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Identification of miR-1256 Signature as a Promising Diagnostic Biomarker in FFPE Tissues of Breast Cancer Patients

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ABSTRACT

MicroRNAs (miRNAs) have emerged as promising biomarkers in cancer diagnosis and prognosis, including breast cancer (BC). In this study, we examine whether miR-1256 may be regarded as a powerful biomarker for predicting the prognosis of BC. The expression of miR-1256 was detected in 30 formalin-fixed paraffin-embedded (FFPE) tissue pairs of tumoral samples and their non-tumoral tissues using qRT-PCR. The clinicopathological characteristics of patients relative to miR-1256 expression, along with fold change analysis employing the 2-ΔΔCT method, were also investigated. All statistical analyses were conducted using GraphPad Prism and MedCalc. We found that the miR-1256 level in BC FFPE tissues is notably increased with $p < 0.006$ and a (2.5367)-fold change compared to control tissues. Also, we analyzed the association between the expression level of miR-1256 and the clinicopathological parameters of BC patients. The overexpression of miR-1256 exhibited no significant correlation with age, tumor grade, tumor size, estrogen receptor (ER) status, human epidermal growth factor-2 (Her-2), or tumor, node, metastasis (TNM) status among patients. The progesterone receptor (PR) status suggested a potential trend ($P = 0.077$), indicating that low miR-1256 expression was more common in PR-positive subjects. Moreover, the p-value, area under the curve (AUC), Std. Error, sensitivity, and specificity are (0.7039, 0.06709, 0.67, and 0.7, respectively), this signifies a moderate capacity of the test to differentiate between tumors with controls. The results of fold change analysis employing the 2-ΔΔCT method indicated a (2.5367)-fold elevation in miR-1256 expression in tumors relative to controls. This suggests that miR-1256 may have an oncogenic role in carcinogenesis, potentially promoting BC development. As a result, we demonstrated that elevated miR-1256 expression correlates with the progression of BC, indicating that miR-1256 may be associated with oncogenic processes in BC tumorigenesis and progression.

1. Introduction

Breast cancer (BC) is the predominant cause of morbidity in women diagnosed with cancer (Afifi et al., 2020). Despite considerable progress in the early diagnosis of BC and the growing availability of treatments, including surgical resection (Moore-Palhares et al., 2024), radiotherapy (Kaidar-Person et al., 2024), endocrine therapy (Ma et al., 2024), and immunotherapy (Michaels et al., 2024), patient's prognosis remains unfavorable due to the elevated incidence of distant metastases associated with BC. In BC, various biomarkers, such as tissue (Zakic et al., 2024) and plasma proteins (Iweala et al., 2024), as well as nucleic acids including miRNAs (Baylie et al., 2024), have been investigated for the early detection of BC, but only a few have been used in clinical practice.

MicroRNAs (miRNAs) are a class of endogenous elements that are widely distributed throughout the genomes of higher eukaryotes (Yadav et al., 2024), with a length of about 20~25 nucleotides (Hou et al., 2024). They participate in gene expression and repression and can modulate various pathways (Kumar and Ranga, 2025). Given that multiple miRNAs can target a specific gene, they collectively form a complex regulatory network, thereby coordinating the intricate system of eukaryotic cellular function (Zhang et al., 2021). Investigations in BC have underscored significant aspects of miRNA participation, including distinct signatures in particular subtypes, their role in the stemness of tumor-initiating cells, and their influence on therapy-resistant BC (Abolhasanzadeh et al., 2024). Consequently, elucidating the function of miRNAs in this cancer presents a viable approach to discovering innovative therapeutics for BC. To achieve this objective, we planned the present investigation to assess miR-1256 expression in paired tumoral and non-tumoral tissues obtained from patients.

MiR-1256 is a newly recognized miRNA encoded by a gene on chromosome 1p36.12, which has only one transcript (NR_031657.1) (based on the NCBI database) (https://www.ncbi.nlm.nih.gov/gene/100302155) (Figure 1). Moreover, it was initially found to be dysregulated in prostate cancer (PCa) by Li et al. (Li et al., 2012). Additionally, the deregulation of miR-1256 was also validated in non-small cell lung cancer (NSCLC) (Liu et al., 2018a), and human colorectal cancer (CRC) (Liu et al., 2018b). These findings indicated that miR-1256 may act as an important regulator in human malignancy.

To the best of our knowledge, this is the first study to provide important clinical evidence that miR-1256 expression was upregulated in tumor tissue compared to non-tumoral adjacent tissue in BC and associated with poor prognosis of BC patients. Additionally, we found no substantial association between miR-1256 expression and clinicopathological parameters, except that PR status suggests that low miR-1256 expression is more common in PR-positive subjects. Furthermore, the results of fold change analysis employing the 2-ΔΔCT method indicated a (2.5367)-fold elevation in the expression level of miR-1256 in tumors relative to controls. This suggests that miR-1256 may have an oncogenic role in carcinogenesis, potentially promoting BC development.

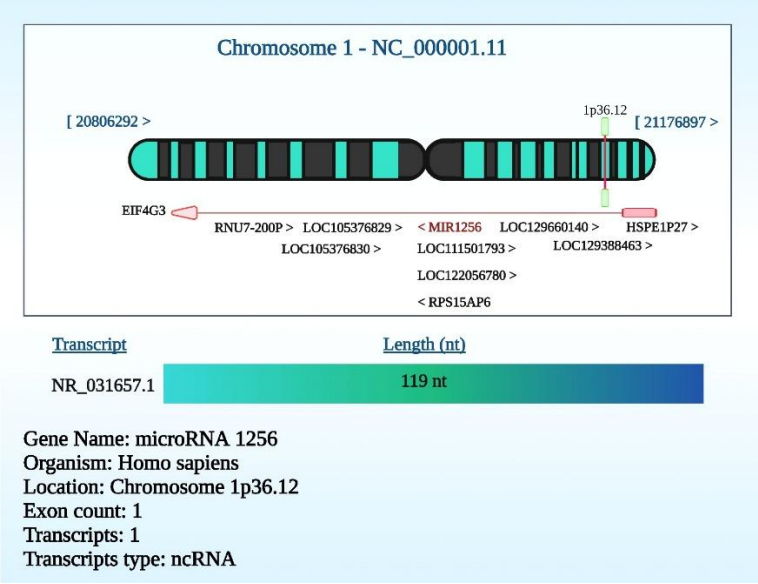

Figure 1 Showed that miR-1256 is located on human chromosome 1. It has only one transcript (NR_031657.1) (based on the NCBI database) (https://www.ncbi.nlm.nih.gov/gene/100302155). This figure drawn by BioRender.

2. Materials and methods

2.1. Cases

Expressions of miR-1256 were quantified in 30 formalin-fixed paraffin-embedded (FFPE) tissue pairs of tumoral samples and their adjacent non-tumoral tissues. Tissues were obtained from the Par Hospital-Erbil, Kurdistan Region, Iraq, during the period from July 2024 to October 2024. All samples were acquired according to the guidelines of Par Hospital's protocol, encompassing patient consent and specimen acquisition. The diagnosis and classification of BC patients were according to the Tumor-Node-Metastasis (TNM) system of the American Joint Committee on Cancer (AJCC). All cases were diagnosed with histologically and clinically confirmed stages I, II, and III of BC. The clinical characteristics of the included patients are listed in (Table 1).

Table 1: Clinical and demographic characteristics of the included patients.

No	Age	Size (mm)	Invasive Carcinoma	Grade	Stage	TNM Stage	Deep margin	ER	PR	Her-2	Lymph Node	Ki67	Breast Lesion	Calcification
1	40	30	Ductal	III	IIIA	pT2 N2 Mx	Excised by 6 mm	+	-	-	+	+	Fibrocystic change	-
2	56	26	Ductal	II	IIB	pT2 N1 Mx	Widely excised	+	-	-	+	-	Fibrocystic change	-
3	55	35	Ductal	II	IIB	pT2 N1 Mx	Excised by 3mm	+	-	-	+	-	Normal breast tissue	N/A
4	57	24	Ductal	II	IIIA	pT2 N2 Mx	Widely excised	+	+	-	+	-	Florid hyperplasia and foci of atypia	N/A
5	58	28	Ductal	II	IIB	pT2 N1 Mx	Widely excised	-	-	+	+	N/A	Fibrosis and benign microcalcifications	N/A
6	60	30	Ductal	I	IIA	pT2 N0 Mx	Excised by 10mm	-	-	-	-	-	N/A	N/A
7	57	22	Ductal	II	IIIA	pT2 N2 Mx	Widely excised	+	+	-	+	-	Nothing significant	-

The clinicopathological parameters and their relation with miR-1256 expression level, categorized as either low or high expression, are shown in (Table 2).

2.2. RNA extraction and reverse transcription

Total RNA was extracted from 30 FFPE tissue pairs of tumoral samples and their adjacent non-tumoral tissues utilizing the miRNeasy FFPE kit (Qiagen, catalog no: 217504) RNA quality and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific), with purity verified by the A260/A280 ratio, ensuring values between 1.8 and 2.0. Reverse transcribed into cDNA utilizing the miScript II RT Kit (cat. nos. 218161).

8	77	30	Ductal	II	IIA	pT2 N0 Mx	Widely excised	+	+	-	-	-	Fibrocystic change	-
9	58	6	Ductal	II	IA	pT1 N0 Mx	Excised by 10mm	-	-	-	-	+	Nothing significant	N/A
10	61	15 & 5	Ductal	II	IIIA	pT1 N2 Mx	Widely excised	+	+	+	+	N/A	Fibrocystic change	+
11	53	40	N/A	N/A	N/A	pTis N0 Mx	Excised by 7mm	-	-	+	-	-	Fibrocystic change and hyperplasia	+
12	60	27	Ductal	II	IIA	pT2 N0 Mx	Widely excised	+	+	-	-	-	Two small fibroadenomas	-
13	62	32	Ductal	II	IIA	pT2 N0 Mx	Excised by >10mm	+	+	-	-	-	Fibrocystic change	+
14	59	25	Both	II	IIIA	pT2 N2 Mx	Widely excised	+	+	-	+	-	Fibrocystic change	-
15	52	40	N/A	N/A	N/A	ypTis N1 Mx	Widely excised	+	+	-	+	-	Fibrocystic change and sclerosing adenosis	+
16	68	25	Ductal	III	IIIA	pT2 N2 Mx	Widely excised	-	-	+	+	+	Fibrocystic change	-
17	66	15	Ductal	II	IA	pT1 N0 Mx	Widely excised	+	+	-	-	+	Nothing significant	-
18	67	28	Ductal	II	IIB	T 2 N1 Mx	Widely excised	+	+	-	+	-	Nothing significant	-
19	49	24	Ductal	II	IIIC	pT2 N3 Mx	Deep	+	+	-	+	-	Nothing significant	-
20	52	70	Ductal	III	IIIA	pT3 N2 Mx	Deep	+	+	+	+	+	Fibrocystic change	+
21	49	25	Ductal	II	IIA	pT2 N0 Mx	Widely excised	+	+	-	-	-	Fibrocystic change, hyperplasia, and adenosis	+
22	45	21	Lobular	III	IIA	pT2 N0 Mx	Excised by 5mm	+	+	-	-	+	Fibrocystic change	-
23	51	55	Ductal	II	IIIA	pT3 N2 Mx	Widely excised	-	-	+	+	+	Fibrocystic change	-

24	54	18	Ductal	I	IIA	pT1 N1 Mx	Widely excised	+	+	-	+	-	Fibrocystic change	+
25	70	24	Ductal	II	IIA	T2 N0 Mx	Widely excised	+	+	-	+	-	Fibrocystic change	-
26	56	45	Ductal	II	IIIC	pT2 N3 Mx	Widely excised	+	+	-	+	-	Fibrocystic change	-
27	72	27	Ductal	II	IIIA	pT2 N2 Mx	Deep	+	+	-	+	-	Nothing significant	-
28	69	17	Ductal	III	IIIA	pT1 N2 Mx	Widely excised	-	-	+	+	+	Nothing significant	-
29	59	30	Ductal	II	IIA	pT2 N0 Mx	Deep	-	+	-	+	-	Fibrocystic change and sclerosing adenosis	+
30	71	30	Lobular	III	IIB	pT2 N1 Mx	Widely excised	-	-	+	+	+	Nothing significant	-

Table 2: Clinicopathological parameters and in relation to the expression level of miR-1256 among 30 cases, categorized as either low or high expression.

miR-1256 Expression level					
Parameters	**Subclasses**	**Cases**	**Low**	**High**	**P-value**
Age (years)	≥60	12	10	2	0.312
	<60	18	12	6	
Tumor grade	I	2	1	1	0.636
	II	20	14	6	
	III	6	5	1	
	N/A	2	2	0	
Tumor size (mm)	≥15	28	20	8	0.377
	<15	2	2	0	
ER status	Negative	9	7	2	0.719
	Positive	21	15	6	
PR status	Negative	11	6	5	0.077
	Positive	19	16	3	
Her2 status	Negative	22	15	7	0.290
	Positive	8	7	1	
TNM Stage	0	11	9	2	0.424
	1-3	19	13	6	

2.3. Real-time quantitative PCR

Two-step quantitative Real-time PCR (qRT-PCR) was performed from Immunogen CENTER according to the manufacturer's guidelines utilizing the miScript SYBR Green PCR kit (Qiagen, catalog no. 218073), with the manufacturer-provided miScript universal primer and miR-1256-specific forward primer. The primers for miR-1256 and the endogenous control U6 are shown in (Table 3), and the characteristics of the selected miRNA for expression assays are shown in (Table 4). The U6 RNA was chosen as an endogenous reference to determine the relative amount of miR-1256 expression in tumor tissues in comparison to control tissues using the 2−ΔΔCt approach, where [ΔCT = CT (a target miRNA) – CT (a reference gene)]. Each reaction was run in triplicate to ensure reproducibility, and no-template controls were included to monitor contamination. PCR amplification was performed under the following cycling conditions: an initial activation step at 95°C for 15 minutes, followed by 40 cycles of 94°C for 15 seconds, 55°C for 30 seconds, and 70°C for 30 seconds.

Table 3: Nucleotide sequences of primers.

miRNA & HKGs	**Sequence of the primers**	**Length of the primers**
MiR-1256	Forward: AACAAGAGGCATTGACTTCTCACT Reverse: GTGCAGGGTCGGAGGT	F: 24 nt R: 16 nt
U6	Forward: GTGCTGCTTGGGCAGCA Reverse: GAAATATGGAACGGTTC	F: 17 nt R: 17 nt

Table 4: Specific characteristics of selected miRNA for expression assays.

ID	Symbol / Gene name	Gene Type	Accession number	Location	Description
100302155	MIR1256 / microRNA 1256	ncRNA	NR_031657.1	1p36.12	MIR1256 is a microRNA associated with several cancer types and has been observed to exhibit uneven expression in patients with PCa (Li et al., 2012), NSCLC (Liu et al., 2018a), and human CRC (Liu et al., 2018b).

2.4. Statistical analyses

All statistical analyses were conducted using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA) and MedCalc version 23.1.7. The comparison of miRNA expression levels between the FFPE tissue of BC patients and the FFPE tissue of BC controls was performed using the Mann-Whitney U test. The Chi-square test (χ^2 test or X^2 test) was employed to examine the association between miR-1256 expression and clinicopathological features. Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of miR-1256 expression, and the area under the curve (AUC) was calculated. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Association of miR-1256 expression with clinicopathological features of BC patients

To examine the association between the overexpression of miR-1256 and the clinical development of BC, we assessed the association between miR-1256 expression levels and the clinicopathological characteristics of BC patients. As shown in (Table 2) and (Figure 2), no discernible difference was detected in age, tumor grade, tumor size, ER (estrogen receptor) status, Her-2, and TNM status between patients with miR-1256 overexpression. However, the PR status indicated a potential trend (P = 0.077), suggesting that low miR-1256 expression was more prevalent in PR-positive cases. Overall, none of the analyzed clinical parameters showed a statistically significant correlation with the expression of miR-1256; however, the association with PR status requires further examination.

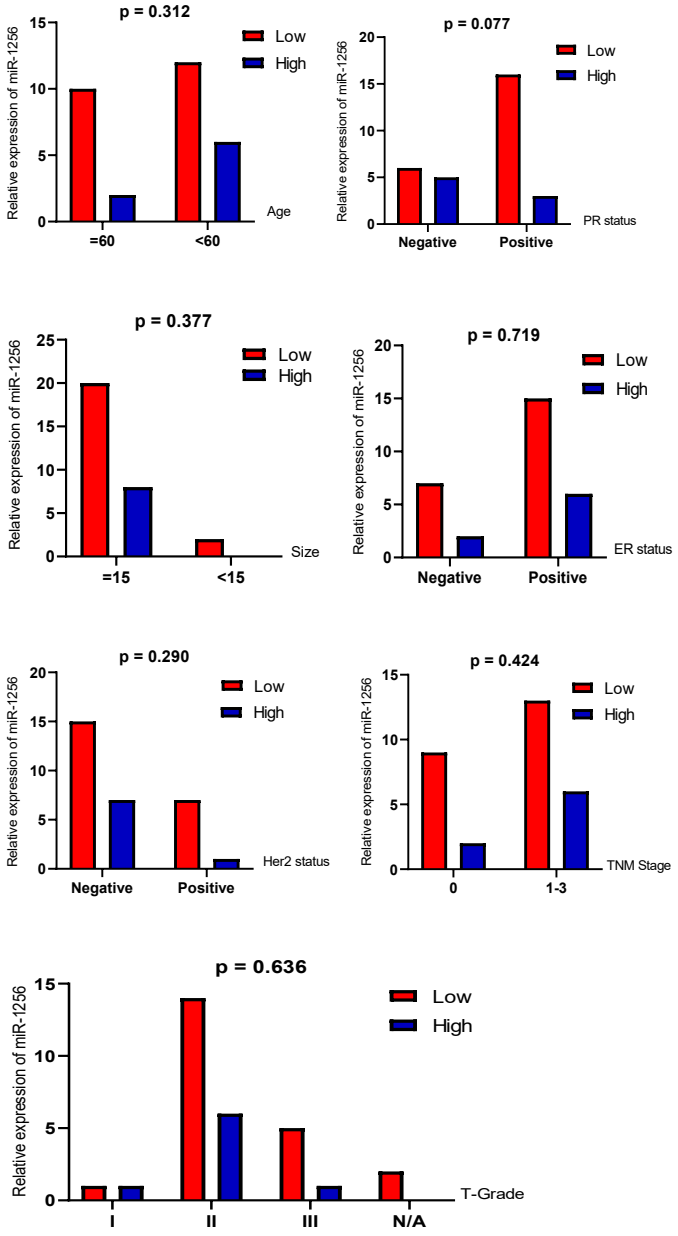

Figure 2 Shows the association between miR-1256 expression and BC clinical parameters.

3.2. Association and expression of miR-1256 and HKGs in FFPE tissues of BC

In the present study, we analyzed the expression level of miR-1256 in the FFPE tissue of BC and compared it with the corresponding expression of their adjacent normal tissues. In

order to investigate the effect of miR-1256 in the progression of BC, a qRT-PCR assay was performed to detect the expression of miR-1256 in 30 pairs of breast tumor tissues and corresponding non-tumor tissues. As shown in (Figure 3A), we found that the expression levels of miR-1256 in breast tumor tissues were significantly higher than those in matched noncancerous tissues ($p < 0.006$), which is statistically significant ($p < 0.05$), and this indicates that there is a significant difference between breast tumor tissues and corresponding non-tumor tissues. Additionally, the median of miR-1256 level was (32.36) and (34.84) in breast tumor tissues and breast control tissues, respectively. The median difference between them was (-2.48); thus, the tumor median was lower than the control median.

Moreover, we explore the expression level of housekeeping genes (HKGs) as a reference gene in our experiment to normalize gene expression data and ensure reliable comparison between the two groups. As shown in (Figure 3 B), we found that the p-value was (0.265), which is not statistically significant ($p < 0.05$), meaning that there is no significant difference between the HKGs of tumor tissue and HKGs of corresponding non-tumor tissue groups. Additionally, the median of HKGs was (35.54) and (33.97) in breast tumor tissues and breast control tissues, respectively. The median difference between the two groups was (1.575); thus, the tumor HKGs median was slightly higher than the control HKGs median.

Additionally, we evaluated the diagnostic power of miR-1256 in distinguishing between breast cancer (BC) tissues and adjacent tissues (Figure 4). The AUC is (0.7039) with the Std. Error of (0.06709), sensitivity (0.67), and specificity (0.7), this signifies a moderate capacity of the test to differentiate between patients with tumors and controls. The 95% confidence interval spans from (0.5724 to 0.8354), indicating fluctuation in the estimate while remaining above (0.5), signifying that the test outperforms random chance. The p-value of (0.007) signifies statistical significance, indicating that the test possesses discriminatory power.

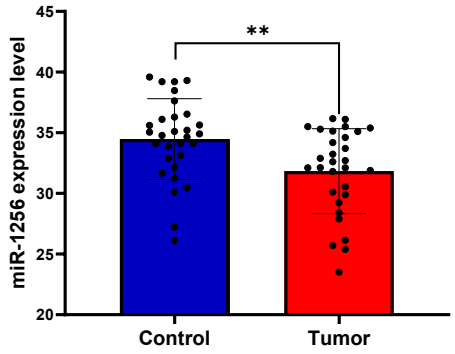

Figure 3A Relative expression level of miR-1256 in 30 FFPE tissue pairings of tumor samples and their corresponding neighboring non-tumor tissues. (**: p-value = 0.006).

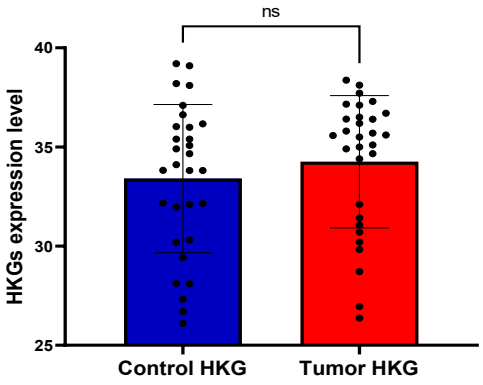

Figure 3B Relative expression level of miR-1256 HKGs in 30 FFPE tissue pairs of tumoral samples and their adjacent non-tumoral tissues. (ns: p-value = 0.265).

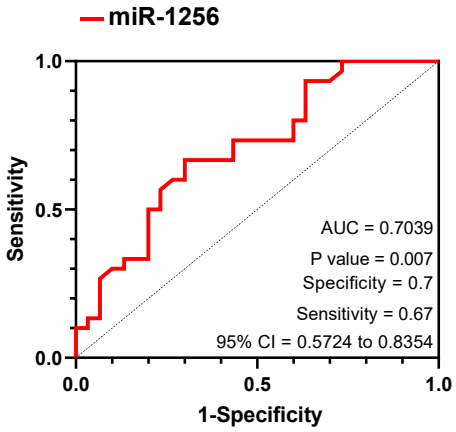

Figure 4 The ROC curve of miR-1256 expression for the differentiation of BC tissues from surrounding normal tissues. AUC indicates area under the ROC curve.

3.3. Folding change of miR-1256 utilizing $2^{-\Delta\Delta CT}$ method for qRT-PCR data analysis

The results of the qRT-PCR indicate that the miR-1256 expression is substantially elevated in BC patients compared to the control. As shown

in (Table 5), the average cycle threshold (CT) value for miR-1256 in tumor tissue was (31.833), and in the control was (33.405), as well as with the reference gene (U6) CT values of (34.252) and (34.481) in both patients and control samples, respectively. Moreover, the ΔCT calculations showed that the BC tumor group and control group had a ΔCT of (-2.419) and (-1.076), respectively. This indicates that the CT value is lower in the tumor samples compared to the control. Additionally, the ΔΔCT value, which compares the expression level of miR-1256 between tumor group and the control group, was calculated as (-1.343), further confirming an upregulation in tumors. The fold change analysis using the 2- ΔΔCT method revealed a (2.5367)-fold increase in the miR-1256 expression level in tumors compared to controls. This suggests that miR-1256 may have an oncogenic function in tumorigenesis, potentially facilitating BC progression.

Table 5: Shows the results of folding expression of miR-1256 utilizing $2^{-\Delta\Delta CT}$ method for qRT-PCR data analysis.

Equations	
ΔCT target miR-1256	ΔCT = CT (a target miR-1256) – CT (a reference gene) ΔCT (target) = 31.833 - 34.252 = -2.419
ΔCT control	ΔCT = CT (Control) – CT (control- reference gene) ΔCT (Control) = 33.405 - 34.481 = -1.076
ΔΔCT	ΔΔCT = ΔCT (a target miR1256) – ΔCT (Control) ΔΔCT = -2.419 – (-1.076) = -1.343
Folding change	Folding change = $2^{-\Delta\Delta CT} = 2^{-(-1.076)} = 2.5367$

4. Discussion

Abnormal expression of miRNAs has been revealed in various tumor tissues, and dysregulation of miRNAs is found to play an important role in the occurrence and development of different cancers (Zhang et al., 2022). BC is one of the most malignant tumors in women, which seriously affects human health and can be fatal (Aghaei, 2023). It is suggested to be a heterogeneous neoplasm involving a variety of profile alterations in both the expression of miRNA and mRNA (Nurzadeh et al., 2021).

A considerable amount of research regarding the abnormal expression levels of different miRNAs and their roles in breast cancer has been documented. For instance, certain miRNAs function as oncogenic and are expressed more frequently in breast cancer. These miRNAs decrease the expression of antioncogenes in apoptosis, metastasis, invasion, and cell proliferation. The miR-10, miR-15, miR-16, miR-17~92 cluster, miR-18, miR-19, miR-20, miR-21 family, miR-92, miR-155, and miR-569 and their families have been identified as carcinogenic miRNAs (Juneja and Shah, 2022). Likewise, elevated levels of miR-21 and diminished levels of miR-125b have been observed in breast cancer patients (Najjary et al., 2020, Wang et al., 2012). Similarly, Zhang et al. revealed that serum exosomal miR-155 and miR-1246 were up-regulated in breast cancer patients (Zhang et al., 2020). Moreover, in PI3K/AKT signaling, aberrant expression of S regulatory proteins leads to unchecked cell division and BC carcinogenesis. Master regulators, such as phosphatase and tension homolog (PTEN), are targets of several miRNAs that can stimulate the PI3K/AKT signaling pathway (Ding et al., 2019). By dephosphorylating PIP3, the phosphatase enzyme PTEN inhibits the activity of PI3K. Although PTEN was discovered to be the target of multiple miRNAs, such as miR-21, which is overexpressed in triple-negative and HER2+ (human epidermal growth factor receptor 2-positive) BCs, leading to the advancement of the tumor progression (Rahmani et al., 2020). However, recent research has shown that miRNAs may serve as ideal candidates for the development of therapeutic targets and novel biomarkers.

This study quantified and statistically evaluated the expression levels of miR-1256 in FFPE tissues from 30 FFPE pairs of BC. We found that miR-1256 showed higher expression levels in BC patients when compared with control tissues ($p < 0.006$), which might imply that miR-1256 might be an onco-miRNA in BC and a good potential candidate for the development of novel biomarkers in BC.

Moreover, the association between miR-1256 expression and the clinicopathological features of BC patients was also examined. No significant

difference was observed in age, tumor stage, size, ER status, and TNM status between patients with and without miR-1256 overexpression, except PR status, which indicated a potential trend (P = 0.077), suggesting that low miR-1256 expression was more prevalent in PR-positive cases. This suggests that high miR-1256 expression may be associated with a high degree of malignancy in BC, which warrants further investigation. Our observations and analyses of miR-1256 expression and clinical characteristics suggest that the absence of association between miR-1256 and clinicopathological features may indicate that the mechanisms underlying miR-1256 overexpression differ from those of other clinical factors in breast cancer (BC), rendering it a potential candidate for an independent diagnostic and prognostic marker distinct from established biomarkers.

In addition, the average CT value for miR-1256 in tumor tissue was lower than in the control group. Thus, the ΔCT calculations showed a higher CT value in the tumor samples compared to the control samples. As a result, the fold change analysis using the 2- ΔΔCT method revealed a (2.5367)-fold increase in the expression level of miR-1256 in tumors compared to controls. This suggests that miR-1256 may have an oncogenic function in BC tumorigenesis.

However, our study has some limitations. First, the sample sizes for FFPE tissues are relatively small. It is challenging to reach a conclusive determination regarding the accuracy of miR-1256 in identifying BC from various sample sources. Thus, additional assessments of the efficacy of miR-1256 from various sample sources as biomarkers for BC detection should be conducted. Second, the investigation of the clinical application of miR-1256 was complicated by the absence of research assessing its diagnostic performance for various ethnic groups. Additional research should be done to assess miR-1256's diagnostic precision for various ethnic groups. Furthermore, there is significant heterogeneity in the detection of BC because all of the cases included for the data pooling include patients with different TNM stages (from stage I to stage IV). It would be extremely challenging to determine whether miR-1256 is a useful marker in the early detection of BC in the absence of patient-level data. Moreover, we acknowledge that the current findings are correlative and do not establish a causal or functional role of miR-1256 in breast cancer progression. Although our data suggest a potential oncogenic implication based on expression patterns, no in vitro or in vivo functional validation was performed to confirm this role. Therefore, we have revised our interpretation to reflect the exploratory scope of the study and recommend future experimental investigations to validate the biological and mechanistic function of miR-1256 in tumorigenesis.

In conclusion, we established that the upregulation of miR-1256 in FFPE tissues may serve as a biomarker for diagnosing BC patients, representing a significant resource for biomarker identification and BC validation. Notable findings will encourage additional research with stringent criteria and extensive study populations to clarify any lingering debate on the diagnostic significance of miR-1256 in BC patients.

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Potential conflicts of interest: The authors declare that they have no conflict of interest.

Authors' contributions: All authors contributed to the study's conception and design. H.J.H. supervised the study. S.R.A. collected the data, wrote the first draft of the manuscript, designed and drew tables, and illustrated the figures. H.J.H. reviewed and edited the draft. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate: The study received ethics approval, and it was carried out according to the standards of Salahaddin University's Ethics Committee (approved no. 45/385; 2025), and the study was conducted according to the principles of the Declaration of Helsinki. All enrolled patients signed informed consent forms. Specimens were collected directly from the hospital.

Data Availability Statement: Applicable.

Research

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