African Journal of Urology (2018) **24**, 347–352

African Journal of Urology

Official journal of the Pan African Urological Surgeon's Association
web page of the journal

www.ees.elsevier.com/afju
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BPH and Prostate Diseases
Original article

microRNA-141 as a diagnostic and prognostic biomarker for prostate cancer in Egyptian population: Pilot study

R. Ali [a], **S. El Tabbakh** [a], **W. El Delgawy** [a], **A. Kotb** [b,*], **M.N. Desouky** [a,*]

[a] *Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Egypt*
[b] *Urology Department, Faculty of Medicine, Alexandria University, Egypt*

Received 26 April 2018; received in revised form 12 July 2018; accepted 29 November 2018; Available online 12 December 2018

KEYWORDS
Prostate cancer;
microRNA;
Prostatic specific antigen

Abstract
Introduction: microRNAs are a family of small non protein-coding RNAs. They are involved in post-transcriptional gene regulation of their target genes. The deregulation of microRNAs has been linked to cancer development and tumor progression. The aim of out study was to look for microRNA-141 as a diagnostic and prognostic biomarker for prostate cancer.
Patients and methods: The study prospectively recruited 30 patients newly diagnosed with prostate cancer; including 13 and 17 patients without and with metastases, respectively. Another 30 patients without prostate cancer diagnosis were included as a control group. Real-time polymerase chain reaction analysis was done for relative quantification of microRNA-141.
Results: The present study showed that microRNA-141 was significantly upregulated in cancerous patients compared to control group. Also it was significantly upregulated in patients with metastatic disease compared to non-metastatic patients. Moreover, it was significantly correlating with serum PSA and Gleason score.
Conclusion: Serum microRNA-141 could be a promising diagnostic and prognostic biomarker for prostate cancer and a good indicative of disease aggressiveness.

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Introduction

Prostate cancer (PCa) is the second most common cancer in men after lung cancer, with nearly 1 million new cases being diagnosed every year and accounts for ~10% of all new cancer cases in men worldwide [1]. Measurements of serum PSA and digital rectal exam-

* Corresponding authors.
E-mail addresses: drahmedfali@gmail.com (A. Kotb), mhmdnd@gmail.com (M.N. Desouky).

Peer review under responsibility of Pan African Urological Surgeons' Association.

https://doi.org/10.1016/j.afju.2018.11.006
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Table 1 Comparison between the prostate cancer patients (PCa) and controls according to different parameters.

	No. (%)	Range	Mean ± SD	Median	IQR	p
Age (years)						
PCa (n = 30)		55–80	67.2 ± 6	67	62.8–70.3	0.389
Controls (n = 30)		55–87	65.4 ± 9.2	63.5	57.8–57.8	
Prostate volume (grams)						
PCa (n = 30)		47–200	88.7 ± 30.1	89	67.8–102	<0.001
Controls (n = 30)		30–112	55.4 ± 23.1	47.5	34.8–74.3	
PSA (ng/ml)						
PCa (n = 30)		4.8–105	37.1 ± 32.4	27	10.2–59.5	<0.001
Controls (n = 30)		0.4–5	2.4 ± 1.2	2.5	1.2–3.3	
miRNA-141						
PCa (n = 30)		1.1–139.1	29.4 ± 43.7	7.2	2.4–67.7	<0.001
Controls (n = 30)		0.2–7	1.5 ± 1.7	1.3	0.6–1.6	
Metastasis						
PCa (n = 30)						
No	13 (43.3%)					–
Yes	17 (56.7%)					
Gleason score						
PCa (n = 30)		6–9				
Low risk (6)	15 (50%)					–
Intermediate risk (7)	6 (20%)					
High risk (≥8)	9 (30%)					

Table 2 Spearman correlation between microRNA-141 and other variables.

	miRNA 141	
	r_s	p
Age (years)	0.070	0.713
Prostate volume (grams)	0.181	0.338
PSA (ng/ml)	0.436*	0.016*
Grading (Gleason score)	0.557*	0.001*

rs: Spearman coefficient.
* Statistically significant at $p \leq 0.05$.

ination (DRE) are still the main triggers for prostatic biopsy [2]. On the other hand; PSA is organ but not cancer specific, and could be elevated by many benign conditions [3]. Furthermore, PCa is detectable in approximately 15% of men with normal PSA values [4], and being subject to genetic variation explaining its low sensitivity and specificity [5].

microRNAs are a family of small non protein-coding RNAs of approximately 22 nucleotides in length. They are involved in post-transcriptional gene regulation of their target genes either by directing mRNA degradation or by inducing translational repression through binding to complementary sequences in the 3′-untranslated region of mRNAs [6].

Each mature microRNA potentially controls many gene targets and each mRNA is regulated by multiple microRNAs. microRNAs are involved in regulation of various biological functions such as cell proliferation, differentiation, apoptosis and metabolism. Previous studies suggested a direct link between microRNAs and cancer, as microRNAs can act as oncogenes or tumor-suppressor genes, depending on the target genes that they regulate [7–9]. Existing data show the potential clinical utility of microRNAs as diagnostic, prognostic and predictive markers for aggressive and metastatic cancers [10].

Importantly, microRNAs have also been detected in plasma, where they are remarkably stable and protected from endogenous RNAase enzymatic activity [11]. These findings raised the possibility that circulating microRNAs can be considered as potential novel markers for the diagnosis and prognosis of many diseases including cancer [12].

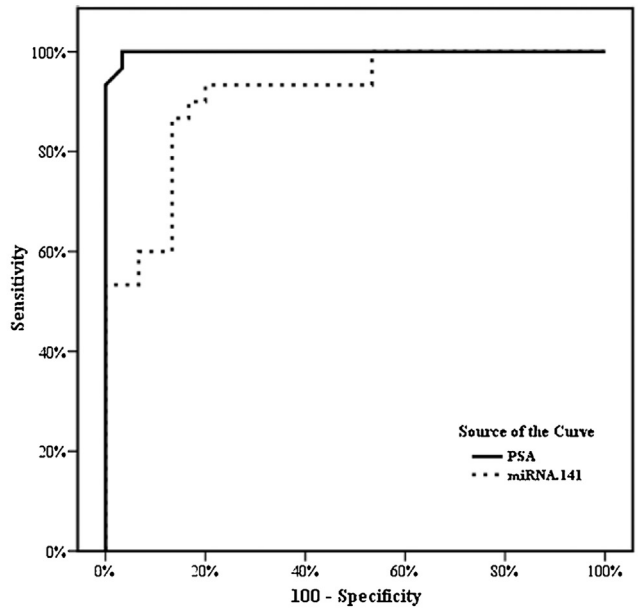
Analysis of microRNA signatures of a large number of tumor samples revealed that microRNA-141 is downregulated in pancreatic [13], esophageal [14], gastric [15] and colorectal cancers [16]. On the other hand; studies on PCa were sparse and with contradictory outcomes.

The objective of the present study was to investigate the role of microRNA-141 for the diagnosis and prognosis of prostate cancer in a group of our patients.

Patients and methods

Ethical committee approval was obtained through Alexandria University, Egypt before conducting the study, and all patients included in the study were notified about our study protocol and its possible future benefits. This study was carried out on 60 individuals: Thirty patients with prostate cancer, this group was further subdivided into; 13 PCa patients without metastasis and 17 PCa patients with metastasis. Thirty healthy individuals of matched age were included as a control group. All patients were recruited through the outpatient clinic of urology department, Alexandria University; Egypt.

All patients included in this study were subjected to detailed history taking, DRE, PSA measurement and Prostate volume measurement using ultrasound abdomen and pelvis. Total serum PSA level was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) technique. The Serum ELISA kit (DRG Instruments GmbH, Germany) was used. Cancerous patients had TRUS guided prostatic biopsies and pelvic MRI. More radiological investigations were done to high risk patients to look for metastases.



	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			L.L	U.L					
PSA(ng/ml)	0.998*	<0.001*	0.994	1.003	>4.2	100.0	96.67	96.8	100.0
miRNA-141	0.912*	<0.001*	0.841	0.984	>1.57	93.33	80.0	82.4	92.3

Figure 1 ROC curve for serum PSA(ng/ml) and miRNA-141 to predict PCa group from control.

Relative quantification of serum miRNA 141 expression levels

Total RNA isolation from serum samples was performed using Qiagen® miRNeasy Mini Kit.

Complementary DNA (cDNA) was synthesized using TaqMan® MiRNA Reverse Transcription Kit with miRNA specific primers (Applied Biosystems, USA).

Real-time PCR was performed using Applied Biosystems StepOne™ Real-Time PCR System using TaqMan® MIRNA141, TaqMan® 2× Universal PCR Master Mix, and Syn-cel-miR-39 miScript miRNA Mimic as a spike in control. Reagents were purchased from Applied Biosystems, USA.

Expression of microRNA-141 was calculated using the comparative cycle threshold (CT) method (2–ΔΔCT) [17].

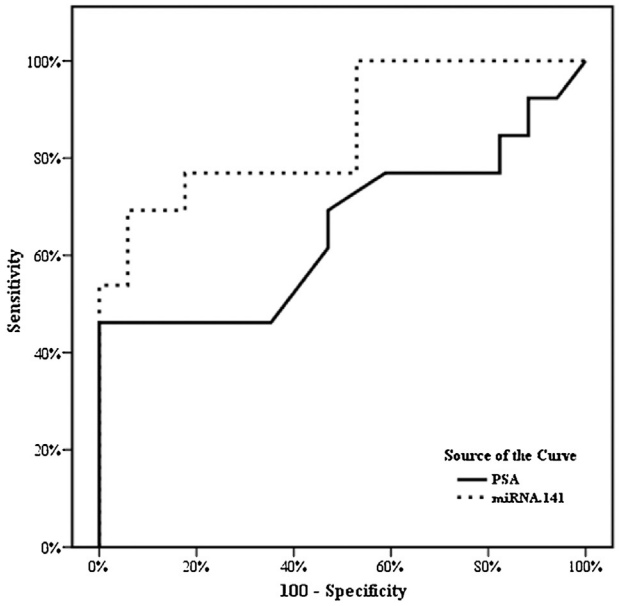
Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Student t-test was used to compare two groups for normally distributed quantitative variables, while ANOVA was used for comparing the three studied groups and followed by Post Hoc test for pairwise comparison. Spearman coefficient was used to correlate between quantitative variables. The receiver operating characteristic (ROC) curve analysis was performed to evaluate performance using the area under the curve and to determine the best cutoff value. The diagnostic sensitivity, specificity and predictive values were calculated by 2×2 tables Significance of the obtained results was judged at the 5% level.

Results

The mean age of cancerous and non-cancerous patients was 67.17 ± 5.99 years and 65.43 ± 9.15 years respectively (p 0.6). The mean serum PSA in non-metastatic PCa, metastatic PCa and control group were 32.1 ± 34.8, 41 ± 31 and 2.4 ± 1.2 ng/ ml respectively ($p < 0.001$), with no statistical difference between the two cancerous groups. The mean prostate volume was significantly lower in control group (55.4 ± 23.1) compared to non-metastatic PCa (79.5 ± 19.8) and metastatic PCa (95.7 ± 35) ($p < 0.001$). All non-metastatic PCa patients had Gleason 6, while metastatic patients had Gleason 6, 7 and >7 in 2, 6 and 9 patients respectively. Table 1 illustrates patients' demographics.

The mean serum microRNA-141 for non-metastatic, metastatic Pca and control groups were 3.9 ± 3.8, 49 ± 50.2 and 1.5 ± 1.7 respectively, with statistical significant difference between the three studied groups ($p < 0.001$). Using Spearman correlation coefficients; there was a significant positive correlation between serum microRNA-141 expression level and each of serum PSA level and Gleason score (Table 2). Serum microRNA-141 expression showed a sensitivity and specificity of 93.33% and 80% at a cut-off value of >1.57 for the diagnosis of PCa (Fig. 1). It also had a sensitivity



	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			L.L	U.L					
PSA(ng/ml)	0.654	0.155	0.438	0.869	≤9.5	46.15	100.0	100.0	70.8
miRNA-141	0.855*	0.001*	0.715	0.995	≤2.79	69.23	94.12	90.0	80.0

Figure 2 ROC curve for serum PSA(ng/ml) and miRNA-141 to predict metastatic from non-metastatic PCa.

and specificity of 69.23% and 94.12% at a cut-off value of 2.79 for predicting metastatic PCa (Fig. 2).

Discussion

Existing data show the potential clinical utility of microRNAs as diagnostic, prognostic and predictive markers for aggressive and metastatic cancers [10]. Alterations in microRNA expression can affect important cellular processes like cell cycle, proliferation or apoptosis, thus providing a direct link to cancer development and progression [18] . Selth et al. proposed that these microRNAs were expressed in prostate cancer cells and were released into the surrounding blood vessels during disease progression [19].

The present study demonstrated that serum microRNA-141 levels were significantly elevated in patients with PCa, compared to controls. ROC analyses for the diagnostic power of serum microRNA-141 yielded an AUC of 0.912 with 93.3% sensitivity and 80% specificity in differentiating patients with PCa from control group. This goes in agreement with Mitchell et al., where they reported that circulating microRNA-141 can distinguish patients with PCa from healthy individuals with sensitivity and specificity of 60% and 100% respectively [11].

In the current study, we also investigated whether microRNA-141 could be used to differentiate non metastatic from metastatic PCa. Our analysis reported overexpression of serum microRNA-141 among PCa with metastasis in comparison to PCa patients without metastases. ROC analyses for the prognostic power of serum miRNA-141 yielded an AUC of 0.855 with 69% sensitivity and 94% specificity in differentiating patients with metastatic PCa from non-metastatic group. This goes in agreement with Brase et al. that demonstrated microRNA-141 to be considerably higher in serum samples from patients with metastasized tumor when compared to those with localized tumour [13]. The surge of microRNA-141 in metastatic cancer could be explained by its role in influencing mesenchymal to epithelial transition (MET), which is a key process in metastatic colonization at distant sites. microRNA-141 allows MET through direct targeting of Zeb1 (zinc finger E-box binding homeobox 1) which is a transcriptional activator of the protein adhesion molecule E-Cadherin. Expression of E-Cadherin reinforces epithelial traits of the cell. Overexpression of microRNA-141 in disseminated tumor cells allows cells to regain epithelial traits, thus enhancing metastatic colonization in secondary organs [20,21] . Zhang et al. found that serum microRNA-141 levels were elevated in patients with bone-metastatic PCa and that patients with higher levels of serum microRNA-141 developed more bone lesions. Furthermore, serum microRNA-141 levels were correlated with serum alkaline phosphatase but not serum PSA [22]. Liu et al. recently published that microRNA-141 is downregulated in metastatic PCa patients [23].

In another cohort of 21 prostate cancer patients, Gonzales et al. reported that changes in microRNA-141 levels are correlated with

variations of other biomarkers of prostate cancer disease, such as prostate specific antigen (PSA), circulating tumor cells (CTC) and lactate dehydrogenase (LDH) [24]. In our study; microRNA-141 was significantly correlating with serum PSA and Gleason score. In contrast to our study, Kachakova et al. did not observe correlation of microRNA-141 with the clinic-pathological characteristics. Also, they showed downregulation of microRNA-141 in a high percent of PCa patients in comparison with BPH controls [25]. Also, Baffa et al. compared 43 primary tumors (colon, bladder, breast and lung) to their matched lymph node metastases showed that microRNA-141 was down-regulated in metastatic cancers [26].

Our study suggests that microRNA-141 may have an important diagnostic role for patients with mildly elevated PSA refusing prostatic biopsies or as a non-invasive tool for patients with high PSA and negative prostatic biopsy to help for future management plans. Its value was much higher than PSA in predicting the presence of metastatic disease which is essential in patients' management and to trigger further radiological studies.

Our study, to our knowledge, is the first study to look for the possible role of microRNA-141 on prostate cancer on Arabic and African populations, however; its weakness represented in the relatively small number of patients. Large scale prospective clinical studies are required to assess prognostic relevance of microRNA-141 to disease outcome and treatment response. The capacity of these microRNA-141 to predict prostate cancer progression warrants further validation also the detection of microRNA-141 levels in serial samples after surgery or treatment are required.

Conclusion

microRNA-141 is a promising non-invasive tool which can help for diagnostic dilemma of prostate cancer in some patients and higher values are significantly correlating with the presence of metastatic disease.

Conflict of interests

All authors have no conflict of interest to declare.

Ethical committee approval

Alexandria University; Egypt.

Authors' contributions

Rasha Ali: The main investigator, meeting patients and collecting data.

Soad El Tabakh: Supervisor, research planning and review.

Waheed El Delgawy: Supervisor, research planning and review.

Ahmed Kotb: Supervisor, research planning and review, help with recruiting patients from urology clinic and manuscript preparation.

Mohamed Desouky: Main corresponding author, research planning and review.

Consent from the patient

Consent was obtained from the patients.

Source of funding

None.

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