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Correlation Between *miR-143-5p*/*miR-744-5p* and *HIF1A* Expression Across Breast Cancer Grades and Treatments in Iraqi Women

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ABSTRACT

Background: Breast cancer (BC) accounts for 25% of all cancer cases and 15% of all cancer-related deaths among women. Hypoxia-inducible factor-1alpha (*HIF1A*) is a crucial regulator of cellular responses to hypoxia. This study aimed to evaluate the expression of miR-*143-5p* and *miR-744-5p* as potential molecular markers and their association with *HIF1A* in BC.

Methods: The study included 100 newly diagnosed cases of BC who attended the Oncology Center in Al-Anbar, Iraq, from July 2024 to January 2025. After RNA extraction and cDNA synthesis, real-time polymerase chain reaction was used to determine microRNA (miR) expression levels for women with BC and healthy controls.

Results: The results showed that *miR-143-5p* exhibited significantly higher expression levels in high-grade after treatment (HAT) and low-grade before treatment (LBT) samples compared with control, high-grade before treatment (HBT), and low-grade after treatment (LAT) samples. *miR-744-5p* did not show significant differences in expression across the different cohorts. *HIF1A* showed no statistically significant correlations with any of the other measured molecules in either the control group (*miR-143-5p*: $r = -0.25$, $P = 0.32$; *miR-744-5p*: $r = -0.32$, $P = 0.23$) or any of the experimental groups (all P values > 0.05). *miR-744-5p* showed moderate potential (area under the curve [AUC] = 0.629). *HIF1A* exhibited high diagnostic potential in the HBT group with an AUC value of 0.774 and moderate diagnostic potential in the HAT group with an AUC value of 0.647, indicating that the *HIF1A* gene may be a good diagnostic marker for certain subgroups of BC.

Conclusion: The findings suggest that *miR-143-5p* may serve as a potential biomarker for BC.

INTRODUCTION

Breast cancer (BC) is a complex disease and the most commonly diagnosed cancer in women worldwide. BC is the primary cause of cancer among women and the leading cause of cancer-related mortality in women in Iraq.[1,2] Additionally, BC is a multifactorial heterogeneous disease.[3,4] MicroRNAs (miRs) are endogenous noncoding RNAs with 21 to 23 nucleotides. They function in posttranscriptional gene regulation through degradation and translational repression of target mRNAs harboring substantial complementarity with miRs.[5] MicroRNAs are

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involved in several cellular processes, such as development, differentiation, growth, proliferation, and apoptosis.[6] They play a significant role as oncogenes and tumor suppressor genes.[7] The role of *miR-143-5p* and its seed region specificity in estrogen receptor–positive BC remains poorly understood.[8] Mechanistically, *miR-143-5p* acts by targeting specific genes in BC cells.[9] *miR-744-5p*, on the other hand, acts as a tumor suppressor as it is able to inhibit BC cell migration and invasion *in vitro*. Hypoxia is defined as an imbalance between rapid tumor growth and inadequate O_2 delivery.[10] The microenvironment of solid tumors, such as BC, can be acidic, causing aggressiveness and treatment resistance. Hypoxia-inducible factor-1alpha (*HIF1A*) is a key transcription factor that regulates cellular responses to hypoxia, a common feature in solid tumors, including BC.[11] Under hypoxic conditions, *HIF1A* stabilizes and activates the transcription of genes involved in angiogenesis, metabolic reprogramming, and cell survival, which are critical for tumor progression and metastasis.[12] *HIF1A* is significantly upregulated in the majority of BC cases but is downregulated in a few cases.[13] The aim of this study is to investigate the role of *miR-143-5p* and *miR-744-5p* in *HIF1A* gene expression in patients with BC.

METHODS

Samples and data collection

This study was approved by the Council of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies at the University of Baghdad, and the study protocol was approved by the Ethics Committee of the Ministry of Health and Environment–Al-Anbar Directorate of Health (AO No. 1812). All procedures of this research, including sample collection, were conducted at the Oncology Center in Al-Anbar Province from July 2024 to January 2025. The study design was cross-sectional, as biomarkers and gene expression were measured simultaneously through convenience sampling. In this research, 100 Iraqi women participated, all of whom were confirmed as newly diagnosed patients with BC aged 30–70 years. All patients with breast cancer underwent all required assessments, including measurement of weight and height and collection of detailed family medical history, which were documented in a dedicated patient admission form at the oncology center. Participants were classified as overweight or obese based on their body mass index (BMI), calculated as weight in kilograms divided by height in meters squared (kg/m^2). Participants were classified as overweight (BMI, 25.0–29.9) or obese (BMI, ≥30.0) based on standard World Health Organization (WHO) criteria. The selected patients were grouped according to specific criteria into 4 groups, each with 25 patients. There were 25 patients with low-grade BC before treatment (LBT) and 25 patients with low-grade BC after treatment (LAT). The remaining 25 patients had high-grade BC before treatment (HBT) and 25 patients had high-grade BC after treatment (HAT). There were 25 healthy individuals who had no history or clinical evidence of BC and no chronic diseases, such as hypertension or diabetes, in the control group. The blood samples were taken before chemotherapy and 6 weeks after treatment for all patients who attended Al-Anbar Oncology Center. A volume of 1.5 mL of blood was collected and transferred into an EDTA anticoagulant tube. After gentle mixing, 0.5 mL of whole blood was placed directly into TRIzol for preservation for the extraction of RNA and kept at −70 °C for molecular analysis.

Inclusion criteria

The study included women with a first-time diagnosis of BC, classified as low or high grade by immunohistochemistry (IHC) and biopsy, who were treatment-naïve and without chronic comorbidities (e.g., hypertension or diabetes).

Exclusion criteria

Women previously treated with any cancer therapy (e.g., chemotherapy), those with other cancers, prior BC diagnoses, or chronic conditions such as hypertension or diabetes were excluded from the study.

Molecular assays

Protocol of RNA extraction

Total RNA, including microRNA, was extracted from patient samples using TRIzol Reagent according to the manufacturer's protocol. Briefly, 200 µL of chloroform was added to homogenized samples to separate the aqueous phase. After centrifugation, the aqueous phase was transferred to a new tube, and 500 µL of isopropanol was added to precipitate the RNA. Then, 500 µL of 70% ethanol was used for RNA washing. Finally, the supernatant was discarded, the pellet was resuspended in 50 µL of nuclease-free water, and the RNA was kept at −70 °C until the RT-qPCR reaction.

Reverse transcription for complementary DNA (cDNA) synthesis

Reverse transcription was performed using RNA extracted from blood. The quantification of miRs by quantitative real-time polymerase chain reaction (qRT-PCR) requires the extension of mature miRs' length by adding poly(A) tails, due to their short sequence. To reverse transcribe miR, the total RNA was first polyadenylated using a Poly(A) Tailing Kit

from Tinzyme Company, China. The cDNA synthesis was carried out using a reverse transcriptase kit (ELK Biotechnology, China). The reverse transcription master mix was placed in a 0.2-mL PCR tube, and the template RNA and cDNA primers were added and gently mixed. For reverse transcription, the thermal cycle settings were 25 °C for 5 minutes, 42 °C for 60 minutes, and 70 °C for 1 minute.

Quantitative real-time polymerase *chain reaction (RT-qPCR)*

Following the manufacturer's instructions, the SYBR Green PCR Kit (Bioer LineGene, China) was used to perform RT-qPCR. The reaction was carried out using a real-time PCR instrument and had a total volume of 20 µL. It contained 10 µL of SYBR Green Master Mix, 1 µL of each specific forward and reverse primer, 3 µL of cDNA, and 6 µL of RNase-free water. The following were the cycling conditions: an initial cycle of 95 °C for 1 minute, followed by 45 cycles of 95 °C for 20 s and 60 °C for 30 s. The relative expression levels of the miRs (normalized to *U6*) and *HIF1A* mRNA (normalized to glyceraldehyde-3-phosphate dehydrogenase [*GAPDH*]) were calculated using the formula $2^{-\Delta\Delta CT}$, presented as fold change. The primer sets used in the current study are listed in Table 1.

Table 1. Primer Sequences Used in This Research

Primer name		Sequence 5′–3′
HIF1A	Forward	GTCTCGAGATGCAGCCAGAT
	Reverse	CCTCACACGCAAATAGCTGA
GAPDH (Housekeeping gene)	Forward	TGCCACCCAGAAGACTGTGG
	Reverse	TTCAGCTCAGGGATGACCTT
miR-143-5p	Forward	AACAGAGGTGCAGTGCTGC
miR-744-5p	Forward	AACAAGTGCGGGGCTAGGG
CR Universal Reverse	Reverse	CAGTGCAGGGTCCGAGGT
U6 (Housekeeping gene)	Forward	GTGCTCGCTTCGGCAGCA
	Reverse	CAAAATATGGAACGCTTC

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF1A, hypoxia-inducible factor 1-alpha; miR, microRNA; U6, U6 small nuclear RNA.
Primers were designed by the researcher in the current study.

Statistical analysis

Data were analyzed using IBM SPSS Statistics (version 26.0) to determine the effect of different factors on the study parameters. The relationship between the variables was assessed using the Pearson correlation coefficient. Categorical data were expressed as numbers and percentages, whereas the quantitative variables were expressed as the median and interquartile range (IQR). Receiver operating characteristic (ROC) curve analysis was employed to calculate the area under the curve (AUC).

RESULTS

The RT-qPCR results for miRs and *HIF1A* were analyzed using the relative quantification of gene expression levels (fold change) based on the cycle threshold (Ct) values. The analyses revealed distinct patterns of *miR-143-5p*, *miR-744-5p*, and *HIF1A* expression in relation to age and BMI and highlighted potential links between these molecules, aging, obesity, and cancer stage–specific factors.

The analysis of the dataset revealed notable distributional disparities across several variables. Family history was unevenly distributed, with the majority of observations (78%) reporting no history of disease compared with 22% with a history. Table 2 presents the demographic and clinical characteristics of the patients in different BC cohorts and the participants in the control group.

The distribution of samples was uneven across categories of family history and other diseases. The control group showed the lowest counts in some clinical categories, such as family history and comorbidities, but not consistently across all variables. Upon analysis of BMI and age across 5 different sample groups, including a control group and 4 cancer-related groups (HAT, HBT, LAT, and LBT), the control group was found to exhibit a median BMI similar to that of the LAT and LBT groups, but it had the narrowest IQR, indicating both a lower median BMI and less variability within this group compared with the cancer-related groups. Compared with the control group (younger, lower BMI, narrower age range), all cancer groups exhibited higher median ages and wider age distributions. The trend of increasing median age from HAT to LBT suggests an association between age, cancer grade, and/or treatment status.

Biomarker expression analysis across age and cancer grades

The study categorized samples into 2 age groups: adult and older adult. The age groups were defined as follows: young adults (18–24 years), adults (25–59 years), and older adults (≥60 years), in alignment with

WHO and commonly accepted epidemiological classifications.

We analyzed multiple biomarkers across different groups (i.e., control, HAT, HBT, LAT, and LBT). The biomarkers examined consisted of microRNAs (*miR-143-5p*, *miR-744-5p*) and *HIF1A*.

Table 2. Demographic and Clinical Characteristics of the Participants

Variables	LBT (n = 25, 20.0%)	LAT (n = 25, 20.0%)	HBT (n = 25, 20.0%)	HAT (n = 25, 20.0%)	Control (n = 25, 20.0%)
Age, y					
<50	10 (40%)	7 (28%)	8 (32%)	8 (32%)	20 (80%)
>50	15 (60%)	18 (72%)	17 (68%)	17 (68%)	5 (20%)
BMI, median (IQR)	32.7 (31.2–34.2)	32.7 (31.2–34.2)	33.5 (32.4–34.2)	33.8 (32.6–34.3)	32.7 (31.8–34.2)
Family history					
Yes	9 (36%)	8 (32%)	4 (16%)	4 (16%)	0 (0%)
No	16 (64%)	17 (68%)	21 (84%)	21 (84%)	25 (100%)
Other diseases					
Yes	0 (0%)	0 (0%)	2 (8%)	2 (8%)	0 (0%)
No	25 (100%)	25 (100%)	23 (92%)	23 (92%)	25 (100%)
Variables	LBT (n = 25, 20.0%)	LAT (n = 25, 20.0%)	HBT (n = 25, 20.0%)	HAT (n = 25, 20.0%)	Control (n = 25, 20.0%)

BMI, body mass index; HAT, high-grade after treatment; HBT, high-grade before treatment; IQR, interquartile range; LAT, low-grade after treatment; LBT, low-grade before treatment.

Analysis of *miR-143-5p* revealed a general trend of decreased expression with age, particularly in HAT and LBT groups. The participants in the control group exhibited relatively low expression levels across both age groups, while HBT and LAT samples showed greater variability, with some instances of high expression in the adult group. The participants in the control group displayed consistently low expression levels across the entire age spectrum. This suggests a potential age-dependent downregulation of *miR-143-5p*, particularly within specific sample types. For *HIF1A*, levels remained relatively stable across age groups in control, HAT, and LBT groups. However, HBT and LAT exhibited greater variability, with some instances of high expression in the adult group, possibly indicative of varying levels of cellular stress or hypoxia. The observed age-related changes in *miR-143-5p* and *miR-744-5p* levels indicate possible dysregulation of these regulatory RNAs, which play crucial roles in gene expression and cellular function. *MiR-744-5p* displayed a more heterogeneous pattern, with some groups showing a slight decrease with age, while others exhibited a slight increase. The LBT and control groups remained relatively stable across the age groups, indicating a more complex and nuanced relationship between age and *miR-744-5p* expression, likely influenced by sample-specific factors (Table 3).

Table 3. Age-Stratified Expression of *miR-143-5p*, *miR-744-5p*, and *HIF1A* in Patients With BC and the Participants in the Control Group

Sample type	Age group	*miR-143-5p*	*miR-744-5p*	*HIF1A*
Control	Adult	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
	Old	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
HAT	Adult	0.65 (0.37–2.89)	0.56 (0.10–1.08)	2.10 (1.46–3.39)
	Old	0.52 (0.19–2.16)	0.52 (0.31–3.07)	2.17 (1.31–2.71)
HBT	Adult	0.48 (0.13–1.01)	0.16 (0.07–0.54)	2.77 (2.01–2.99)
	Old	0.11 (0.05–0.34)	0.10 (0.01–0.16)	2.87 (2.43–3.23)
LAT	Adult	0.63 (0.08–0.85)	0.08 (0.07–0.79)	1.10 (0.85–1.48)
	Old	0.04 (0.01–0.10)	0.08 (0.02–0.26)	0.86 (0.73–1.19)
LBT	Adult	18.92 (0.55–37.90)	0.48 (0.08–6.84)	0.60 (0.55–0.70)
	Old	3.43 (0.86–21.71)	0.22 (0.04–3.63)	0.78 (0.66–0.82)

BC, breast cancer; HAT, high-grade after treatment; HBT, high-grade before treatment; HIF1A, hypoxia-inducible factor 1-alpha; IQR, interquartile range; LAT, low-grade after treatment; LBT, low-grade before treatment; miR, microRNA.
Data are presented as median (IQR).

Biomarker expression analysis across BMI and cancer grades

This study examined biomarker expression levels, which included 2 microRNAs (*miR-143-5p*, *miR-744-5p*) and *HIF1A* in individuals classified by BMI as either overweight or obese, with further stratification by sample type: control, HAT, HBT, LAT, and LBT. BMI was categorized based on the

WHO criteria: underweight (BMI < 18.5), normal weight (18.5–24.9), overweight (25.0–29.9), and obese (≥30.0). Analysis of *miR-143-5p* expression revealed higher levels in obese compared with overweight participants, particularly in HAT and LBT groups, while control samples showed consistently low expression. In contrast, *miR-744-5p* exhibited a complex pattern: expression was higher in obese participants for HBT and LAT groups but lower in HAT groups, with controls consistently low. These nuanced patterns suggest a variable relationship with BMI. *HIF1A* expression was elevated in obese participants, most notably in HBT and LAT groups, with low expression in controls (Figures 1 and 2).

miR-143-5p showed substantially elevated expression levels in HAT and LBT samples compared with control, HBT, and LAT samples. This observation suggests a potential role for *miR-143-5p* in the biological processes or characteristics specific to HAT and LBT. In contrast, *miR-744-5p* did not show discernible differences in expression across the different groups. This suggests that the expression of *miR-744-5p* is likely not influenced by the factors that differentiate these sample groups. *HIF1A* exhibited markedly higher expression in HBT samples compared with control and LAT samples. While HBT and LBT samples also showed higher *HIF1A* levels compared with the control group, these differences did not reach statistical significance.

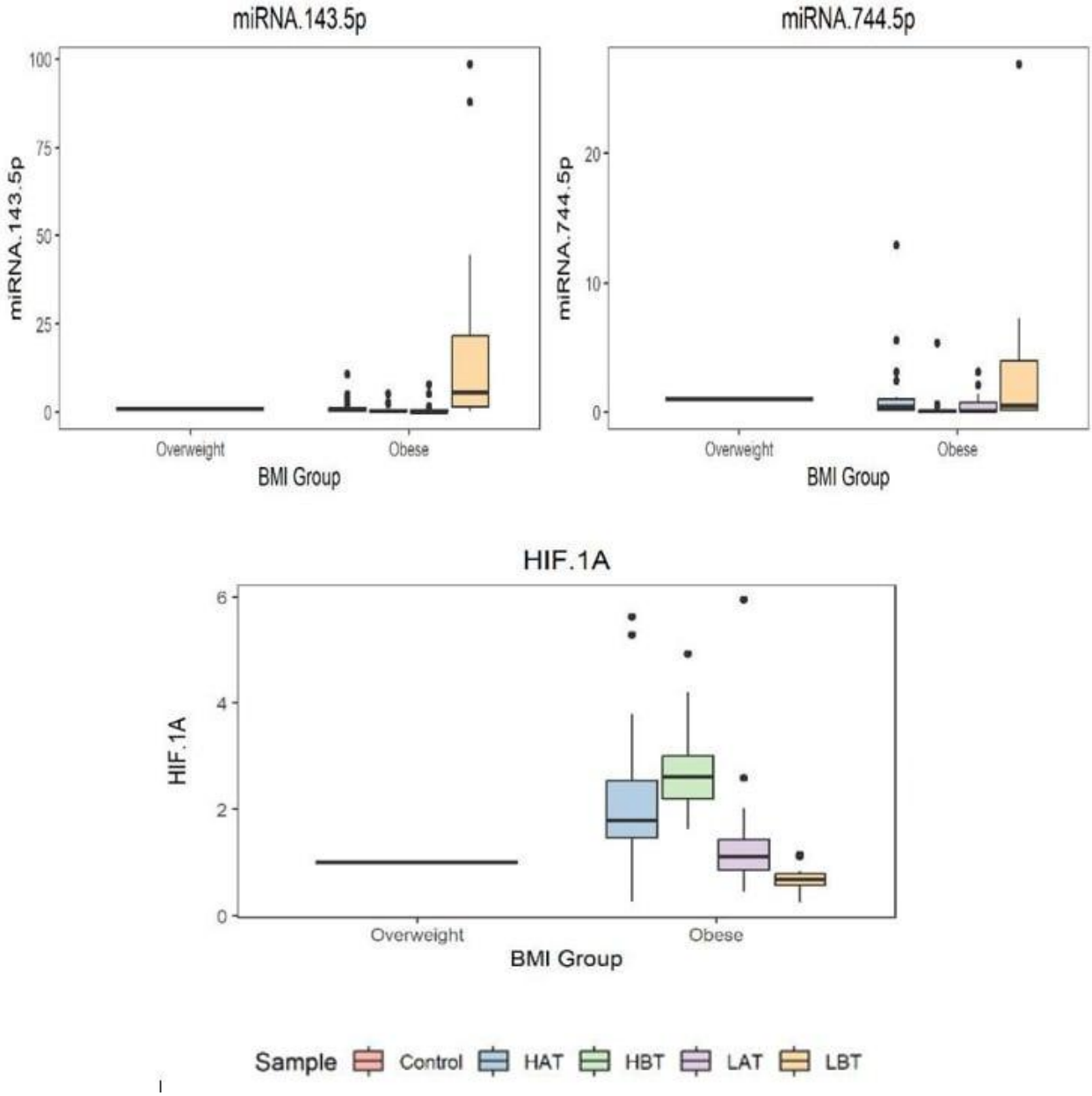

Figure 1. Biomarker expression across BMI values and cancer grades

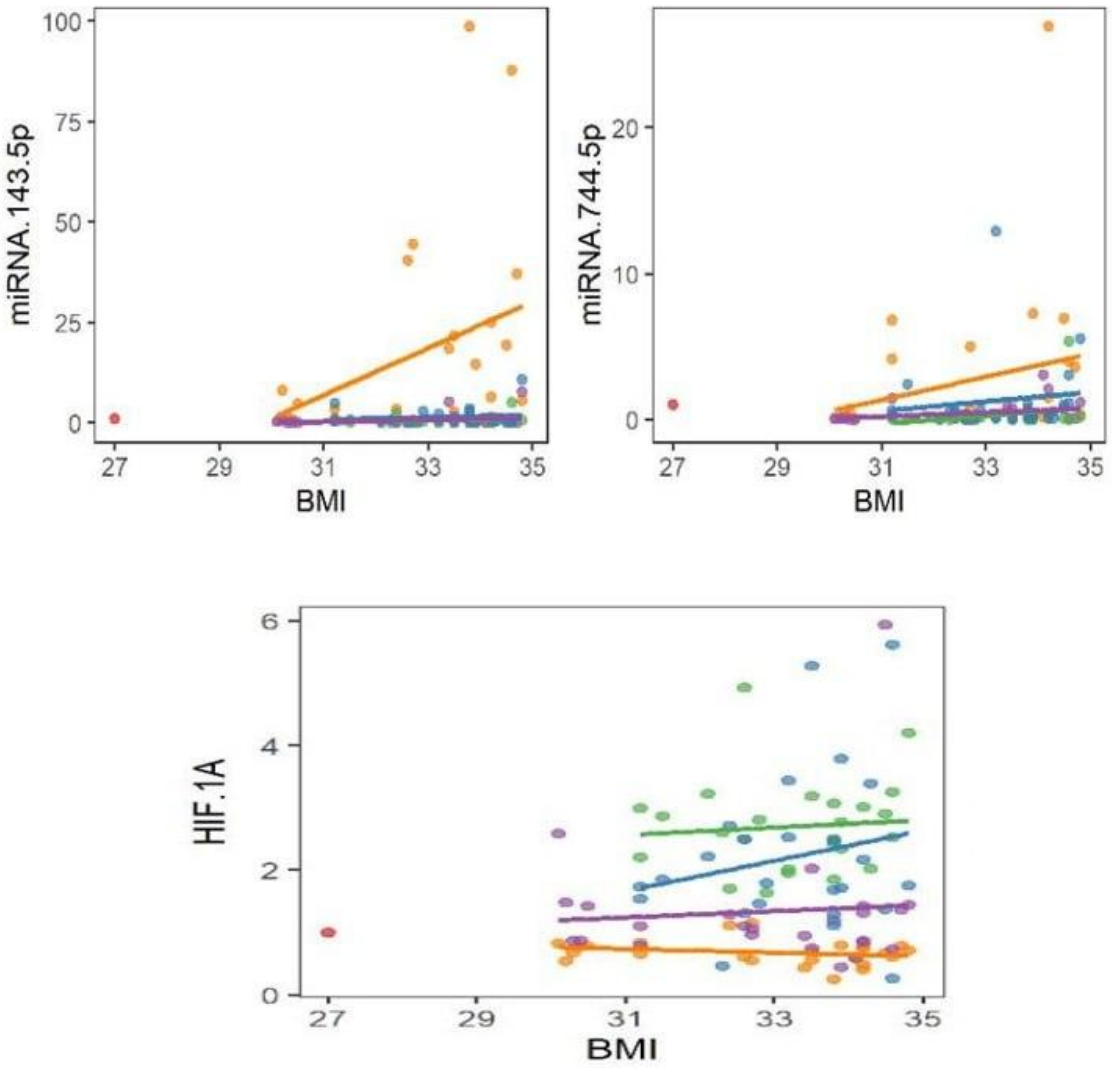

Figure 2. Expression of biomarkers across different sample types, categorized by BMI. Panel A shows the expression of miR-143-5p, Panel B shows miR-744-5p, Panel C shows HIF-1A

Correlation analysis of biomarkers across BC cohorts

In the LBT group, the correlation between *miR-143-5p* and *HIF1A* was −0.52, suggesting a negative correlation between them. In the control group, *miR-143-5p* exhibited a strong, highly significant positive correlation with *miR-744-5p* ($r = 0.88$, $P < 0.001$), suggesting co-expression. However, no significant correlations were found with *HIF1A* (control: $r = -0.32$, $P = 0.23$). Correlation analysis within the LAT group revealed a moderate positive correlation between *miR-143-5p* and *miR-744-5p* ($r = 0.22$). The correlations between *miR-143-5p* and *HIF1A* ($r = 0.01$) and between *miR-744-5p* and *HIF1A* ($r = -0.03$) were negligible and not statistically significant ($P = 0.89$). In contrast, a strong positive correlation was observed between *miR-143-5p* and *miR-744-5p* in the HBT group ($r = 0.835$). A strong positive correlation was observed between *miR-143-5p* and *miR-744-5p* in the HAT group ($r = 0.83$), indicating that their expression levels tend to change in the same direction. In contrast, correlations between *HIF1A* and both miRNAs were negligible and not statistically significant in all groups analyzed (HAT group: *miR-143-5p* vs *HIF1A*, $r = 0.10$; *miR-744-5p* vs *HIF1A*, $r = -0.03$, $P = 0.89$; control group: *miR-143-5p* vs *HIF1A*, $r = -0.25$, $P = 0.32$; *miR-744-5p* vs *HIF1A*, $r = -0.32$, $P = 0.23$).

Diagnostic Performance of BC Biomarkers

Our analysis revealed strong diagnostic performance for specific biomarkers in distinct patient subgroups. *MiR-143-5p* in the LBT group demonstrated exceptional potential as a standalone diagnostic test (AUC = 0.83). *HIF1A* in the HBT group also showed reliable performance (AUC = 0.77). While slightly lower than the top biomarkers in Table 4, its strength suggests it would be a valuable contributor to a multi-biomarker panel for BC diagnosis.

miR-143-5p (AUC = 0.66) and *HIF1A* (AUC = 0.65) in the HAT group, along with *miR-744-5p* in the LBT and HAT groups (AUC = 0.63 and 0.60, respectively), demonstrated only moderate discriminatory power. Clinically, such markers are generally not suitable for standalone diagnosis. However, they should not be dismissed entirely, as they could still contribute valuable information to a multivariate diagnostic algorithm where the combination of several moderate markers can achieve high overall accuracy.

Table 4. Comparative Evaluation of *miR-143-5p*, *miR-744-5p*, and *HIF1A* in BC

Comparison	Biomarker	AUC	95% CI
LBT vs Control	*miR-143-5p*	0.83	0.68–0.94
	miR-744-5p	0.63	0.44–0.80
	HIF1A	0.15	0.04–0.31
LAT vs Control	*All Biomarkers*	<0.40	NA
HBT vs Control	*miR-143-5p*	0.44	0.26–0.62
	miR-744-5p	0.33	0.16–0.52
	HIF1A	0.77	0.61–0.91
HAT vs Control	*miR-143-5p*	0.66	0.47–0.82
	miR-744-5p	0.60	0.41–0.78
	HIF1A	0.65	0.46–0.81

AUC, area under the curve; BC, breast cancer; CI, confidence interval; HAT, high-grade after treatment; HBT, high-grade before treatment; HIF1A, hypoxia-inducible factor 1-alpha; LAT, low-grade after treatment; LBT, low-grade before treatment; miR, microRNA; NA, not applicable.

DISCUSSION

This study aimed to analyze the expression of a panel of biomarkers, including *miR-143-5p*, *miR-744-5p*, and *HIF1A*, across different BC cohorts (HAT, HBT, LAT, and LBT) and to identify potential associations between these biomarkers and specific cancer grades. Crucially, miRNA expression patterns are not uniform across different breast tumor stages (luminal A, luminal B, basal-like, and $HER2^{+}$), highlighting the importance of cancer stage classification in miRNA studies.[14] Histological grade has also been identified as a major source of variation in BC miRNA expression, potentially even more influential than other factors.[15] Our findings revealed distinct patterns of expression for these biomarkers in relation to age, BMI, and cancer grade, suggesting complex regulatory mechanisms and potential roles in the biological characteristics of each subgroup. *MiR-143-5p* expression revealed higher levels in the obese group, particularly in the HAT and LBT sample types. In contrast, the control samples exhibited consistently low expression. This suggests a potential positive correlation between BMI and *miR-143-5p* expression.[16] *miR-744-5p* exhibited higher expression in specific sample types (HBT and LAT) within the obese group, while other samples (HAT) and the control group showed lower expression levels. Previous studies have reported alterations in microRNA expression in several diseases, including BC and obesity.[17]

Regarding BMI and age, the control group had the lowest median BMI and age, with narrower IQRs, while BC cohorts showed higher median BMIs and ages with broader IQRs. The age distribution of patients with BC in this study is comparable to previous Iraqi research[18,19], which identified age ≥50 years as a significant risk factor. According to these findings[20,21], the risk of BC can occur at any age, but it increases in middle age and beyond the age of 50. *HIF1A* exhibited higher expression in the obese group, most notably in HBT and LAT samples, suggesting a potential link between hypoxia and this specific cohort (high-grade before treatment). This is a crucial finding, as it suggests HBT tumors may possess a more hypoxic microenvironment, potentially driving their aggressive behavior. In contrast, *HIF1A* levels were stable in control, HAT, and LBT samples but exhibited greater variability in HBT and LAT groups. Obesity-related increased expression of *HIF1A* in HBT and LAT samples suggests a potential link between obesity and increased cellular stress or hypoxia. In this study, *HIF1A* expression showed moderate significance with (AUC > 0.60) in high-grade cancer before and after treatment. This partially agrees with the results of another study[22], which indicated that the *HIF1A* gene can be a good diagnostic marker. Our analysis showed significant distributional disparities. Family history was found to be imbalanced, with 78% reporting no disease history and 22% reporting a disease history. The "other diseases" variable was similarly skewed, with 97% indicating no comorbidities. The noticeable difference in counts between family history categories suggested that family history plays a significant role in diagnosis and treatment of BC.[23]

Our correlation analysis revealed distinct co-expression patterns for *miR-143-5p*, *miR-744-5p*, and *HIF1A* across patient subgroups, suggesting differential regulatory mechanisms based on disease state and treatment. Notably, we observed a very strong, significant positive correlation between *miR-143-5p* and *miR-744-5p* in the control ($r = 0.80$, $P < 0.001$), HBT ($r = 0.835$), and HAT ($r = 0.83$) groups. This consistent synchrony suggests a potential co-regulation or shared regulatory pathway for these 2 miRNAs in the absence of advanced disease or following certain treatments.

In stark contrast, these relationships were attenuated in the LAT group ($r = 0.22$), implying that treatment and/or tumor grade may disrupt this coordinated expression. Furthermore, correlations between the miRNAs and *HIF1A* were consistently negligible or very weak across all groups (e.g., $r = 0.01$ to 0.10) and were not statistically significant. The lack of a strong linear relationship suggests that *HIF1A* expression is likely independent of these specific miRNAs in this cohort or that its regulation involves more complex, nonlinear interactions.

Significantly higher levels of *miR-143-5p* observed in LBT (low-grade before treatment) and HAT (high-grade after treatment) point to a possible function in the pretreatment condition or in response to treatment. *miR-744-5p* demonstrated a moderate diagnostic potential in LBT and HAT comparisons, with AUC values exceeding 0.60, suggesting potential diagnostic utility when combined with other markers. Increasing evidence has shown that miRNAs function as oncogenes or anti-oncogenes and are involved in all types of important physiological processes in cancer initiation, progression, treatment, and drug resistance.[24] MicroRNAs have emerged as important posttranscriptional regulators of *HIF1A*. Several studies have demonstrated that specific miRNAs can directly target the 3′ untranslated region (3′ UTR) of *HIF1A* mRNA, thereby modulating its expression and activity. Among these, *miR-143-5p* has been shown to function as a tumor suppressor by downregulating *HIF1A*. Previous studies have suggested that certain miRNAs may influence hypoxia responses through posttranscriptional regulation of *HIF1A*.

Analysis of AUC values revealed significant differences in the diagnostic potential of 3 biomarkers across 4 sample comparisons in each cancer group compared with controls. *miR-143-5p* showed high diagnostic accuracy, with an AUC value of 0.83 in the LBT group (AUC > 0.70 indicates good diagnostic accuracy). *MiR-744-5p* showed a moderate potential in the LBT vs control comparison (an AUC of 0.63). *HIF1A* exhibited a high diagnostic potential in the HBT group with an AUC value of 0.774 and a moderate diagnostic potential in the HAT group with an AUC value of 0.647.

The remaining measurements of biomarkers across the other sample comparisons displayed a low diagnostic accuracy, with AUC values below 0.50, indicating poor discriminatory ability.

CONCLUSION

The current study showed the importance of evaluating different miRNAs and *HIF1A* expression as potential predictive diagnostic markers for the early detection of BC. In addition, we evaluated the role of these biomarkers in different grades and treatments. Relative gene expression analysis (fold change) suggested that *miR-143-5p*, *miR-744-5p*, and *HIF1A* could potentially form reliable diagnostic panels for BC, particularly when combined with other biomarkers.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests.

ETHICAL CONSIDERATIONS

This study was approved by the Council of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies at the University of Baghdad, and the study protocol was approved by the Ethics Committee of the Ministry of Health and Environment–Al-Anbar Directorate of Health (AO No. 1812).

FUNDING

The research is self-funded.

DATA AVAILABILITY

The data related to the study are presented in the article.

AI DISCLOSURE

No AI tool was used for preparing the manuscript of this study.

AUTHOR CONTRIBUTIONS

HMH: Data curation, Methodology, Investigation, Writing – original draft, Visualization. MNR: Supervision, Resources, Validation, Writing – review & editing. AAS: Conceptualization, Project administration, Supervision, Writing – review & editing.

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