

Evaluation of miRNA-21 and miRNA-155 Expressions in Breast Carcinoma: Promising Diagnostic Tumor Markers: A Cross-Sectional Study

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Abstract

Objective: This study aimed to evaluate the expression of miRNA-21 and miRNA-155 in breast cancer in patients as well as their potential value as molecular biomarkers in Duhok Government, Kurdistan Region, Iraq.

Methods: Levels of miRNA-21 and miRNA-155 were assessed in pre-operative sera, neoplastic and adjacent non-neoplastic tissue samples from breast cancer patients, and sera of healthy controls using quantitative real-time PCR (qRT-PCR). Relative expression was calculated. Receiver operating characteristic (ROC) curve analysis was done for testing the diagnostic accuracy of each miRNA separately and together.

Results: Both miRNA-21 and miRNA-155 were significantly upregulated in sera of cancer patients compared to control sera and neoplastic tissue compared to adjacent non-neoplastic tissue (*P*-value: sera: 0.002 and ≤ 0.0001 ; tissue: 0.015 and ≤ 0.001 , respectively). ROC curve analysis demonstrated good diagnostic performance for each of miRNA-21 and miRNA-155 in sensitivity and specificity for sera (89.7%, 82.8%; 70.0%, 90.0%) and tissue (96.4%, 50.0%, 42.9%, 100%) respectively.

Conclusion: The significant overexpression of both biomarkers in sera sample compared to controls and neoplastic compared to non-neoplastic tissues supports their potential role as diagnostic biomarkers; however, these two combined analyses may provide a more accurate non-invasive approach to early detection of breast cancer.

Keywords: miRNA-21, miRNA-155, breast carcinoma, tumor biomarkers, diagnostic markers

Introduction

In most countries around the globe, breast cancer is the most frequent cancer among women. In 104 countries across the world, breast cancer ranks as one of the top killers of women from malignant tumors.¹ It is the fifth most common cause of cancer deaths in the world, with about 685,000 fatalities each year.²

As per the latest update from the Iraqi Cancer Registry, breast cancer constituted 21.2% of all cases, thereby being the most frequently reported malignancy among all cancers in Iraq.³

The targets of miRNA-21 include a set of tumor suppressor genes like TPM1, TIMP3, RECK, the p53 network, Cdc25A, PTEN, PDCD4, and Maspin. This miRNA-21 has been extensively researched in cancer and is known to be a possible biomarker for diagnosis, prognosis, and prediction across various types of cancer, particularly breast cancer.⁴ miRNA-21 can initiate the growth and transformation of breast cells by inhibiting the translation of the tumor suppressor gene PDCD4, which plays a role in regulating apoptosis.

The expression of miRNA-21 in breast cancer tissues has shown some variability; however, it tends to be more stable when found in blood circulation. miRNA-155 is among the key oncomiRs in human cancers, including breast cancer.⁵ In breast cancer tissues, the expression level of miR-155 was significantly higher compared to normal tissues. Similarly, the level of plasma miR-155 in breast cancer patients was significantly elevated compared to healthy controls.⁶

Oncogenic miRNA-155 has been linked to clinicopathologic markers, tumor subtype, and poor survival in breast cancer. It has also been implicated in the invasiveness and recurrence of breast tumors. More specifically, an increase in the level of expression of miRNA-155 is related to a high

tumor grade, an advanced stage of the disease, and lymph node metastasis.⁷

However, it is important to recognize that these miRNAs lack high specificity for diagnostic use, while the miRNAs offer advantages, including stable quality, straightforward sample collection, and abundant sources of samples.⁸

This study is considered the first study conducted on miRNAs, particularly miRNA-21 and miRNA-155, as breast cancer tumor biomarkers in Duhok Government, Kurdistan region, Iraq, also evaluating the level of both miRNA-21 and miRNA-155 in fresh tissue samples in comparison to sera samples, in addition to performing combined ROC analysis for both miRNAs to clarify their clinical applicability.

This study aimed to assess and evaluate miRNA-21 and miRNA-155 as diagnostic tumor biomarkers in patients with breast carcinoma in the Kurdistan region of Iraq.

Method and Material

This study was a cross-sectional one performed at the University of Duhok, College of Medicine, Department of Medical Chemistry. The Medical Ethics Committee of the College of Medicine and the Research Ethics Committee at the Duhok Directorate General for Health approved it (reference number 29052024-4-1), in addition to an informed consent obtained from all participants prior to enrollment in this study.

Total samples used in this investigation were 160 samples collected from 40 breast carcinoma patients (including 40 fresh neoplastic tissues, 40 adjacent non-neoplastic tissues, and 40 preoperative serum samples) and 40 serum samples from apparently healthy controls between September 1, 2024, and May 31, 2025.

Inclusion Criteria: pre-operative sera and fresh samples taken during lumpectomy, partial or total mastectomy, from patients previously diagnosed with malignant breast carcinoma, preserved directly in TRIzol.

Exclusion Criteria: Tru-cut biopsy cases and other cases with inadequate information were excluded.

Patients' information included clinical data and histological data (type, grade, stage, lymphovascular invasion, lymphocytic infiltration, perineural invasion, and dermal invasion). Of the included patients, 24 (60.0%) received neoadjuvant chemotherapy; 16 (40.0%) did not.

The molecular study consisted of many steps, including the preservation of serum and tissue samples by transferring samples to Eppendorf tubes containing 500 µl TRIzol (Invitrogen, USA) and keeping them at -80°C . Extraction of RNA proceeded by using the TRIzol[®] LS Reagent according to the procedure provided by the manufacturing company (Invitrogen, USA). Then, miRNA quantitation by Qubit 4 using the Qubit[™] RNA HS Assay kit (ThermoFisher, USA). The results of this study indicated that the RNA concentration for included samples ranged in sera samples from 18 to 23 ng/ml, while in the tissue samples it was 150–180 ng/ml. Next came complementary DNA (cDNA) synthesis using total RNA as a template with the ProtoScript[®] First Strand cDNA Synthesis Kit (ProtoScript[®] First Strand cDNA Synthesis Kit-UK). The single-stranded primers are used for the synthesis of cDNA.

miRNA-155 (Reverse Transcription)

GTCGTATCCAGTGCAGGGTCCGAGGTATTCG-
CACTGGATACGACTCAACA

miRNA-21 (Reverse Transcription)

GTCGTATCCAGTGCAGGGTCCGAGGTATTCG-
CACTGGATACGACTCAACA,

Quantitative Real-time-PCR (qRT-PCR) (Bior-Japan) was performed to amplify the complementary DNAs simultaneously with specific primers pertinent to each particular target miRNA. Primers used in this study were from Macrogen[®] (S. Korea).

miRNA-155:

F3' CTCAGACTCGGTTAATGCTAATCGTGATAGG5'
R 3' GCTGTGGCAGTGGAAGCGTGATTTATT 5'

miRNA-21:

F 3' CGGCTAGCTTATCAGACTGA 5'
R 3' GTGCAGGGTCCGAGGT 5'

Two PCR tubes were prepared per sample, one for each target miRNA and the other for U6, which is used as a house-keeping gene. Because of its comparatively stable expression in a variety of tissues and experimental settings, U6 small nuclear RNA (U6 snRNA) is frequently employed as an internal house-keeping control in miRNA quantification by real-time PCR. In order to enable precise relative quantification of miRNA expression levels, its function is to normalize variations in RNA input, reverse transcription efficiency, and amplification. Because U6 is abundant, constitutively expressed, and structurally similar to miRNAs, it is especially useful for improving the accuracy of comparative analysis.⁹

All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS, version 27). Continuous variables were expressed as mean $\pm$ standard deviation (SD), and differences between 2 groups were assessed by an independent samples t-test, while differences between 3 groups

were assessed by using an ANOVA test. A P -value of <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves measured areas under the curve (AUC), sensitivities, and specificities of these miRNAs.

This study included 160 samples from female breast carcinoma patients and apparently healthy controls, aged 29–69 years. Demographical characteristics, clinical data, and histopathological diagnoses compared with miRNA-21 and miRNA-155 expressions were shown in Tables 1 and 2.

There was an overall increase in the fold of expression of miRNA-21 by 3.21 times in serum samples of patients relative to controls; likewise, it appeared that the same miRNA was overexpressed in neoplastic tissue samples compared to the adjacent non-neoplastic tissues by 3.39 times (Table 3).

The results of this study show that there was an overall increase in the fold of expression of miRNA-155 by 3.09 times in serum samples of patients relative to controls; similarly, it seemed that an overexpression by 2.35 times of this miRNA was also noticed in neoplastic tissue samples compared to the adjacent non-neoplastic tissues (Table 4).

It was found that the mean fold change of miRNA-21 in the serum samples was 3.78 ± 2.08 and 1.42 ± 1.22 and in miRNA-155 was 3.52 ± 1.76 and 1.29 ± 0.83 for the patients and controls, respectively, and these values differed statistically significantly (P -values of 0.002 and ≤ 0.001). Moreover, in the tissue samples, the mean fold change of miRNA-21 and miRNA-155 showed statistically significant differences (P -values of 0.015 and ≤ 0.001) when the values of the neoplastic tissue (3.62 ± 1.31 and 3.52 ± 2.68) were compared to those of the non-neoplastic tissue (2.42 ± 1.70 and 1.34 ± 1.03) (Table 5 and Figure 1).

The results of this study showed that the cut-off values of miRNA-21 and miRNA-155 in serum samples were >1.59 and >1.895 , respectively. The AUCs were 85.0 and 88.3, indicating a very good discriminatory power of the two variables. The P -values for the three variables were very highly statistically significant (P -value ≤ 0.001) (Figure 2).

Figure 2 shows the high sensitivity and specificity for both sera of miRNA-21 (89.7% and 70%) and miRNA-155 (82.8% and 90%), respectively, where the AUC is about 0.85 and 0.88 with a very highly significant P -value of less than 0.001.

Moreover, the cut-off values of the same variables in tissue samples were >1.7824 and >3.23 , respectively. The discriminatory power of miRNA-21 was fair, while that of miRNA-155 was well represented by 69.9 and 75.3, respectively, with P -values as follows: 0.03 and ≤ 0.001 (Table 5 and Figure 3).

Figure 3 shows the high sensitivity and specificity for sera of miRNA-21 are 96.4 and 42.9, respectively, and for miRNA-155, 50 and 100, respectively. Also, the AUC is about 0.699 and 0.753 with very highly significant P -values of 0.03 and 0.001, respectively.

Multivariate logistic regression identified P -values in serum miRNA-21 (≤ 0.001), serum miRNA-155 (≤ 0.001), tissue miRNA-21 (0.03), and tissue miRNA-155 (≤ 0.001) as independent predictors of breast cancer. The combined model demonstrated excellent discrimination with an AUC of 0.997 (95% CI: 0.903–1.000), outperforming serum miRNA-21 (AUC 0.85), serum miRNA-155 (AUC 0.883), tissue miRNA-21 (AUC 0.699), and tissue miRNA-155 (AUC 0.753) alone (Figure 4 and Table 6).

Table 1. Demographical and clinicopathological features of breast carcinoma patients and their relation to miRNA-21

Variables	Group	N (%)	miRNA-21			
			Serum	P-value	Tissue	P-value
Age (years)	≥49	19 (47.5)	3.6392	0.741 ^{NS}	3.4825	0.678 ^{NS}
	<49	21 (52.5)	3.9118		3.7556	
Family history	+ve	33 (84)	3.9786	0.009**	3.7143	0.923 ^{NS}
	-ve	7 (16)	2.0614		3.2275	
Side	Right	25 (62.5)	3.7527	0.504 ^{NS}	3.7795	0.919 ^{NS}
	Left	15 (37.5)	3.8325		3.3982	
Marital status	Married	36 (90)	3.6823	0.379 ^{NS}	3.5099	0.154 ^{NS}
	Single	4 (10)	4.6288		4.5384	
NAC	Yes	24 (60)	3.9566	0.189 ^{NS}	3.3793	0.861 ^{NS}
	No	16 (40)	3.4451		4.1137	
Type	Ductal	29 (72.5)	3.6955	0.5 ^{NS}	3.9061	0.2 ^{NS}
	Lobular	11 (27.5)	4.0027		2.8185	
Grades	1	15 (37.5)	3.9092	0.721 ^{NS}	3.3245	0.647 ^{NS}
	2	10 (25)	4.0674		4.1881	
	3	15 (37.5)	3.5184		3.5289	
Stage	1	15 (37.5)	3.0558	0.210 ^{NS}	3.0712	0.373 ^{NS}
	2	17 (25)	4.7112		4.3553	
	3	8 (37.5)	3.2191		3.2052	
LVI	+ve	8 (20)	3.0752	0.58 ^{NS}	2.6433	0.14 ^{NS}
	-ve	32 (80)	3.9642		3.8471	
LI	+ve	14 (35)	4.5807	0.05*	3.4723	> 0.999 ^{NS}
	-ve	26 (65)	3.2910		3.7135	

NAC, neoadjuvant chemotherapy; LVI, lymphovascular invasion; LI, lymphocytic infiltration. P-value: NS: non-significant, significant ≤ 0.05, *significant ≤ 0.01, **highly significant.

Discussion

miRNA-21 is one of the most consistently reported oncogenic miRNAs in breast cancer. In this study, serum miRNA-21 expression was significantly elevated in breast carcinoma patients, showing a 3.21-fold overexpression compared with healthy controls (*P*-value 0.002), which indicates high statistical significance, supporting the strength of the association between serum miRNA-21 and breast carcinoma. This aligns with evidence that elevated circulating miRNA-21 levels can diriment breast cancer patients from healthy individuals.¹⁰ In one study performed by Savitri and his colleagues, median miRNA-21 expression in the breast cancer group was elevated 4.92-fold compared to the control group (*P*-value 0.001).¹¹

The elevation of circulating miRNA-21 may reflect tumor burden and active secretion via extracellular vesicles, which has been documented across multiple cancers, including breast cancer. The *P*-value (0.002) indicates high statistical significance, supporting the strength of the association between serum miRNA-21 and breast carcinoma.¹²

In this work and at the tissue level, miRNA-21 expression was also significantly higher in breast carcinoma samples than in normal breast tissue, with a 3.39-fold overexpression (*P*-value 0.0015). Gareev et al. mentioned that tissue overexpression of miRNA-21 is a hallmark of oncogenic activity,

promoting proliferation and invasion through downregulation of tumor suppressor targets such as PDCD4 (programmed cell death) and reported that miRNA-21 expression in the breast cancer tissue was 4.77 ± 1.36 , significantly higher than 4.17 ± 1.01 in the adjacent normal tissue ($t = 2.921$, *P*-value 0.004).¹³

miRNA-21 promotes breast cancer progression and metastasis due to its suppression of LZTFL1, which acts on the beta-catenin protein signaling pathway, inactivating the epithelial-mesenchymal transition (Wang et al., 2019). miRNA-21 also has an inhibitory action on PDCD4 programmed cell death.¹³ TIMP3, TPM1, and RECK. All these have protein products with known anti-tumorigenic, anti-proliferative, anti-invasive, and anti-angiogenic actions.¹⁴ These statements are in concordance with the low *P*-value (< 0.0015) estimated in the present study and further confirm that tissue overexpression of miRNA-21 is very unlikely to be due to random fluctuation and reflects a robust biological difference.

Circulating miRNA-155 has a potential in the diagnosis of BC.¹⁵ In one study, the expression of miRNA-155 in BC patients was 1.68 ± 0.66 times greater than that in the control group (*P*-value < 0.0001).¹⁶ These findings are similar to ours; miRNA-155 was significantly overexpressed in both serum and tissue of breast carcinoma patients compared with healthy individuals. Serum miRNA-155 exhibited a 3.09-fold

Table 2. **Demographical and clinicopathological features of breast carcinoma patients and their relation to miRNA-155**

Variables	Group	N (%)	miRNA-155			
			Serum	P-value	Tissue	P-value
Age (years)	≥49	19 (47.5)	3.1798	0.372 ^{NS}	2.9907	0.483 ^{NS}
	< 49	21 (52.5)	3.8327		3.7629	
Family history	+ve	33 (84)	3.5524	0.082 ^{NS}	3.6505	0.352 ^{NS}
	-ve	7 (16)	3.2153		2.2496	
Side	Right	25 (62.5)	3.8210	0.05*	3.0859	0.73 ^{NS}
	Left	15 (37.5)	2.9408		3.8350	
Marital status	Married	36 (90)	3.5614	0.886 ^{NS}	3.3810	0.735 ^{NS}
	Single	4 (10)	3.1366		3.4716	
NAC	Yes	24 (60)	3.4240	0.633 ^{NS}	3.2722	0.713 ^{NS}
	No	16 (40)	3.6951		3.6288	
Type	Ductal	29 (72.5)	3.2986	0.173 ^{NS}	3.4418	0.894 ^{NS}
	Lobular	11 (27.5)	4.0921		3.2461	
Grades	1	15 (37.5)	3.6778	0.031* G2 Vs G3	3.6916	0.76 ^{NS}
	2	10 (25)	2.4079		3.5083	
	3	15 (37.5)	3.9254		3.0085	
Stage	1	15 (37.5)	3.4370	0.622 ^{NS}	4.0148	0.089 ^{NS}
	2	17 (25)	3.3466		4.0462	
	3	8 (37.5)	3.9254		1.1505	
LVI	+ve	8 (20)	3.3630	0.92 ^{NS}	3.4425	0.934 ^{NS}
	-ve	32 (80)	3.5578		3.3794	
LI	+ve	14 (35)	3.6625	0.431 ^{NS}	3.7375	0.509 ^{NS}
	-ve	26 (65)	3.4289		3.1873	

NAC, neoadjuvant chemotherapy; LVI, lymphovascular invasion; LI, lymphocytic infiltration. P-value; NS, non-significant, significant ≤ 0.05, *significant ≤ 0.01.

Table 3. **The overall fold of miRNA-21 expression in patients' samples relative to controls**

Sample	Group	Ct miRNA-21	Ct U6	Δ Ct	2 ^{-ΔCt}	Fold
Serum	Patients	38.46	25.78	12.68	0.00015253	3.21
	Controls	37.33	22.97	14.36	0.00004759	1.00
Tissue	Neoplastic tissue	22.44	19.42	3.02	1.23E-01	3.39
	Non-neoplastic tissue	25.81	21.03	4.78	3.64E-02	1.00

Table 4. **The overall fold miRNA-155 expression in patients' samples relative to controls**

Sample	Group	Ct miRNA-155	Ct U6	Δ Ct	2 ^{-ΔCt}	Fold
Serum	Patients	22.90	25.51	-2.61	6.08609664	3.09
	Controls	24.25	25.23	-0.98	1.971719780	1.00
Tissue	Neoplastic tissue	25.03	19.82	5.21	0.02699365	2.35
	Non-neoplastic tissue	27.18	20.74	6.44	0.01150633	1.00

Table 5. The mean fold comparisons of miRNA-21 and miRNA-155

Variables	Group	Mean fold	Std. deviation	P-value
miRNA-21 (serum)	Patients	3.78	2.08	0.002**
	Controls	1.42	1.22	
miRNA-155 (serum)	Patients	3.52	1.76	≤0.001***
	Controls	1.29	0.83	
miRNA-21 (tissue)	Neoplastic tissue	3.62	1.31	0.015*
	Non-neoplastic tissue	2.42	1.70	
miRNA-155 (tissue)	Neoplastic tissue	3.52	2.68	≤0.001***
	Non-neoplastic tissue	1.34	1.03	

P-value *significant ≤0.05, **highly significant at ≤0.01, ***very highly significant at ≤0.001.

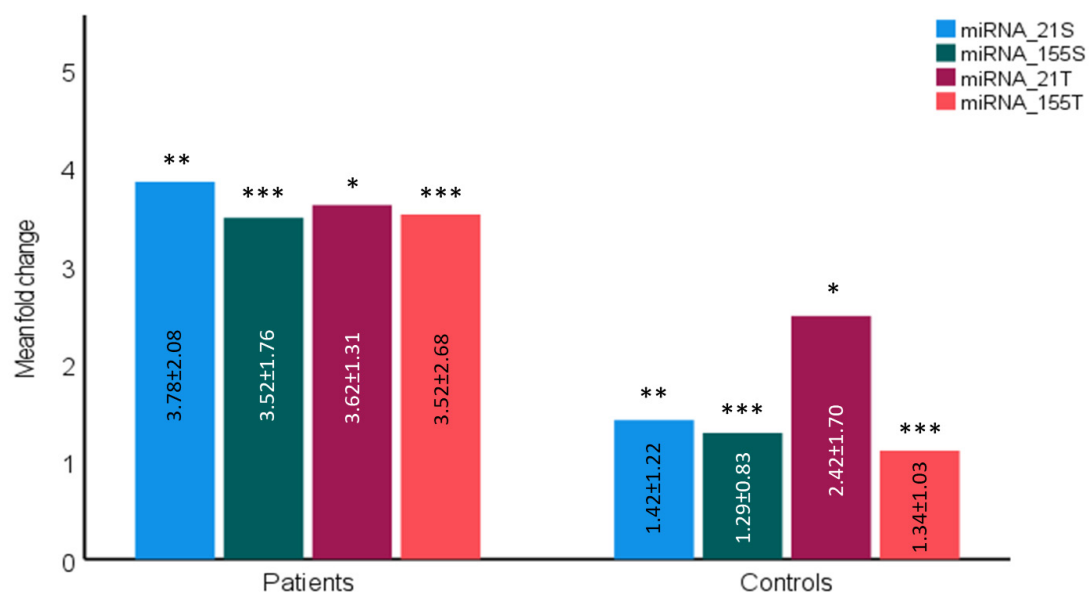


Fig. 1 The mean fold change comparisons of miRNA-21 and miRNA-155 in serum and tissue samples of patients and controls. (miRNA-21S: miRNA-21 serum, miRNA-21T: miRNA-21 tissue; miRNA-155S: miRNA-155 serum; miRNA-155T: miRNA-155 tissue), P-value significant ≤0.05, *highly significant ≤0.01, and ***very highly significant at $P \leq 0.001$.

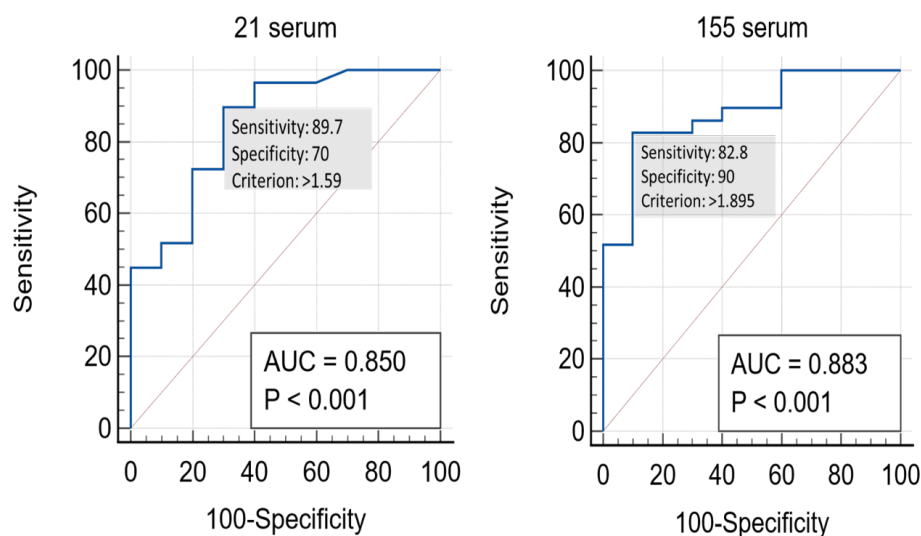


Fig. 2 The ROC curve analysis of miRNA-21 and miRNA-155 in serum samples.

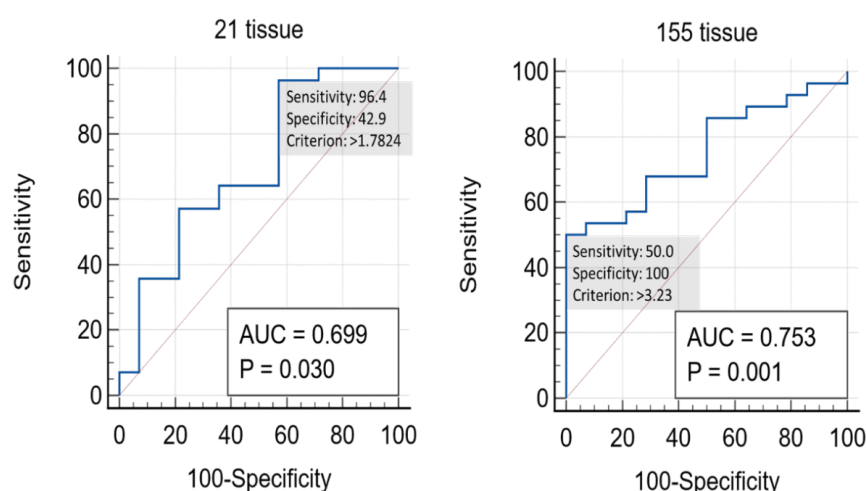


Fig. 3 The ROC curve analysis of miRNA-21 and miRNA-155 in tissue samples.

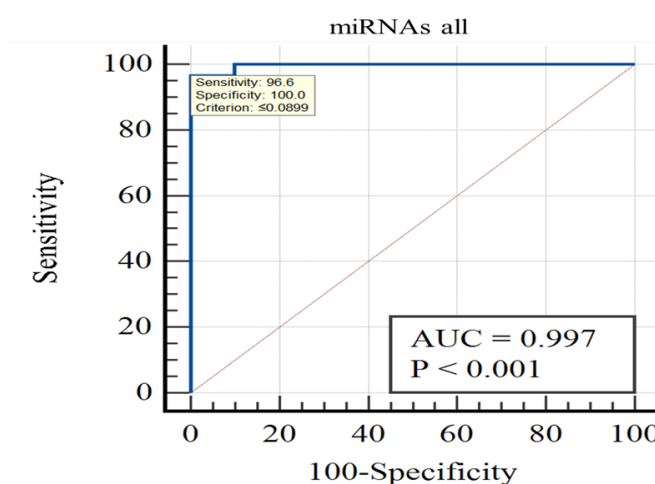


Fig. 4 The ROC curve analysis of miRNA-21 and miRNA-155 in serum samples, combined model of both miRNA analysis shows high sensitivity and specificity 96.6, 100% respectively, where AUC 0.997 and highly significant P -value less than 0.001.

Table 6. The ROC analysis of the miRNAs under investigation

Variables	Cut-off	Sensitivity	Specificity	Accuracy	AUC	95% C.I	P -value
miRNA-21 (Serum)	>1.59	89.7	70	59.0	85.0	0.699–0.944	$\leq 0.001^{***}$
miRNA-155 (Serum)	>1.895	82.8	90	72.76	88.3	0.739–0.963	$\leq 0.001^{***}$
miRNA-21 (Tissue)	>1.7824	96.4	42.9	39.29	69.9	0.538–0.831	0.03*
miRNA-155 (Tissue)	>3.23	50.0	100.0	50.0	75.3	0.595–0.872	$\leq 0.001^{***}$
Combined model	≤ 0.0899	96.6	100.0	96.55	99.7	0.903–1.000	$\leq 0.001^{***}$

AUC, area under the curve; CI, confidence intervals; P -value; *significant ≤ 0.05 , ***very highly significant at ≤ 0.001 .

increase (P -value 0.001), and this very low P -value confirms that serum miRNA-155 overexpression is statistically significant and unlikely to be a chance finding, indicating a strong association with malignant disease.

In this study, miRNA-155 expression in neoplastic tissue demonstrated a 2.35-fold overexpression compared with healthy breast tissue (P -value 0.001). A finding that is in consistence with that of Altrawy et al., who demonstrated a 2.73-fold overexpression with a highly significant difference (P -value < 0.001*).¹⁷ Both studies support the notation of

Tian et al., who concluded that miRNAs are very often considered to be potential biomarkers in cancer.¹⁸ The statistically significant P -value affirms the presence of a meaningful difference in miRNA-155 tissue expression between neoplastic and non-neoplastic tissues.

In the present study, the ROC curve is a graphical presentation of screening properties to determine the diagnostic power of the variables of interest. In the current study, the AUC, sensitivity, and specificity of serum miRNA-21 were found to be 85%, 89.7%, and 70%, respectively (P -value ≤ 0.001).

The above values were somehow similar to what has been reported by de Miranda et al. (2024), who found ROC curve analysis of plasma miRNA-21 as follows: AUC = 79.8%, 62.2%, 94.1% sensitivity, and specificity, respectively (P -value ≤ 0.001).¹⁹ However, Desouky et al. (2023) reported a better diagnostic performance of serum miRNA-21 than what has been mentioned in the present work, as they showed that the AUC was 91.8% (P -value ≤ 0.001) with 90% sensitivity and 100% specificity.²⁰ Furthermore, values of AUC of 96.5% with 95.7% sensitivity and 85% specificity for serum levels of miRNA-21 were also obtained by another group of researchers.²¹

The findings of this study in terms of the AUC analysis of tissue miRNA-21 (AUC = 69.9%, sensitivity 96.4%, specificity 42.9% (P -value ≤ 0.001)) were below those recorded by others for the same miRNA, AUC 98.2%, specificity of 97.6%, and sensitivity of 95.4%.²²

The results of this study concerning the AUC, sensitivity, and specificity of serum miRNA-155 were found to be 88.3%, 82.8%, and 90%, respectively (P -value ≤ 0.001), and were comparable to the values of the same parameters reported by a previous study (AUC = 0.89%, sensitivity 77.78%, and specificity 88.89%).¹⁶ Furthermore, Wang et al. (2024) yet obtained greater values, compared to those of the present investigation, for the same parameters describing miRNA-155 discriminatory power (AUC = 0.97%, sensitivity 94%, specificity 89%).¹⁵

The AUC, sensitivity, and specificity of tissue miRNA-155 obtained in the current work were 75.3%, 50%, and 100%, respectively (P -value ≤ 0.001); the above values were far less than those of another study (AUC of 99.8%, with outstanding sensitivity and specificity of 96.67% and 93.33%, respectively, and a very highly statistically significant difference (P -value ≤ 0.001)), demonstrating that miRNA-155 significantly discriminates between patients and the control

group.¹⁷ However, Alnoaimi and Ozaslan found that the AUC was calculated to be 74.6%, sensitivity 51.9%, and specificity 92.3%. The P -value associated with the AUC was very highly statistically significant (P -value ≤ 0.001);²³ the outcomes of the present investigation were nearly similar to the later findings.

Limitation: small sample size due to cross-sectional study design during short period of time, no specific designated center for breast carcinoma patients, neoadjuvant chemotherapy may have confounding effect on these miRNAs' expressions, and lack of follow-up data.

Conclusion

In conclusion, the significant overexpression of miRNA-21 and miRNA-155 in breast carcinoma patients compared with healthy controls supports their oncogenic roles and highlights their potential as non-invasive biomarkers for breast cancer diagnosis. The consistently statistically significant low P -values across both serum and tissue samples for miRNA-21 and miRNA-155 indicate that these associations are unlikely to be due to random variation; both miRNA-21 and miRNA-155 are significantly overexpressed in the serum of breast cancer patients and show promising discrimination from healthy controls in ROC analysis. Moreover, when integrated into a multi-marker panel, the combined use of these miRNAs yields a higher area under the ROC curve (AUC), greater sensitivity, and improved specificity. Therefore, the combined assessment of miRNA-21 and miRNA-155 could strengthen breast cancer diagnostic strategies.

Conflict of Interest

The authors declare that they have no conflict of interest. ■

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