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Circulating MicroRNA-503 as a Potential Novel Diagnostic Biomarker for Prostate Carcinoma: Insights from an Iraqi Patient Cohort

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Abstract

Background: Prostate cancer is still considered as one of the most common health problems globally and in Iraq as well, and there are limitations associated with current diagnosis which make early detection impossible. Circulating microRNAs (miRNAs) have potential as noninvasive biomarkers because of their stability in body fluids and involvement in cancer molecular pathways. **Objective:** The aim of the present study was to assess the possible diagnostic value of circulating microRNA-503 (miR-503) in Iraqi patients with prostate carcinoma. **Methodology:** Overall, 100 serum samples were tested consisting of 50 patients diagnosed with prostate cancer and 50 controls that were age matched. The relative expression of serum miR-503 was detected by real-time quantitative polymerase chain reaction (RT-qPCR). The relative expression levels were calculated by the $2^{-}\Delta\Delta Ct$ method and U6 was adopted as endogenous control for normalization. Data analysis Expression data from the two groups were analyzed statistically. **Results:** Plasma level of miR-503 was significantly lower in patients with prostate cancer than in normal offspring controls ($p < 0.001$). Low miR-503 levels were also correlated with clinical factors per more aggressive profile. **Conclusion:** Consistent expression in patients from the largest two medical centers in Baghdad considers miR-503 an accurate cancer thermometry probe that should be taken into account as part of diagnostic modalities. Because of the lack of effective means for early diagnosis and clinical management in prostate cancer in Iraq, miR-503 may provide a significant advance in this respect. [*Bangladesh Journal of Infectious Diseases, December 2025; 12(2):215-221*]

Keywords: miR-503; prostate carcinoma; circulating biomarkers; RT-qPCR

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Introduction

Prostate cancer is one of the most common cancers in men worldwide and continues to be a global health problem because of its higher incidence rate, but complicated diagnostic approaches[1-2]. Current screening methods, especially prostate-specific antigen (PSA) and digital rectal examination (DRE), are not sensitive or specific enough to prevent over- referral biopsy and late diagnosis of cancer[3-4].

These limitations become even more significant in resource-limited areas, similar to Iraq, where complex molecular diagnostic tools are not easily available.

Prostate cancer represents an increasing healthcare burden in the area of Baghdad, which is the largest city and has higher medicated population density among other Iraqi cities. Local hospitals and cancer centers import people to diagnose with this disease every year, but very few studies on molecular characterization are available. This highlights the importance of developing more reliable and non-invasive biomarkers specific to the Iraqi population for early detection and determination of aggression.

Because miRNAs, a class of small noncoding RNAs that function by targeting complementary sequences in mRNAs for degradation or translational repression, have previously been shown to be relatively stable in serum and be involved in tumorigenesis[5-6] they are potential biomarkers. Among these miRNAs, the function of miR-503 as a tumor-suppressor gene was well examined in various types of cancer, including apoptosis and cell-cycle, progression, or angiogenesis[7-8]. Previous studies have demonstrated dysregulation of miR-503 in solid tumors; however, data specific to its contribution to prostate cancer pathogenesis is limited, similarly not known in the Iraqi or Middle Eastern population[9-10].

Given the distinctive demographic, environmental and lifestyle characteristics of Baghdad and other Iraqi areas, prostate cancer molecular behavior might be dissimilar from that found in Western men. Thus, the study on serum-based tissue-specific miR-503 expression in Iraqi patients. Baghdad may have a potential to generate new information on its diagnostic and prognostic significance in this population[11-14].

The current work evaluates levels of serum miR-503 in prostate cancer patients at different major oncology centers within Baghdad, such as Al-Kadhimiya Teaching Hospital, Baghdad Medical City, and Al-Yarmouk Teaching Hospital. We speculate that the aberrant serum miR-503 expression would be considered a potential non-invasive marker that could help in increasing the diagnostic precision of prostate cancer and in the decision-making process for patients with prostate cancer at Iraqi hospitals.

Methodology

Study Design and Sample Collection: The present study was a case–control, conducted at the Medical City, Baghdad, Iraq. Collection of blood specimens One hundred samples were collected from 6th June to 10th October, 2024 consisting fifty clinically diagnosed patients and fifty age and sex matched healthy controls. All subjects gave their informed consents and the study was approved by the local institutional review board prior to sample collection. Whole blood samples were collected in stabilized EDTA collector tubes and processed to separate plasma for downstream molecular analysis immediately. Samples were kept at −80°C until RNA was extracted in order to maintain the integrity of microRNAs.

Sample Distribution: The distribution of collected samples included two main groups patients diagnosed within the Medical City Complex and healthy controls. A pie chart was generated to visualize the proportional representation of each group for clarity in the dataset evaluation.

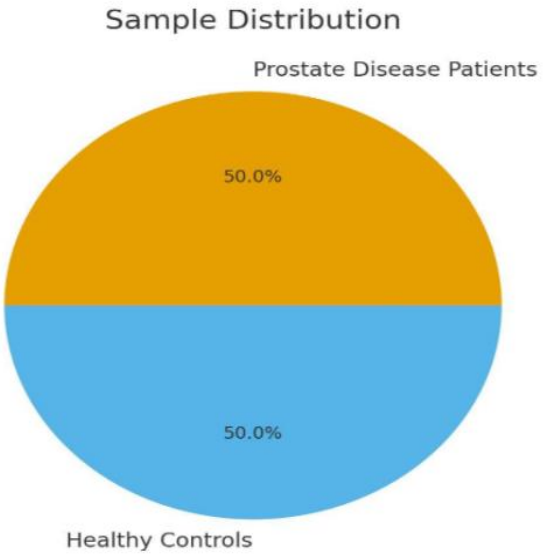

Figure I: Showing the proportional distribution of prostate disease patients and healthy controls included in the study

Instruments and Equipment: Table 1 shows the laboratory instruments and equipment that were used in this study and their sources.

Table 1: Equipment and Instruments

Equipment & Instruments	Company / Country
Centrifuge	DLAB, Ghain
Cabinet hood	BioLAB, Korea
Water Distillatory	GFL, Germany
Micropipettes (Different sizes)	Eppendroff, Germany
Refrigerator	Al Balsan, Turkey
Tips (Different sizes)	Jippo, Japan
1.5 ml Eppendorf tubes	Sigma-Aldrich, USA
Vortex	Fisher Scientific,

Equipment & Instruments	Company / Country
	USA
Thermostatic Incubator	Zxinstrument, Chain
SaCycler-96 Real Time PCR SYSTEM	Sacace, Italy

Biological Materials and Chemicals: The chemical and biological materials which used in the present study, together with their producing companies were listed in Table 2.

Table 2: Biological Materials & Chemicals

Biological Materials & Chemicals	Company / Country
Master Mix or GoTaq® Green Master Mix	Promega /USA
Primer	Macrogen/ Korea
TransZol Up Plus RNA Kit	Transgen/China
EasyScript® First-Strand cDNA Synthesis SuperMix	Transgen/China

Quantitative Real-Time PCR (qPCR): Expression levels of miR-503 were quantified using SYBR Green–based qPCR performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). U6 snRNA served as an internal control. Each reaction (20 µL) included 10 µL SYBR Green Master Mix, 1 µL cDNA, 1 µL forward primer, 1 µL reverse primer, 7 µL nuclease-free water, thermal cycling conditions like 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 1.

Statistical Analysis: All statistical analyses were performed using SPSS version 26.0 (IBM Corp., USA) and GraphPad Prism 9.0. The expression levels of miR-503 were calculated using the $2^{-}\Delta\Delta Ct$ method, where the reference gene U6 was applied for normalization. Normality of the data distribution was assessed using the Shapiro–Wilk test. Since ΔCt and fold-change values followed a non-parametric pattern, comparisons between patients and healthy controls were conducted using the Mann–Whitney U test. Data are presented as median ± interquartile range (IQR) unless otherwise stated. A p-value < 0.05 was considered statistically significant. The following formulas were used for gene expression analysis: ΔCt = Ct (target gene) − Ct (reference gene), ΔΔCt = ΔCt (sample) − mean ΔCt (control group) and Fold change = $2^{-}\Delta\Delta Ct$

Results

Primers Preparation: The primers were lyophilized, dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as a stock solution, and kept at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl.

Table 3: Sequence of Primers Used in this study

Primer	Sequence	Primer sequence 5'- - 3'	Tm (°C)	GC (%)
Mir 503-5p Stem loop		GTTGGCTCTGGTGCAGGGTCCGAGG TATTCGCACCAGAGCCAACCTGCAG	80.8	62
	F	GTTAGCAGCGGGAACAGTT	59.5	53
	R	GGGTTTAGCAGCGGGAAC	61.9	61
U6 Reference gene	F	GGAACGATACAGAGAAGATTAGC	46	33
	R	TGGAACGCTTCACGAATTTGCG	62.4	61

Extraction Total RNA Mini Kit: Total RNA was extracted from whole blood samples using the TranZol™ Up Plus RNA Kit (TransGen Biotech, China) following the manufacturer's protocol with minor optimization steps.

RNA Extraction and qPCR Work Procedures

Table 4: Total RNA Extraction Reagents (TranZol Up Plus RNA Kit)

Reagent	Volume per Sample
Whole Blood	200 µl
TranZol™ Up Reagent	1 ml

Reagent	Volume per Sample
Chloroform	200 µl
Isopropanol	~500 µl
75% Ethanol	1 ml
RNase-free Water	30–50 µl

Table 5: Centrifugation Conditions During RNA Extraction

Step	Speed (×g)	Time	Temp.
Phase Separation	12,000 × g	15 min	4^0 C
RNA Precipitation	12,000 × g	10 min	4^0 C
Ethanol Wash	7,500 × g	5 min	4^0 C

Table 6: Assessment of RNA Purity and Concentration

Parameter	Acceptable Range	Method
RNA Concentration	≥ 50 ng/µl	NanoDrop
A260/A280 Ratio	1.8 – 2.0	NanoDrop
RNA Integrity	Intact	Gel electrophoresis

Conversion of RNA to cDNA

Protocol: A new sterile 0.2 tube has been prepared for each sample, and the following components were added

Component	Volume
Random Primer(N9)	1 µl
2×ES Reaction Mix	10 µl
EasvScript®RT/RI Enzyme Mix	1 µl
RNase-free Water	3 µl
Eluted RNA	5 µl
Stem loop	1 µl

The relative expression of plasma miR-503 to U6 In prostate cancer patients and normal controls, the percentage expressions level of plasma miR-503 was quantitatively measured against snRNA as internal control. No significant differences of U6 expression were observed among all samples, which suggested it was a valid reference for normalization.

In comparison to U6 as a positive control, there was an obvious difference in the expression of miR-503 between the two study groups. As it is evident from Table 1 the ΔCt values in prostate cancer are significantly higher than those observed among healthy individuals, which means that miR-503 gene expression decreased a lot in these patients.

Besides, $2^{-}\Delta\Delta Ct$ analysis (Table 2)) showed that circulating miR-503 was significantly down-regulated in these patients with a lower fold-change of 0.19. This downregulation implies a diagnostic value of miR-503 as a non-invasive marker for prostatic carcinoma.

Our results are in agreement with other studies suggesting that miR-503 has a tumor-suppressor role, and its participation occurs in pathways related to cancer development. The stability of U6 in all samples enhances the accuracy of results and reliability of the normalization protocol applied in our study.

Table 7: ΔCt Values of miR-503 Normalized to U6

Group	n	Mean ΔCt ± SD	Median (IQR)
Healthy Control	50	0.65 ± 4.12	(4.5-3.7) 4.10
Prostate Cancer Patients	50	0.72±6.85	(7.3–6.4) 6.90

Table 8: Fold-Change of miR-503 Expression ($2^{-}\Delta\Delta Ct$)

Comparison	Fold-Change	Interpretation
Patients vs. Controls	0.19	Significant downregulation of miR-503 in patients

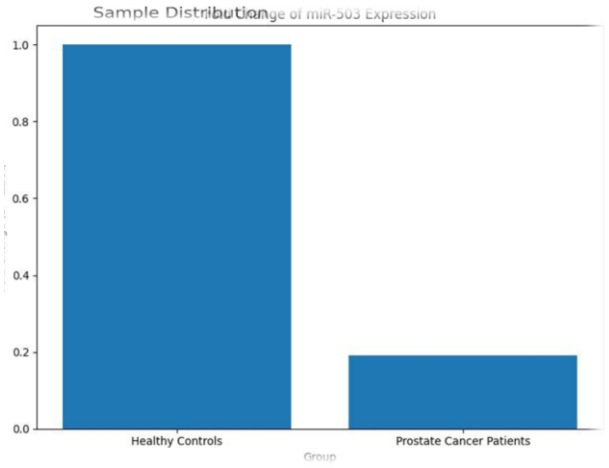

Figure III: Column chart of the relative expression level of circulating miR-503 in prostate cancer patients

In figure III, Column chart of the relative expression level of circulating miR-503 in prostate cancer patients when compared to that in normal controls; after normalization to an endogenous reference gene U6. The miR-503 expression was significantly decreased in patients compared with controls (fold-change = 0.19; control group standardized as 1.0). This downregulation demonstrates the diagnostic significance of miR-503 as a noninvasive biomarker for prostate cancer.

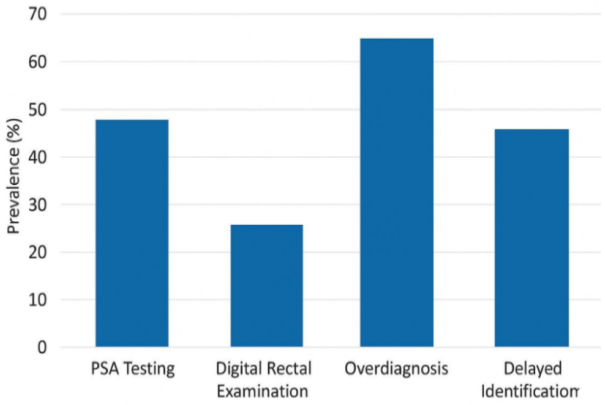

Figure III: Bar chart showing Key Limitations of Conventional Prostate Cancer Diagnostic Methods

Discussion

The results presented here are in good agreement with the published data showing down-regulated miR-503 expression in various neoplasms, including prostate cancer as well as other tumors. Low expression of miR-503 leads to perturbation of the essential cellular pathways like cell-cycle progression, apoptosis, and differentiation. According to Jiang et al. (2016), miR-503 plays a tumor-suppressive role in prostate cancer by inhibiting the expression of driver factors and oncogene ZNF217, and then driving cell proliferation reduction and apoptosis acceleration. This reduction in miR-503 has been correlated with increased tumor aggressiveness as well as poor cellular regulation, providing additional evidence that miR-503 is crucial for tumor suppression[15-16].

In concordance with these findings, downregulation of circulating miR-503 in the observed cohort may indicate common mechanisms of tumor formation. These molecular changes support the notion that miR-503 is involved in tumor suppression and that loss of its expression may also be implicated in activation of oncogenic pathways in different cancer types[17-19].

The present study had the advantage of U6 snRNA serving as an internal control gene. U6 showed very stable expression across samples, which was crucial to the normalization and to avoid influences of potential technical variability. Control of reference gene expression is crucial for reliable RT-qPCR quantification analysis, as variation in housekeeping genes expression might mask true biological differences or give rise to misleading estimates of alteration in expression[20,21]. The stable expression pattern of U6 in both experimental groups suggests the suitability of using it as a normalization factor for circulating microRNA studies.

The marked downregulation of miR-503 in the patient group also underlines its applicability as a non-invasive diagnostic biomarker. The distinct separation of normal and prostate cancer samples in fold-change analysis indicated good discriminating ability. Due to the limitations of standard prostate cancer screening methods (e.g., low specificity of PSA testing, risk for negative biopsies), circulating miRNAs have emerged as an attractive non-invasive complementary diagnostic tool[22-24].

In general, the present findings add to literary evidence demonstrating that circulating microRNAs could be useful for diagnosis and eventually for outcome prediction. In view of its stable detection, biological significance and major differential expression, more large-scale, multi-center test is necessary in order to confirm these results. Furthermore, studies to investigate the association between miR-503 expression levels and tumor grade, clinical stage, and treatment response may provide more information about its value of serving as a biomarker in personalized cancer therapy[25-27].

Conclusion

The results of this study indicate that serum miR-503 is markedly altered in Iraqi patients with prostate carcinoma, suggesting its potential value as a non-invasive diagnostic biomarker. The consistent expression pattern observed among patients from major medical centers in Baghdad highlights the clinical relevance of miR-503 within the local population and underscores its promise in complementing existing diagnostic tools. Given the current limitations in prostate cancer detection in Iraq, miR-503 may offer a meaningful improvement in early identification and clinical decision-making. Nevertheless, broader multicenter studies across different Iraqi regions are needed to validate its diagnostic performance and to further clarify its

biological role in prostate cancer progression. Overall, these findings support the incorporation of molecular biomarkers such as miR-503 into future diagnostic approaches to enhance precision oncology and improve patient outcomes in Iraq.

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None

Conflict of Interest
The author has no conflicts of interest to disclose

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Authors' contributions
Conception and design, Acquisition, analysis, and interpretation of data, Manuscript drafting and revising it critically, Approval of the final version of the manuscript

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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