

Research Article

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miR-221, miR-650 and miR-4534 as diagnostic markers in prostate cancer and their relationship with lymphatic invasion

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Abstract

Objectives: This study aimed to examine the role of miR-221, miR-650, and miR-4534 expressions in the development, pathogenesis, and early diagnosis of prostate cancer.

Methods: The study included 83 participants: 37 patients with PCa, 31 patients with BPH, and 15 healthy subjects. MiRNA expressions in plasma samples was evaluated by quantitative RT-PCR.

Results: Plasma miR-221 and miR-4534 levels were significantly upregulated in the PCa and BPH groups compared to the control group. A significant difference was determined between the presence of lymph node metastasis and the expressions of miRNAs. In the ROC analysis of the miRNAs, it was determined that the AUC for miR-221 was 0.737 with a sensitivity of 57% and specificity of 100%, AUC for miR-650 was 0.706 with a sensitivity of 62% and specificity of 93% and AUC for miR-4534 was 0.800 with a sensitivity of 73% and specificity of 93%.

Conclusions: Overexpression of miR-221, miR-650, and miR-4534 may distinguish PCa and BPH from healthy controls, but seems to be insufficient in differentiating PCa from BPH when used alone or in combination. However, these oncogenic miRNAs may have a role in determining the development and progression of the disease by suppressing the tumor suppressor genes they target.

Keywords: benign prostatic hyperplasia; biomarker; lymphatic invasion; microRNA; prostate cancer.

Introduction

Prostate cancer (PCa) is the second most common cancer among men [1]. Although it is known to be a multifactorial disease caused by genetic predisposition, hormonal variables, and environmental exposures, the pathogenesis of the disease is still unclear [2]. Prostate specific antigen (PSA) is still the most commonly employed serum biomarker that provides information about the risk, extent, and prognosis of PCa [3]. However, since serum PSA levels may vary in all prostate pathologies, it is not a cancer-specific marker and accordingly may cause false positives in cases of infection, inflammation, and benign prostatic hyperplasia (BPH) [4]. In addition, the poor correlation between PSA levels and disease states leads to overdiagnosis and unnecessary treatment [5]. Therefore, non-invasive biomarkers with higher sensitivity and specificity are required for the early diagnosis of PCa.

Genetic and epigenetic alterations significantly contribute to the pathogenesis and development of PCa. These alterations in the genome affect cancer-related pathways such as cell cycle, angiogenesis, proliferation, and apoptosis [6]. Disruption of microRNA (miRNA) expression and function is one of the epigenetic alterations [7]. The upregulation and downregulation of miRNA expressions in different cancer types indicate that miRNAs can act as oncogenes or tumor suppressors during tumorigenesis. These deregulations of miRNA expressions have

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been strongly associated with metastasis, carcinogenesis, and the prognosis of prostate carcinomas [8].

PCa-specific miRNAs are released directly from cancer cells or the tumor cellular microenvironment and enter into circulation [9]. Previous studies have shown that miR-221, which is overexpressed in PCa, suppresses p27, leading to cell cycle progression from G1 to S-phase, resulting an increased in clonogenicity [6]. It has also been demonstrated that increased oncogenic miR-650 expression level in PCa suppresses cellular stress response 1 (CSR1) expression, increases colony formation and induce the entry of the cell to the S-phase [10]. Another oncogenic miRNA that is overexpressed in PCa is miR-4534 which is associated with survival without poor prognosis and increased PSA. It has been reported that miR-4534 is hypermethylated in normal cells and tissues compared to PCa cells and exerts an oncogenic effect by suppressing the tumor suppressor PTEN gene [11].

In this study, we examined the expression patterns of oncogenic miR-221, miR-650, and miR-4534, which are involved in cell cycle arrest, in PCa, BPH, and healthy controls. We analyzed the correlation between circulating levels of these miRNAs and clinicopathological features (serum PSA, Gleason score, TNM staging). We also evaluated the diagnostic utility of these miRNAs in PCa and BPH patients.

Materials and methods

Patients and samples

The research protocol was designed in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Süleyman Demirel University, Faculty of Medicine (dated 17.11.2020, numbered 374). A total of 83 individuals (37 untreated PCa, 31 untreated BPH, and 15 age-matched healthy controls) applied to the Urology Outpatient Clinic of Süleyman Demirel University, Research and Training Hospital were included in the study. All patients provided written informed consent. PCa inclusion criteria were being a patient with PCa histopathology, age over 45 and without other type of cancer history. BPH inclusion criteria was having negative prostate biopsy. BPH exclusion criteria were having urinary infection, bladder stones and catheterization. The Gleason score was calculated for patients diagnosed with PCa. The PCa group was evaluated with the TNM staging system. Patients with chronic diseases other than PCa and BPH, other malignancies, and autoimmune diseases were excluded from the study. Untreated patients were selected in all study groups. The chemiluminescence immunochemical method (Beckman Coulter) was used to determine serum concentrations of total prostate specific antigen (tPSA) and free PSA (fPSA). For genetic analysis, 4cc venous blood was taken from the patients into EDTA-containing tubes. The blood samples were centrifuged for 10 min at 15,000 rpm at 4 °C (Thermo, Mega Fuge 16R) within 1 h and the plasma was placed into 1.5 mL RNase and DNase free Eppendorf tubes and kept at –80 °C until miRNA isolation.

MicroRNA isolation and cDNA synthesis

For miRNA isolation, the samples at –80 °C were thawed at room temperature and a short spin was performed. RNA extraction from plasma samples was performed using a RiboEx-LS solution (Gene All Biotechnology, Seoul, Korea). The extraction steps were followed in accordance with the manufacturer's instructions. The purity and concentration of the obtained miRNAs were determined using a Thermo Fisher NanoDrop™ spectrophotometer. Samples with an A260/280 ratio of less than 1.8 or an A260/230 ratio of less than 2.0 were not included in the study. 2 µL (10 ng) of RNA was used for cDNA synthesis. cDNA was obtained using a WizScript™ cDNA Synthesis Kit (Gene All Biotechnology, Seoul, Korea). A separate stem-loop primer was used for each miRNA. Reverse transcription was performed according to the manufacturer's instructions using the SimpliAmp Thermal Cycler (Thermo Fisher Scientific, US). The cDNA samples obtained were stored at –80 °C until Real-Time PCR analysis.

Expression analysis of selected miRNAs in plasma samples

Quantification of miRNA molecules was performed with a Rotor-Gene Q (Qiagen, Germany) Real-Time PCR device using iTaq SYBR Green (Bio-Rad). Two negative controls, no template control (NTC) and no reverse transcriptase (NRT) were used in qRT-PCR. cDNA concentrations, primer efficiency, and PCR conditions were optimized. U6 small nuclear RNA (RNU6B) was chosen as the endogenous control for data normalization. qRT-PCR steps were performed according to the manufacturer's instructions. Relative expression was calculated using the comparative threshold cycle (CT) method [12]. Each PCR reaction was performed in triplicate. Ct values for RNU6B were detected to be stable in our study groups. The fold change (FC) in the miRNA expression level was calculated ($\text{fold change} = 2^{-\Delta\Delta C_t}$) to determine the relative quantitative levels of each target miRNA. The sequences of PCR primers were as follows: for miR-221 (MIMAT0000278): 5'-AGCTACATTGTCTGCTGGTTTC-3' (sense), 5'-CGAGGAAGAAGACGGAAGAAT-3' (antisense); for miR-650 (MIMAT0003320): 5'-AGGAGGCAGCGCTCTCAGGAC-3' (sense), 5'-CGAGGAAGAAGACGGAAGAAT-3' (antisense); for miR-4534 (MIMAT0019073): 5'-GGATGGAGGAGGGGTCT-3' (sense), 5'-CGAGGAAGAAGACGGAAGAAT-3' (antisense); and 5'-GCTTCGGCAGCACATATACTAAAT-3' (sense), 5'-CGCTTCACGAATTTCGTGTCAT-3' (antisense) for RNU6B (NR_002752).

Statistical analysis

Continuous variables were expressed as mean $\pm$ SD. A normality test was performed using Kolmogorov-Smirnov test. In the comparison of two independent groups, the independent sample t-test was used for normally distributed data and the Mann-Whitney U test for abnormally distributed data. One-way analysis of variance (ANOVA) was used in the comparison of three independent groups. The Dunnett test was used for post hoc analysis. Specificity and sensitivity values were obtained by receiver operating characteristic (ROC) curve analysis, and areas under the curves (AUCs) were reported. To combine biomarkers, logistic regression analysis was applied. MiRNA expression differences were given as fold changes using the $2^{-\Delta\Delta C_t}$ equation. Statistical analysis was performed using the PASW (Predictive Analytics Software) 19th version. The significance level was defined as $p < 0.05$.

Results

Clinical characteristics

To identify biomarker candidates in PCa, we examined a cohort of 37 patients with PCa, 31 patients with BPH, and 15 healthy individuals. We analyzed miRNA expression levels in the plasma samples of totally 83 individuals. The pre-treatment PSA value, Gleason score, and TNM status were evaluated as clinical variables. There was no difference in the age distribution between the groups ($p=0.100$). Serum PSA level in the BPH group was lower than in the PCa group $p<0.001$. The Gleason score was 6 (3 + 3) for 13 (35%) of the patients, 7 (3 + 4, 4 + 3) for 10 (27%) of the patients, 8 (5 + 3, 4 + 4) for 7 (19%) of the patients, 9 (4 + 5) for 5 (14%) of the patients, and 10 (5 + 5) for 2 (5%) of the patients. The demographic and clinicopathological characteristics of the study groups are given in Table 1.

Expression of miRNAs

Three oncogenic human miRNAs considered to have diagnostic potential, miR-221, miR-650, and miR-4534, were chosen in accordance with the literature [2, 11, 13, 14]. RNU6B was chosen as a reference gene for the normalization of miRNA data. The values given were determined according to the relative expression levels in the gene expression. The distribution of the plasma miRNA levels is shown in Figure 1.

The mean ΔCt values in the PCa, BPH, and HC groups were -8.54 , -8.91 , and -6.53 for miR-221; -9.91 , -10.08 , -8.79 for miR-650; and -11.17 , -11.42 , -9.27 for miR-4534. Analysis of variance revealed that the plasma levels of miR-221 and miR-4534 were significantly different between the groups ($p=0.042$; $p=0.040$, respectively). Dunnett's post hoc analysis was performed to identify the group that made the difference. Accordingly, a significant difference was found between the

Table 1: Clinicopathological features and characteristics of the study groups.

	PC	BPH	HC	p-Value ^a
n	37	31	15	–
Mean age, years	66.11 ± 7.77	65.77 ± 7.21	61.47 ± 5.89	0.100
Mean PSA, ng/mL	30.44 ± 36.12	7.75 ± 15.83	3 ± 0.79	0.001 ^a
miR-221 ΔCt	-8.59 ± 3.52	-8.92 ± 3.01	-6.53 ± 1.44	0.042 ^a
miR-650 ΔCt	-9.91 ± 3.44	-10.14 ± 2.15	-8.78 ± 0.79	0.263
miR-4534 ΔCt	-11.34 ± 3.54	-11.56 ± 2.81	-9.27 ± 1.09	0.040 ^a
Serum PSA, n (%)				
<4 ng/mL	0	16 (51.6%)	15 (100%)	0.001 ^a
4–10 ng/mL	17 (45.9%)	13 (41.9%)		
>10 ng/mL	20 (54.1%)	2 (6.5%)		
Gleason score, n (%)				
≤ 7	23 (62.2%)			
> 7	14 (37.8%)			
Pathologic T stage, n (%)				
T1c	1 (2.7%)			
T2a	5 (13.5%)			
T2b	5 (13.5%)			
T2c	6 (16.2%)			
T3a	5 (13.5%)			
T4	15 (40.5%)			
Lymphatic invasion, n (%)				
N0	26 (70.3%)			
N1	11 (29.7%)			
Metastasis				
M0	22 (59.5%)			
M1	15 (40.5%)			

Data are expressed as mean $\pm$ SD; ^a $p<0.05$, statistically significant; PCa, prostate cancer; BPH, benign prostatic hyperplasia; HC, healthy control; PSA, prostate specific antigen; T, tumor; N, node; N0, no lymph node involvement; N1, there is lymph node involvement; M, metastasis; M0, no metastases; M1, there is metastasis.

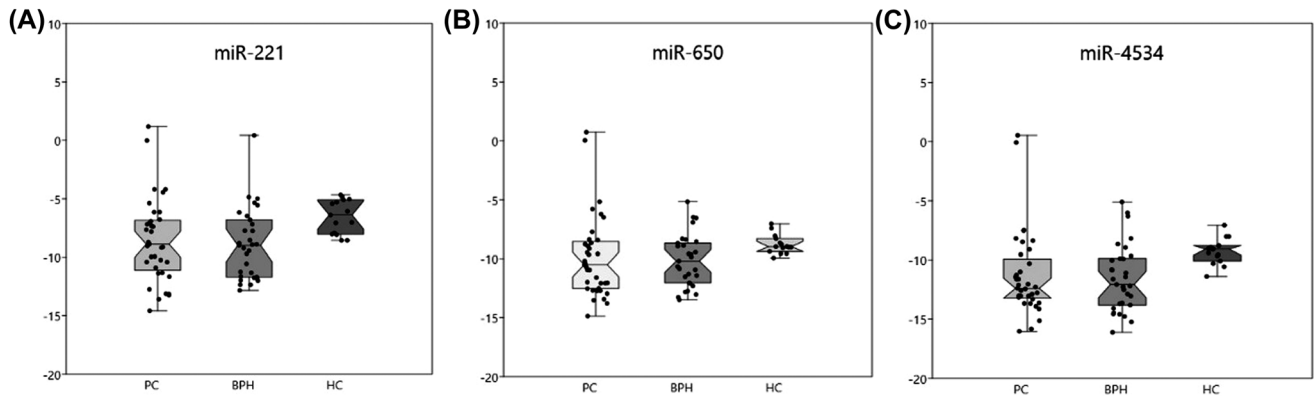


Figure 1: Differential expression of miR-221 (A), miR-650 (B) and miR-4534 (C) in PCa, BPH and HC.

The expression levels are examined by real-time QPCR and calculated using $\Delta\Delta C_t$ method. Presented data are the mean $\pm$ SD.

PCa and HC groups ($p=0.026$), and between the BPH and HC groups ($p=0.013$) for miR-221. Also, a significant difference was found between the PCa and HC groups ($p=0.021$), and between the BPH and HC groups ($p=0.014$) for miR-4534.

Fold changes were calculated using the $2^{-\Delta\Delta C_t}$ equation. Compared to the control group, the plasma miR-221, miR-650, and miR-4534 levels in the PCa group were found to be upregulated 4.01-fold, 2.19-fold, and 3.75-fold, respectively. Compared to the control group, the plasma miR-221, miR-650, and miR-4534 levels in the BPH group were found to be upregulated 5.20-fold, 2.45-fold, and 4.47-fold, respectively. The coefficient changes in the plasma levels of miR-221, miR-650, and miR-4534 for all three groups are shown as bar graphs in Figure 2.

miRNA expression and clinicopathological characteristics

The correlation between the miRNAs examined and the clinical characteristics of the PCa patient group are

shown in Table 2. There was no significant difference between miRNA expression levels and age, serum PSA, Gleason score, tumor stage, and metastasis status in the PCa group ($p>0.05$). A significant difference was determined between lymph node involvement and the plasma miR-221, miR-650, and miR-4534 expression levels ($p=0.028$, $p=0.023$, $p=0.015$, respectively). In addition, no correlation was found between the miRNA expression levels and age and serum PSA level in the PCa group ($p>0.05$).

miRNAs as diagnostic markers

To evaluate the diagnostic power of the miRNAs, we performed intergroup comparisons using ROC analysis (Table 3). In the comparison of the PCa and control groups, we found that the AUC for miR-221, miR-650 and miR-4534 was 0.737, 0.706 and 0.800, respectively ($p=0.008$, $p=0.021$ and $p=0.001$, respectively).

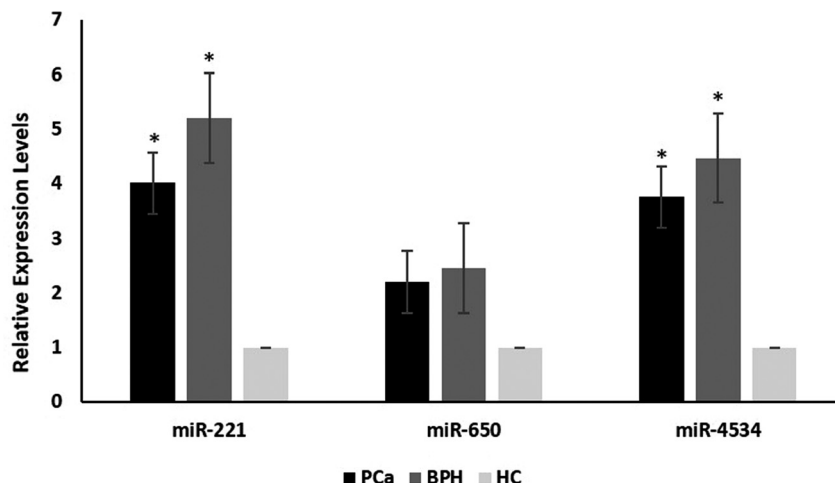


Figure 2: Relative miRNA expressions of PCa and BPH compared to HC.

The relative expression value of the control group was accepted as 1. Data are presented as a median of normalized miRNA expression in $\log_2(2^{-\Delta\Delta C_t})$. p values obtained from one-way analysis of variance (ANOVA). * $p<0.05$ vs. control group.

Table 2: Relationship between clinical characteristics and miRNA expression levels of PCa patients.

Characteristics	Patients	miR-221	p-Value ^a	miR-650	p-Value ^a	miR-4534	p-Value ^a
Age							
≤65 years	18	-9.6 ± 2.9	0.084	-10.8 ± 2.7	0.111	-12.2 ± 2.3	0.138
>65 years	19	-7.6 ± 3.8		-9 ± 3.9		-10.5 ± 4.3	
Serum PSA							
<4 ng/mL	0	–	0.743	–	0.862	–	0.293
4–10 ng/mL	17	-8.8 ± 2.7		-10 ± 2.6		-12 ± 2.6	
>10 ng/mL	20	-8.4 ± 4.1		-9.8 ± 4.1		-10.8 ± 4.2	
Gleason score							
≤7	23	-8.7 ± 2.7	0.803	-10 ± 2.4	0.858	-11.8 ± 2.5	0.360
>7	14	-8.4 ± 4.7		-9.8 ± 4.8		-10.7 ± 4.8	
Tumor stage							
T1	1	-4.2	0.449	-5.8	0.451	-7.5	0.242
T2	16	-9.3 ± 2.5		-10.5 ± 2.3		-12.4 ± 2.2	
T3	5	-8.9 ± 2.3		-10.7 ± 1.6		-12.2 ± 0.9	
T4	15	-8 ± 4.6		-9.3 ± 3.4		-10.2 ± 4.8	
Lymphatic invasion							
N0	26	-9.4 ± 2.7		-10.7 ± 2.4		-12.2 ± 2.1	
N1	11	-6.7 ± 4.5	0.028	-7.9 ± 4.7	0.023	-9.2 ± 5.2	0.015
Metastasis							
M0	22	-9 ± 2.6	0.403	-10.4 ± 2.3	0.357	-12.1 ± 2.2	0.120
M1	15	-8 ± 4.6		-9.3 ± 4.7		-10.2 ± 4.8	

miRNA relative expression levels was calculated using $\Delta\Delta C_t$ method; ^ap<0.05, statistically significant; T, tumor; N, node; N0, no lymph node involvement; N1, there is lymph node involvement; M, metastasis; M0, no metastases; M1, there is metastasis.

Table 3: Diagnostic efficiency of miRNAs and PSA in the discriminating of patient groups.

miRNA	Groups	AUC (95% CI)	Sensitivity, %	Specificity, %	p-Value ^a
miR-221	PCa vs. HC	0.737 (0.605–0.869)	57	100	0.008
	BPH vs. HC	0.787 (0.659–0.915)	61	100	0.002
	PCa vs. BPH	0.472 (0.333–0.611)	41	61	0.689
miR-650	PCa vs. HC	0.706 (0.569–0.844)	62	93	0.021
	BPH vs. HC	0.725 (0.580–0.870)	65	93	0.014
	PCa vs. BPH	0.516 (0.378–0.654)	41	74	0.820
miR-4534	PCa vs. HC	0.800 (0.681–0.919)	73	93	0.001
	BPH vs. HC	0.789 (0.659–0.920)	68	93	0.002
	PCa vs. BPH	0.502 (0.360–0.644)	54	58	0.975
PSA	PCa vs. HC	1	100	100	0.001
	BPH vs. HC	0.671 (0.518–0.824)	58	87	0.062
	PCa vs. BPH	0.857 (0.767–0.947)	89	68	0.001
miR-221+ miR-650+ miR-4534	PCa vs. HC	0.748 (0.607–0.889)	65	67	0.005
	BPH vs. HC	0.770 (0.628–0.912)	74	66	0.003
	PCa vs. BPH	0.539 (0.400–0.677)	60	55	0.584

PCa, prostate cancer; BPH, benign prostatic hyperplasia; HC, healthy control; AUC, area under the curve; PSA, prostate specific antigen; ^ap<0.05, statistically significant.

In the comparison of the BPH and control groups, we found that the AUC for miR-221, miR-650, and miR-4534 was 0.787, 0.725, and 0.789, respectively (p=0.002, p=0.014 and p=0.002, respectively). Since the sensitivity rates of the miRNAs examined were higher than the specificity rates, we determined that their power to distinguish

the disease was higher. However, the diagnostic power and significance value of miR-4534 was found to be more significant than other miRNAs.

In the comparison of PCa and BPH groups, we found that the AUC for miR-221, miR-650, and miR-4534 was 0.472, 0.516, and 0.502, respectively (p>0.05). The diagnostic power of

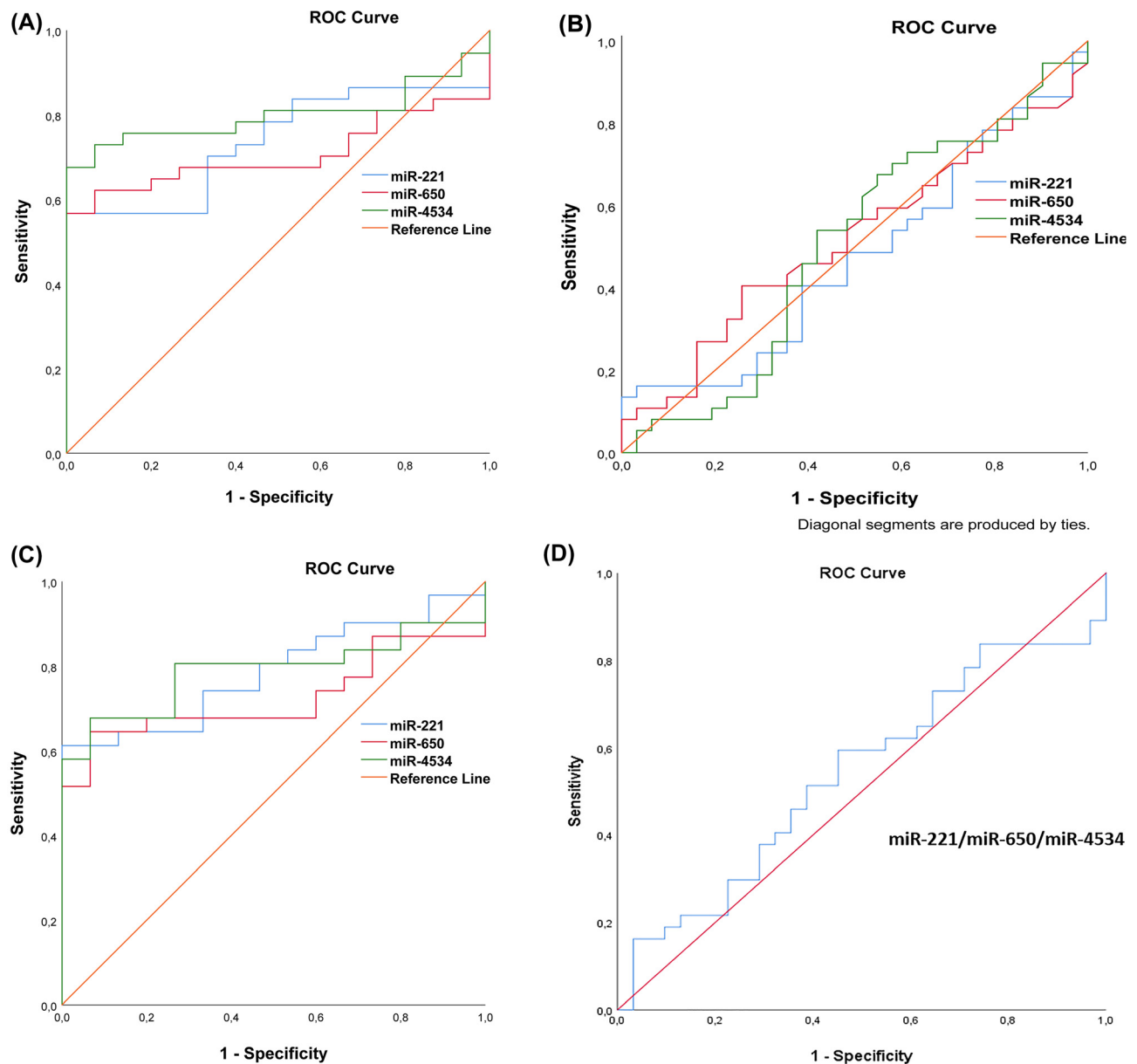


Figure 3: ROC curve showing expression levels of differential miRNAs.

ROC analyses for the discrimination of miRNA levels in patients with PCa and HC (A), in patients with PCa and BPH (B), in patients with BPH and HC (C), multimarker ROC analysis in patients with PCa and BPH (D). ROC curves were created using the ΔCt method.

these miRNAs was low in distinguishing the PCA group from the BPH group.

Multimarker ROC curve analysis was performed with combinations of miR-221, miR-650 and miR-4534 in order to differentiate PCa and BPH from healthy controls and PCa from BPH. In group comparisons, AUC values were 0.748, 0.770, and 0.539, respectively ($p=0.005$; $p=0.003$; $p=0.584$, respectively). The power of serum PSA in differential diagnosis between the groups was higher than in

miRNAs ($p<0.05$). Intergroup ROC analysis graphs are given in Figure 3.

Discussion

In this study, miR-221 and miR-4534 levels were found to be significantly upregulated in the PCa and BPH groups compared to the HC. In addition, the power of miR-221,

miR-650 and miR-4534 to be an independent biomarker, alone or in combination, was not as high as serum PSA. In addition, the expression levels of these miRNAs were associated with lymph node involvement. According to the literature review, our study is the first study to have revealed the association of these miRNAs with lymph node involvement in PCa.

MiR-221 has been reported to show increased expression in many cancers (e.g. thyroid, breast, CLL) and appears to be oncogenic due to suppression of the p27 tumor suppressor gene [15]. In some types of cancer, miR-221 levels have been reported to be increased compared to healthy tissues [16]. Dülgeroğlu et al. reported that miR-221 levels in the serum of the patients with PCa did not change compared to the control group [17]. Another study demonstrated that overexpressing miR-221 plays a role in the development of the castration-resistant PCa (CRPC) [18]. Ağaoğlu et al. found higher levels of miR-221 in the serum of PCa patients compared to the healthy controls [19]. In our study, miR-221 levels were found to be significantly upregulated in the PCa group compared to the control group. The high expression of miR-221 in PCa patients suggests that this miRNA has an oncogenic role in this disease. The increased expression of miR-221 made us consider that it may have an oncogenic effect by suppressing the expression of tumor suppressor genes.

In an *in vitro* experiment, it was reported that suppression of miR-4534 impaired the viability and proliferation of PCa cells and exerted an anti-tumorigenic effect by inducing apoptosis and leading to G0/G1 cell cycle arrest. In contrast, it has been suggested that overexpression of miR-4534 exerts an oncogenic effect by downregulating the tumor suppressor PTEN gene [20]. miR-4534 expression levels in the plasma samples of patients with PCa have not been previously evaluated. The upregulation of miR-4534 in this study confirms that it has an oncogenic effect in PCa. We consider that this oncogenic effect may be achieved by disrupting the function of the PTEN gene therefore inducing tumor development. Considering all this, miR-4534 may be an important therapeutic target in prostate cancer.

There are limited number of study in the literature comparing the plasma levels of miR-221, miR-650, and miR-4534 with clinicopathological characteristics in PCa. In a study reporting that miR-221 expression is decreased in aggressive and metastatic PCa, it was suggested that this deregulation was associated with clinicopathological parameters, including Gleason score, and predicts clinical recurrence [21]. Zheng et al. observed that decreased miR-221 expression increases the risk of recurrence [16]. On the other hand, Spahn et al. demonstrated a progressive downregulation of miR-221 in tissue samples from patients

with PCa with lymph node metastases. The study also reported that downregulation of miR-221 was associated with Gleason score, tumor stage, and clinical recurrence [21]. In another study, with a study group consisting of 10 PCa and 10 BPH patients, no significant relationship was found between Gleason score and overexpressed miR-221 [22]. İbrahim et al. suggested that miR-221 levels, which they found overexpressed in PCa serum samples, were correlated with metastasis and demonstrated that this might play a role in the progression of the disease [2]. Regarding miR-650, the other miRNA which we investigated, it has been reported in animal experiments and *in vitro* studies that miR-650 has an oncogenic effect by being upregulated in PCa cells [6, 10, 23]. Upregulation of miR-650 in PCa cells was found to be associated with poor differentiation of cancer and a higher PCa recurrence rate [10]. High expressions of miR-4534, which has been shown to be of clinical significance as an independent risk factor in PCa, have been reported to be positively correlated with poor overall and recurrence-free survival. It has been stated that this effect is achieved by deactivating the PTEN gene and inducing tumorigenesis [24]. In the current study, miRNA expressions were found to be significantly different between groups with and without lymph node metastases, but there was no significant difference between groups separated according to the presence of distant metastases. In cancers, lymph node metastasis usually occurs earlier than distant metastasis. The expression of miRNAs included in our study may be increased in the earlier stages of the disease before distant metastasis and then decreased.

In this study, the individual and combined diagnostic capabilities of miR-221, miR-650 and miR-4534 expression levels in the serum of PCa, BPH and the healthy individuals were investigated, as well. Our data revealed that the power of miRNAs to distinguish the patients from the healthy individuals was lower than that of PSA. The miRNAs we examined did not have independent diagnostic power to discriminate between PCa and BPH patients. Furthermore, combined ROC analysis was used to evaluate the joint diagnostic value of these three miRNAs. It was observed that this combination did not change the individual specificity and sensitivity values significantly and did not positively encourage the ability to distinguish between PCa and BPH. Akbayır et al. demonstrated that the diagnostic power of the combination of miR-16 and f/T PSA was better in prostate cancer patients compared to the control group [25]. Another study suggested that in differentiating PCa and BPH, the combination of miR-223-3p and -223-5p increased the diagnostic power compared to individual sensitivity and specificity [26]. Kotb et al. found that serum levels of miR-221 were upregulated in PCa in their study

group consisting of 10 patients with PCa and 10 BPH, and they calculated the specificity and sensitivity for miR-221 as 80% in differentiating PCa from BPH [27]. In the study of Kurul et al., low expression of miR-221 was associated with recurrent PCa (sensitivity: 70%, specificity: 71%) [15]. In another study, overexpression of miR-221 was reported to have 100% sensitivity and 92.9% specificity in distinguishing the low-risk PCa group from the metastatic PCa group [2]. Nip et al. found an AUC value of 0.90 in ROC analysis, in which they evaluated the ability of miR-4534 expression to discriminate between malignant and non-malignant samples. Also, they reported that miR-4534 could be used as a diagnostic marker for PCa [24]. In our study, the diagnostic power and significance level of miR-4534 was more significant compared to the other miRNAs. These findings suggested that miR-4534 may discriminate between malignant and healthy individuals and thus may have the potential to be used as an early diagnostic marker for PCa over other miRNAs we examined, although it should be validated in a larger independent cohort.

There are some limitations of the current study. Among these are an inadequate number of cases for subgroup analysis, the evaluation of a small number of miRNAs, and studying only plasma samples. In addition, it should be noted that in miRNA expression studies, results may be contradictory due to differences in methodology, population size, population diversity, and sample preference (urine, tissue, plasma, serum, and biopsy). In summary, the miRNAs we studied have the power to distinguish PCa and BPH patients from healthy controls, but they are insufficient to distinguish PCa patients from BPH patients, although the number of stage I PCa patients is low.

Based on the data we obtained from our study, we believe that individual or combined use of miR-221, miR-650 and miR-4534 in the early diagnosis of prostate cancer may be insufficient, but they may play a role in the pathogenesis of prostate cancer. Finally, we suggest conducting the current study in a larger multicenter cohort to evaluate the potential diagnostic and prognostic role of circulating miR-221, miR-650 and miR-4534 as non-invasive biomarkers for PCa.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was approved by the Ethics Committee of Süleyman Demirel University, Faculty of Medicine (dated 17.11.2020, numbered 374).

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