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# Inverse correlation of miR-196a and HOXB13 expression in Iraqi patients with prostate cancer

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**Abstract**

Prostate cancer (PCa), a significant health concern in aging men, is influenced by the HOXB13 gene, a key regulator of prostate development and cell differentiation. Dysregulation of HOXB13, including genetic variants and altered expression, is related to PCa risk and progression. Importantly, the expression of HOXB13 is modulated by microRNAs, particularly miR-196a. The miR-196a controls expression of genes via mRNA binding, causing degradation or inhibiting translation. It targets HOX genes, crucial for development, and exhibits variable activity in cancers, including PCa. Therefore, the interplay between PC, HOXB13, and miR-196a contributes to the understanding of the molecular basis of PCa and identifies potential therapeutic strategies. This study involved a case-control design, comprising 120 blood samples divided into 60 PCa patients and 60 controls. Molecular analyses were performed on these samples, involving total RNA extraction followed by purification via a commercial kit. Subsequently, complementary DNA was synthesized. A quantitative real-time polymerase chain reaction was used to measure the expression levels of the HOXB13 gene and miR-196a. Relative expression levels of both HOXB13 and miR-196a were determined using established quantification methodologies. Statistical analyses were conducted using SPSS and GraphPad Prism software. Our findings demonstrated a significant increase in HOXB13 gene expression ($p \leq 0.01$), specifically a four-fold elevation in PCa patients compared to healthy individuals. In contrast, miR-196a expression exhibited a significant decrease ($p \leq 0.01$), suggesting a potential inverse regulatory correlation with HOXB13. This study reveals a significant inverse correlation between HOXB13 and miR-196a expression in PCa patients. Specifically, the HOXB13 expression level was upregulated, while the miR-196a level was downregulated. These findings suggest that miR-196a may be used as a prospective tumor suppressor in PCa by negatively regulating HOXB13, thereby inhibiting cell proliferation and invasion. Consequently, miR-196a may emerge as a promising diagnostic molecular target for prostate cancer.

**Keywords:** Homeobox B13, miR-196a, Prostate cancer, Gene expression.

## 1. Introduction

Prostate cancer is among the most predominant malignancies affecting men, particularly those over the age of 50, and represents a significant public health concern due to its high incidence and substantial impact on quality of life. The prostate is a small gland situated below the bladder and anterior to the rectum, responsible for seminal fluid production that supports and transports sperm. The PCa arises when abnormal cells within the prostate proliferate uncontrollably, leading to the formation of a malignant tumor [1]. Several risk factors have been identified in relation to prostate cancer. Age is a primary determinant, with incidence rates rising markedly after the age of 50. Family history also plays a critical role; individuals with a first-degree relative (such as a father or brother) diagnosed with PCa face a significantly elevated risk. Inherited genetic mutations, including those in BRCA1, BRCA2, and HOXB13, further contribute to susceptibility. Additionally, race and ethnicity are important considerations, as prostate cancer exhibits a higher incidence and tends to be more aggressive among African-American men [2].

Early detection of PCa is critical for improving patient outcomes and overall survival rates [3]. Several diagnostic methods are commonly employed. The prostate-specific antigen (PSA) test measures the concentration of PSA, a protein produced by the prostate, in the bloodstream. Elevated PSA levels may suggest the presence of prostate cancer, although they can also result from benign conditions such as prostatitis or benign prostatic hyperplasia. The digital rectal examination (DRE) is a physical assessment in which a clinician palpates the prostate through the

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rectal wall to identify abnormalities such as nodules or areas of increased firmness. If clinical suspicion remains, a prostate biopsy is performed, involving the collection of tissue samples for histopathological evaluation to confirm malignancy. Furthermore, genetic testing may be indicated in patients with a strong family history or those diagnosed with aggressive forms of prostate cancer, to detect hereditary mutations such as BRCA1, BRCA2, or HOXB13 [4].

MicroRNAs play a vital role in the progression of carcinogenesis by negatively regulating the expression of tumor-associated genes, which are crucial in various types of cancer. They block the post-transcriptional expression of specific target genes, functioning as oncogenes or tumor suppressors[5]. In particular, miR-196a exists in two forms (miR-196a-1 and miR-196a-2) and is involved in gene regulation post-transcriptionally. It plays significant roles in development, cell differentiation, and disease processes.miR-196a functions by binding to complementary mRNA sequences, resulting in mRNA degradation or translational repression[6]. Its major targets include HOX genes, which are critical for proper developmental processes. In various cancers, including prostate cancer, miR-196a exhibits context-dependent behavior.

While in some cancers it can act as an oncogene by promoting proliferation, invasion, and metastasis, there are contexts where it may exhibit tumor-suppressive properties [7].In the case of prostate cancer, miR-196a has been observed to be dysregulated, which is associated with the progression of the disease. This dysregulation suggests that miR-196a could be a potential biomarker for prostate cancer prognosis. Given its involvement in disease progression, targeting miR-196a may offer promising therapeutic avenues for managing prostate cancer [8, 9]. The HOXB13 gene is part of the homeobox gene family, which plays a vital role in development, particularly in the formation of the body plan and organ differentiation. HOXB13 encodes a transcription factor important for male-specific development, particularly in the prostate and reproductive system[10]. It is involved in regulating genes implicated in various developmental processes and has been shown to affect cell differentiation and proliferation. It has gained attention due to its association with prostate cancer. Variants in the HOXB13 gene, such as the G84E mutation, have been linked to an increased risk of hereditary prostate cancer[11].

Dysregulation of HOXB13 expression has been observed in PCa tissues, suggesting it plays a role in tumor progression. Also, it is part of the homeobox gene family, which plays a vital role in development, particularly in the formation of the body plan and organ differentiation [12]. Furthermore, it encodes a transcription factor important for male-specific development, particularly in the prostate and reproductive system [13]. The HOXB13 gene is vital for development and is intricately linked to prostate cancer. The regulation of HOXB13 by microRNAs is an important aspect of its function and could provide insights into new therapeutic strategies and biomarkers for cancer management[14]. Understanding the interplay between HOXB13 and miRNAs can shed light on the molecular mechanisms underlying prostate cancer and enhance our treatment approaches.

## 2. Methods and materials

### 2.1. Subjects

A total of 120 blood samples were collected and categorized into two groups: the first group comprised 60 patients diagnosed with prostate cancer, while the control group included 60 healthy individuals without a history of malignancy. Sample collection was conducted between October 2022 and May 2023 at Al-Amal Hospital and affiliated private diagnostic laboratories in Baghdad, Iraq. Prior to sample collection, each participant provided informed consent, and comprehensive medical histories were obtained using a standardized and validated questionnaire to ensure consistency and reliability of clinical data. The study protocol was considered and approved by the Institutional Review Board (IRB) of the Biology Department, College of Science, Mustansiriyah University, and ethical clearance was granted under approval number [BCSMU/12523/00063M].

### 2.2. Blood collection

Adequate volumes of blood samples were obtained from all participants in EDTA tube under strictly aseptic conditions to ensure sample integrity and prevent contamination. The collected samples were intended for the relative-quantification (RQ) of *miR-196a* and *HOXB13* gene expression. Following collection, the samples were stored in appropriate tubes at −20 °C until further molecular analysis.

### 2.3. Expression of HOXB13 and RQ of *MiR-196a*

Quantitative real-time polymerase chain reaction (qRT-PCR) was utilized to evaluate the *HOXB13* and *miR-196a* expression levels. Total RNA was extracted from peripheral blood samples obtained from both PCa patients and healthy controls using the EasyPure® TransZol Up Plus RNA Kit (ER501-01, TransGen Biotech, China) [15]. For the specific isolation of miRNA, the EasyPure® miRNA Kit (ER601-01, TransGen Biotech, China) was employed, as previously described [16, 17]. The concentration and purity of the extracted RNA samples were assessed using the NanoDrop OneC spectrophotometer (Thermo Fisher Scientific, USA). The measured RNA purity ranged from 2.00 to 2.01, while the concentrations were within the range of 25–37 ng/µL. These values indicate that the RNA was of sufficient quality for subsequent analysis by RT-qPCR.

Subsequently, complementary DNA (cDNA) synthesis was carried out using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (AE311-01, TransGen Biotech, China), in accordance with the manufacturer's instructions [18, 19]. The conditions for the heat cycler steps of cDNA reverse transcription are shown in

**Table 1.** The PCR thermal conditions for cDNA reverse transcription synthesis.

| cDNA Synthesis | First Step | second Step | third Step |
|---|---|---|---|
| Temperature (°c) | 25 | 42 | 85 |
| Time | 10 min | 15 min | 5 sec |
| Reaction | Random Primer (N9) binding | Anchored Oligo (dT)18 binding | Inactivate reverse transcriptase enzyme |

Table 1.

### 2.4. Primers

The primers used in this study were obtained through the NCBI bioinformatics program. (Table 2) lists the primers and their sequences that were used in this study. Primers were synthesized by Alpha DNA Company (Canada) and supplied in lyophilized form. Upon receipt, stock solutions were prepared by dissolving each primer in nuclease-free water to a final concentration of 100 µM. Working solutions were subsequently prepared by diluting the stock solutions to a concentration of 10 µM using nuclease-free water, and these were used in RT-qPCR reactions[20].

### 2.5. Real-time qPCR analysis of gene expression

Gene expression analysis was performed using RT-qPCR with the TransStart® Top Green qPCR SuperMix (AQ131-01, TransGen Biotech, China), adhering strictly to the manufacturer's recommended protocol [21]. The reaction was performed in a total volume of 20 µL, comprising 2 µL of cDNA as the template, 1 µL each of forward and reverse primers, and 6 µL of nuclease-free water to complete the final volume. The thermal cycling conditions included an initial denaturation at 94 °C for 60 seconds (1 cycle), followed by 40 cycles of denaturation at 94 °C for 10 seconds, annealing at 58 °C for 15 seconds, and extension at 72 °C for 20 seconds. A dissociation curve (melting curve) analysis was then conducted from 65 °C to 95 °C with temperature increments every 1 second. All reactions were performed in duplicate to ensure reproducibility and accuracy of the results.

For normalization of gene expression data, GAPDH was used as the internal control for HOXB13, while U6 small nuclear RNA (U6 snRNA) served as the reference gene for miR-196a. Relative gene expression levels were calculated using the ΔCt method, where the cycle threshold (Ct) value of the target gene was subtracted from that of the corresponding reference gene, as follows: ΔCt (test) = Ct_target – Ct_reference. Subsequently, the relative expression levels were determined using the $2^{\wedge-\Delta Ct}$ method to obtain the normalized expression ratio for comparison between groups [22]. This approach allowed for consistent and quantitative assessment of transcript levels across all samples.

### 2.6. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26. The mean ± standard error (SE) of the cycle threshold (Ct) values for target and reference genes was calculated. An independent t-test was used to determine statistical significance (p-values), while the chi-square test was applied for percentage comparisons. GAPDH and U6 served as reference genes for HOXB13 and miR-196a, respectively. Gene expression levels were quantified using the ΔCt method [23]. Expression ratios were determined using the 2^–ΔCt formula. For comparative analysis across samples, the 2^–ΔΔCt method was employed, where ΔΔCt = ΔCt (test sample) – ΔCt (calibrator sample) [24]. A p-value ≤ 0.05 was considered statistically significant, and p ≤ 0.01 was considered highly significant.

## 3. Results

### 3.1. PCa patients characteristics

The study's age distribution of prostate cancer patients reveals a distinct pattern, with men between the ages of 50 and 59 accounting for the majority of cases. The observed distribution is marginally significant, according to the p-value of 0.052, suggesting that increasing age may increase the risk of PCA. These findings support the need for targeted screening and prevention strategies, especially for men over 50, and highlight the significance of age as a major risk factor for prostate cancer. Table 3 illustrates the results**.**

Table 4 demonstrates the distribution of cancer patients across four stages (I, II, III, and IV) within four distinct age groups (20-39, 40-49, 50-59, and 60-70). Statistical analysis indicated no significant differences in cancer stage distribution across or within age groups (p > 0.05). Stage II exhibited the highest percentage of cases overall (36.67%), while Stage IV had the lowest (13.33%). The distribution of total cancer cases across age groups approa-

**Table 2.** Primers and their sequences.

| Primers | Sequence (5′→3′ direction) |
|---|---|
| HOXB13 Gene | |
| Forward | CAG CAG CAG CAG CAG CAG AA |
| Reverse | TCC TTG TCC TCG TCC TCG TT |
| GAPDH Gene | |
| Forward | TGAGAAGTATGACAACAGCC |
| Reverse | TCCTTCCACGATACCAAAG |
| miRNA | |
| *MiR-169a* F.P. | TTAAGGCACGCGGTGAATGCCA |
| U6 snRNA F.P. | AGAGAAGATTAGCATGGCCCCT |
| MiR-universe R.P. | GCGAGCACAGAATTAATACGAC |

**Table 3.** Age-related distribution of prostate cancer patients.

| Age | No. of prostate cancer group |
|---|---|
| 20-39 | 8 (13.33%) |
| 40-49 | 18 (30%) |
| 50-59 | 22 (36.67%) |
| 60-70 | 12 (20%) |
| p-value = 0.052 | |

**Table 4.** Frequency and percentage of prostate cancer stages according to age interval.

| Age Group | Stage I | Stage II | Stage III | Stage IV | Total | P-Value |
|---|---|---|---|---|---|---|
| 20-39 | 2 (3.33%) | 3 (5%) | 2 (3.33%) | 1 (1.67%) | 8 (13.33%) | 0.8 NS |
| 40-49 | 5 (8.33%) | 7 (11.67%) | 4 (6.67%) | 2 (3.33%) | 18 (30%) | 0.4 NS |
| 50-59 | 6 (10%) | 8 (13.33%) | 5 (8.33%) | 3 (5%) | 22 (36.67%) | 0.5 NS |
| 60-70 | 3 (5%) | 4 (6.67%) | 3 (5%) | 2 (3.33%) | 12 (20%) | 0.8 NS |
| Total | 16 (26.67%) | 22 (36.67%) | 14 (23.33%) | 8 (13.33%) | 60 (100%) | 0.08 NS |
| P-value | 0.5 NS | 0.4 NS | 0.7 NS | 0.8 NS | 0.05 * | 0.9 NS |

NS mean non-significant, * mean significant.

ched statistical significance (p=0.05), suggesting a significant difference; specifically, the 50-59 age group showed the highest percentage of total cases (36.67%), and the 20-39 age group showed the lowest (13.33%). Furthermore, no significant association was found between age group and cancer stage (p=0.9). Therefore, while the statistical analysis recommends a largely constant distribution of cancer stages, the observed percentage variations permit careful consideration and potentially further investigation.

### 3.2. Assessment of GAPDH Ct value variation in prostate cancer and healthy control groups

Housekeeping genes are typically assumed to exhibit consistent expression across the cells or tissues under study when used in molecular research. *GAPDH* is one of the most universally used housekeeping genes for gene expression analysis. In fact, GAPDH has been employed as a reference gene in 72 distinct types of normal human tissues in qRT-PCR studies examining the expression of 1,718 genes [19]. When applied in clinical research, GAPDH serves as a reliable method for normalization in qRT-PCR assays. To refine this approach, the $2^{\wedge-\Delta Ct}$ value and the ratio of the $2^{\wedge-\Delta Ct}$ values between the study groups and the control group were utilized to evaluate the variability in GAPDH expression across the groups, as shown in Table 5. The $2^{\wedge-\Delta Ct}$ value for the healthy group was 5.7024E-06, while for the prostate cancer group, it was 5.21103E-06. The fold change in gene expression among the PCa and control groups was calculated to be 0.913831.

### 3.3. Fold change in *HOXB13* expression based on the $2^{\wedge-\Delta Ct}$ method

The study contributors were divided into two categories including healthy controls and prostate cancer patients. The relative quantitation approach was used to determine the quantitative expression of the *HOXB13gene* using qRT-PCR. For the prostate cancer group, the calculated *HOXB13* fold expression ratio was 4.06, while it was 1.00 for the healthy groups. According to the results, the prostate cancer group's CT values were lower than those of the control group. (Table 6) [25]. Fig. 2 illustrates the amplification curve of HOXB13.

### 3.4. Evaluation of Ct value of U6 snRNA among study groups

The Ct value of U6 snRNA, the housekeeping gene in the present study, is shown in Table 7. The healthy group (control group) had a Ct value for U6 snRNA that ranged from 29.14 to 0.99 (30.05±0.47). In the group with PCa, it varied between 28.00 and 30.31 (29.9±1.12). The mean Ct value of U6 snRNA did not differ significantly between these groups (P = 0.912). Housekeeping genes are used in molecular studies with the underlying assumption that their levels in the cells or tissue being studied stay constant. U6 snRNA is one of the housekeeping genes that is most frequently used in the context of gene expression data. To further enhance this, the 2-ct value and the ratio of the 2-Ct of the various study groups to that of the control group

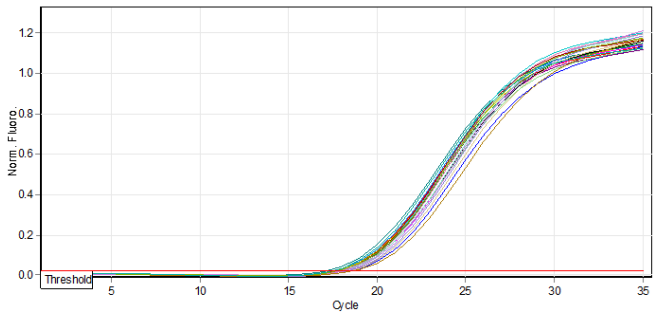


**Fig. 1.** The amplification curve analysis of GAPDH.

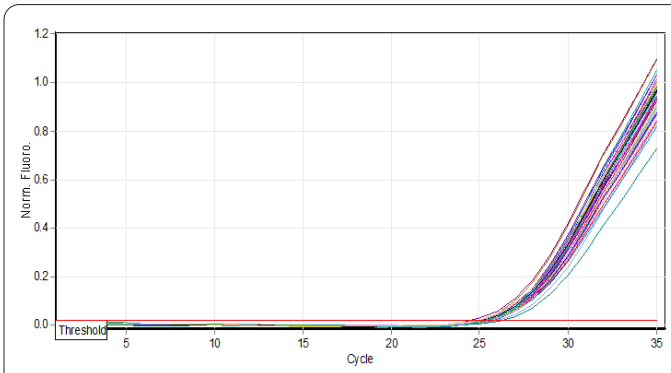


**Fig. 2.** The analysis of *HOXB13* amplification curve.

**Table 5.** Comparison between the different studied groups in the Ct value of GAPDH (Mean ± SD).

| GAPDH | PCa Patients | Control |
|---|---|---|
| Mean ± SD of Ct value | 17.42±0.87 | 17.55±0.84 |
| 2-Ct | 5.7024E-06 | 5.21103E-06 |
| Experimental group/ Control group | 5.7024E-06/ 5.21103E-06 | 5.21103E-06 / 5.21103E-06 |
| Fold of gene expression | 1.094 | 1.00 |
| Range | 17.20-17.54 | 17.12-17.89 |
| P- value | 0.831 NS | |

**Table 6.** Fold of HOXB13 expression depending on $2^{-\Delta Ct}$ Method.

| HOXB13 | PCa Patients | Control |
|---|---|---|
| Means of Ct HOXB13 | 23.23 | 25.12 |
| Means of Ct GAPDH | 17.55 | 17.42 |
| ΔCt (Means of Ct HOXB13 - Means Ct of GAPDH) | 5.68 | 7.7 |
| $2^{-\Delta Ct}$ | 0.019505 | 0.004809 |
| Experimental group/ Control group | 0.019505/0.004809 | 0.004809/0.004809 |
| Fold of gene expression | 4.055838 | 1.00 |
| P- value | $p \leq 0.01$ | |

**Table 7.** Comparison between different groups in Ct value of U6 snRNA.

| U6 snRNA | PCa Patients | Control |
|---|---|---|
| Mean ± SD of Ct value | 29.9±1.12 | 30.05±0.47 |
| $2^{-\Delta Ct}$ | 9.98E-10 | 9E-10 |
| Experimental group/ Control group | 9.98E-10/9E-10 | 9E-10/9E-10 |
| Fold of gene expression | 1.1096 | 1.00 |
| Range | 28.00-30.31 | 29.14-30.99 |
| P- value | 0.912 NS | |

**Table 8.** Fold of miR-196a expression depending on the 2-ΔCt method.

| miR-196a | PCa Patients | Control |
|---|---|---|
| Means of Ct miR-196a | 22.09 | 20.35 |
| Means of Ct U6 SNRNA | 29.9 | 30.05 |
| ΔCt (Means of Ct miR-196a - Means Ct of U6 SNRNA) | -7.81 | -9.7 |
| 2-Ct | 224.4111 | 831.7465 |
| Experimental group/ Control group | 224.4111/831.7465 | 831.7465/831.7465 |
| Fold of gene expression | 0.269807 | 1.00 |
| P- value | $p \leq 0.01$ | |

were used to examine the variation of the overall change in expression of U6 snRNA in each study group, even though there was a non-significant difference in the mean Ct value between groups in the current study [26]. Figure 3 elucidates the amplification curve of U6 snRNA.

The control group's $2^{-Ct}$ value was 9E-10, while the prostate cancer group's was 9.98E-10. For the control groups, the calculated gene fold expression ratio was 1.00, while for the prostate cancer group, it was 1.10956947. Because the study groups' gene fold expression differs slightly, the U6 snRNA acts as a useful control gene. These results are consistent with researchers Kozera, B., & Rapacz, M. U6 expression is unsuitable as an internal reference gene in the research of circulating miRNAs due to large fluctuations and changes in expression with repeated freezing and thawing cycles [27].

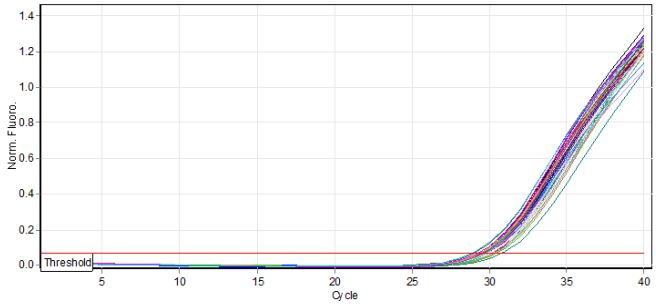


**Fig. 3.** The result of amplification curve for U6 snRNA gene.

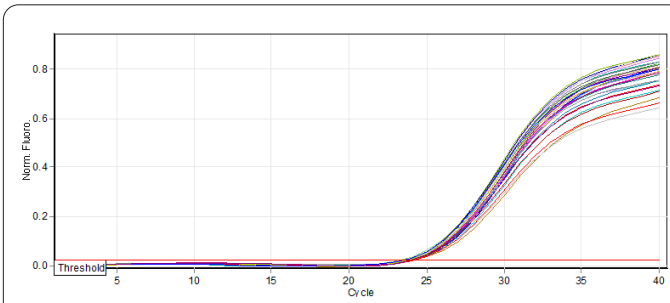


**Fig. 4.** The *miR-196a* qPCR amplification curve analysis result.

### 3.5 Quantification of *miR-196a* expression fold change using the $2^{-\Delta Ct}$ method

The expression of miR-196a in the PCA group was analyzed using a qRT-PCR approach. Relative quantification was employed to evaluate the fold change in gene expression. To normalize the expression levels, the ΔCt was calculated by subtracting the mean Ct value of the internal control (U6 snRNA) from the mean Ct value of miR-196a amplification for each sample. The resulting $2^{\wedge-\Delta Ct}$ values were then compared between the prostate cancer group and the healthy control group to determine the relative fold change in gene expression (Table 8). The analysis revealed that miR-196a expression in the prostate cancer group was approximately 0.269807-fold lower than that observed in the healthy control group, indicating a marked downregulation of *miR-196a* in prostate cancer cases. These findings show that groups' expression of the miR-196a gene has significantly decreased. According to these findings, the gene expression of the prostate cancer group is significantly lower. With high expression denoting a fold change larger than one and low expression denoting a fold change smaller than one, all study groups were divided into two subgroups: high expression and low expression. When compared to the healthy group, the prostate cancer group's elevated expression was detectable, suggesting that there was a significant difference between the study groups and highlighting the importance of rigorous analysis. The miR-196a amplification curve is made clear in Fig. 4.

## 4. Discussion

Prostate cancer is one of the most common malignancies among men, with an estimated 1.5 million new cases and 397,000 deaths worldwide and classified as second most frequent cancer and the fifth leading cause of cancer death among men in 2022, ranking it as the most diagnosed cancer in males across 118 out of 185 countries[28]. This rise is largely attributed to aging populations and urbanization, while mortality has declined in high-income countries due to early detection efforts[29]. In the Middle East, including Iraq, incidence and mortality remain relatively lower but show an upward trend, emphasizing the need for improved health infrastructure and awareness[30, 31].

Our results revealed a significant increase in the mRNA expression level of *HOXB13* in PCa patients compared to healthy controls, suggesting a potential role for this gene in promoting tumor growth or progression [32]. *HOXB13* is a key regulatory gene within the HOX family and has been previously linked to the normal development of prostate tissue. However, its overexpression may be associated with pathological cellular changes[13]. On the other hand, analysis of *miR-196a,* known to negatively regulate HOXB13 [33], showed a marked decrease in its expression in the same cancerous samples. This suggests a possible inverse association between *miR-196a* and *HOXB13*, whereby the downregulation of miR-196a could release its suppression on *HOXB13*, leading to its upregulation. These findings are consistent with previous studies reporting that miR-196a acts as a negative regulator of certain *HOX* genes and may function as a tumor suppressor in specific biological contexts[34–36].

The findings show that HOXB13 is substantially overexpressed in prostate cancer as opposed to healthy tissue, as demonstrated by the cancer group's lower CT values and fold expression ratio of 4.055838. This finding aligns with Kim, T. H., et al. (2018) and Brechka et al. (2017). The HOXB13 gene has emerged as a critical player in prostate cancer biology, with studies showing that it is constitutively activated in over 40% of prostate cancers. This activation contributes to tumor progression by deregulating downstream target genes involved in key processes such as proliferation, angiogenesis, and epithelial-mesenchymal transition (EMT). Further research is needed to fully understand its role and potential as a biomarker for diagnosis, prognosis, and targeted therapy [14, 37]. According to previous investigations about prostate cancer [38], *HOXB13* is detected in 51.7% of cases with varying expression levels. Its expression is associated with advanced disease stages, high Gleason scores, lymph node metastasis, increased Androgen Receptor (AR) expression, reduced prostate-specific antigen (PSA) levels, and early disease recurrence. A subset of tumors with high HOXB13 and AR but low PSA expression showed a particularly poor prognosis. HOXB13 is a promising biomarker for prostate cancer diagnosis, either alone or in combination with AR and PSA. Patel et al. (2024) demonstrated that HOXB13, while slightly reduced in high-grade tumors, remains significantly expressed across all prostate cancer stages, including advanced metastatic castration-resistant prostate cancers (CRPCA). They also established HOXB13 as a superior diagnostic marker for prostatic origin, highlighting its persistent role in prostate cancer progression and its potential as a valuable biomarker [39].

Furthermore, HOXB13 acts as a key driver of prostate cancer malignancy by overexpressing and consequently lowering intracellular zinc levels. This reduction, achieved through increased ZnT transporter activity, triggers NF-κB signaling, promoting cancer cell invasion and metastasis. The inverse relationship between HOXB13 and IκBα further supports this role in enhancing cancer progression[40]. While Barashi *et al* 2024 [41] investigated Patients with Benign Prostatic Hyperplasia (BPH) who later developed PCa (BPH/PCa) and showed significant upregulation of HOXB13. Yao et al.,2019, exhibited that HOXB13 promotes PCa metastasis by coordinately upregulating mitotic kinases and downregulating HSPB8 [42]. HOXB13 demonstrates consistent upregulation in prostate cancer progression. Specifically, increased HOXB13 expression correlates with heightened MYC and Androgen Receptor (AR) activity, likely through an inverse relationship with MEIS1 [43].

The previous study [44] found miR-196a upregulated in PCAa, acting as an oncogene and promoting tumor progression via p27kip1 suppression. This contradicts our finding of miR-196a downregulation, suggesting a potential tumor suppressor role in our patient cohort. A previous study, using PCa cell lines, reported upregulated miR-196a in prostate cancer, associating it with aggressive tumor characteristics. This contrasts with our results, which demonstrated downregulated miR-196a in blood samples of PCa [45]. Rodríguez et al. (2017), consistent with the current finding, demonstrate a significant downregulation of miR-196a-5p in urinary exosomes from prostate cancer patients, reinforcing its potential as a promising non-invasive biomarker and highlighting the value of urinary exosomes as a source for microRNA analysis in prostate cancer detection[46].

The results may show a negative correlation between miR-196a and HOXB13 expression levels, suggesting that miR-196a suppresses HOXB13. Alternatively, a positive correlation could indicate that miR-196a enhances HOXB13 expression, contributing to tumor aggressiveness. The study found that miR-196a directly or indirectly regulates the expression of HOXB13 in prostate cancer patients.MiR-196a acts as a tumor suppressor; it downregulates HOXB13, thereby inhibiting prostate cancer progression. Conversely, if miR-196a acts as an oncogene, it may upregulate HOXB13, promoting tumor growth and metastasis [25]. Taken together, these findings indicate that the miR-196a/HOXB13 axis may represent a critical molecular mechanism in the regulation of prostate cancer development, highlighting it as a promising target for future research in diagnosis or gene-based therapy.

However, this study has several limitations. First, only a single miRNA (miR-196a) was selected for analysis, which, although supportive of the findings, may not fully represent the broader regulatory network influencing HOXB13. Second, the potential effects of HOXB13 and miR-196a on hormone levels were not assessed and warrant further investigation. Third, the study focused exclusively on gene expression analysis without conducting functional validation through in vitro or in vivo experiments. Future research involving larger patient cohorts and mechanistic studies is essential to elucidate the regulatory relationship between miR-196a and HOXB13 and to evaluate their potential as diagnostic or therapeutic targets in prostate cancer.

A molecular investigation into the association between *miR-196a* and *HOXB13* expression in Iraqi prostate cancer patients provides valuable insights into the regulatory mechanisms contributing to *HOXB13* overexpression and, subsequently, prostate cancer progression. The study revealed a significant upregulation of *HOXB13* alongside a noticeable downregulation of *miR-196a* in PCa patients, suggesting a potential inverse correlation between the two. These findings underscore the potential of both *miR-196a* and *HOXB13* as diagnostic, prognostic, and therapeutic biomarkers, offering promising avenues for the development of personalized medicine strategies in PCa management. The *miR-196a/HOXB13* regulatory axis may represent a critical molecular pathway in PCa pathogenesis and

warrants further exploration. Continued research is essential to validate these results and facilitate their translation into clinically applicable tools aimed at improving patient outcomes.

## Author declaration

### Conflicts of interest

The authors declare no conflicts of interest.

### Originality of figures and tables

All figures and tables presented in this manuscript are original to this investigation.

### Clinical trial information

This study was not a clinical trial and therefore did not require a trial number.

### Funding

No external funding was received for this research.

### Competing interests

The authors confirm there are no competing interests.

### Human ethics and consent

All participants provided informed consent before enrollment in the study.

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