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Potential Role of Melatonin-Modulated Circulating miR-24, miR-152, and miR-497 as Early Liquid Biopsy Biomarkers in Egyptian Breast Cancer Patients.

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ABSTRACT:

Background: Liquid biopsy shows huge prospects for breast cancer (BC). MicroRNA (miRNA) dysregulation is implicated in breast cancer (BC) development, and thus, their role as early BC biomarkers is under investigation. Melatonin has been studied for its roles in cancer development via miRNAs modulation. Here, we investigate the expression of some miRNAs previously reported to be related to BC pathogenesis and to be linked to melatonin, aiming to assess their potential as early BC biomarkers.

Methods: 35 newly diagnosed BC patients and 15 healthy controls were included. Clinicopathological characteristics were assessed for all BC patients. Plasma miR-24, miR-152, and miR-497 were evaluated via quantitative real-time polymerase chain reaction (qRT-PCR). Serum melatonin was measured using enzyme-linked immunosorbent assay (ELISA).

Results: We found significant upregulation of miRNAs and higher melatonin levels in BC patients compared to control subjects. We showed a positive correlation between miR-152 and miR-497. Receiver operating characteristic curve (ROC) demonstrated the utility of target miRNAs for distinguishing early BC, both as standalone or together in a diagnostic panel.

Conclusions: The investigated miRNAs demonstrated good discriminatory power in distinguishing BC patients from healthy subjects.

Keywords miRNA-24, miRNA-152, miRNA-497, Melatonin, Early Breast Cancer, non -invasive biomarkers, Liquid biopsy.

1. INTRODUCTION

Early detection has substantial effects on both morbidity and mortality of Breast cancer (BC). (Giaquinto et al., 2024). The currently used techniques for early BC diagnosis, including breast imaging, tissue biopsy, and tumor markers, have limitations and lack the required sensitivity and specificity for reliable screening. (Ali et al., 2022) This calls for directing research efforts to identify novel early detection BC biomarkers to improve patient outcomes. (Taleb RSZ et al., 2025) Compared to tissue sampling, the detection of potential cancer biomarkers in liquid biopsy has emerged as a reliable noninvasive diagnostic tool. In BC, liquid biopsy can be combined with standard screening methods to aid early diagnosis. It also enables multiple repetitions and easy monitoring of disease progression, while limiting unnecessary tissue biopsies and their associated disadvantages. (Jang et al., 2021)

MicroRNAs (miRNAs) are short non-coding RNAs (18–24 nucleotides in length) that control the expression of messenger RNA (mRNA) targets post-transcriptionally. (Santosh et al., 2015) Increasing evidence demonstrates the role of miRNA dysregulation in BC pathogenesis via several mechanisms. (Kumar., 2025) Through the negative regulation of H2A histone family member X (H2AX) expression, the relationship of miR-24 was established with BC tissues and cell lines. An overexpression of miR-24 leads to downregulation of H2AFX and BCL-2, impairing DNA repair and promoting apoptosis. (Srivastava et al., 2011) Cell proliferation, invasion, and angiogenesis in BC have been associated with the underexpression of miR-152 by directly targeting and downregulating the ROCK1 gene, which is known to promote tumor growth and metastasis. By inhibiting ROCK1, miR-152 disrupts pathways essential for cancer cell movement and division, thereby reducing the aggressiveness of BC cells.

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(Maimaitiming et al., 2020) An overexpression of miR-497 was found to inhibit phase transition G0/G1 in the MCF-7 BC cell line. miR-497 directly binds to the 3′ -untranslated region (3′ -UTR) of cyclin E1 mRNA, as shown by dual-luciferase reporter assays, resulting in decreased Cyclin E, a key regulator of the cell cycle, and its downregulation by miR-497 contributes to the observed G1 arrest and reduced proliferation. (Luo et al., 2013) Additionally, inhibition of miR-497 increased the colony formation ability of BC cells and lowered apoptosis. (Wei et al., 2015) The stability of miRNA expression in liquid biopsy was also demonstrated, establishing them as potential non-invasive biomarkers. Research has focused on identifying specific miRNA signatures in blood that can distinguish early BC from healthy controls. (Jang et al., 2021)

Melatonin (N-acetyl-5-methoxytryptamine) is formed mainly by the pineal body from L-tryptophan. (Ribeiro et al., 2025) In addition to its well-known role as a regulator of the circadian rhythm, it has been reported to have antineoplastic activities. (Samanta et al., 2020) The various roles of melatonin in regulating cancer, including BC, are a growing field of research. (Sadoughi et al., 2022) The interaction of melatonin with various miRNAs implicated in BC was also reported. (Chuffa et al., 2020)

Research indicates that melatonin can inhibit cancer cell proliferation and migration partly by downregulating miR-24. Long-term melatonin treatment decreased miR-24 levels post-transcriptionally, leading to broader regulation of genes associated with cell proliferation, DNA damage response, RNA metabolism, and cellular transformation. It was also suggested that melatonin's effect on miR-24 was due to downregulation of hnRNP A1, a protein essential for miRNA processing. (Mori et al., 2016) In triple-negative BC, melatonin treatment was found to increase miR-152 expression, which in turn led to reduced protein levels of key angiogenic factors such as IGF-IR, HIF-1α, and VEGF, both in cell cultures and animal models. (Marques et al., 2018) Melatonin has also been shown to modulate the expression of miR-497, influencing key cellular processes in different disease contexts. (Liu et al., 2018 & Kim et al 2017)

In our work, a panel of 3 miRNAs (miR-24, miR-152, and miR-497) previously reported to be linked to BC pathogenesis was assessed for diagnostic potential as liquid biopsy biomarkers in newly diagnosed Egyptian BC patients. Based on the apparent link between melatonin and both BC pathogenesis and miRNA expression, we also assayed serum melatonin levels to test its possible miRNA regulatory role in the development of BC. The assessed parameters were correlated with the clinicopathological features of BC

Methods

Data Collection

The sample size in this study was calculated using the G-power software and based on a previous study comparing melatonin levels in cancer and non- cancer patients: the effect size was d = 1.39, and applying power = 80%, α = 0.05, and control: case = 1:3, the total estimated sample size was 24 divided as 18 in the cancer group (n1) and 6 in the control group (n2). (Stanciu et al., 2020) Accordingly, we conducted our work on 50 subjects allocated into 2 groups: Group I (control group) comprised 15 age-matched healthy women selected based on the absence of breast neoplasm or family history of BC, as well as freedom from other solid cancers and immune disorders. Group II (BC group) consisted of 35 newly diagnosed BC patients between 42 and 74 years old, selected from those admitted to the Experimental and Clinical Surgery Department, Medical Research Institute, Alexandria University, from 23-02-2022 to 30-11-2022. All studied patients had Invasive Ductile Carcinoma No Special Type (IDC NST). Patients who had previously started their treatment programs (chemotherapy and/or radiotherapy), those at an advanced disease (stage IV), and those with metastasis were excluded from the study.

Thorough assessment for all participants included relevant history taking (age, menopausal status, marital status, previous use of oral and hormonal contraceptives). Review of patients' records was done to retrieve relevant data such as values of CA 15-3. Sleep disturbance was assessed via an Arabic translated version of the Pittsburgh Sleep Quality Index (PSQI). (Swann et al., 2018) Physical examination and breast imaging via bilateral breast ultrasound were performed, and an ultrasound-guided core biopsy of breast lesions was done for pathological evaluation. Histopathological analysis was conducted to determine tumor type and evaluate hormone receptor status. The pathological stage was defined according to the 8th edition of the TNM staging system. (www.pathologyoutlines.com/topic/breastmalignantstaging.html).

This study was approved by the local ethical committee of the Medical Research Institute (IRB 00010526), Alexandria University. Informed consent was obtained from all the participants in the present study.

Immunohistochemical Staining (IHC)

Serial 5 µm thick formalin-fixed paraffin sections from each case were subjected to immunohistochemical staining for TRPS1, GATA3, ER, PR, HER2, and Ki-67 as follows: Mounting on coated Super frost glass slides (Menzel Glaeser, Germany), dewaxing with xylene gradual rehydration in descending grades of alcohol (100% to 70%) followed by rinsing in distilled water. Next, blocking of endogenous peroxidase activity was performed by incubating for 30 minutes in 3% H2O2, followed by 15 minutes of antigen retrieval using a microwave oven at 800W in citrate buffer (pH 6.0). To prevent drying during the incubation process, we added buffer periodically to the container. We cooled the slides for 20 minutes in buffer to room temperature, then washed twice in phosphate-buffered saline (PBS). The immunohistochemical reaction employed primary polyclonal rabbit antibodies against TRPS1 (Q9UHFV) at a dilution of 1:50-1:200, and the sections were incubated with the antibodies overnight at room temperature. Next, incubation with biotinylated secondary antibodies and streptavidin-peroxidase conjugates was performed, each for 30 minutes at room temperature. Slides were rinsed with PBS (pH 7.0) 3 times between the previous steps. DAB (Diaminobenzidine tetrahydrochloride) was used as a chromogen, applied to the slides for 5-15 minutes in the dark at room temperature to detect the reaction product. Finally, all sections were

counterstained with hematoxylin, dehydrated in alcohol, cleared in xylene, and examined using the light microscope. Interpretation of ER and PR was done according to the Allred system of scoring for estrogen receptor and progesterone receptor. (Allred et al., 1990) Interpretation of HER2 was done according to the American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guidelines 2018. (Wolff et al., 2018)

Blood Sampling:

Five mL of blood was drawn by peripheral venipuncture from all participants. 2mL of blood were taken in an Ethylenediaminetetraacetic acid (EDTA) tube to separate plasma. The remaining 3 mL of blood was left for 15 minutes at room temperature before centrifugation to collect serum. Plasma was stored (-80°C) and serum was frozen (-20°C) until used according to the manufacturer's protocol for the determination of miRNA targets in plasma samples by RT-PCR and melatonin in serum samples by ELISA.

Plasma expression of miRNA targets by qRT-PCR:

Extraction of total RNA was done utilizing the miRNeasy Mini Kit (Qiagen, CA). (ID: 217004). Total RNA concentrations were determined on the Thermo Scientific ND2000 Nanodrop Spectrophotometer, and extracts were frozen (−80°C). Single-stranded cDNA was reverse transcribed by miRCURY® LNA® RT Kit on SimpliAmp™ Thermal Cycler (Applied Biosystems), and the cDNA was frozen (−20°C) until qPCR experiments. miRCURY LNA SYBR® Green PCR Assays were used to detect and quantify miRNA targets on the Rotor-Gene Q real-time PCR system. The miRCURY LNA miRNA PCR Assays used were hsa-miR-24-3p, hsa-miR-152-3p, hsa-miR-497-5p, and as endogenous control, we used hsa-miR-103a-3p (ID: 339306). Table 1. We finally calculated the relative quantitation of the gene expression (RQ) using the comparative cycle threshold ($2^{-\Delta\Delta CT}$) method. (Livak et al., 2001)

Table 1: MicroRNA sequences

miRNA	Gene Globe ID	miRNA sequence
hsa-miR-24-3p	YP00204260	5′-UGGCUCAGUUCAGCAGGAACAG-3′
hsa-miR-152-3p	YP00204294	5′-UCAGUGCAUGACAGAACUUGG-3′
hsa-miR-497-5p	YP00204354	5′-CAGCAGCACACUGUGGUUUGU-3′
hsa-miR-103a-3p	YP00204063	5′-AGCAGCAUUGUACAGGGCUAUGA-3′

Serum melatonin levels measured by ELISA:

Quantitative measurement of melatonin in serum samples was done via competitive enzyme immunoassay using the Fine Test ELISA Kit provided by Wuhan Fine Biotech Co., Ltd., Wuhan, China (Cat. No. EH3344). The microtiter plate is pre-coated with melatonin. Standards and samples were then added to microtiter plate wells, along with biotin-labeled antibodies. Melatonin in the serum or standard competed with a fixed amount of melatonin on the solid-phase support for sites on the biotinylated detection antibody specific to melatonin. We then washed excess conjugate and unbound serum or standards from the plate. Next, we added streptavidin-horseradish peroxidase (HRP) to each well and incubated the plate. Then, Tetramethylbenzidine (TMB) substrate was added to each well. The reaction was terminated by adding sulfuric acid stop solution, and the color change was measured at a wavelength of 450 nm spectrophotometrically. Melatonin concentration was determined by comparing the samples' Optical Density (OD) to the standard curve.

Statistical analysis of the data:

The statistical analysis was performed using IBM SPSS software version 20.0 (Armonk, NY: IBM Corp). Qualitative data were summarized as counts and percentages. The Shapiro-Wilk test was used to assess whether the data followed a normal distribution. Quantitative variables were presented as range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). Statistical significance was determined at the 5% level. The analyses included the chi-square test, Fisher's exact test or Monte Carlo correction, Student's t-test, Mann-Whitney test, Kruskal-Wallis test, and Spearman correlation coefficient. The cut-off value for the biomarkers was determined using a Receiver Operating Characteristic (ROC) curve analysis, along with its validity parameters, namely sensitivity and specificity. The area under the ROC curve (AUC) was calculated to assess the diagnostic performance of melatonin and miRNAs (miR-24, miR-152, and miR-497) in BC. The diagnostic accuracy of each biomarker was interpreted according to AUC values as follows: AUC > 0.9: excellent diagnostic accuracy, AUC between 0.7 and 0.9: good diagnostic accuracy, AUC between 0.5 and 0.7: poor diagnostic accuracy, and AUC < 0.5: no diagnostic value. The optimal cut-off points for each biomarker were selected based on the coordinate that maximized Youden's Index (sensitivity + specificity – 1), indicating the best balance between sensitivity and specificity. For all statistical tests, a p-value of < 0.05 was considered statistically significant. (Kirkpatrick et al., 2014)

Results

Patient Characteristics

As demonstrated in Table 2, no significant differences were recorded between the studied groups regarding age, marital status, breastfeeding history, number of children, menopausal status, or previous use of oral contraceptives. Regarding sleep disturbance, according to the Pittsburgh Sleep Quality Index (PSQI) questionnaire, a score of 5 or above is highly effective in detecting sleep disorders, with a sensitivity of 89.6% and specificity of 86.5%. In this study, 13.3% of the control

group and 37.1% of the BC group recorded a score equal to or higher than 5 (implying evidence of sleep disturbance). However, this difference did not reach statistical significance (P = 0.176).

Table 2: Comparison between the two studied groups according to demographic data, reproductive history, and sleep disturbance.

	Control (n = 15)	Patient (n = 35)	p-value
Age (years) Mean ± SD.	58.60 ± 10.40	57.57 ± 8.59	0.739t
Marital status			
Unmarried	0 (0.0)	3 (8.6)	0.545FET
Married	15 (100.0)	32 (91.4)	
Breastfeeding			
Yes	15 (100.0)	31 (88.6)	1.000 FET
No	0 (0.0)	1 (2.9)	
No of children			
Min. – Max.	2.0 – 5.0	1.0 – 6.0	0.331U
Median (IQR)	3.0 (3.0 – 3.0)	3.0 (2.0 – 5.0)	
Menopause status			
Premenopausal	4 (26.7)	8 (22.9)	1.000 FET
Menopausal	11 (73.3)	27 (77.1)	
Previous Use of Oral Hormonal Contraceptives			
Yes	6 (40.0)	15 (42.9)	0.851χ2
No	9 (60.0)	20 (57.1)	
Sleep Disturbance			
Yes	2 (13.3)	13 (37.1)	0.176χ2
No	13 (86.7)	22 (62.9)	

t: Student t-test, χ^2: Chi-square test, FET: Fisher Exact test, U: Mann-Whitney test, IQR: Interquartile rang**e**, p: p-value, SD: Standard deviation

CA 15-3 assessment

Table 3: Comparison between the two studied groups regarding CA 15-3.

CA 15-3 (IU/mL)	Control (n = 15)	Patient (n = 35)
Minimum	6.8	5.3
Maximum	32.4	162.2
Median	17.85	21.26

1. Tumor size, tumor site, and vascular invasion

Tumor size between 2 and 5 cm was recorded for 25 patients (71.4%), 8 patients (22.9%) had a tumor size less than 2 cm, and 2 patients (5.7%) had a tumor size greater than 5 cm. The left breast was the primary site of the tumor in 54.3% of patients, while 45.7% of patients had tumors on the right breast. Vascular invasion was positive in 34 patients and negative in one, as shown in Fig.1.

2. Tumor staging, grading, and hormonal receptors

According to the tumor stage, Fig. 2 shows 14.2% were stage I, 42.9% were stage II, and 42.9% were stage III. Regarding grading, 62.9% of patients were classified as grade II, and 37.1% of patients were classified as grade III. Concerning hormonal receptor status, 33 patients (94.3%) were estrogen positive, and 2 patients (5.7%) were estrogen negative, while 32 patients (91.4%) were progesterone positive, and 3 patients (8.6%) were progesterone negative. Regarding HER2 receptors, 16 patients (45.7%) were HER2 positive, and 19 patients (54.3%) were HER2 negative, as demonstrated in Fig. 3.

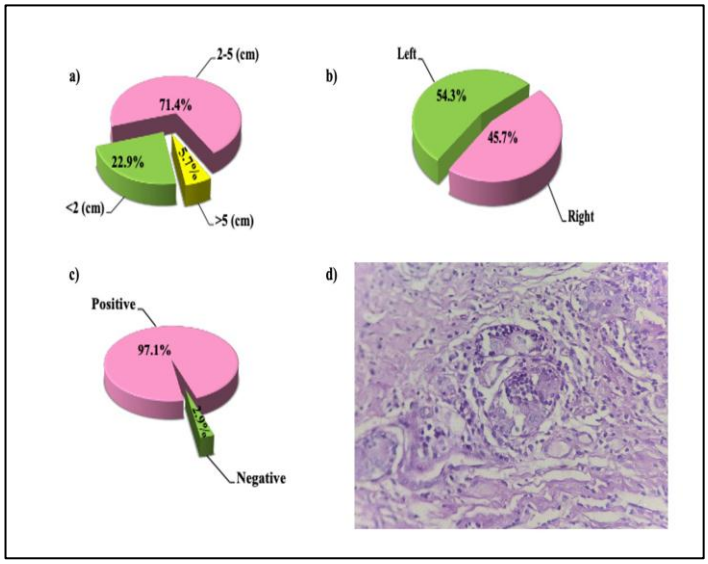


Fig. 1: Comparison between BC patients according to a) tumor size, b) tumor site, and c) vascular invasion d) H&E staining showing the presence of lympho-vascular invasion (H&E, x400).

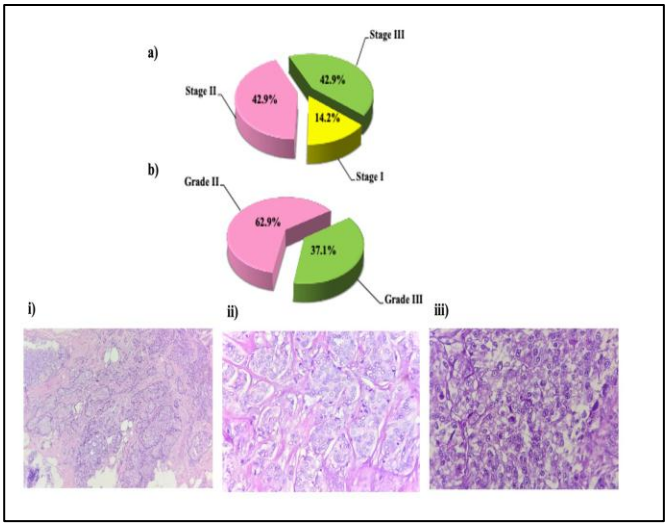


Fig. 2: Comparison between BC patients according to clinicopathological characteristics a) staging, b) grading: i) IDC NST grade II showing irregular nests and trabeculae formation (tubules in 10-75% of tumor area) (H&E, X100), ii) IDC NST grade II (2,2,2) showing malignant ductal cells with moderate tubular formation, moderate nuclear pleomorphism and moderate mitosis (10-19/10 HPFs) (H&E, X400). iii) IDC NST grade III showing large pleomorphic ductal cells with marked atypia and mitosis (H&E, X400).

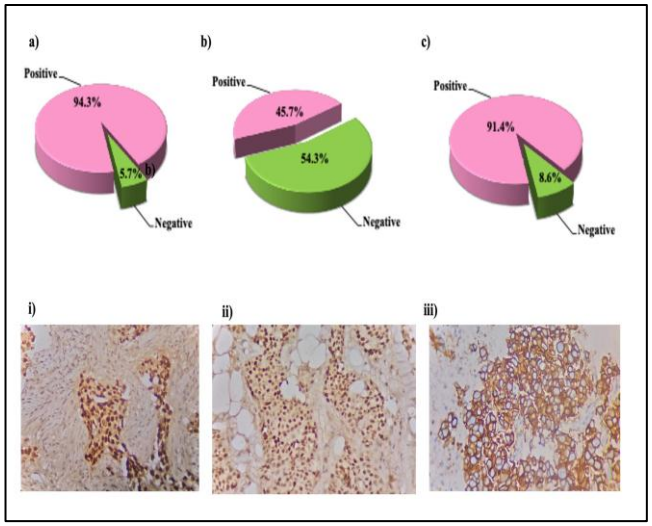


Fig. 3: Comparison between BC patients according to hormonal receptors status a) estrogen receptors b) progesterone receptors and c) HER2 receptors. i) IHC staining of ER showing strong nuclear positivity in malignant ductal cells (IHC, X 400). ii) IHC staining of PR showing strong positive nuclear staining of the malignant ductal cells (IHC, X 400). iii) HER2 immunostaining showing strong complete membrane staining in malignant ductal cells (score 3) (IHC, X 400)

Plasma miRNA expression and serum melatonin level

We show in Fig. 4 that the median values of miR-24, miR-152, and miR-497 fold change were significantly higher in BC patients compared to the control subjects' group (P <0.001 for miR-24 and miR-152, and P = 0.001 for miR-497). Also, significantly higher melatonin levels were recorded in BC patients compared to the control group. (P = 0.016).

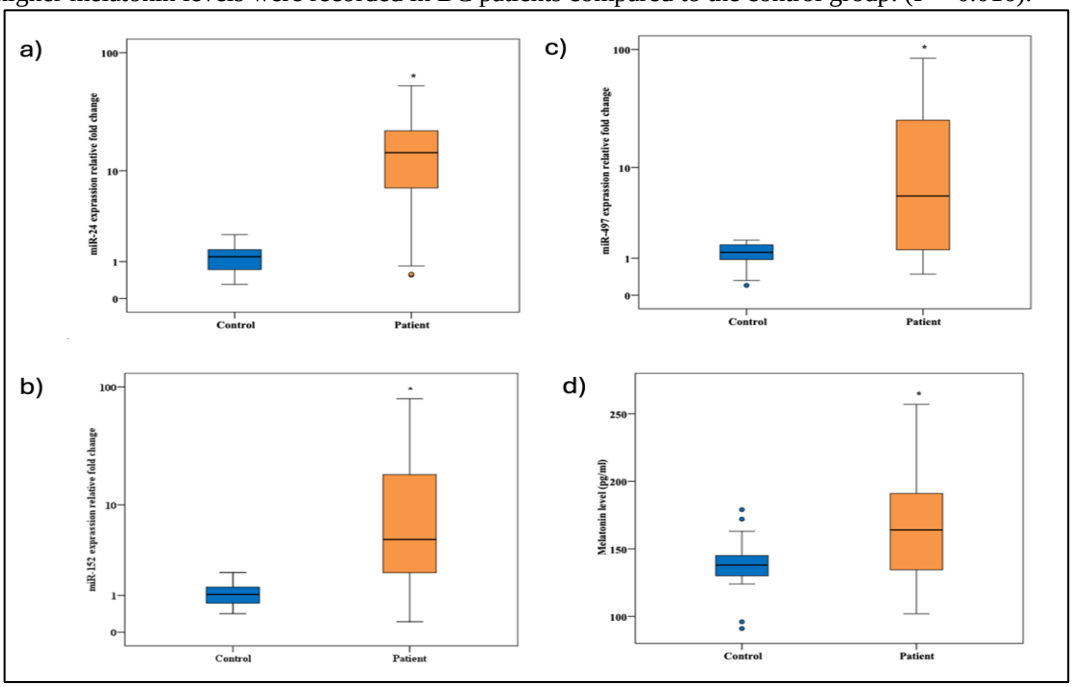


Fig. 4: Plasma miRNA expression and serum melatonin levels in Control and BC patient groups. a) miR-24, b) miR-152, c) miR-497 and d) Melatonin.

Plasma miRNA expression and serum melatonin level in relation to clinicopathological parameters

None of the studied parameters showed a significant relation to tumor grade or stage.

Correlations between plasma miRNA expression and serum melatonin levels

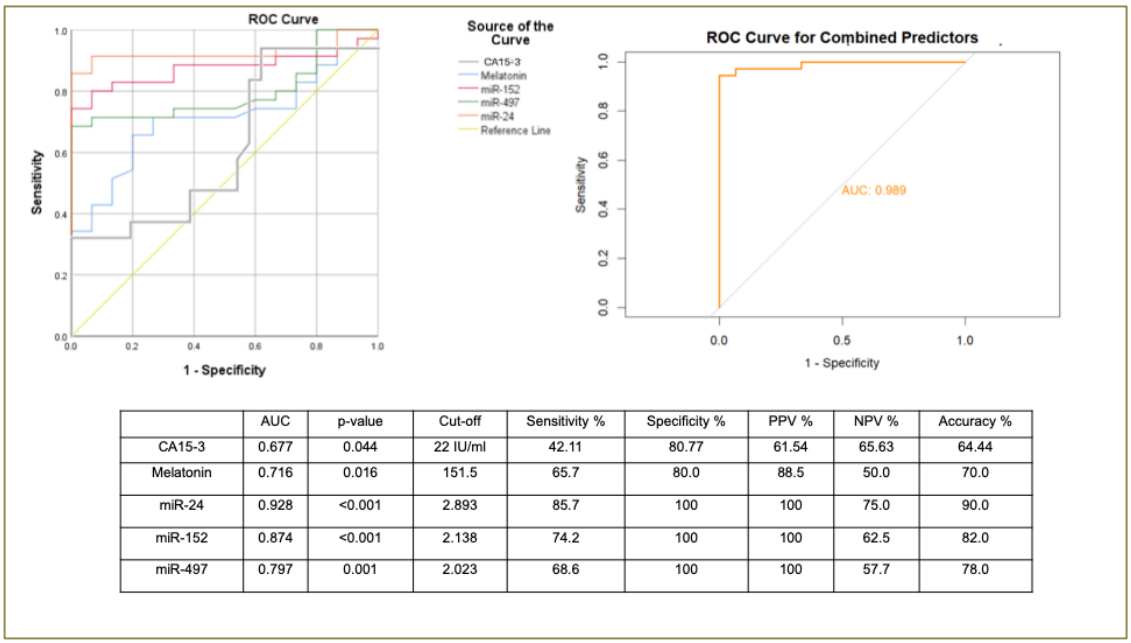
In BC cases, as shown in the correlation matrix in Table 3, a significant positive correlation was observed between miR-152 and miR-497 (r = 0.516). (Table 3)

Table 4: Correlation matrix for serum melatonin levels and plasma miRNA expression in BC cases

Cases group (n = 35)		miR-24	miR-152	miR-497
Melatonin	r_s	-0.066	0.113	0.162
	P	0.707	0.519	0.354
miR-24	r_s		0.270	0.218
	P		0.117	0.209
miR-152	r_s			0.516*
	P			0.001*

Diagnostic performance of melatonin and the studied miRNAs in BC

The Receiver operating characteristic (ROC) curve analysis shown in Fig. 5 demonstrates the AUC of melatonin and the studied miRNAs in patients with BC against the control group. The standard diagnostic marker for BC used was as CA 15-3 at Cut-Off value of 22 U/mL. An excellent diagnostic accuracy was recorded for miR-24 with an AUC=0.928, sensitivity = 85%, and specificity 100%. The AUC for miR-152, miR-497, and melatonin were 0.874, 0.797, and 0.716, respectively, showing good diagnostic accuracy. The highest discriminative power in distinguishing BC from control subjects was recorded for combining all three miRNAs in a panel with AUC = 0.989.



	AUC	p-value	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
CA15-3	0.677	0.044	22 IU/ml	42.11	80.77	61.54	65.63	64.44
Melatonin	0.716	0.016	151.5	65.7	80.0	88.5	50.0	70.0
miR-24	0.928	<0.001	2.893	85.7	100	100	75.0	90.0
miR-152	0.874	<0.001	2.138	74.2	100	100	62.5	82.0
miR-497	0.797	0.001	2.023	68.6	100	100	57.7	78.0

Fig. 5: ROC curve analyses showing the AUC of the studied miRNAs and melatonin in BC group against the control group

Discussion

We show an upregulation of the three studied miRNAs in plasma samples of BC patients compared to controls. As a promising biomarker for early BC, circulating miR-24 was shown to be significantly overexpressed in patients at diagnosis compared to healthy controls. Notably, miR-24 levels decrease following surgical tumor removal and further decline with adjuvant therapy, suggesting a strong link between its concentration, tumor presence, and response to treatment. This was reported by Sochor et al. who demonstrated a significant overexpression in the serum miR-24 of early BC patients compared to healthy controls at the time of diagnosis and a decrease in its levels after surgical resection and further with therapy. This dynamic change highlights the potential of miR-24 for monitoring treatment efficacy and possibly detecting minimal residual disease or early relapse. (Sochor et al., 2014) Furthurmore, research indicates that miR-24, especially when combined with other markers like Ki-67, can predict relapse risk in early BC patients, offering additional prognostic information beyond traditional clinical factors. (Bašová et al., 2017) Additionally, multiple studies have found that plasma levels of miR-152 are significantly elevated in BC patients compared to healthy controls. One study using RT-qPCR showed that miR-152 was increased in the plasma of women with BC relative to controls ($p < 0.05$), even though miR-152 was decreased in tumor tissue, highlighting a distinct pattern between tissue

and plasma expression. (Lopes et al., 2021) However, several works consistently reporting a downregulation of miR-497 in BC patients compared to healthy controls contradict our findings. The downregulation of miR-497 in early BC patients reported in previous literature suggested its potential as a noninvasive biomarker for early detection, with studies reporting moderate sensitivity and high specificity for distinguishing BC patients from healthy individuals. Biologically, miR-497 was reported to function as a tumor suppressor, with lower levels associated with increased cancer cell proliferation, migration, and invasion. (Xiao et al., 2020) Experimental upregulation of miR-497 in cell lines inhibits these malignant behaviors. (Adhami et al., 2018) The reduction of miR-497 in BC may be driven by epigenetic mechanisms, such as increased methylation mediated by lncRNA H19, which further promotes tumor progression and chemoresistance. (Tao et al., 2024)

In agreement with our results, ROC curve analyses in multiple studies have demonstrated that panels of circulating miRNAs can effectively differentiate early BC patients from healthy individuals, often outperforming single-biomarker approaches. In this context, A study analyzing plasma samples from 226 breast cancer patients and 146 healthy controls identified nine candidate miRNAs associated with BC including miR-24. Combinations of these miRNAs, rather than single markers, provided superior diagnostic accuracy. Notably, a panel of four miRNAs (miR-1246, miR-206, miR-24, miR-373) achieved 98% sensitivity, 96% specificity, and 97% overall accuracy in distinguishing early BC from controls. (Jang et al., 2021) Lopes et al performed ROC curve analysis of plasma miR-152 levels and showed an AUC of 0.77 (95% CI: 0.61–0.93, p=0.0105), indicating acceptable discrimination between BC patients and healthy controls. At a cut-off value of 0.252, sensitivity is 80% and specificity is 72.7%, with a positive predictive value of 86.9% and a negative predictive value of 61.6%. (Lopes et al., 2021)

Several works reported an alteration in melatonin levels in BC patients, suggesting a potential link between melatonin and BC risk. Previous studies indicate that serum melatonin levels are generally lower in BC patients. (Kubatka et al., 2018 & Travis et al., 2003) Other studies demonstrated no effect between BC and melatonin production for example, a study by the Women's Health Initiative reported no significant correlation between the risk of BC and urine melatonin levels. (Schernhammer ES et al., 2005) Interestingly, a meta-analysis that included multiple prospective studies reported no strong connection between breast cancer (BC) risk and melatonin levels. (Wong et al., 2021)

Disruptions in sleep quality were shown to influence melatonin production. In this regard, Reiter et al. reported that altered sleep-wake cycles suppressed melatonin production, increasing the risk for BC development. (Erren et al., 2008)We assessed sleep disturbance in all subjects via the Pittsburgh Sleep Quality Index (PSQI), which indicated higher evidence of sleep disturbance amongst BC patients compared to healthy controls. Still, these results did not reach statistical significance.

The discriminative value of melatonin using ROC curve analysis was previously reported in cancer. (Stanciu et al., 2020) While melatonin shows promise as a biological marker, there is currently insufficient evidence to support its use as a diagnostic tool for early BC based on ROC curve analysis. Our ROC curve analysis results demonstrate that melatonin, compared to miRNAs, showed the lowest discriminative power in distinguishing BC from control subjects with AUC= 0.716.

The crosstalk between miRNAs, which may influence similar signaling pathways, including those involved in cell proliferation, apoptosis, and metastasis, has been reported. Due to their central role in these processes, miRNAs are being investigated as biomarkers for cancer diagnosis and as potential targets for novel cancer therapies (Xu et al., 2017) Also, the role of melatonin in altering the expression of our target miRNAs was previously studied. Therefore, we tested the correlation between our target miRNAs and melatonin. A positive correlation was shown for miR-152 with miR-497 in our study, highlighting a possible common functional pathway. Studies showed that both miR-152 and miR-497 can be co-expressed in certain cancers, such as ovarian cancer and gastric cancer, both have shown altered expression patterns, and their levels can be correlated., but there is no direct, consistently literature reported positive correlation between them. Instead, they are part of families with related functions and often share common target genes or pathways, leading to co-expression.

Conclusions

Taken together our findings highlight the functional connection between our target miRNAs and the pathogenesis of BC and their potential utility as liquid biopsy markers for early-stage BC. Future larger studies considering all factors affecting melatonin secretion are warranted to elicit its modulatory role on the studied miRNAs and to validate their reproducibility as a specific signature in blood that can distinguish early BC from healthy controls.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors Contributions

NK performed laboratory and qPCR experiments, wrote, reviewed and edited the manuscript. IM analyzed and interpreted the patient data and wrote the first draft of the manuscript. AY analysed patient data and reviewed and

edited the manuscript. TE conceptualized the study, validated the methodology and reviewed and edited the manuscript. AA conceptualized the study and validated the methodology. ME performed histopathological examination. All authors read and approved the final manuscript

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