Received: 17 January 2019 | Revised: 13 June 2019 | Accepted: 27 June 2019

DOI: 10.1111/and.13384

ORIGINAL ARTICLE

First International Journal of Andrology ANDROLOGIA WILEY

Diagnostic significance of miR-21, miR-141, miR-18a and miR-221 as novel biomarkers in prostate cancer among Egyptian patients

Noha H. Ibrahim[1] | Mona S. Abdellateif[2] | Samar Hassab-Allah Kassem[3] | Mohamed Ahmed Abd El Salam[4] | Mosaad M. El Gammal[5]

[1]Department of Clinical and Chemical Pathology, National Cancer Institute (NCI), Cairo University, Cairo, Egypt

[2]Medical Biochemistry and Molecular Biology, Cancer Biology Department, National Cancer Institute (NCI), Cairo University, Cairo, Egypt

[3]Biochemistry Department, Faculty of Applied Medical Sciences, 6th October University, Giza, Egypt

[4]Department of Andrology, Sexology & STDs, Faculty of Medicine, Cairo University, Cairo, Egypt

[5]Medical Oncology Department, National Cancer Institute (NCI), Cairo University, Cairo, Egypt

Correspondence
Mohamed Ahmed Abd El Salam, Faculty of Medicine, Cairo University, (9) Montaser Buildings, St. Gamal AbdEl Naser, Hadayek Helwan, Cairo 11731, Egypt.
Emails: moh_756@yahoo.com; moh_756@cu.edu.eg

Abstract

Prostate cancer (PC) is considered as the fifth cause of cancer deaths worldwide. The exact etiopathogenesis is unclear; however, genetic predisposition, hormonal influencers, lifestyle and environmental factors act as major contributors. It has been found that several miRNAs may play a crucial role in cancer initiation and progression. Here, in this study, we evaluated the peripheral blood levels of miR-21, miR-141, miR-221 and miR-18a expression among 80 prostate cancer patients (50 localised and 30 metastatic) and 30 benign prostatic hyperplasia patients compared to 50 normal control subjects, using RT-PCR. Our results of analysis of miR-21, miR-141, miR-18a and miR-221 in the plasma of PC patients showed that miR-18a is a powerful discriminator of PC patients from healthy controls as it had the highest AUC (0.966; 95% CI, 0.937–1.000), while miR-221 provided better differentiation of metastatic from localised PC (sensitivity was 92.9% at 100% specificity), and when we combine miR-18a and miR-221 for differentiating patients with MPC, it will increase the sensitivity to 96.4% at a specificity of 100% (AUC, 0.997; 95% CI, 0.988–1.0) ($p < .000$). This current study recommends that analysis of these miRNAs might have clinical value in enhancing PSA testing.

KEYWORDS

biomarkers, diagnostic yield, miRNAs, prognosis, prostate cancer

1 | INTRODUCTION

Prostate cancer (PC) is listed as the second common male cancer following skin cancer and the fifth cause of cancer mortalities (Daniyal et al., 2014). Unfortunately, the pathogenesis of PC is still obscure; however, it has been found that it is a multifactorial disease including genetic predisposition, hormonal influences and various environmental exposure risks (Attard et al., 2016). Hormones play a crucial role in the development of PC, whereas testosterone and its potent metabolite, DHT, act on prostatic epithelium. Therefore, administration of anti-androgens or even castration was found to be effective in apoptosis and involution of the prostate (Hsing, Reichardt, & Stanczyk, 2002).

Clinical manifestations in most of the cases are asymptomatic. However, obstructive and irritative urinary symptoms may be encountered that may be unfortunately misdiagnosed with benign prostatic enlargement that is prevalent among elderly people. Previously, annual DRE, biochemical screening tests and TRUS have been used in assessing prostate cancer in its early stage (Mettlin, Lee, Drago, & Murphy, 1991). Currently, assessment of PSA is considered as the most commonly used biomarker able to correlate with PC risk, extent and prognosis. Unfortunately, it has been found that patients may

develop PC in spite of low levels of PSA. Besides that, several factors such as benign prostatic enlargement, prostatitis or manipulation as well as some drugs may raise the PSA levels that could be misdiagnosed with PC during screening (Pron, 2015). Thus, it was essential to identify other alternative biomarkers, such as miRNA, to enhance cancer diagnosis, prognosis and evaluation of treatment outcomes.

miRNAs are noncoding RNA nucleotides of small size that control gene expression and post-transcriptional events as they degenerate, or hinder target mRNAs. Additionally, they play several roles in cellular development, differentiation, proliferation, cell-cycle regulation, apoptosis and metabolism (Bartel, 2004). Chromosomal abnormalities such as structural deletions, amplifications and mutations, promoter methylation, and regulation of transcription could influence expression as well as regulatory functions of miRNA. Also, a single miRNA can control the expression of several messenger RNAs (mRNAs). Therefore, alterations in miRNA could lead to dysregulated expression of several mRNAs and proteins that may precipitate in occurrence of various human cancers (Calin & Croce, 2006; Kim & Kim, 2013). In addition, several researches and clinical trials are being conducted to identify the exact pathogenic role of miRNA in PC as well as evaluating its diagnostic and prognostic roles.

2 | PATIENTS/SUBJECTS AND METHODS

This is a prospective cohort study including 160 participants: 50 patients with localised prostate cancer (LPC) before radical prostatectomy, 30 patients with metastatic prostate cancer (MPC), 30 patients diagnosed with benign prostatic hyperplasia (BPH) diagnosed by histopathological examination of conventional transrectal ultrasound-guided template biopsy specimens of the prostate, and 50 healthy normal controls (NC). Cancer patients presented to the National Cancer Institute (NCI) for diagnosis and treatment during the period between June 2015 and September 2017. This study received approval of the ethical committee of the NCI, Cairo University (Egypt), and was conducted in accordance with the 2011 Helsinki Declaration, and written informed consents were obtained from each patient before enrolment into the study.

All patients were subjected to routine laboratory investigations and imaging diagnoses. All patients with severe comorbidity or prostatitis were excluded. In addition, blood samples from patients who did not receive hormonal ablative or cytotoxic treatments were used, as they may affect the level of circulating miRNAs. PC aggressiveness was evaluated using the Gleason histopathological grading; patients with a score of ≤7 were considered low-grade and those with a score of >7 were considered high-grade. On the other hand, clinical staging of PC was done using the TNM system that assesses the tumour extent (T), lymph node involvement (N) and distant metastases (M) (Sobin & Wittekind, 2002). Whole-blood samples (7 ml) were aseptically divided into two tubes: the first tube contained k2EDTA and the second was the serum collection tube. Within 30 min of blood draw, the blood samples on k2EDTA were centrifuged at room temperature using Thermo Scientific Megafuge 16R Centrifuge for 10 min at 1,340 *g*. The collected samples were aliquoted and frozen at −80°C until microRNA extraction, while the serum collection tube was left for 30 min to undergo clotting; then, the samples were centrifuged at 1,790 *g* for 10 min yielding serum that was used for the determination of serum concentrations of total prostate-specific antigen (tPSA) and free prostate-specific antigen (fPSA) by chemiluminescent assays (ARCHITECT i1000SR Immunoassay Analyzer; Abbott).

2.1 | RNA extraction

Total RNA was extracted from 200 μl of plasma using the miRNeasy Mini Kit (cat. no. 217004). The concentration and purity of RNA samples were assessed using NanoDrop 1000 (Thermo Scientific NanoDrop™ Spectrophotometer ND 1000). Then, the RNA was eluted in 40 μl of RNase-free water and was stored at −80°C until reverse transcription (RT) reactions.

2.2 | Reverse transcription (RT)

The total RNA (100 ng) was reverse-transcribed using miScript II RT Kit (catalogue no. 218161), and complementary DNA (cDNA) synthesis was completed in a thermal cycler [IGEM: MIT/2005/Thermo Cycler]; then, the cDNA was stored at −80°C in anticipation of use.

2.3 | Quantitative real-time polymerase chain reaction (RT-PCR)

A 2.5 μl of cDNA was amplified using 10 μl of TaqMan 2X Universal PCR Master Mix II (Applied Biosystems; Thermo Fisher Scientific), 1 μl of gene-specific primers of the target miRNA and 6.5 μl of nuclease-free water in a final volume of 20 μl. qPCR was run on the StepOne Real-Time PCR System (Applied Biosystems), and the reaction mixtures were incubated at 95°C for 10 min to stimulate HotStarTaq DNA polymerase, followed by 40 cycles of initial denaturation at 94°C for 15 s, annealing at 55°C for 30 s and final extension at 70°C for 30 s. The expression of selected miRs in the blood was normalised to the expression of U6 small nuclear RNA (RNU6B). The data obtained from the miRNA expression levels were calculated and evaluated by the cycle threshold (*Ct*) method, which is the number of cycles required for the fluorescent signal to cross the threshold in RT-PCR (Schmittgen & Livak, 2008). Notably, Δ*Ct* was calculated by subtracting the *Ct* values of RNU6B from the *Ct* values of the target miRNA. ΔΔ*Ct* was then calculated by subtracting the average Δ*Ct* of the healthy control samples from the Δ*Ct* of the case samples (LPC, MPC and BPH). The fold change in the miRNA expression level was calculated (fold change = $2^{-\Delta\Delta Ct}$) to determine the relative quantitative levels of target miRNA (Livak & Schmittgen, 2001).

2.4 | Statistical analysis

All data were assorted and then statistically analysed using SPSS software version 21 (IBM SPSS). Data are presented as median and range. Comparisons between patient groups were analysed using

the Kruskal–Wallis H test and Mann–Whitney test. Receiver operating characteristic (ROC) curves were used to assess miRs as biomarkers, and the area under the curve (AUC) was reported. Multivariate logistic regression analysis was used to assess the association of target miRNAs' expression with LPC, MPC and BPH. $p < .05$ (two-tailed) was considered to indicate a statistically significant difference.

3 | RESULTS

In this prospective cohort study, 50 patients with localised prostate cancer (LPC), 30 patients with metastatic prostate cancer (MPC) and 30 cases with benign prostate hyperplasia (BPH) were included and then compared to 50 normal controls (NC).

3.1 | Patients' characteristics

The mean age of the PC patients was 56.8 ± 12.18, while the mean age of BPH patients was 53 ± 9.5, and the mean age of control group was 54 ± 8.93; there was no significant difference between the studied groups regarding the age ($p > .05$), so the three groups were well matched for age. Of all cancer patients, 33 cases were stage I, 17 were stage II, and 30 were stage IV. Digital rectal examination (DRE) was palpable in 25.6% of all participants. Gleason score was 6 in 21 patients, 7 in 29 patients, 8 in 20 patients and 9 in 7 patients, while only 3 patients had Gleason score 10. Prostate volume was more than 50 g in 71.25% of patients. 42.5% of the patients had lymph node metastasis, and 37.5% of the patients had distant metastasis, as shown in Table 1a, b.

3.2 | Plasma expressions of miRNAs and PSA

There was a statistically significant difference among all patient groups regarding tPSA and fPSA plasma expression ($p < .001$). However, regarding f/tPSA, there was no statistically significant difference between the NC and BPH groups ($p = .56$), while there was a significant difference among the NC, LPC and MPC groups ($p < .001$). miRNA expression in the PC and BPH groups and healthy controls was determined relative to the endogenous control, RNU6B, in the peripheral blood of PC and BPH patients and healthy control subjects using qPCR. Values are expressed as the relative median fold difference in gene expression. Target miRNAs and RNU6B yielded reliable Ct values in all samples from PC and BPH patients and control subjects. There was no statistically significant difference between the NC and BPH groups regarding plasma expression of miR-21, miR-141, miR-221 and miR-18a ($p = .186, .083, .704$ and $.684$ respectively). However, there was a significant difference among the LPC, MPC and nonmalignant groups (NC and BPH) regarding plasma expression of miR-21, miR-221 and miR-18a ($p < .001$). miR-141 showed no significant difference between the LPC and nonmalignant groups (NC and BPH) ($p = .283$); meanwhile, it showed a significant difference between the LPC and MPC groups ($p < .001$), as shown in Table 2 and Figures 1–4.

3.3 | ROC curve analysis for the tested miRNAs in localised prostate cancer and nonmalignant cases

As relative median miRNA expression was differentially expressed in the peripheral blood of PC patients, BPH patients and control subjects, the potential of peripheral blood oncogenic miRNAs as biomarkers was evaluated based on a ROC analysis. ROC curve analysis was performed for miR-21, miR-221 and miR-18a to differentiate patients with LPC and those with BPH and NC. miR-21 showed AUC of 0.959 (95% CI, 0.886–1.000; $p < .0001$), and miR-221 showed the lowest AUC (0.872; 95% CI, 0.772–0.97; $p < .0001$), while miR-18a showed the highest AUC (0.996; 95% CI, 0.987–1.000; $p < .0001$), as mentioned in Table 3. The sensitivity of miR-21, miR-221 and miR-18a was 90.9%, 45.5% and 95.5% respectively at 100% specificity, and when we added miR-21 to miR-18a for detecting patients with LPC, it did not affect its diagnostic power as it achieved the same sensitivity (95.5%) at a specificity of 100% (AUC, 0.973; 95% CI, 0.921–1.0; $p < .0001$), as shown in Figure 5.

3.4 | ROC curve analysis for the tested miRNAs in localised prostate cancer and metastatic cases

ROC curve analysis was performed for miR-21, miR-221, miR-141 and miR-18a to differentiate patients with LPC and those with MPC, and it showed that miR-221 showed the highest AUC (0.982; 95% CI, 0.951–1.000; $p < .0001$), while miR-18a showed AUC of 0.966 (95% CI, 0.922–1.000; $p < .0001$) and miR-141 showed AUC of 0.925 (95% CI, 0.854–0.996; $p < .0001$). miR-21 showed the lowest AUC (0.761; 95% CI, 0.630–0.893; $p = .002$), as shown in Table 4. The sensitivity of miR-21, miR-141, miR-221 and miR-18a was 32.1%, 53.6%, 92.9% and 60.7% respectively at 100% specificity. By combining miR-18a and miR-221 for differentiating patients with MPC, it will increase the sensitivity to 96.4% at a specificity of 100% (AUC, 0.997; 95% CI, 0.988–1.0; $p < .0001$), as shown in Figure 6.

3.5 | Multivariate logistic regression analysis

Multivariate statistical analysis revealed a strong association between LPC and higher expression of miR-21 (odds ratio [OR], 0.018; 95% confidence interval [CI], 0.003–0.118; $p = .001$) and miR-18a (OR, 0.003; 95% CI, 0.000–0.043; $p < .001$), fPSA (OR, 0.056; 95% CI, 0.012–0.259; $p = .01$) and f/tPSA (OR, 90; 95% CI, 11.462–706.71; $p < .001$). However, no significant association was found between LPC and miR-221 (OR, 0.074; 95% CI, 0.017–0.326; $p = .513$), as shown in Table 5.

3.6 | Associations between miR-21, miR-221, miR-141 and miR-18a and clinicopathological features of the patients

All tested miRNAs (miR-21, miR-221, miR-141 and miR-18a) were significantly associated with increased tPSA, fPSA, Gleason score, and

TABLE 1 (a) Demographic data of individual groups

	Diagnosis				
	Normal (50)	Benign prostate lesion (30)	Localised prostate cancer (50)	Metastatic prostate cancer (30)	*p*-Value
DRE (160)					
Not felt	50 (100%)	30 (100%)	14 (28%)	27 (90%)	<.001
Felt	0 (0%)	0 (0%)	36 (72%)	3 (10%)	
Gleason_score (80)					
6	0 (0%)	0 (0%)	21 (42%)	0 (0.0%)	<.001
7	0 (0%)	0 (0%)	29 (58%)	0 (0.0%)	
8	0 (0%)	0 (0%)	0 (0%)	20 (66.7%)	
9	0 (0%)	0 (0%)	0 (0%)	7 (23.3%)	
10	0 (0%)	0 (0%)	0 (0%)	3 (10%)	
PR_vol (80)					
<50 g	0 (0%)	0 (0%)	23 (46%)	0 (0%)	.001
≥50 g	0 (0%)	0 (0%)	27 (54%)	30 (100%)	
T (80)					
1	0 (0%)	0 (0%)	8 (16%)	0 (0%)	.001
2	0 (0%)	0 (0%)	33 (66%)	0 (0%)	
3	0 (0%)	0 (0%)	9 (18%)	28 (93.3%)	
4	0 (0%)	0 (0%)	0 (0%)	2 (6.7%)	
N (80)					
No	0 (0%)	0 (0%)	46 (92%)	0 (0%)	<.001
Yes	0 (0%)	0 (0%)	4 (8%)	30 (100%)	
M (80)					
No	0 (0%)	0 (0%)	50 (100%)	0 (0.0%)	<.001
Yes	0 (0%)	0 (0%)	0	30 (100%)	
Stage (80)					
1	0 (0%)	0 (0%)	33 (66%)	0 (0%)	<.001
2	0 (0%)	0 (0%)	17 (34%)	0 (0%)	
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
4	0 (0%)	0 (0%)	0 (0%)	30 (100%)	

(b) Descriptive data of the patients' characteristics

Parameter	Number (%)	Parameter	Number (%)
Age		*Pathological staging (80)*	
Mean ± *SD* in the PC group	56.8 ± 12.18	T1	8 (10%)
Mean ± *SD* in the BPH group	53 ± 9.5	T2	33 (41.25%)
Mean ± *SD* in the NC group	54 ± 8.93	T3	37 (46.25%)
Diagnosis		T4	2 (2.5%)
NC	50	*Lymph node metastasis (80)*	
LPC	50	Yes	34 (80) 42.5%
MPC	30	No	46 (80) 57%
BPH	30	*Distant metastasis (80)*	
DRE (160)		Yes	30 (80) 37.5%
Palpable	41 (25.6%)	No	50 (80) 62.5%
Not palpable	119 (74.4%)	*Stage (80)*	
Gleason score (80)		I	33 (41.25%)
6	21 (26.25%)	II	17 (21.25%)
7	29 (36.25%)	III	0 (0%)
8	20 (25%)	IV	30 (37.5%)
9	7 (8.75%)		
10	3 (3.75)		
Prostate volume (80)			
Less than 50 g	23 (28.75)%		
More than 50 g	57 (71.25)%		

Abbreviations: BPH, benign prostatic hyperplasia; DRE, digital rectal; LPC, localised prostate cancer; MPC, metastatic prostate cancer; NC, normal control.

TABLE 2 Serum expression of miRNAs and PSA in different patient groups

	Normal control	Benign prostatic hyperplasia	Localised prostate cancer	Metastatic prostate cancer	*p*-Value
tPSA	2.4 (0.2–4.7)* A†	5 (1.7–8.9) b	9.75 (5.80–29.7) c	77 (26–223) D	<.001
fPSA	0.7 (0.1–1.8) a	1.65 (0.6–2.7) b	1.6 (0.8–2.8) b	5.95 (2.2–12.8) C	<.001
f/tPSA	0.33 (0.13–0.64) a	0.33 (0.15–0.41) a	0.12 (0.08–0.26) b	0.07 (0.04–0.11) C	<.001
miR-21	0.04 (0–0.14) a	0.03 (0–0.08) a	1.4 (0.01–3.8) b	2.3 (0.5–9) C	<.001
miR-141	0.01 (0.00–0.09) a	0.01 (0.00–0.09) a	0.02 (0.00–0.09) a	0.35 (0.03–1.1) b	<.001
miR-221	0.03 (0.00–0.09) a	0.03 (0.00–0.09) a	0.09 (0.01–0.48) b	1.09 (0.22–2.90) c	<.001
miR-18a	1.3 (0.4–2) a	1.25 (0.3–2) a	3.1 (1.9–8.4) b	8.5 (7.9–10.1) c	<.001

Abbreviations: fPSA, free PSA; tPSA, total PSA.
*Data are expressed as median and range.
†Data having the different letters (a, b, c, d) in the same row are statistically different.

pathological stages of the patients, lymph node metastasis and distant metastasis. However, miR-221, miR-141 and miR-18a were significantly associated with decreased f/tPSA (p = .0001). Only miR-221 and miR-18a were significantly associated with DRE (p = .011 and .030 respectively), and prostate volume was significantly associated with miR-221, miR-18a and miR-21 (p = .001, .005 and .017 respectively).

4 | DISCUSSION

PC is one of the commonly encountered urogenital tumours especially among elderly people, whereas it is the second most commonly diagnosed malignancy and the fifth major cause of cancer-associated mortalities. Unfortunately, BPH is frequently misdiagnosed with PC, thus leading to invasive prostate biopsies to differentiate between them without actual need (Hoffman, Gilliland, Adams-Cameron, Hunt, & Key, 2002). Thus, finding alternative noninvasive biomarkers became crucial, helping in early detection of PC, as well as differentiating between patients with malignant and benign prostatic conditions and reducing the need for invasive biopsies. Furthermore, these new biomarkers such as miRNAs may act as targets for future therapies of cancer prostate. miRNAs are a group of 18– to 25–nucleotide-long noncoding RNAs that control gene expression and post-transcriptional events (Nadler, Humphrey, Smith, Catalona, & Ratliff, 1995) that control various cellular functions, such as proliferation, differentiation and apoptosis (Kloosterman & Plasterk, 2006). Remarkably, they are dysregulated in many cancers playing important roles in tumour development due to their capacity to act as oncogenes and regulators of tumour suppressor genes (Kent & Mendell, 2006). Mitchell et al in 2008 have described, for the first time, tumour-derived miRNAs as biomarkers circulating in the peripheral blood; besides that, they suggested that these biomarkers

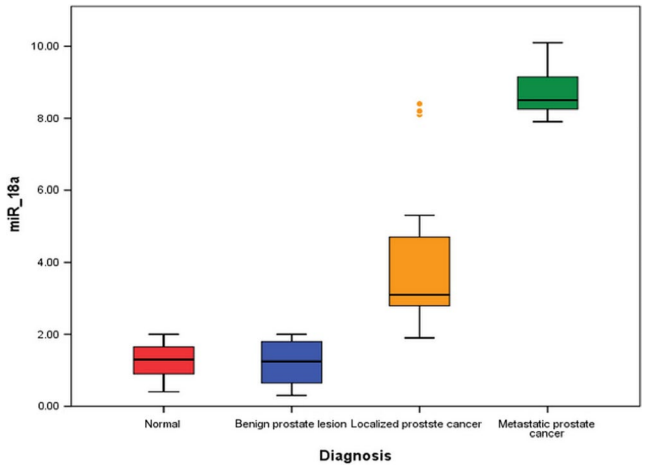

FIGURE 1 Expression of miR-18a in all studied groups

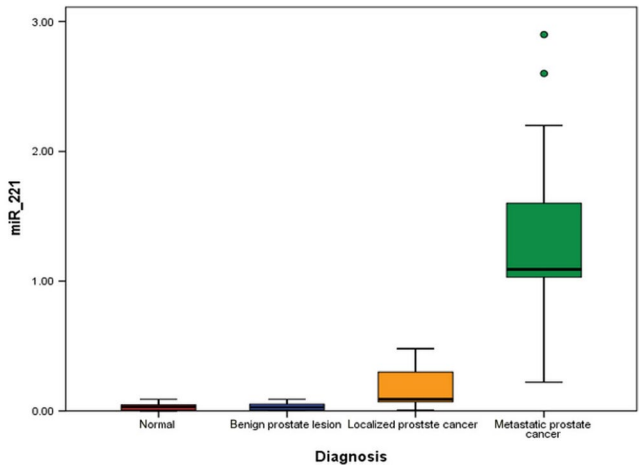

FIGURE 2 Expression of miR-221 in all studied groups

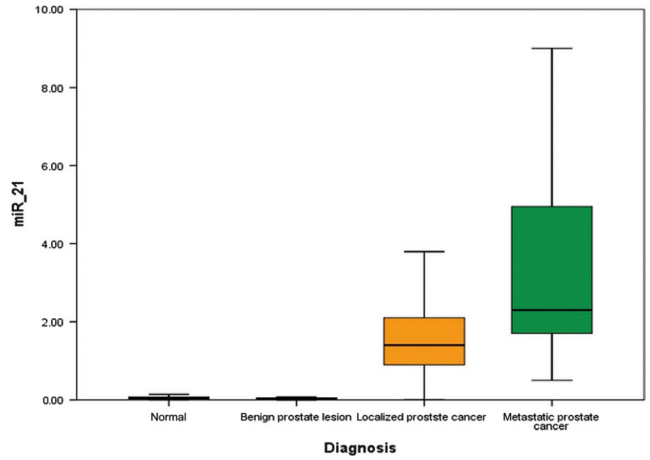

FIGURE 3 Expression of miR-21 in all studied groups

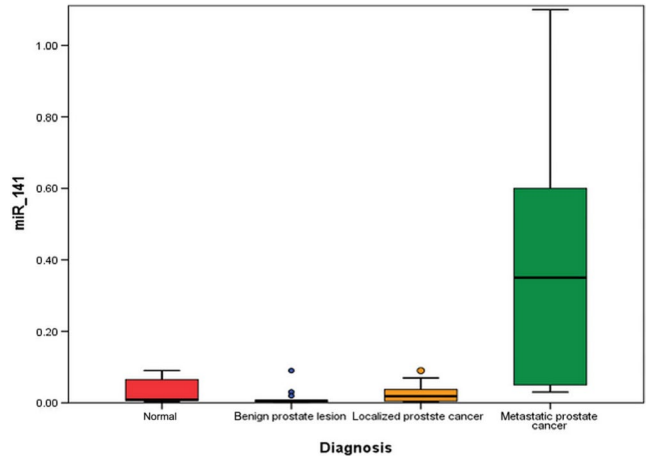

FIGURE 4 Expression of miR-141 in all studied groups

could be innovative noninvasive markers for screening patients with PC (Mitchell et al., 2008). Later on, researchers have found that tumour-derived miRNAs can be isolated from the circulation despite being generated from epithelial cancer cells. Besides, they described that miRNAs are stable as they could tolerate prolonged room temperature incubation and multiple cycles of freezing-thawing as they were included in lipid or lipoprotein complexes protecting them from endogenous RNase (Kosaka, Iguchi, & Ochiya, 2010). Therefore, circulating miRNAs have been found as ideal and noninvasive biomarkers for many cancer types (Chen et al., 2008; Keller et al., 2011; Liu et al., 2011; Meder et al., 2011; Medina-Villaamil et al., 2014; Roth et al., 2011; Schrauder et al., 2012; Wang et al., 2010; Yaman Agaoglu et al., 2011; Zheng et al., 2013).

The purpose of this study is to spot miRNAs which could help in early detection of LPC and follow-up of its progression. Hence, we evaluated the expression of four cancer-related miRNAs (miR-21, miR-141, miR-18a and miR-221) in plasma localised and metastatic subgroups of PC, in comparison with BPH and NC. A closer look at the data indicates that there was a statistically significant difference among the LPC and MPC groups compared to nonmalignant groups (NC and BPH) regarding plasma expression of miR-21, miR-221 and miR-18a ($p < .001$). On the contrary, miR-141 showed no statistically significant difference between the same subgroups with $p = .283$. However, it showed a statistically significant difference between LPC and MPC ($p < .001$); thus, it could be a useful prognostic rather than diagnostic biomarker. On the other hand, our results were in line with the previous studies as miR-21 has been found to be one of those oncogenic miRNAs (Cannistraci, Pace, Maria, & Bonci, 2014), whereas overexpressed miR-21 may stimulate tumour angiogenesis by affecting PTEN and triggering AKT and ERK1/2 intracellular signalling pathways, thus enhancing the expression of HIF-1a and VEGF. Notably, HIF-1a is an essential downstream target for miR-21 in the process of tumour angiogenesis (Liu et al., 2011); besides that, overexpression of miR-221 has been detected in different types of tumours and in primary PC cell lines (Galardi et al., 2007; Mercatelli et al., 2008). Our finding that miR-141 showed no statistically significant difference between the LPC and nonmalignant groups with $p = .283$, and that was not going with previous reports which suggested that miR-141 could differentiate between PC patients and healthy controls (Mitchell et al., 2008). However, it is in line with two other studies that have sample size twice that of the previous study (Nguyen et al., 2012; Yaman Agaoglu et al., 2011).

Regarding miR-18a, several studies have suggested that it could have a role as an oncogenic miRNA as it was highly overexpressed in PC tissues. In addition, it was found that it was overexpressed and detected in the peripheral blood in higher levels among PC patients compared with BPH patients as well as healthy controls; besides that, its overexpression could correlate with PC progression (Al-Kafaji, Al-Naieb, & Bakhiet, 2015). Therefore, evaluation of plasma levels of overexpressed miR-18a could be a noninvasive biomarker for PC screening as well as differentiating between PC and BPH consolidating our results in the current study.

There is a rapidly growing premise that miRNAs could differentiate localised prostate cancer from nonmalignant cases, and when we compared them, we found miR-18a had the highest AUC (0.996; 95% CI, 0.987–1.000), while the other miR-221 showed the lowest AUC

TABLE 3 ROC curve analysis for different miRNAs in localised prostate cancer and metastatic cases

Test variables	AUC	Cut-off value	Sensitivity (%)	Specificity (%)
miR_21	0.761	1.80	71.4	68.2
miR_141	0.925	0.047	92.9	86.4
miR_221	0.982	0.44	92.9	100
miR_18a	0.966	8.05	100	86.4

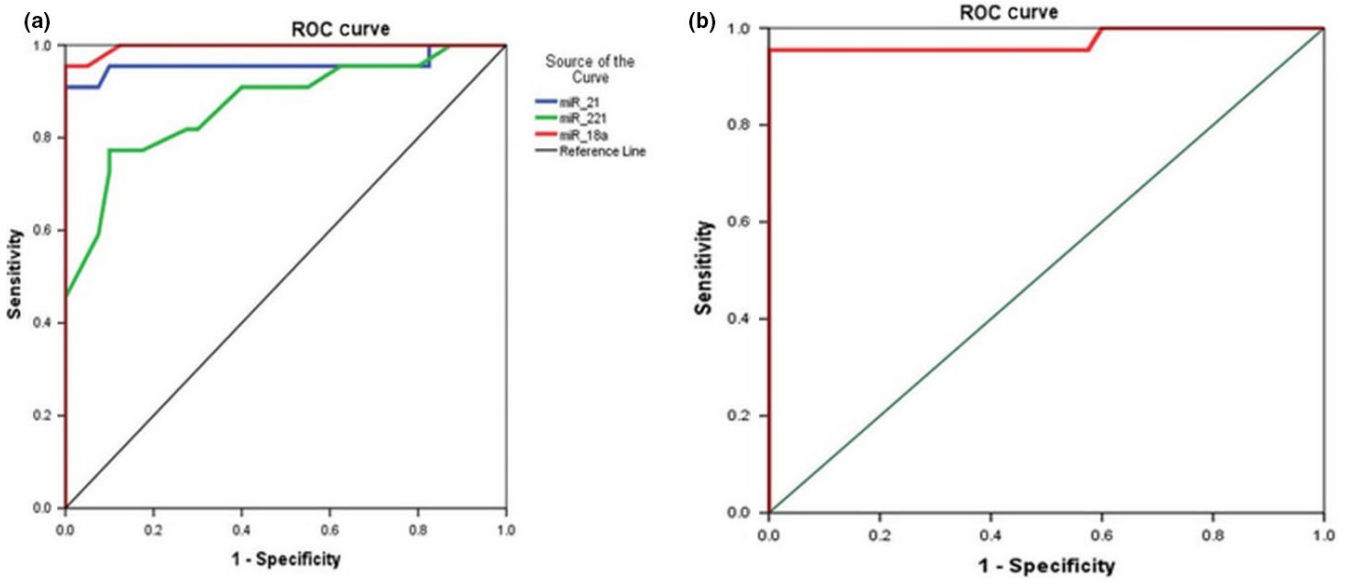

FIGURE 5 (a) ROC curve for localised prostate cancer and nonmalignant cases; (b) combined miR-21 and miR-18a for localised prostate cancer and nonmalignant cases

(0.872; 95% CI, 0.772–0.97). miR-21 showed AUC of 0.959 (95% CI, 0.886–1.000), and when we added miR-21 to miR-18a for detecting patients with LPC, it did not affect its diagnostic power as it achieved the same sensitivity (95.5%) at a specificity of 100% (AUC, 0.973; 95% CI 0.921–1.0; $p < .0001$).

Our study showed that low-risk LPC patients and MPC cancer patients have different circulating miRNA patterns and suggested that evaluation of plasma miRNA levels could be a potential predictor of PC as well as assessing the tumour aggressiveness. Notably, miR-21, miR-141, miR-221 and miR-18a were significantly overexpressed in patients with MPC in comparison with low-score LPC patients. The sensitivity of miR-21, miR-141, miR-221 and miR-18a was 32.1%, 53.6%, 92.9% and 60.7% respectively at 100% specificity, and when we combine miR-18a and miR-221 for differentiating patients with MPC, it will increase the sensitivity to 96.4% at a specificity of 100% (AUC, 0.997; 95% CI, 0.988–1.0; $p < .000$). These findings are accepted and explained by the significant association between all tested miRNAs (miR-21, miR-221, miR-141 and miR-18a) and clinicopathological variables of PC such as increased tPSA, fPSA, Gleason score, and pathological staging of the patients, lymph node metastasis and distant metastasis. However, only miR-221, miR-141 and miR-18a were significantly associated with decreased f/tPSA, and this needs further validation to understand the different relationships between varied miRNAs and f/tPSA ratio. Only miR-22 and miR-18a were significantly associated with DRE ($p = .011$ and .030 respectively). Meanwhile, PR volume was significantly associated with miR-221, miR-18a and miR-21 ($p = .001$, .005 and .017 respectively).

On the contrary, our research is in contradiction to the most recent meta-analysis that was conducted by Greco et al. (2019) which demonstrated statistically significant difference in plasma expression of miR-221 between the NC and BPH groups. Additionally, our study showed no statistically significant difference among both subgroups in plasma expression of miR-21, miR-141, miR-221 and miR-18a with *p*-values of .186, .083, .704 and .684 respectively. Furthermore, the diversity among the results of various reports could be relayed to the use of different methodologies or use of either serum or plasma for evaluation because serum contains higher amounts of nucleic acids compared to plasma (Umetani, Hiramatsu, & Hoon, 2006). Finally, we recommend the conduction of the current trial in a larger cohort of populations at a multicentric level to evaluate the potential diagnostic and prognostic role of circulating miR-18a, miR-21, miR-221 and miR-141 expressions as noninvasive biomarkers for PC.

5 | CONCLUSION

The current results demonstrated that expressions of circulating miR-18a, miR-21 and miR-221 are increased in the peripheral blood

TABLE 4 ROC curve analysis for different miRNAs in localised prostate cancer and nonmalignant cases

Test variables	AUC	Cut-off value	Sensitivity (%)	Specificity (%)
miR_21	0.959	0.085	95.5	90
miR_221	0.872	0.048	81.8	72.5
miR_18a	0.996	2.2	95.5	100

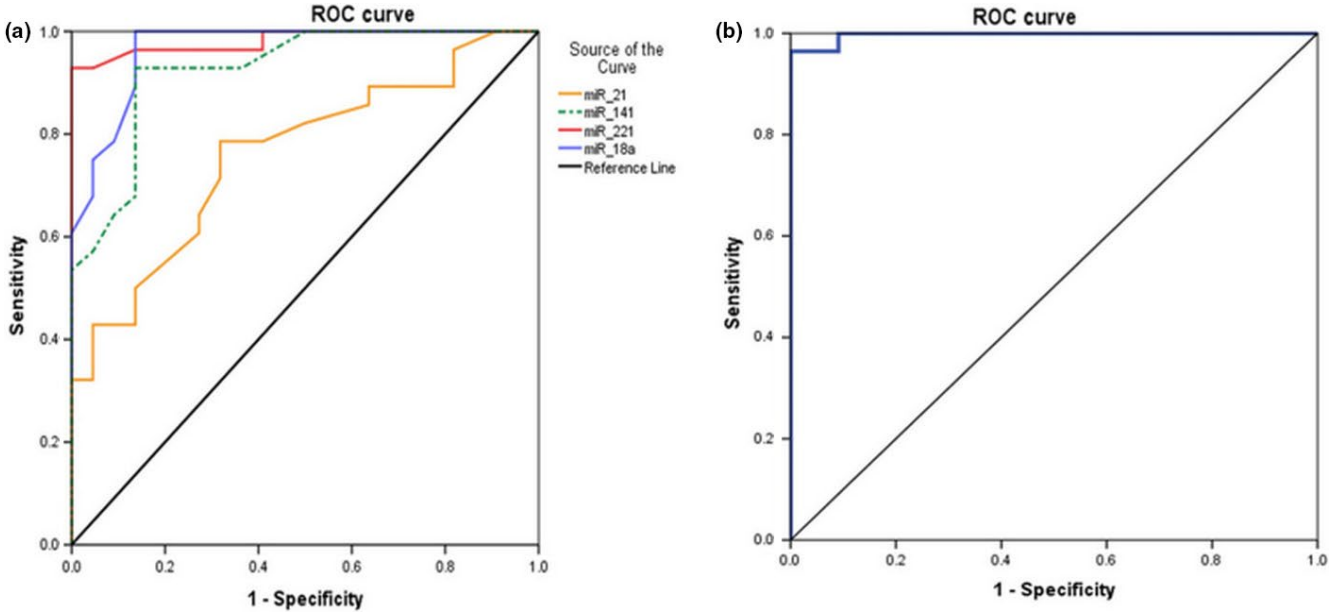

FIGURE 6 (a) ROC curve for localised prostate cancer and metastatic prostate cancer; (b) combined miR-122 and miR-18a for localised prostate cancer and metastatic cases

TABLE 5 Multivariate analysis for diagnosis of localised prostate cancer

Test variables	OR	*SE*	95% confidence interval		*p*-Value
miR-21	0.018	0.971	0.003	0.118	.001
miR-221	0.074	0.756	0.017	0.326	.513
miR-18a	0.003	1.449	0.000	0.043	<.001
fPSA	0.056	0.786	0.012	0.259	.010
f/tPSA	90	1.051	11.462	706.711	<.001

of patients with PC compared with BPH patients and healthy controls and that higher miR-21, miR-141, miR-221 and miR-18a expressions are correlated with PC progression. Thus, these circulating oncogenic miRNAs may be recommended as innovative noninvasive biomarkers for PC that can discriminate between PC and BPH.

CONFLICT OF INTERESTS

All authors have declared no conflict of interests.

ORCID

Noha H. Ibrahim https://orcid.org/0000-0001-6221-1477
Mona S. Abdellateif https://orcid.org/0000-0002-5510-4435
Samar Hassab-Allah Kassem https://orcid.org/0000-0003-3095-3278
Mohamed Ahmed Abd El Salam https://orcid.org/0000-0002-7694-3852
Mosaad M. El Gammal https://orcid.org/0000-0002-4210-839X

REFERENCES

Al-Kafaji, G., Al-Naieb, Z., & Bakhiet, M. (2015). Increased oncogenic microRNA-18a expression in the peripheral blood of patients with prostate cancer: A potential novel non-invasive biomarker. *Oncology Letters*, *11*(2), 1201–1206. https://doi.org/10.3892/ol.2015.4014

Attard, G., Parker, C., Eeles, R. A., Schröder, F., Tomlins, S. A., Tannock, I., … de Bono, J. S. (2016). Prostate cancer. *The Lancet*, *387*(10013), 70–82. https://doi.org/10.1016/S0140-6736(14)61947-4

Bartel, D. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, *116*(2), 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Calin, G., & Croce, C. (2006). MicroRNA signatures in human cancers. *Nature Reviews Cancer*, *6*(11), 857–866. https://doi.org/10.1038/nrc1997

Cannistraci, A., Di Pace, A., De Maria, R., & Bonci, D. (2014). MicroRNA as new tools for prostate cancer risk assessment and therapeutic intervention: Results from clinical data set and patients' samples. *BioMed Research International*, *2014*, 1–17. https://doi.org/10.1155/2014/146170

Chen, X. I., Ba, Y. I., Ma, L., Cai, X., Yin, Y., Wang, K., … Zhang, C.-Y. (2008). Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Research*, *18*(10), 997–1006. https://doi.org/10.1038/cr.2008.282

Daniyal, M., Siddiqui, Z., Akram, M., Asif, H., Sultana, S., & Khan, A. (2014). Epidemiology, etiology, diagnosis and treatment of prostate cancer. *Asian Pacific Journal of Cancer Prevention*, *15*(22), 9575–9578. https://doi.org/10.7314/APJCP.2014.15.22.9575

Galardi, S., Mercatelli, N., Giorda, E., Massalini, S., Frajese, G., Ciafrè, S., & Farace, M. G. (2007). miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. *Journal of Biological Chemistry*, *282*(32), 23716–23724.

Greco, F., Inferrera, A., La Rocca, R., Navarra, M., Casciaro, M., Grosso, G., … Mirone, V. (2019). The potential role of microRNAs as biomarkers in benign prostatic hyperplasia: A systematic review and meta-analysis. *European Urology Focus*, *5*(3), 497–507.

Hoffman, R., Gilliland, F., Adams-Cameron, M., Hunt, W., & Key, C. (2002). Prostate-specific antigen testing accuracy in

community practice. *BMC Family Practice*, *24*(3), 19. https://doi.org/10.1186/1471-2296-3-19

Hsing, A., Reichardt, J., & Stanczyk, F. (2002). Hormones and prostate cancer: Current perspectives and future directions. *The Prostate*, *52*(3), 213–235. https://doi.org/10.1002/pros.10108

Keller, A., Leidinger, P., Bauer, A., ElSharawy, A., Haas, J., Backes, C., … Meese, E. (2011). Toward the blood-borne miRNome of human diseases. *Nature Methods*, *8*(10), 841–843. https://doi.org/10.1038/nmeth.1682

Kent, O., & Mendell, J. (2006). A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene*, *25*(46), 6188–6196. https://doi.org/10.1038/sj.onc.1209913

Kim, W., & Kim, W. (2013). MicroRNAs in prostate cancer. *Prostate International*, *1*(1), 3–9. https://doi.org/10.12954/PI.12011

Kloosterman, W., & Plasterk, R. (2006). The diverse functions of microRNAs in animal development and disease. *Developmental Cell*, *11*(4), 441–450. https://doi.org/10.1016/j.devcel.2006.09.009

Kosaka, N., Iguchi, H., & Ochiya, T. (2010). Circulating microRNA in body fluid: A new potential biomarker for cancer diagnosis and prognosis. *Cancer Science*, *101*(10), 2087–2092. https://doi.org/10.1111/j.1349-7006.2010.01650.x

Liu, L. Z., Li, C., Chen, Q., Jing, Y., Carpenter, R., Jiang, Y., … Jiang, B. H. (2011). MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1alpha expression. *PLoS ONE*, *6*(4), e19139.

Livak, K., & Schmittgen, T. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Meder, B., Keller, A., Vogel, B., Haas, J., Sedaghat-Hamedani, F., Kayvanpour, E., … Rottbauer, W. (2011). MicroRNA signatures in total peripheral blood as novel biomarkers for acute myocardial infarction. *Basic Research in Cardiology*, *106*(1), 13–23. https://doi.org/10.1007/s00395-010-0123-2

Medina-Villaamil, V., Martínez-Breijo, S., Portela-Pereira, P., Quindós-Varela, M., Santamarina-Caínzos, I., Antón-Aparicio, L. M., & Gómez-Veiga, F. (2014). Circulating MicroRNAs in blood of patients with prostate cancer. *Actas Urológicas Españolas (English Edition)*, *38*(10), 633–639. https://doi.org/10.1016/j.acuroe.2014.10.002

Mercatelli, N., Coppola, V., Bonci, D., Miele, F., Costantini, A., Guadagnoli, M., … Ciafrè, S. A. (2008). The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. *PLoS ONE*, *3*, e4029.

Mettlin, C., Lee, F., Drago, J., & Murphy, G. (1991). The American cancer society national prostate cancer detection project. Findings on the detection of early prostate cancer in 2425 men. *Cancer*, *67*(12), 2949–2958.

Mitchell, P., Parkin, R., Kroh, E., Fritz, B., Wyman, S., Pogosova-Agadjanyan, E., … Tewari, M. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(30), 10513–10518.

Nadler, R., Humphrey, P., Smith, D., Catalona, W., & Ratliff, T. (1995). Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *The Journal of Urology*, *154*(2 Pt 1), 407–413.

Nguyen, H., Xie, W., Yang, M., Hsieh, C., Drouin, S., Lee, G., & Kantoff, P. W. (2012). Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. *The Prostate*, *73*(4), 346–354.

Pron, G. (2015). Prostate-Specific Antigen (PSA)–based population screening for prostate cancer: An evidence-based analysis. *Ontario Health Technology Assessment Series*, *15*(10), 1–64.

Roth, P., Wischhusen, J., Happold, C., Chandran, P., Hofer, S., Eisele, G., … Keller, A. (2011). A specific miRNA signature in the peripheral blood of glioblastoma patients. *Journal of Neurochemistry*, *118*(3), 449–457.

Schmittgen, T., & Livak, K. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, *3*(6), 1101–1108.

Schrauder, M., Strick, R., Schulz-Wendtland, R., Strissel, P., Kahmann, L., Loehberg, C., … Fasching, P. A. (2012). Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection. *PLoS ONE*, *7*(1), e29770.

Sobin, L. H. & Wittekind, C. H. (Eds.) (2002). *TNM classification of malignant tumours* (6th ed., pp 184–187). New York, NY: Wiley-Liss.

Umetani, N., Hiramatsu, S., & Hoon, D. (2006). Higher amount of free circulating DNA in serum than in plasma is not mainly caused by contaminated extraneous DNA during separation. *Annals of the New York Academy of Sciences*, *1075*(1), 299–307. https://doi.org/10.1196/annals.1368.040

Wang, G.-K., Zhu, J.-Q., Zhang, J.-T., Li, Q., Li, Y., He, J., … Jing, Q. (2010). Circulating microRNA: A novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *European Heart Journal*, *31*(6), 659–666. https://doi.org/10.1093/eurheartj/ehq013

Yaman Agaoglu, F., Kovancilar, M., Dizdar, Y., Darendeliler, E., Holdenrieder, S., Dalay, N., & Gezer, U. (2011). Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. *Tumor Biology*, *32*(3), 583–588. https://doi.org/10.1007/s13277-011-0154-9

Zheng, H., Zhang, L., Zhao, Y., Yang, D. A., Song, F., Wen, Y., … Chen, K. (2013). Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. *PLoS ONE*, *8*(11), e77853. https://doi.org/10.1371/journal.pone.0077853

How to cite this article: Ibrahim NH, Abdellateif MS, Kassem SH-A, Abd El Salam MA, El Gammal MM. Diagnostic significance of miR-21, miR-141, miR-18a and miR-221 as novel biomarkers in prostate cancer among Egyptian patients. *Andrologia*. 2019;51:e13384. https://doi.org/10.1111/and.13384