20 ng/mL or Gleason score 8-10.

miRNA extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

miRNA was extracted from the whole blood samples using the Hybrid-RTM miRNA Isolation Kit (*GeneAll, Seoul, Korea*) specially optimized for small RNA (< 200 nt). During the homogenization step, 750 µL of RiboEx reagent was added to 250 µL of whole blood, and subsequent steps were performed according to the manufacturer's instructions.

The quantity and quality of the extracted miRNA were assessed using the BioTek Synergy-H1 Hybrid Reader with the Take3 Multi-Volume Plate (*BioTek Instruments, USA*).

Following isolation, *complementary DNA* (cDNA) synthesis was performed using the A.B.T.™ miR cDNA Synthesis Kit (*A.B.T., Turkey*), according to the manufacturer's protocol. Each reaction had a final volume of 20 µL and included miRNA-specific stem-loop primers as shown in Table 1. During the study, miRNA samples were stored at -80°C, and cDNAs were stored at -20°C until further analysis.

miRNA expression with quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Expression levels of miR-107, miR-134-5p, miR-149-5p, miR-370-3p, miR-221-5p were measured by qRT-PCR using A.B.T 2X miRqGreen MasterMix (ABT, Türkiye). The reaction was performed with cDNA samples, master mix, miRNA-specific forward primer and universal reverse primer, according to the manufacturer's instructions. The miRbase code and miRNA forward primer sequences used in qRT-PCR are listed in Table 1. The U6 transcript was used as a normalization signal. The PCR was performed on the Roche LightCycler 96 real-time PCR instrument (*Roche Diagnostics Spa, Italy*). Expression levels of each miRNA were calculated using the comparative *cycle threshold* (ct) method according to the formula 2-ΔΔCt (23). The fold change ratio of each miRNA was determined and compared with that of the control.

Statistical analysis

All statistical analysis and graphs were performed using GraphPad Prism-5 (version 9.0) and SPSS 21.0 software. The normal distribution of the data was evaluated with the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation (x ± SD) or median [*Interquartile range* (IQR)] as appropriate. Categorical variables were compared using the chi-square test. Analyses of miRNA expressions were performed using the Kruskal-Walli's rank or Mann Whitney-U test and Spearman's test was used for correlation analysis. The role of miRNAs as biomarkers in prostate cancer was investigated by drawing the *receiver operating characteristic* (ROC) curves and the area under the ROC curve (AUC), cut-off point, sensitivity and specificity for each miRNA was calculated. Statistical significance was taken as $p < 0.05$.

RESULTS

Clinical and demographic assessments

The study included twenty PCa patients, seventeen BPH patients and twenty healthy volunteers. Demographic characteristics and clinical evaluations of the groups are summarized in Table 2. Median values were used for serum PSA (ng/ml) since the data were not normally distributed. There was no significant difference in PSA levels between the BPH and PCa groups ($p > 0.05$); however, the serum PSA levels of both groups were significantly higher compared to the control group ($p < 0.0001$). In prostate cancer patients, the PSA level (ng/mL) was 7.40 (IQR: 6.15, 36.05), with considerable variability observed within the group. All PCa and BPH patients had above 4 ng/mL serum PSA levels.

Among the PCa patients included in the study, 60% had a Gleason score < 7, 10% had a Gleason score = 7 and 30% had a Gleason score > 7. When reclassified according to the *International Society of Urological Pathology* (ISUP) Grade Group system, 60% were categorized as Grade Group 1, 5% as Grade Group 2, 5% as Grade Group 3, 25% as Grade Group 4, and 5% as Grade Group 5. In terms of D'Amico risk stratification, 45% of patients were classified as low-risk, 20% as intermediate-risk, and 35% as high-risk.

Figure 1 presents the histopathological images of BPH and PCa prostate tissue.

Differences in miRNA expression levels between the groups and correlation analysis

Expression of five microRNAs (miR-107, miR-134-5p, miR-149-5p, miR-370-3p and miR-221-5p) were studied using RT-qPCR. MiRNA expression levels were compared

Table 1.
Real-time PCR primers of miRNAs.

miRNA	MiRbase *	Forward primer sequence(s)	miRNA stem-loop sequence(s)
hsa-miR-107	MI0000114	AGCAGCATTGTACAGGGCTATCA	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTCGCCTTCTTTCTGATAGTG
hsa-miR-134-5p	MIMAT0000447	TGTGACTGGTTGACCAGAGGGG	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTCGCCTTCTTTCCCCCTCTG
hsa-miR-149-5p	MIMAT0000450	TCTGGCTCCGTGTCTTCACTCCC	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTCGCCTTCTTTCGGGAGTGA
hsa-miR-370-3p	MIMAT0000722	GCCTGCTGGGGTGGAACCTGGT	GAAAGAAGGCCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTCGCCTTCTTTCACCAGGTT
hsa-miR-221-5p	MIMAT0004568	ACCTGGCATACAATGTAGATTT	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAAGAATGTGCGTCTCGCCTTCTTTCAAATCTAC
U6 snRNA	NCBI: X07425.1	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAC

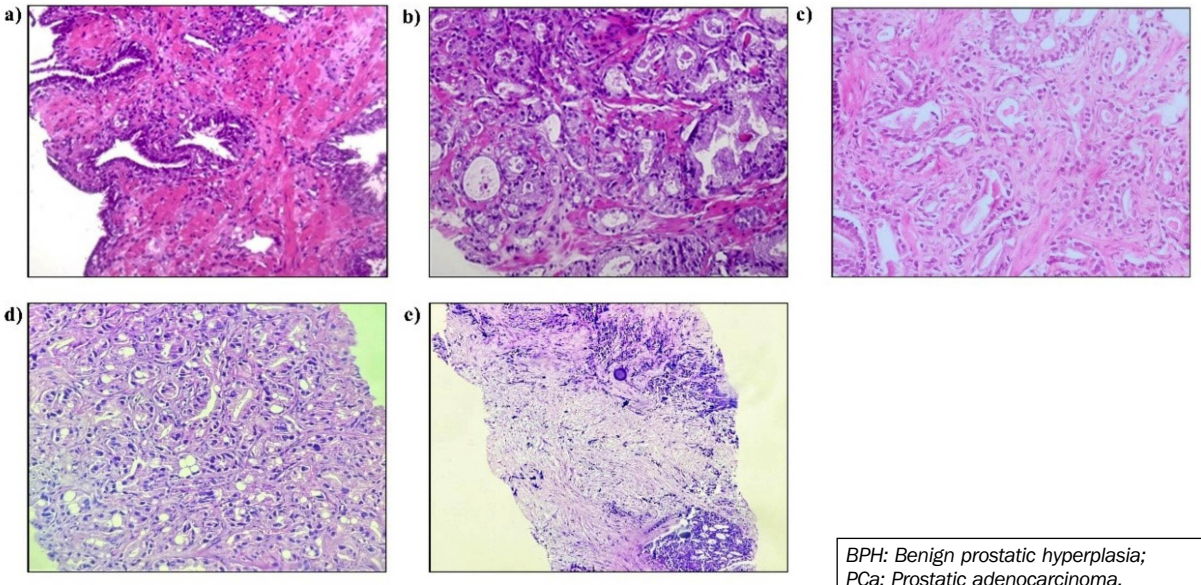
* *mirBase accession number.*

Table 2.
Clinical demographics of the study population.

Variables	Patients with PCa (n = 20)	Patients with BPH (n = 17)	Control (n = 20)	P values
Age (Mean ± SD)	64.35 ± 6.66	63.94 ± 6.67	64.75 ± 7.72	0.9414
Prostate Volume, mL (Mean ± SD)	46.8 ± 14.9	49.2 ± 15.7	44.7 ± 13.8	0.21
PSA, ng/mL (Median, IQR)	7.4 (29.9)	7.2 (3.13)	0.69 (1.06)	< 0.0001
Number of patients with PSA range, n(%)				< 0.0001*
< 4 ng/mL	0 (0%)	0 (0 %)	20 (100%)	
4-10 ng/mL	13 (65%)	16 (94,12%)	0 (0%)	
> 10 ng/mL	7 (35%)	1 (5.9 %)	0 (0%)	
Histopathological Grade Group, n (%)				
Gleason Score < 7	12 (60%)	N/A	N/A	
Gleason Score = 7	2 (10%)	N/A	N/A	
Gleason Score > 7	6 (30%)	N/A	N/A	
ISUP grade groups				
Grade group 1	12 (60%)	N/A	N/A	
Grade group 2	1 (5%)	N/A	N/A	
Grade group 3	1 (5%)	N/A	N/A	
Grade group 4	5 (25%)	N/A	N/A	
Grade group 5	1 (5%)	N/A	N/A	
D'Amico Risk, n (%)				
Low risk	9 (45%)	N/A	N/A	
Intermediate risk	4 (20%)	N/A	N/A	
High risk	7 (35%)	N/A	N/A	

PCa: prostate cancer, BPH: Benign Prostate hyperplasia, PSA: prostate-specific antigen; ISUP: International Society of Urological Pathology; N/A: not applicable, SD: Standard deviation; IQR: Interquartile range. Group differences were evaluated using one-way ANOVA or Kruskal–Wallis test.
**: p value applies to comparison among all PSA subgroups (< 4, 4-10, and > 10 ng/mL).*

Figure 1.
Histopathology of BPH and PCa. a) BPH (X20, H&E), b) PCa, Gleason score 3+3 = 6 (X20, H&E), c) PCa, Gleason score 3+4 = 7 (X20, H&E), d) PCa, Gleason score 4+4 = 8 (X20, H&E), e) PCa, Gleason score 4+5 = 9 (X40, H&E).



BPH: Benign prostatic hyperplasia; PCa: Prostatic adenocarcinoma.

among the three groups (Figure 2) and subsequently between the non-cancer (BPH and healthy) and cancer (PCa) groups (Figure 3).
When the PCa group was compared with the BPH group, levels of miR-107, miR-134-5p, miR-149-5p and miR-370-3p were found to be increased 3-fold, on average ($p < 0.05$). When the PCa group was compared with the healthy control group, levels of miR-134-5p, miR-149-5p and miR-370-3p were found to be increased 3.5-fold, on average (Figure 2) ($p < 0.05$). Similarly, when cancer

Figure 2.
miRNA expression levels between PCa, BPH and control groups. a) miR-107, b) miR-134-5p, c) miR-149-5p, d) miR-370-3p and e) miR-221-5p expressions.

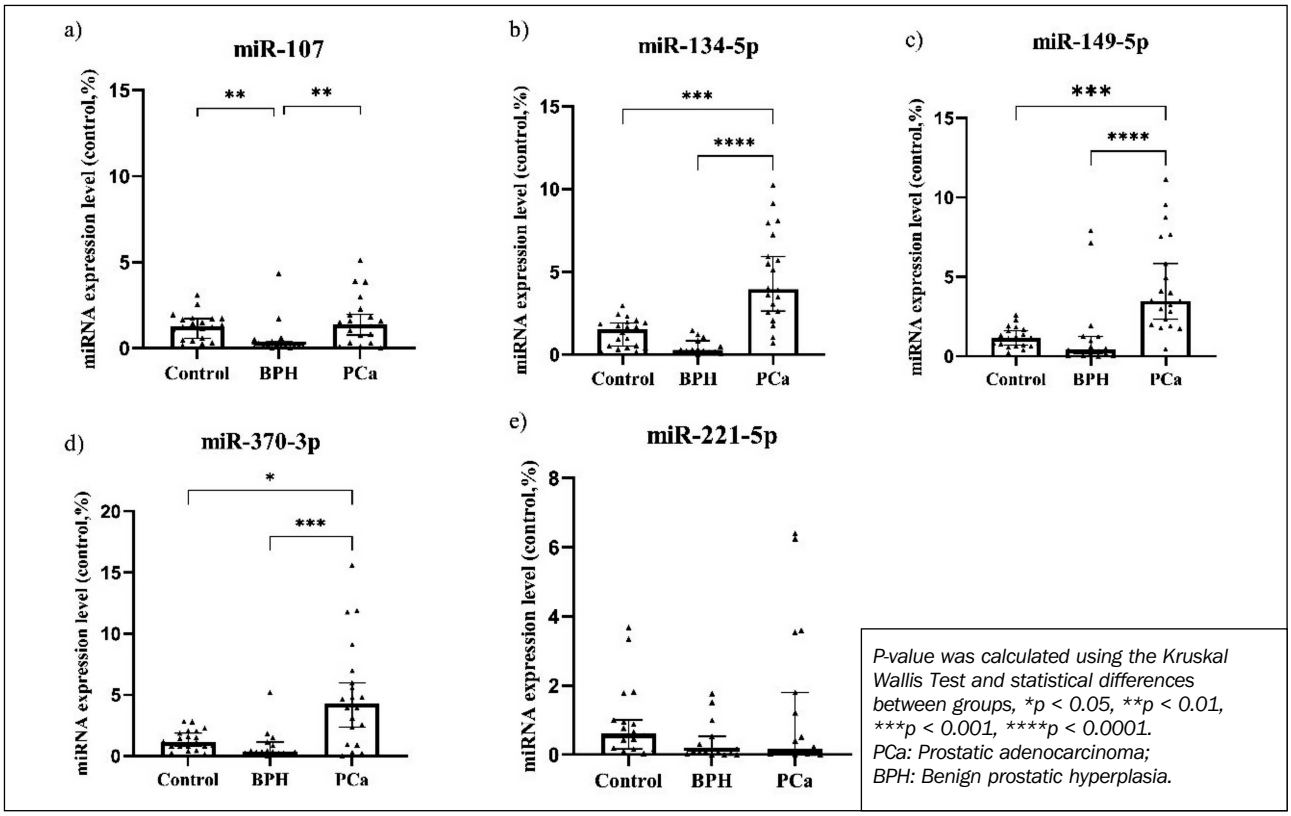


Figure 3.
Boxplots of the five microRNAs' relative expression comparing (a-e) PCa cancer patients and non-cancer (healthy subjects and BPH) patients groups in blood.

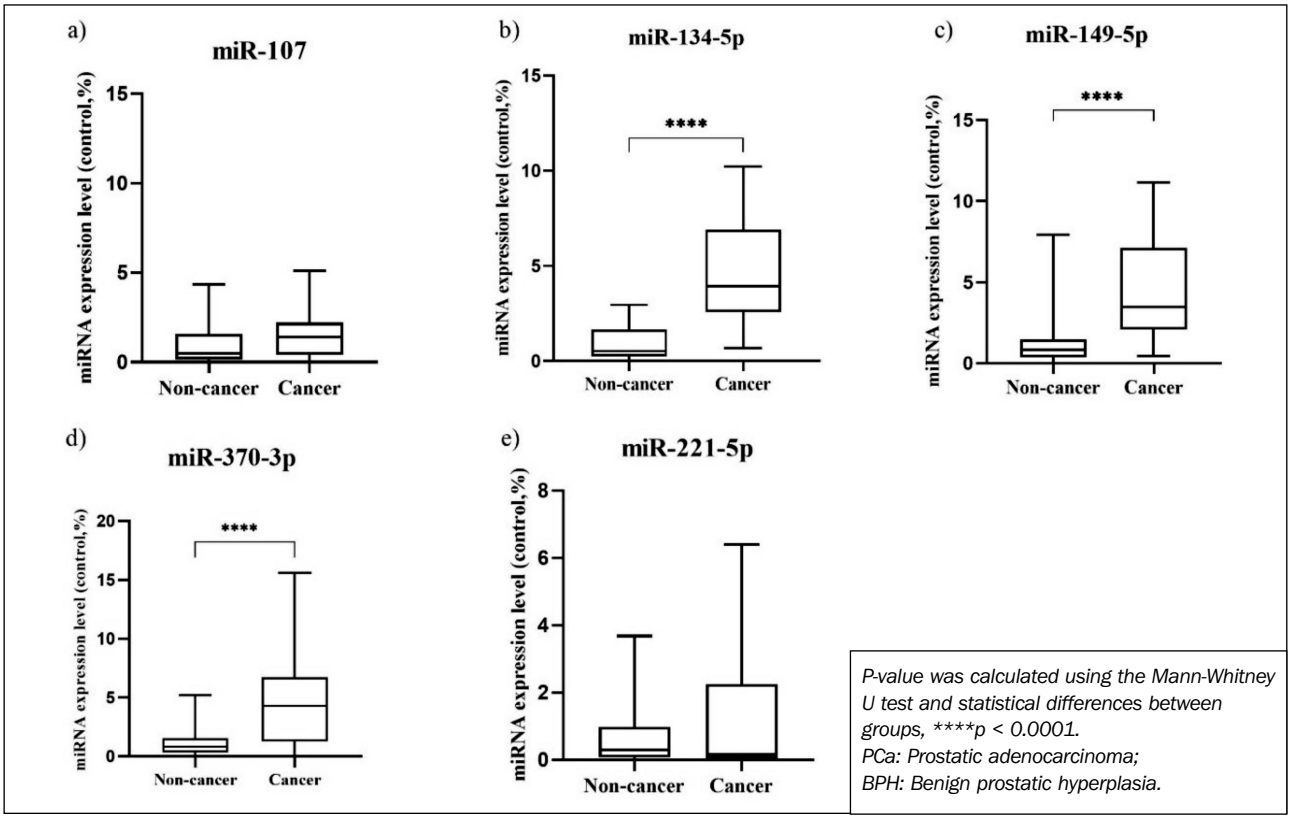


Table 3.
Correlation of PSA with miRNA expression in all data (n = 57) and correlation with Gleason score in PCa group (n = 20).

Variables	miR-134-5p	miR-149-5p	miR-370-3p	miR-221-5p	miR-107
PSA, ng/ml (N = 57)					
Sig. (2-tailed)	0.2086	0.0484*	0.3876	0.0823	0.3142
Spearman correlation (r)	0.1722	0.2627	0,1177	-0.2457	-0.1357
Gleason score (N = 20)					
Sig. (2-tailed)	0.4753	0.5500	0.1218	0.8920	0.1218
Spearman correlation (r)	-0.1694	-0.1421	0.3575	0.0344	0,3575

PSA: Prostate Specific Antigen, Correlations were calculated using Spearman's rank correlation test.
**: p < 0.05 indicates statistical significance*

(PCa) and non-cancer groups (BPH + healthy control) were compared, these miRNAs increased 3.5-fold (Figure 3) ($p < 0.0001$).

Additionally, a positive correlation was observed between miR-149-5p expression and PSA levels, with statistical significance ($p < 0.05$, $r = 0.2627$), as shown in Table 3. No significant correlation was found between PSA levels and the expression levels of the other miRNAs ($p > 0.05$). Furthermore, no association was detected between Gleason scores and miRNA expression levels ($p > 0.05$).

Evaluation of diagnostic efficiency of target miRNAs in the differential diagnosis of prostate cancer

ROC analyses were conducted to evaluate the diagnostic performance of the miRNAs in distinguishing between serum samples from cancer and non-cancer groups, as presented in Table 4 and illustrated in Figure 4.

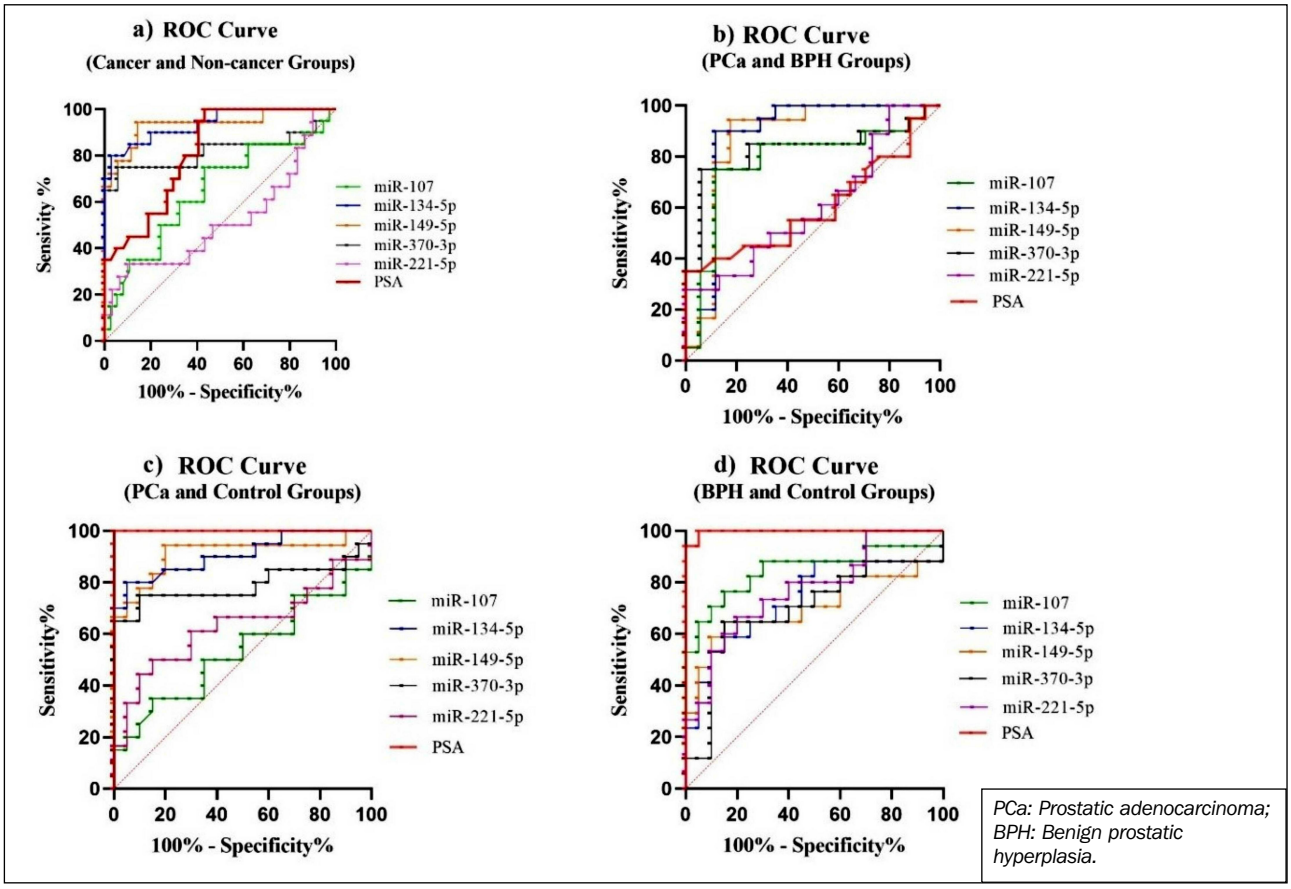
According to these results, in comparison between cancer and non-cancer groups, miR-134-5p, miR 149 5p and miR-370-3p exhibited the highest AUC values: 0.94 (95% CI 0.87-1.00), 0.93 (95% CI 0.86-1.00), 0.82 (95% CI

Table 4.
Diagnostic efficiency of miRNAs and PSA in discriminating groups from each other.

Groups	Parameters	AUC (95%CI)	p-value	cut-off	sp (%)	se (%)
Cancer-Noncancer	PSA	0.82 (0.71-0.92)	< 0.0001 ****	7.350	81.08	55.00
	miR-107	0.65 (0.49-0.80)	0.0723	1.507	75.68	50.00
	miR-134-5p	0.94 (0.87-1.00)	< 0.0001 ****	2.490	97.14	80.00
	miR-149-5p	0.93 (0.86-1.00)	< 0.0001 ****	1.716	85.71	94.44
	miR-370-3p	0.82 (0.67-0.96)	< 0.0001 ****	2.302	94.29	75.00
	miR-221-5p	0.52 (0.33-0.70)	0.848	0.2094	53.33	50.00
PCa-BPH	PSA	0.60 (0.42-0.79)	0.2931	7.350	58.82	55.00
	miR-107	0.78 (0.62-0.94)	0.0038 **	0.284	70.59	85.00
	miR-134-5p	0.88 (0.74-1.00)	< 0.0001 ****	1.601	88.24	90.00
	miR-149-5p	0.87 (0.72-1.00)	0.0002 ***	1.499	82.35	94.44
	miR-370-3p	0.78 (0.62-0.94)	0.0009 ***	2.094	93.75	75.00
	miR-221-5p	0.61 (0.41-0.80)	0.2944	0.090	53.33	55.56
PCa-Control	PSA	1.00 (1.00-1.00)	< 0.0001 ****	3.880	100.00	100.00
	miR-107	0.53 (0.35-0.72)	0.7353	1.507	65.00	50.00
	miR-134-5p	0.91 (0.82-1.00)	< 0.0001 ****	2.182	85.00	80.00
	miR-149-5p	0.91 (0.81-1.00)	< 0.0001 ****	1.716	80.00	94.44
	miR-370-3p	0.79 (0.63-0.95)	0.0017 **	2.302	90.00	75.00
	miR-221-5p	0.63 (0.44-0.82)	0.1694	0.533	60.00	66.67
BPH-Control	PSA	0.10 (0.99-1.00)	< 0.0001 ****	4.0	100.00	94.12
	miR-107	0.85 (0.71-0.99)	0.0003 **	0.533	75.00	82.35
	miR-134-5p	0.74 (0.56-0.91)	0.0148 *	0.881	65.00	70.59
	miR-149-5p	0.70 (0.52-0.89)	0.0355 *	0.619	85.00	64.71
	miR-370-3p	0.69 (0.51-0.88)	0.0443 *	0.437	85.00	64.71
	miR-221-5p	0.77 (0.61-0.93)	0.0069 **	0.165	80.00	66.67

PCa: prostate cancer; BPH: Benign Prostate hyperplasia; AUC: Area under the curve; CI: Confidence interval; sp: specificity; se: sensitivity.
*Bold values indicate statistically significant results at *: p< 0.05; ** p< 0.01; *** p < 0.001; and **** p < 0.0001 levels.*

Figure 4.
Receiver-operating characteristic (ROC) curve analysis and area under the curve (AUC) with 95% confidence interval of miRNAs analyzed in blood. a) Comparison between Cancer and Non-cancer group. b) Comparison between PCa and BPH groups. c) The PCa and control groups were compared. d) Comparison between BPH and Control groups.



0.67-0.96), respectively ($p < 0.0001$). The specificity and sensitivity values of these miRNAs were 97.14% and 80% for miR-134-5p, 85.71% and 94.44% for miR-149-5p, 94.29% and 75.00% for 370-3p, respectively and all these values were higher than those for PSA results ($p < 0.0001$). In addition, the diagnostic performance of miRNAs in distinguishing between PCa, BPH and healthy control subgroups was comprehensively evaluated using ROC analysis. The results of these subgroup comparisons are detailed in Table 4, and the corresponding ROC curves are illustrated in Figure 4. In this context, it was determined that miR-134-5p and miR-149-5p showed the best performance, particularly in differentiating PCa and BPH patients from each other; AUCs for miR-134-5p and miR-149-5p were 0.88 (95% CI 0.74-1.00) and 0.87 (95% CI 0.72-1.00), respectively ($p < 0.0001$ and $p < 0.001$) (Figure 4b). In discriminating the PCa group from the BPH group, specificity and sensitivity values for miR-134-5p were 88.24% and 90% and for miR-149-5p, were 82.35% and 94.44%, respectively.

Discussion

miRNAs play critical roles in the pathogenesis of various cancers. Their detectability in body fluids, such as urine and blood, also reveals their potential as diagnostic biomarkers (7, 9, 24). In this study, we investigated the expression profiles of miR-107, miR-134-5p, miR-149-5p, miR-370-3p, and miR-221-5p in PCa and evaluated their potential utility in the diagnosis of PCa.

PSA remains the cornerstone biomarker for PCa screening; however, recent studies consistently indicate that it cannot reliably distinguish between PCa and BPH. Up to 70% of men with PSA values between 4-10 ng/mL have negative prostate biopsy results (3, 6, 7, 9, 25). In our study, we included PCa and BPH patients with PSA levels above 4 ng/mL to assess the diagnostic value of the identified miRNAs. Accordingly, although PSA levels were significantly elevated in both patient groups compared to controls, no significant difference was observed between the PCa and BPH groups. Furthermore, our ROC analysis confirmed that PSA had low sensitivity and specificity in distinguishing between PCa and BPH. Consistent with previous studies, these findings indicate that PSA alone is neither sufficiently discriminatory nor reliable as a sole diagnostic marker to differentiate BPH from PCa.

In this preliminary study, serum miRNA levels were compared across three clinically distinct groups: histologically confirmed prostate cancer, histologically confirmed benign prostatic hyperplasia, and a healthy control group with normal DRE findings, serum PSA levels below 4 ng/mL, and no history or clinical suspicion of prostate disease. This design was intended to explore how miRNA expression differs not only between malignant and benign prostate

conditions, but also between disease and health. Although the inclusion of a control group with PSA < 4 ng/mL and normal DRE findings may introduce minor biological heterogeneity, it provides a more realistic representation of the clinical spectrum encountered in daily urologic practice.

Circulating miRNAs offer unique advantages for biomarker development due to their stability, ease of detection, and association with tumor biology (9, 11, 12). In the literature, miR-107 (14), miR-134-5p (15, 26, 27), miR-149-5p (17), miR-221 (18), and miR-370-3p (19) have been reported to exhibit tumor suppressor or oncogenic effects across various cancer types. However, research on these miRNAs in prostate cancer is limited and their roles in PCa remain incompletely understood. Additionally, the findings from existing studies are highly contradictory. In this study, RT-qPCR results showed that miR-134-5p, miR-149-5p and miR-370-3p expression levels were significantly elevated in the PCa group compared to the BPH and healthy control groups. When analysed between cancer and non-cancer groups, these miRNAs displayed significantly increased levels in cancer patients.

If we evaluate them respectively, miR-107 has been reported to exhibit both oncogenic and tumor-suppressive functions depending on the context. Some studies have shown an increase in serum, urine, or tissue samples of PCa patients, proposing it as a potential diagnostic biomarker (14, 24, 28). In contrast, *Zhang et al.* observed downregulation in prostate tissues and cell lines (29). In our study, circulating miR-107 levels were increased in PCa compared to BPH but also elevated in healthy controls, suggesting potential variability and limited standalone diagnostic utility. Additionally, ROC curve analysis indicated that miR-107 exhibits strong diagnostic performance in distinguishing PCa from BPH. Although our results suggest an increase of miR-107 in PCa, the underlying molecular mechanisms remain largely unexplored in this context. In other cancer types, miR-107 has been implicated in oncogenic processes through various pathways. For instance, in colorectal cancer, it has been shown to promote metastasis via the CAB39-AMPK-mTOR signalling axis or by targeting genes such as *death-associated protein kinase* (DAPK) and *Krüppel-like factor 4* (KLF4) (30, 31). Similarly, in gastric cancer, it has been shown to promote proliferation, migration, and invasion via the *neurofibromin 1* (NF1) pathway (32). Future studies investigating the involvement of these molecular pathways in PCa may provide further insight into the oncogenic role of miR-107.

Pelka et al. showed that miR-134 was downregulated in both PCa and adjacent tissues (13), while *Ngalame et al.* reported that, in addition to its downregulation in PCa, miR-134 was negatively correlated with RAS oncogenes (33). Conversely, some researchers have reported upregulation of miR-134 in the serum of PCa patients (15, 34). In the present study, we demonstrated that miR-134-5p was increased in PCa blood samples and exhibited high diagnostic performance. As previously mentioned, discrepancies among studies may stem from differences in tissue-derived versus circulating miRNA levels or from methodological variations. Therefore, standardized approaches and well-designed studies are urgently needed in this field.

There are limited studies on miR-149-5p regarding PCa in human samples. *Ma et al.* reported that downregulation of miR-149-5p in PCa led to reduced suppression of RGS17, resulting in increased malignancy and proliferation (35). Additionally, reports with varying results have been published, indicating that miR-149 is either increased or decreased in different cell culture studies (16, 19). In contrast, *Wang et al.* reports with varying elevated levels of miR-149-5p in the serum of patients with PCa as well as in bioinformatic screenings, highlighting its potential diagnostic value in distinguishing PCa from BPH (36). In the present study, we found miR-149-5p increased in PCa blood samples, consistent with these findings and it showed a positive correlation with PSA levels. ROC analysis results indicated that miR-149-5p had the highest AUC value of 0.93 (95 % CI:0.86-1.00) among the miRNAs we investigated, making it the most diagnostically effective miRNA in our study. These divergent findings underscore the need for further detailed investigations into the role of miR-149-5p in PCa.

Although miR-221 is generally recognized for its oncogenic role in cancer, contradictory data has emerged from various studies on PCa, leaving its specific role in this cancer type unclear (18). *Shao et al.* demonstrated that miR-221-5p promotes cancer progression through MAPK/ERK signalling by targeting the tumor suppressor SOCS1 (37). Other studies have indicated that miR-221 is increased and plays a role in tumor growth (38, 39). Conversely, *Leidinger et al.* reported a decrease in miR-221 expression in PCa patient sera compared to the BPH group (40). *Kiener et al.* further showed that overexpression of miR-221-5p reduced prostate cancer cell proliferation and colony formation in both in vitro and in vivo models (18). In our study, miR-221 expression was slightly reduced, although not statistically significant. Additionally, miR-221-5p levels showed marked heterogeneity in PCa patients, consistent with reports of both upregulation and downregulation in the literature. Our data found no correlation between miR-221 expression and Gleason scores or PSA levels.

Although the role of miR-370-3p in PCa is not fully established, some studies have shown it to be upregulated (19). miR-370 has been shown to enhance proliferation and facilitate tumor development in cell culture studies by suppressing the FOXO1 and p21Cip1/p27Kip1 signaling pathways, thereby modulating cell cycle progression (41, 42). However, to the best of our knowledge, no previous studies have investigated circulating miR-370 in the context of prostate cancer. Our findings support this data, demonstrating that miR-370-3p is expressed at higher levels in the PCa group compared to the BPH and control groups. Furthermore, according to ROC analysis, it achieved an AUC of 0.78 (95% CI: 0.62-0.94), indicating its potential diagnostic ability to distinguish between the BPH and PCa groups.

The miRNAs included in our study raise important unanswered questions in the literature. However, there are also certain limitations regarding the use of circulating miRNAs as diagnostic biomarkers. In many studies, results obtained from serum, plasma, or urine may not accurately reflect the tissue-specific profiles. Some of the main reasons for this discrepancy include methodological differences and variations in normalization strategies. Moreover, the broad heterogeneity among prostate cancer

patients and the complexity of tumor-microenvironment interactions also contribute to this uncertainty (9, 13, 28). Therefore, it is essential to clearly define the limitations and standardize the methodologies in future studies within this field.

The present study has several limitations. The study involved a relatively small sample size without an independent validation cohort, which may limit the generalizability and statistical power of the findings. Nevertheless, an a priori power analysis based on ROC AUC values indicated sufficient statistical power (1-β = 0.80) for the comparisons made.

Additionally, the ROC curves and AUC values were generated using GraphPad Prism, which provided 95% confidence intervals for the AUC estimates. Given the small sample size in ROC analysis, the high AUC values observed for certain miRNAs may be influenced by overfitting; therefore, our findings should be interpreted with caution and confirmed in larger independent cohorts using appropriate cross-validation or external validation techniques.

Moreover, miRNA extraction was performed from whole blood samples, which are known to be sensitive to pre-analytical factors such as processing time, storage, hemolysis, and normalization method. Standardized procedures should be implemented in future studies to improve reproducibility.

Finally, the definition of the control group represents an additional methodological limitation. Histological BPH is common in men over 60 years of age, and some degree of benign hyperplasia could be expected even among individuals with normal PSA and DRE findings. However, these participants were included as controls because prostate biopsy was not ethically indicated in the absence of clinical suspicion or laboratory abnormalities. Thus, the control group was intended to represent a clinically healthy reference population rather than histologically verified normal prostates. This distinction should be considered when interpreting intergroup comparisons of serum miRNA levels, as subtle histological changes undetectable by clinical assessment might still influence circulating miRNA expression. Nevertheless, this design reflects real-world clinical practice, enhancing the translational relevance of our findings.

Conclusions

Circulating miRNAs are remarkable biomarkers in the diagnosis of PCa. A critical aspect of this process is to prevent erroneous biopsy decisions. Our study identified five miRNAs with conflicting roles in PCa, as reported in the literature. Notably, we found that miR-134-5p, miR-149-5p and miR-370-3p in whole blood samples were significantly increased in PCa patients compared to BPH and control groups. No relationship between these miRNAs and Gleason scores was observed, possibly due to our limited cohort size. Our results may serve as a foundation for future large-scale studies aimed at validating the clinical utility of these miRNAs in prostate cancer diagnostics

Declarations

Ethical approval and consent for participate: *The Alanya Alaaddin Keykubat University Faculty of Medicine Clinical Research Ethics Committee gave the study ethical approval (Approval No. 13.02.2020/16-24). The study was conducted according to the guidelines of the Declaration of Helsinki. Clinical Trial Registration: NCT06726070.*

Consent for publication: *Not applicable.*

Availability of data and material: *The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.*

Competing interests: *The authors declare that they have no competing interests.*

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Authors' contributions: *AA: principal investigator; conceptualized the study, analysed the results, and drafted the main manuscript. HES: conducted the literature review, contributed to data interpretation, and assisted in formulating the conclusions. HES, FY, ÖCG, and MU: actively participated in patient recruitment, sample processing, and data collection. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.*

Informed consent: *All participants were fully informed about the purpose, scope, and procedures of the study. Written informed consent was obtained from all individuals prior to their inclusion in the study.*

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