Diagnostic value of miR-21 and miR-221 as potential biomarkers for early diagnosis of prostate cancer

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Abstract. Prostate cancer (PCa) is globally the second most diagnosed malignancy in men, with >1.5 million new cases reported in 2020. Given the limitations of classical detection methods, the discovery of new predictive PCa biomarkers is critical. MicroRNAs (miRs), which are small, single-stranded, non-coding RNA molecules, have emerged as potential biomarkers for cancer diagnosis and prognosis. The present study aimed to evaluate the diagnostic value of miR-21 and miR-221 in PCa and their association with clinicopathological parameters. The expression of miR-21 and miR-221 was assessed using reverse transcription-quantitative PCR in 50 tumour and 50 control tissue samples. The results demonstrated that miR-21 and miR-221 were significantly upregulated in PCa tissues compared with that of the normal control tissues. Receiver operating characteristic curve analysis revealed that miR-21 had an area under the curve (AUC) of 0.90, with a sensitivity of 70% and a specificity of 96%. Similarly, miR-221 demonstrated an AUC of 0.89, with a sensitivity of 86% and a specificity of 78%. High expression of miR-21 and miR-221 was also demonstrated to be associated with higher Gleason scores and advanced tumour stages. The findings of the present study indicate the potential role of miR-21 and miR-221 as biomarkers in the diagnosis of PCa. However, further studies in non-invasive samples such as serum, blood and urine are needed to support the results of the present study.

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Introduction

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in men, following lung cancer, with >1.5 million new cases reported globally in 2020. It remains one of the leading causes of cancer-related mortality in males, accounting for >375,000 deaths worldwide in the same year (1). Early and accurate detection of PCa is critical for improving clinical outcomes and reducing mortality. However, conventional diagnostic tools such as prostate-specific antigen (PSA) testing have marked limitations in sensitivity and specificity. PSA levels can be influenced by non-malignant conditions, including infections or benign prostatic hyperplasia, leading to false-positive results, overdiagnosis and unnecessary interventions. These limitations underscore the urgent need for more reliable biomarkers to enhance diagnostic accuracy and provide improved risk stratification for patients with PCa (2).

Recent advancements in molecular biology have identified microRNAs (miRNAs/miRs) as promising diagnostic and prognostic biomarkers in cancer. miRNAs are small, single-stranded, non-coding RNA molecules, typically 15-27 nucleotides in length, that regulate gene expression at the post-transcriptional level. They serve pivotal roles in several biological processes, including cell proliferation, differentiation, migration and apoptosis. Aberrant miRNA expression has been associated with oncogenesis, tumour progression and metastasis in several cancers, including PCa (3). Calin *et al* (4) was the first to propose the involvement of miRNAs in cancer development and progression in 2004, identifying a genomic region at 13q14 that includes miR-15a and miR-16-1, which is often deleted in leukaemia. Since then, abnormal expression of miRNAs has been observed in several cancers, driven by both genetic alterations and epigenetic mechanisms.

The initial comprehensive profiling study of miRNA expression in PCa, published in 2007, signified a pivotal advancement in comprehending their function in oncogenesis (5). Certain miRNAs act as oncogenes (oncomiRs) or tumour suppressors, depending on their target genes. For instance, miR-21, one of

the most studied oncomiRs, is consistently upregulated in multiple cancers, including PCa. It promotes tumour progression by targeting key tumour suppressor genes such as PTEN and programmed cell death 4 (PDCD4), thereby enhancing cell proliferation and inhibiting apoptosis (6). Furthermore, the expression of miR-21 in the PCa DU145 cell line has been reported to enhance the expression of hypoxia-inducible factor-1α and vascular endothelial growth factor, which are pivotal factors in tumour growth and angiogenesis (7). Similarly, miR-221 has been reported to be upregulated in PCa PC3 cells, where it serves a notable role in promoting cell proliferation. One of its mechanisms involves targeting cyclin dependent kinase inhibitor 1B (p27 Kip1), a key cyclin-dependent kinase inhibitor that regulates the cell cycle. By inhibiting p27 Kip1, miR-221 facilitates progression through the cell cycle, leading to increased cellular proliferation. This process underscores the potential of miR-221 as an oncogenic factor in PCa development and progression (8). Overexpression of miR-221 exerts a pivotal influence on the growth potential of LNCaP cells, inducing a notable transition from the G1 to the S phase of the cell cycle. This transition accelerates cell cycle progression, which in turn enhances cellular proliferation. Furthermore, the colony-forming potential of LNCaP cells in soft agar is markedly increased by miR-221 overexpression (9). Other oncogenic miRNAs, such as miR-125b, contribute to cancer cell survival and growth by directly repressing the tumour suppressor gene TP53. TP53, often referred to as the 'guardian of the genome', serves a critical role in regulating cell cycle arrest, DNA repair and apoptosis in response to cellular stress or DNA damage. By downregulating TP53, miR-125b enables cancer cells to evade these protective mechanisms, promoting increased cell survival, uncontrolled proliferation and resistance to apoptosis (10).

Conversely, several miRNAs function as tumour suppressors in PCa. For example, miR-34a inhibits cancer cell proliferation and induces apoptosis by targeting oncogenes such as c-Myc and BCL-2. By downregulating these oncogenes, miR-34a effectively disrupts the pathways that promote uncontrolled cell proliferation and survival, thereby inducing apoptosis in cancer cells (11,12). Similarly, miR-200c suppresses epithelial-to-mesenchymal transition (EMT) and metastasis in PCa by downregulating zinc finger E-box binding homeobox (ZEB)1 and ZEB2, key transcription factors involved in EMT (13). The stability of miRNAs in body fluids and tissues, along with their differential expression patterns between cancerous and non-cancerous tissues, highlights their potential as minimally invasive diagnostic biomarkers for PCa.

The association between miR-21 and miR-221 in PCa and clinicopathological parameters has not been previously assessed in Moroccan men, to the best of our knowledge. The aim of the present study was to evaluate the diagnostic potential of miRNAs, specifically miR-21 and miR-221, as biomarkers for PCa and their association with clinicopathological parameters in Moroccan men. From the analysis of tissue samples, the findings of the present study offer valuable insights that advance diagnostic strategies and enhance the understanding of PCa progression.

Patients and methods

Study subjects. Between April 2023 and March 2024, patients with a PCa diagnosis were recruited from the Urology Department of Mohammed V Military Hospital (Rabat, Morocco). Control cases without a history or current symptoms related to PCa were also recruited during this period. The inclusion criterion for the patients with PCa was histologically-confirmed PCa. Patients with PCa who were receiving chemotherapy and/or radiotherapy, as well as those with any other malignant diseases, were excluded from the present study. Controls were age-matched to the PCa cases and had no history of malignancy; however, they were diagnosed with benign inflammatory disorders.

A total of 50 tumor tissues from patients with diagnosed PCa and 50 control tissues were included in the present study. After the collection of tissue samples, samples were placed in RNAlater™ Stabilization Solution (Thermo Fisher Scientific, Inc.) and were kept at -80˚C until RNA extraction. Biopsies were obtained by physicians directly performed according to standard protocols. The clinical, histological and epidemiological characteristics of the recruited patients were also obtained. The clinical characteristics of the patients in the PCa and control groups are provided in Table I. Informed consent was obtained from all participants, and the study was approved by the Moroccan Biomedical Research Ethics Committee (approval no. 3/2018; April 30, 2018).

RNA extraction. Total RNA, including miRNAs, was extracted using the mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. The purity and concentration of extracted RNA were assessed using a NanoDrop™ 2000 Spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.), with A260/A280 ratios >2.0 indicating high RNA purity. Extracted RNA was stored at -80˚C until used.

cDNA synthesis. For miRNA detection, cDNA was synthesized using the TaqMan™ MicroRNA assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) alongside reagents from the TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used for miR-21 and miR-221 detection are provided in Table SI.

The reverse transcription reactions were performed using 5 μl total RNA in a 10-μl reaction volume according to the manufacturer's instructions. The reaction mixture composed of 0.15 μl of 100 mM dNTPs mix, 1 μl of 50 U/μl reverse transcriptase, 1.5 μl of 10X reverse transcriptase buffer, 0.19 μl of 20 U/μl RNase inhibitor, and 3 μl of 5X RT miR primers. The reaction volume was made up to a total of 15 μl with nuclease-free water (4.16 μl ultra-pure H_2O). Reactions were incubated at 16˚C for 30 min, 42˚C for 30 min and 85˚C for 5 min using the GeneAmp® PCR System 2400 (Scientific Support, Inc.). cDNA was stored at -20˚C until further use.

Reverse transcription-quantitative PCR (RT-qPCR) of miR-21 and miR-221. RT-qPCR was performed in a volume of 20 μl composed of 10 μl TaqMan™ Universal PCR Master Mix (2X) (Applied Biosystems; Thermo Fisher Scientific, Inc.), 1 μl TaqMan miRNA Assay (20X), 7 μl Nuclease Free Water, and 2 μl cDNA used a StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The internal control U6 small nuclear (sn) RNA was used. The

Table I. The clinical characteristics of the patients in the prostate cancer and control groups.

Clinical characteristics	Cases, n (%)	Controls, n (%)
Total cases	50	50
Age at diagnosis/surgery		
<60 years	13 (26)	15 (30)
≥60 years	37 (74)	35 (70)
Prostate-specific antigen (ng/ml)		
<2.5	2 (4)	35 (70)
2.5-10	12 (24)	15 (30)
≥10	36 (72)	0 (0)
Pathological Gleason score		
≤6	14 (28)	NA
>6	36 (72)	NA
Pathological T-stage		
T1	17 (34)	NA
T2 X	29 (58)	NA
T3 X	1 (2)	NA
T4	3 (6)	NA
Alcohol consumption		
Yes	28 (56)	20 (40)
No	12 (24)	20 (40)
Weaned	10 (20)	10 (20)
Smoking		
Yes	32 (64)	20 (40)
No	11 (22)	25 (50)
Weaned	7 (14)	5 (10)

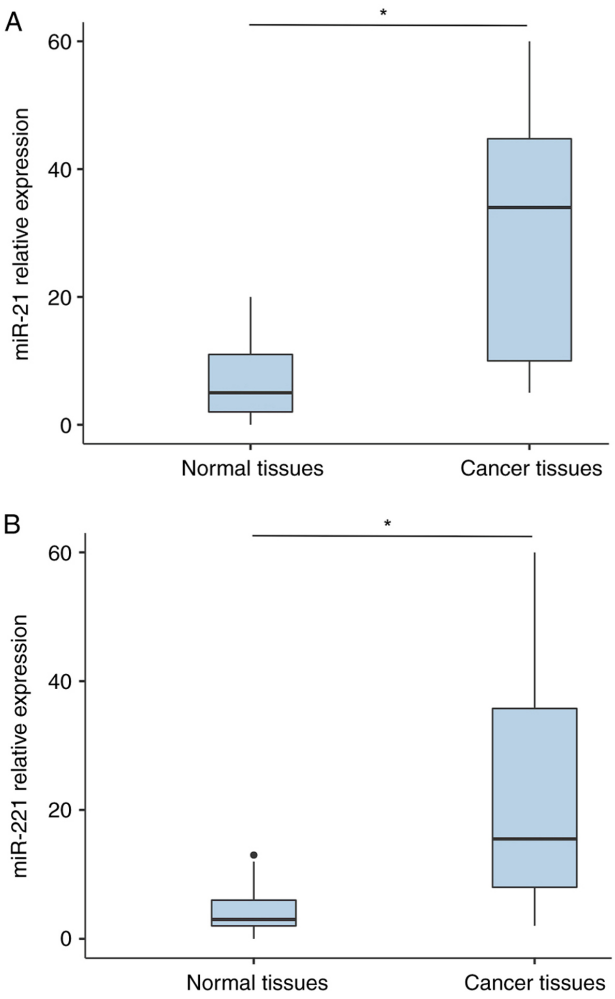

Figure 1. Relative expression levels of (A) miR-21 and (B) miR-221 in patients with prostate cancer vs. controls. *P<0.001. miR, microRNA.

thermal cycling conditions were as follows: Initial activation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec, then 60˚C for 1 min.

The expression levels of the 2 miRNAs candidates, relative to the internal control, U6 snRNA (reference gene), were calculated using the $2^{-\Delta\Delta Cq}$ method (14). Negative controls were used to detect any possible contamination in the reagents.

Statistical analysis. Statistical analysis was performed using jamovi software 2022 (www.jamovi.org). The difference in miRNA expression between the tumour and normal tissues was evaluated using one-way ANOVA, followed by Tukey's post-hoc test. Moreover, the association between miRNA expression and several clinicopathological characteristics was assessed using the χ^2 test or Fisher's exact test. The analysis plotted sensitivity (true positive rate) against 1-specificity (false positive rate) across different thresholds of miRNA expression. The Youden index was used to determine the optimal cut-off values. The sensitivity analysis measured the ability to correctly identify cancerous tissues, whilst the specificity analysis measured the ability to correctly identify normal tissues. The area under the curve (AUC) was calculated to estimate overall diagnostic accuracy, and higher AUC values reflected an improved performance of the test. P<0.05 was considered to indicate a statistically significant difference.

Results

Study population. In the present prospective cohort study, 50 patients with PCa were included and compared with 50 control subjects. The median age of the PCa group was 68 years (range, 55-82 years), whilst the median age of the control group was 63 years (range, 52-78 years).

Expression levels of miR-21 and miR-221. RT-qPCR analysis revealed that the expression level of miR-21 in PCa tissues was significantly higher than that in normal tissues (P<0.001; Fig. 1A). Similarly, miR-221 expression level was significantly higher in patients with PCa compared with that of controls (P<0.001; Fig. 1B).

Diagnostic performance of miR-21 and miR-221. A receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of miR-21 and miR-221 as PCa biomarkers. Both miRNAs were revealed to have significant diagnostic value for differentiating cancer from non-cancer tissues. For miR-21, sensitivity and specificity were demonstrated to be

Table II. Receiver operating characteristic curves tested for specificity and sensitivity and the AUC of miR-21 and miR-221.

miRNA	AUC	Sensitivity	Specificity	P-value	Youden index
miR-21	0.90	70	96	<0.001	0.66
miR-221	0.89	86	78	<0.001	0.64

miRNA or miR, microRNA; AUC, area under the curve.

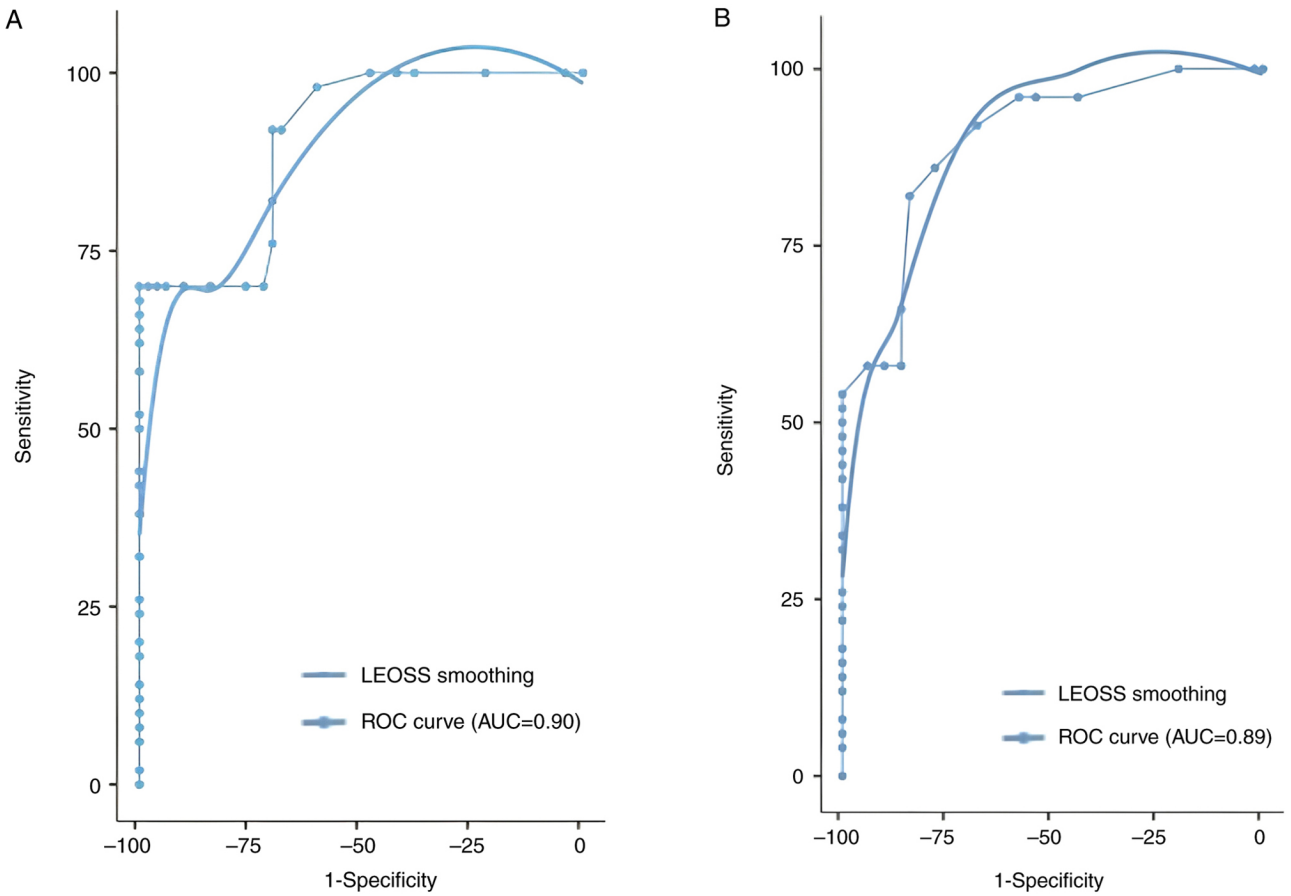

Figure 2. ROC curve analysis was conducted to evaluate the ability of (A) miR-21 and (B) miR-221 to differentiate between cancerous and normal tissues. ROC, receiver operating characteristic; miR, microRNA; LOESS, Locally Estimated Scatterplot Smoothing.

70 and 96%, respectively, and an AUC of 0.90 (P<0.001). miR-221 also presented an important diagnostic performance, with sensitivity and specificity demonstrated to be 86 and 78%, respectively, and an AUC of 0.89 (P<0.001). The cut-off value was determined on the basis of Youden's index (maximum=sensitivity + specificity-1) to maximize both sensitivity and specificity for each ROC analysis (Table II; Fig. 2).

Association with clinicopathological parameters. Patients were categorized into two groups based on high and low miRNA expression levels. The high expression levels of miR-21 and miR-221 were further analysed. An assessment of the association between these miRNA expression levels and clinicopathological parameters revealed that although both miR-21 and miR-221 were significantly associated with higher pathological Gleason scores (>6) and earlier tumour stages (T2X and T1) (P<0.01), their expression levels did not demonstrate a statistically significant association with PSA levels (P>0.05). Notably, for patients with PSA levels of ≥10 ng/ml, the expression levels of miR-21 and miR-221 were markedly higher compared with those with lower PSA levels, although this association was not statistically significant. Additionally, no significant associations were observed between miRNA expression levels and other clinical factors, including age at diagnosis, smoking status or alcohol consumption (P>0.05; Table III).

Discussion

miRNAs are small regulatory molecules involved in post-transcriptional gene silencing, which serve an essential

Table III. Association of miRNAs expression with various clinicopathological parameters of patients with prostate cancer.

Clinicopathological parameters	Number of cases	High miR-21 expression (n=35)	Low miR-21 expression (n=15)	P-value	High miR-221 expression (n=30)	Low miR-221 expression (n=20)	P-value
Age at diagnosis/surgery							
<60 years	13	8	5	0.44	6	7	0.24
≥60 years	37	27	10		24	13	
Prostate-specific antigen (ng/ml)							
<2.5	2	1	1	0.63	0	2	0.13
2.5-10	12	8	4		6	6	
≥10	36	26	10		24	12	
Pathological Gleason score							
≤6	14	6	8	0.009	4	10	0.009
>6	36	29	7		26	10	
Pathological T-stage							
T1	17	8	9	0.007	5	12	0.001
T2 X	29	24	5		22	7	
T3 X	1	1	0		1	0	
T4	3	2	1		2	1	
Alcohol consumption							
Yes	28	20	8	0.54	17	11	0.79
No	12	7	5		8	4	
Weaned	10	8	2		5	5	
Smoking							
Yes	32	24	8	0.43	10	22	0.16
No	11	6	5		7	4	
Weaned	7	5	2		3	4	

miRNA or miR, microRNA.

role as modulators of key biological processes such as cell proliferation, apoptosis or differentiation (15). However, this may depend on the targets they regulate. In cancer, miRNAs can function as either tumour suppressors or oncogenes (16). Currently, miR-21 and miR-221 have emerged as two critical upregulated oncomiRs in PCa that promote tumorigenesis through their high expressions in cancerous tissues (17,18).

By using RT-qPCR, it was demonstrated that the expression of miR-21 and miR-221 was upregulated in PCa tissues compared with that in normal control tissues, which is consistent with other published studies. Thus, these high levels of expression suggest that miR-21 and miR-221 may serve as potential biomarkers for both diagnosis and prognosis prediction of PCa. Previous studies have highlighted the role of miR-21 as a potential candidate biomarker for early detection of PCa. miR-21 downregulates a number of important tumour suppressors, such as *PTEN* and PDCD4, leading to increased proliferation and resistance to apoptosis (19). Wang *et al* (20) and Gunawan *et al* (21) reported that miR-21 was differentially expressed in patients with PCa and therefore they concluded that it could be useful as a potential biomarker for PCa. Similarly, Agaoglu *et al* (22) demonstrated that miR-21 levels in patients with PCa were significantly higher than in controls ($P<0.001$). Other research on miRNA-221 indicated its expression was increased in PCa PC3 cells and it may promote cell growth by targeting p27 Kip1, which blocks the cell cycle (9). Similarly, Kachris *et al* (23), Sun *et al* (24) and Song *et al* (25) reported that miR-221 was upregulated in PCa. However, downregulation of miR-221 has also been reported (26,27).

The findings from the present study demonstrated that associations between the levels of miR-21 and miR-221 in PCa tissues vary significantly between key clinicopathological parameters, particularly Gleason score and tumour stage. It was revealed that higher expression of both miR-21 and miR-221 was significantly associated with increased Gleason scores and tumour stages ($P<0.05$), suggesting that these miRNAs could be significant in the progression and aggressiveness of PCa. These results align with previous research linking miR-21 and miR-221 with PCa progression. For instance, Guan *et al* (28) reported an association between increased miR-21 expression and higher Gleason scores. Moreover, Ibrahim *et al* (29) and a meta-analysis by Stafford *et al* (30) concluded that miR-21 expression was associated with higher Gleason scores as well as advanced tumour stages, supporting the notion that miR-21 is involved in promoting tumour aggressiveness in PCa. Furthermore, miR-221 has been reported to be upregulated in castration-resistant PCa, a more advanced and treatment-resistant form of the disease, further indicating its role in cancer progression (31). Certain studies have also identified an association between high expression of miR-221 and higher Gleason scores (29). However, another study reported that the level of miR-221 was not associated with a high Gleason score or other clinicopathological parameters (32).

Although PSA continues to be an important biomarker for PCa screening, the present study identified no association between PSA levels and the expression of these miRNAs. This suggests the potential for enhancing the precision of PCa evaluation through the incorporation of miR-21 and miR-221 into diagnostic or prognostic models. These findings are consistent with the findings of Porzycki *et al* (33), which highlights the potential for the use of miRNAs as independent markers for tumour progression. The absence of associations between the expression of these miRNAs and other indicators, such as age, smoking status and alcohol consumption, suggests that miR-21 and miR-221 are likely independent of these variables. This suggests that they may serve as specific biomarkers for these indicators, offering insights into PCa progression.

The diagnostic potential of miR-21 and miR-221 demonstrated in the present study is in agreement with that reported in previous studies. The meta-analysis by Zhou and Zhu (34) revealed that miR-21 had an AUC of 0.95, with a pooled sensitivity and specificity of 91 and 88%, respectively. Likewise, Purnomo *et al* (35) reported a sensitivity and specificity of 91 and 89%, respectively, with an AUC of 0.97. On the other hand, Ibrahim *et al* (29) reported an AUC of 0.872 for miR-221, with sensitivity and specificity values of 82 and 72%, respectively, indicating its potential as a diagnostic biomarker. In another study, increased expression of miR-221 was associated with a sensitivity of 57% and a specificity of 100%, with an AUC of 0.74 in ROC analysis (36). These results support the findings of the present study and highlight the clinical importance of miR-21 and miR-221 in the diagnosis of PCa.

Studies on miR-21 as a biomarker for diagnosis and prognosis are widespread for other cancers such as breast, gastric and endometrial cancer. In breast cancer, a meta-analysis evaluating the diagnostic value of miR-21 as a serum biomarker reported a sensitivity of 79%, specificity of 85% and an AUC of 0.89 (37). In gastric cancer, miR-21 demonstrated diagnostic potential with a sensitivity of 82.9%, a specificity of 85.7% and an AUC of 0.96 (38). In endometrial cancer, miR-21 demonstrated a sensitivity of 84.51% and specificity of 86.79%, with an AUC of 0.925 (39).

miR-221 has also been demonstrated to be upregulated in several cancers, including breast and thyroid cancer, targeting tumour suppressor genes such as p27 Kip1 and oestrogen receptor-α in breast cancer (40). In breast cancer, ROC curve analysis revealed an AUC of 0.769, with a sensitivity of 61.29% and a specificity of 85.19% (41). For thyroid cancer, a meta-analysis of 16 studies reported a pooled sensitivity of 82%, specificity of 84% and an AUC of 0.88 (42). However, the reasons for the variable expression of these miRNAs in different cancer cell types remain unclear.

The results of the present study highlight the potential of miR-21 and miR-221 as diagnostic and prognostic biomarkers for PCa. These miRNAs serve not only as predictive markers for PCa but also as important indicators of cancer aggressiveness. In particular, the present study demonstrated that elevated expression of miR-21 and miR-221 was significantly associated with early-stage PCa and higher Gleason scores, which are indicative of more aggressive disease. This finding reinforces their potential not only in the early detection of PCa but also in assessing prognosis. By focusing on the relationship between miRNA expression, tumour staging and clinical outcomes, the present study highlights their value in predicting disease progression and guiding treatment decisions.

In the present study, the control group was defined as individuals with benign inflammatory disorders, reflecting the common occurrence of low-level inflammation in the general population, particularly with aging, a phenomenon

known as 'inflammaging' (43). The procurement of completely healthy, inflammation-free tissue presents significant ethical challenges and practical difficulties, and it could introduce potential selection bias (44,45). By employing controls with benign inflammatory conditions, our objective was to emulate real-world scenarios, as inflammation is frequently observed in tumour microenvironments and contributes to cancer progression (46).

Despite the promising results of the present study, there are certain limitations. First, the sample size, of 50 patients with PCa and 50 controls is relatively small, which may affect the degree to which the results apply to a larger population. Additionally, the cross-sectional design of the study restricts the ability to track miRNA expression changes over time and establish a direct cause-and-effect relationship between miR-21 and mirR-221 and cancer progression across different disease stages. Lastly, RT-qPCR was used but more advanced techniques, such as western blotting and flow cytometry, could improve the precision and reliability of biomarker investigations. While these findings are encouraging, further research is needed to validate the potential of miR-21 and miR-221 as biomarkers, and larger and more diverse cohorts are needed to confirm the generalizability of the results. Future research should also utilise non-invasive samples, such as blood or urine, to assess the use of less invasive diagnostic approaches and pave the way for personalized and effective PCa management. Furthermore, integrating miR-21 and miR-221 with other molecular markers into a multi-biomarker panel could enhance diagnostic accuracy and refine risk stratification, ultimately improving outcomes for patients with PCa.

In conclusion, the significant overexpression of miR-21 and miR-221 in PCa tissues compared with control tissues, coupled with their association with critical clinicopathological parameters such as Gleason score, tumour stage and patient prognosis, emphasizes their pivotal role in both the initiation and progression of PCa. These miRNAs are not only integral to key cellular processes such as cell cycle regulation, apoptosis and metastasis but also act as potential modulators of the tumour microenvironment, further highlighting their relevance in cancer biology. Furthermore, given their stable presence in body fluids and tissues, miR-21 and miR-221 hold potential as non-invasive biomarkers for the early detection, prognosis and monitoring of disease progression. Their ability to serve as molecular signatures for aggressive forms of PCa provides a unique opportunity to improve the sensitivity and specificity of current diagnostic tools, surpassing the limitations of traditional methods such as PSA testing. Furthermore, the identification of these miRNAs could enhance risk stratification, offering a more personalized approach to treatment planning. However, as the field of miRNA research continues to evolve, it is crucial to delve deeper into the molecular mechanisms through which miR-21 and miR-221 exert their oncogenic effects, as well as their potential interactions with other biomarkers and therapeutic targets. Understanding these interactions could lead to the development of miRNA-based therapies, potentially inhibiting tumour progression or sensitizing tumours to existing treatments. Ultimately, the findings of the present study could contribute to a paradigm shift in PCa management, fostering the development of more accurate, effective and personalized diagnostic and therapeutic strategies that improve patient outcomes and reduce mortality rates globally.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

IM conceptualized the study, developed methodology, wrote the manuscript and performed formal analysis. KAT performed formal analysis, reviewed and edited the manuscript. MA conducted investigation and conceptualized the study. AL, AA, KE and AEG contributed to sample collection and patient data acquisition. MME supervised the study, performed project administration, developed methodology and validated analysis and interpretation of data. All authors read and approved the final version of the manuscript. MA and KAT confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Informed consent was obtained from all participants. The present study was approved by the Moroccan Biomedical Research Ethics Committee (approval no. 3/2018; approval date: April 30, 2018; Rabat; Morocco).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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