Biochemistry and Biophysics Reports 27 (2021) 101060

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

miRNA-148a and miRNA-30c expressions as potential biomarkers in breast cancer patients

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ARTICLE INFO

Keywords:
Breast cancer
miRNA-148a
miRNA-30c

ABSTRACT

Background: Breast cancer is an extensively identified malignant tumor and is a prime cause of cancer mortalities in females. It has been shown that alteration of miRNAs expression (up or down regulation) can affect the initiation and progression of many malignancies. We aimed to evaluate the role of circulating miRNA-148a and miRNA-30c in female patients with breast cancer and estimate their usage as potential biomarkers in the diagnosis, prognosis and survival of breast cancer.

Methods: This study included 75 breast cancer female patients.They were compared with 55 apparently healthy female subjects. miRNAs expression analysis was assessed via real-time PCR.

Results: To discriminate breast cancer patients from controls, miR-30c showed the best performance at a cut off value of $\leq$20.6 (AUC = 0.998, 97.33% sensitivity, 96.36% specificity, $p < 0.001$), followed by miR-148a (AUC = 0.995, 94.67% sensitivity, 90.91% specificity, $p < 0.001$ at a cut off value of $\leq$0.1), CA 15-3 (AUC = 0.930, 88.0% sensitivity, 81.82% specificity, $p < 0.001$ at a cut off value of >21.3), and finally CEA (AUC = 0.751, 70.67% sensitivity, 63.64% specificity, $p < 0.001$ at a cut off value of >2.5).

Conclusion: miRNA-148a and miRNA-30c expressions were down regulated in female patients with breast cancer and might be considered as potential blood biomarkers. Both also might have rule in disease treatment and selection of therapeutic targets. Future studies are needed to improve their role in predicting response to treatment and prognosis.

1. Introduction

Breast cancer is one of the most common causes of cancer-related mortalities in females worldwide. The incidence rates of breast cancer keep to expand by approximately 0.5% per year, about 281,550 cases of female breast cancer will be reported in 2021 in the United States [1].

Indeed, even patients with a localized tumor seem to be restrained to the breast, most patients will experience metastases and/or potentially tumor recurrence [2].

Different factors either genetic or environmental could affect the initiation and the progression of breast cancer. Additionally, biochemistry biomarkers (i.e. measurement of enzymes, hormones, and expression profiles of microRNAs (miRNAs)) have been emerged as new diagnostic and therapeutic biomarkers for breast cancer patients [3].

MicroRNAs (miRNAs) are groups of endogenous non-coding RNAs which could suppress gene expression via directly binding to the 3′untranslated region (3′UTR), leading to translation inhibition or mRNA degradation [4]. Additionally, miRNAs have likewise reported to bind to the 5′ UTR and gene promoters [5]. Also, miRNAs dysregulation might impact various vital cellular processes; leading to the enhancement of tumor progression by affecting cellular proliferation and apoptosis, promoting the tumor invasiveness and development of metastasis [6,7]. They are involved in virtually the most vital progressions like, cell cycle regulation and cellular differentiation [8].

It has been identified that alteration in miRNAs expression (up or down regulation) can affect the initiation and progression of different malignancies [9]. Additionally, some miRNAs have been correlated with breast cancer progression and development of metastasis [10].

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https://doi.org/10.1016/j.bbrep.2021.101060
Received 28 May 2021; Received in revised form 13 June 2021; Accepted 18 June 2021

Epithelial–mesenchymal transition (EMT) is a significant element in the development of breast cancer metastasis. EMT is recognized to include loss of E-cadherin expression which might activate the Wnt/β-catenin signaling pathway [11]. The WNT-1, one of the ligands of Wnt/β-catenin pathway, is a direct target of miR-148a. The dysregulation of miR-148a affects the WNT-1 concentration metalloproteinase-7 (MMP-7) expression level [12]. Also, miR-148a suppresses the migration of breast cancer cells by affecting MMP-13 [13].

The miRNA-148a belongs to the mir-148/mir-152 family, which involves three highly conserved miRNAs. The miRNA-148a has 68 nucleotide sequences on chromosome 7. Previously, assessed down regulation of miRNA-148a has been implicated in breast cancer [14,15]. The miRNA-30c is a member of family consists of six diverse miRNAs. It is encoded by genes located on chromosome 1, 6 and 8 [16]. Most of cases of breast cancer respond to treatment therapies, such as chemotherapy and radiation. Though, both therapies have constraints with reported resistance and tumor recurrence [17]. The role of miRNA-30c as a regulator of response to chemotherapy in breast tumors has been implicated. miRNA-30c expression has been related to endocrine therapy resistance, which is often linked to chemotherapy resistance in advanced estrogen receptors (ER)-positive patients [18].

In this current study, we aimed to evaluate the role of circulating miRNA-148a and miRNA-30c in female patients with breast cancer and estimate their usage as potential biomarkers in diagnosis, prognosis and survival of patients.

2. Material and methods

The current study was performed via cooperation between the Medical Biochemistry and Molecular Biology and Clinical Oncology and Nuclear Medicine Departments, (Faculty of Medicine) and Organic Chemistry and Biochemistry Departments, (Faculty of Science). Menoufia University. Egypt.

This study included 75 breast cancer female patients chosen from Clinical Oncology and Nuclear Medicine Department, Hospital of Menoufia University from January 2019 to April 2020. The cancer breast female patients were compared with 55 apparently healthy female subjects. Cases were diagnosed by histopathology. Patients with associated heart failure, renal failure or liver failure were excluded. Staging workup was done (chest X-ray and pelvi-abdominal ultrasound for early stages) and (chest, abdomen, and pelvis CT, contrast study and bone scan or PET/CT scan for advanced stages). Tumor staging depends on Tumor Node Metastasis (TNM) classification [19] and the grading was dependent upon the criteria of Nottingham modification in the Bloom-Richardson system [20]. Determination of molecular subtypes of breast cancer was based on status of estrogen receptor (ER), progesterone receptor (PR), Her2/neu and Ki 67 [21].

This current study was conducted in accordance with the Declaration of Helsinki. We obtained informed written consent from all the participants, which was approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

2.1. Data collection

All studied subjects were subjected to full history taking, general examination, clinical examination, and laboratory investigations.

2.2. Blood samples and laboratory tests

Seven milliliters of blood were withdrawn from each participant. Three milliliters were collected in a plane tube and the serum was isolated for the assessment of tumor markers and Four milliliters were obtained in an EDTA tube for miRNA analysis.

Carbohydrate antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA) were measured by enzyme-linked immunosorbent assay (ELISA) using a kit supplied by Chemux BioScience, Inc, USA for CA 15-3 and the Human CEA/Carcino Embryonic Antigen PicoKine™ Fast ELISA kit for CEA (Boster Biological Technology, USA).

2.3. miRNA expression profiling

Purification and cDNA synthesis: The miRNeasy® Mini Kit (QIAGEN, Germany; cat. #217004) was used for the purification of miRNA from whole blood. Both the yield and the purity of RNA were assessed using a NanoDrop instrument (Thermo Scientific, USA). Purified miRNA was stored at −80 °C. Complementary DNA (cDNA) was obtained via reverse transcription by the miScript II RT Kit (QIAGEN, Germany; cat. #218161). Each reaction was performed on ice with total volume of 20 μl; 4 μl of the miScript HiSpec RT buffer, 2 μl of miScript Nucleics Mix, 2 μl of the miScript™ reverse transcriptase, and 2 μl of nuclease-free water were pipetted into every well, followed by 10 μl of extracted miRNA. Analysis was done in a 2720 Applied Biosystems thermal cycler (Singapore) for one cycle: 37 °C for 60 min and 95 °C for 5 min to inactivate the reverse transcriptase. The cDNA produced was preserved at −20 °C.

Amplification by real-time PCR (miScript® SYBR® Green PCR Kit [QIAGEN, Germany; cat. #218073])**:** Before amplification, the cDNA samples were diluted with nuclease-free water at a ratio of 1:5. A total volume of 25 μl was used (12.5 μl of SYBR Green Master Mix, 3.5 μl of nuclease-free water, 4 μl of diluted cDNA, 2.5 μl of the miScript universal primer, and 2.5 μl of the miScript primer). The miRNA RNU6 was used as the reference miRNA. The miScript primer assay containing miRNA-specific forward primers was used to detect mature miRNA-148a and miRNA-30c (miScript Primer Assay Kit, QIAGEN, Germany). The data was analyzed via an ABI 7500 real-time PCR instrument with software version 2.0.1, with the following cycling conditions: initial activation step at 95 °C for 15 min and then 40 cycles (94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s). The expression levels of miRNA- 148a and miRNA- 30c were normalized to those of RNU6 and calculated via the comparative $2^{-\Delta\Delta Ct}$ method to achieve the relative quantification of each miRNA.

2.4. Statistical analysis

The data was analyzed using SPSS version 20 (SPSS Inc., released 2011; IBM SPSS Statistics for Windows, version 20.0, Armonk, NY: IBM Corp.). The quantitative data was described by using range (minimum and maximum), mean, standard deviation (SD), and median. Student's t-test was used to compare the two groups regarding the quantitative variables, chi-square (χ^2) test for qualitative data, and the Mann–Whitney test for the nonparametric variables. For comparisons between more than two groups, ANOVA and the Kruskal–Wallis test were used for normally and not normally distributed variables, respectively. A receiver-operating characteristic (ROC) curve was used to determine the predictive performance of the miRNAs. The p-value of ≤ 0.05 was considered to be statistically significant.

3. Results

There were no statistically significant differences between breast cancer patients and controls regarding age, parity, and menstrual status. However, a statistically significant difference ($p = 0.021$) in the family history was observed between the studied groups. In terms of biochemical analysis, ALT, AST, and ALP did not differ significantly between the participants groups. Breast cancer patients exhibited significant higher serum values of urea ($p = 0.019$), creatinine ($p < 0.001$), CEA ($p < 0.001$), and CA15-3 ($p < 0.001$), while they displayed significant lower values of Hb ($p < 0.001$), WBCs ($p < 0.001$), and platelets count ($p < 0.001$) compared to the controls. Expression assessment of the circulating levels of miR-148a and miR-30c revealed statistically significant lower values ($p < 0.001$) among patients in comparison to their values in the control group (Table 1).

Table 1
Comparison between the studied groups according to variable parameters.

	Cases (n = 75)	Control (n = 55)	p
Age group			
≤50	39 (52%)	31 (56.4%)	$^{\chi^2}$**p** = 0.622
>50	36 (48%)	24 (43.6%)	
Age (years)	49.87 ± 11.43	48.25 ± 10.45	^{t}**p** = 0.412
Parity			
Nullipara	3 (4%)	2 (3.6%)	$^{\chi 2FE}$p = 1.000
Para	72 (96%)	53 (96.4%)	
Menstrual status			
Premenopausal	40 (53.3%)	33 (60%)	$^{\chi^2}$**p** = 0.449
Postmenopausal	35 (46.7%)	22 (40%)	
Family history			
Negative	68 (90.7%)	55 (100%)	$^{\chi 2FE}$p =
Positive	7 (9.3%)	0 (0%)	0.021*
ALT (U/L) Mean ± SD	26.88 ± 7.59	25.20 ± 4.33	^{t}**p** = 0.113
AST (U/L) Mean ± SD	27.28 ± 5.89	26.55 ± 3.59	^{t}**p** = 0.380
ALP			
Min. – Max.	33–210	33–81	^{U}**p** = 0.376
Median (IQR)	58 (47.50–68.50)	62 (54–66)	
Urea (mg/dl)	28.32 ± 6.91	25.78 ± 5.22	^{t}**p** = 0.019*
Creatinine (mg/dl)			
Min. – Max.	0.40–1.80	0.30–1.50	^{U}**p** < 0.001*
Median (IQR)	0.90 (0.70–1.20)	0.70 (0.50–0.80)	
Hb (g/dl) Mean ± SD	10.12 ± 1.77	11.90 ± 0.60	^{t}**p** < 0.001*
WBCs ($\times 10^3$/μl) Mean ± SD	6.63 ± 1.79	7.85 ± 1.28	^{t}**p** < 0.001*
Platelets ($\times 10^3$/μl) Mean ± SD	181.79 ± 557	311.91 ± 54.11	^{t}**p** < 0.001*
CEA value			
Min. – Max.	0.50–60	0.40–4	^{U}**p** < 0.001*
Median (IQR)	4.30 (25–7.80)	2.30 (1.55–2.95)	
CA15–3			
Min. – Max.	9–251	5.70–26.90	^{U}**p** < 0.001*
Median (IQR)	41.60 (26.80–75)	13.90 (11.80–18.70)	
Mir-148a			
Min. – Max.	0.0–0.17	0.10–2.53	^{U}**p** < 0.001*
Median (IQR)	0.03 (0.01–0.07)	1.01 (0.51–1.76)	
Mir-30c			
Min. – Max.	0.02–24.87	19.97–382.31	^{U}**p** < 0.001*
Median (IQR)	1.89 (0.85–5.62)	42.54 (32.16–78.16)	

χ^2: Chi square test MC: Monte Carlo FE: Fisher Exact U. Mann Whitney test.
t: Student t-test IQR: Inter quartile range.
*: Statistically significant at $p \leq 0.05$.
ALT: Alanine transaminase AST: Aspartate transaminase.
ALP: Alkaline phosphatase.
Hb: Hemoglobin WBC: White blood cells.
CEA: Carcinoemberionic antigen.
CA15-3: Carbohydrate antigen 15-3.

Clinicopathological parameters of patients with breast cancer were enlisted in Table 2. According to the performance status, patients were classified into 85.3% in status 0, 13.3% in status 1, and 1.3% in status 2.38 (50.7%) patients had the tumor on the right side, while 37 (49.3%) patients had it on the left side. Regarding the pathological subtype, IDC represented 86.7% of all cases. Pathological stage 1, 2, 3, and 4 accounted for 8%, 40%, 34.7%, and 17.3% respectively. 22 (29.3%) patients experienced distant metastasis while the rest (70.7%) had localized tumors. As for the tumor grade, we found that most patients (76%) suffered from grade II tumor, followed by grade III tumor (20%), and finally grade I tumor in only 4% of patients. As per tumor stages, T1, T2, T3, and T4 were represented as follows; 12%, 52%, 21.3%, and 14.7% respectively, while nodal status N0, N1, N2, and N3 were found in 30.7%, 40%, 13.3%, and 16% of patients, respectively. 65 (86.7%) patients were ER positive, 63 (84%) patients were PR positive, while 23 (30.7%) patients were HER2/neu positive. Breast cancer patients exhibited different modalities of treatment. 66 (88%) patients underwent curative surgery, 57 (76%) patients received chemotherapy, 50

Table 2
Distribution of the studied cases (n = 75) according to different variables.

	No.(%)
Performance status ECOG	
0	64 (85.3%)
1	10 (13.3%)
2	1 (1.3%)
Comorbidities	
No	50 (66.7%)
Hepatic	5 (6.7%)
HTN	9 (12%)
DM	5 (6.7%)
Multiple	6 (8%)
Tumor side	
Right	38 (50.7%)
Left	37 (49.3%)
Pathological subtype	
IDC	65 (86.7%)
ILC	4 (5.3%)
Mixed IDC & ILC	1 (1.3%)
Other	5 (6.7%)
Pathological stage	
Stage 1	6 (8%)
Stage 2	30 (40%)
Stage 3	26 (34.7%)
Stage 4	13 (17.3%)
Metastasis status	
No	53 (70.7%)
Yes	22 (29.3%)
Grade	
Grade I	3 (4%)
Grade II	57 (76%)
Grade III	15 (20%)
PT status	
T1	9 (12%)
T2	39 (52%)
T3	16 (21.3%)
T4	11 (14.7%)
PN status	
N0	23 (30.7%)
N1	30 (40%)
N2	10 (13.3%)
N3	12 (16%)
ER	
Negative	10 (13.3%)
Positive	65 (86.7%)
PR	
Negative	12 (16%)
Positive	63 (84%)
HER2 neu	
Negative (0, +1)	52 (69.3%)
Positive (+3)	23 (30.7%)
Curative surgery	
Not done	9 (12%)
Done	66 (88%)
Chemotherapy status	
No	18 (24%)
Yes	57 (76%)
Radiotherapy status	
No	25 (33.3%)
Yes	50 (66.7%)
Hormonal ttt	
No	13 (17.3%)
Yes	62 (82.7%)
Biological treatment	
No	59 (78.7%)
Yes	16 (21.3%)
Relapse or progression status	
No	56 (74.7%)
Yes	19 (25.3%)
Died	
No	61 (81.3%)
Yes	14 (18.7%)

ECOG: Eastern Coopetative Oncology Group.
Performance state: 0 means fully active, 1 means unable to do strenuous activities, 2 means able to walk and manage self-care but cannot work).

HTN: Hypertention DM: Diabetis mellitus.
PT status: Tumor status PN status: Nodal status.
ER: Estrogen receptor PR: Progesterone receptor.
IDC: Invasive duct carcinoma ILC: Intralobular carcinoma.

(66.7%) patients received radiotherapy, hormonal treatment was delivered to 62 (82.7%) patients and only 16 (21.3%) patients were treated with biological therapy. By the end of the follow up duration, 19 (25.3%) were progressed and 14 (18.7%) patients were dead.

To validate the diagnostic ability of both miRNAs (miR-148a and miR-30c) in relation to the ordinary breast cancer tumor markers (CEA and CA 15-3), we applied the ROC curve (Table 3, Fig. 1) to discriminate breast cancer patients from controls, miR-30c showed the best performance at a cut off value of ≤20.6 (AUC = 0.998, 97.33% sensitivity, 96.36% specificity, $p < 0.001$), followed by miR-148a (AUC = 0.995, 94.67% sensitivity, 90.91% specificity, $p < 0.001$ at a cut off value of ≤0.1), CA 15-3 (AUC = 0.930, 88.0% sensitivity, 81.82% specificity, $p < 0.001$ at a cut off value of >21.3), and finally CEA (AUC = 0.751, 70.67% sensitivity, 63.64% specificity, $p < 0.001$ at a cut off value of >2.5). Additionally, miR-30c exhibited a good discriminative ability to differentiate metastatic breast cancer from non-metastatic ones (miR-30c: AUC = 0.970, 95.45% sensitivity, 94.34% specificity, $p < 0.001$ at a cut off value of ≤1.05) and miR-148a (AUC = 0.892, 81.82% sensitivity, 79.25% specificity, $p < 0.001$ at a cut off value of ≤0.02).

In breast cancer patients, the expression level of miR-148a was positively correlated with Hb ($r = 0.346$, $p = 0.002$), platelets count ($r = 0.368$, $p = 0.001$), and miR-30c ($r = 0.821$, $p < 0.001$), while it was negatively correlated with CEA ($r = -0.594$, $p < 0.001$) and CA 15-3 ($r = -0.736$, $p < 0.001$). Moreover, miR-30c displayed a positive correlation with Hb ($r = 0.263$, $p = 0.022$) and a negative correlation with CEA ($r = -0.765$, $p < 0.001$) and CA 15-3 ($r = -0.749$, $p < 0.001$) (Table 4).

The relations of the expression levels of miR-148a and miR-30c and different pathological parameters of breast cancer patients were illustrated in Table 5. A significant lower expression levels of miR-148a were detected in advanced pathological stages ($p < 0.001$), presence of distant metastasis ($p < 0.001$), widespread type of metastasis ($p = 0.004$), T3 and T4 stages ($p < 0.001$), and nodal metastasis ($p < 0.001$). As regards miR-30c, it was found to be significantly decreased in advanced pathological stages ($p < 0.001$), distant metastasis ($p < 0.001$), tumor grade ($p < 0.001$), T3 and T4 stages ($p < 0.001$), nodal metastasis ($p < 0.001$), and positive HER2/neu ($p < 0.022$).

The log rank (Mantel-Cox) of Kaplan-Meier survival curve analysis was employed in patients with breast cancer. We noticed that the lower expression levels of miR-148a were significantly associated with lower overall survival ($p < 0.001$, upper limit (UL) = 18.99, lower limit (LL) = 16.95, mean = 17.97; 95% CI(Fig. 2 a), and poor progression free survival ($p < 0.001$,UL = 17.44, LL = 13.96, mean = 15.70; 95% CI(Fig. 2 c). Decreased expression level of miR-30c was not significantly related to the overall survival ($p < 0.071$, UL = 21.98, LL = 18.63, mean = 20.30; 95% CI(Fig. 2 b), however it was associated significantly with poor progression free survival ($p < 0.001$, UL = 18.87, LL = 14.46, mean = 16.66; 95% CI(Fig. 2 d).

4. Discussion

Breast cancer is an extensively identified malignant tumor and is a prime cause of cancer mortalities among females. The particular reason of breast cancer is indistinct, but it is assumed that both aspects; genetic and environmental participate in the tumor genesis and advancement of this cancer [22]. The miRNAs have a role as tumor suppressor or oncogenic factors in malignant cells and may be utilized as potential markers in diagnosis, identification and determination of the therapeutic protocols of various diseases as malignant tumors [23]. Previous studies of miRNAs have used tissue specimens. Recently, there has been a trend to use cell-free circulating miRNAs as potential biomarkers for various cancers with the benefits of less invasive procedures and possible samples repeating [24].

In the current investigation, we meant to evaluate the role of both circulating miRNA-148a and miRNA-30c in breast cancer and assess their usage as potentially biomarkers in the diagnosis, prognosis and survival of breast cancer.

In this current analysis, we detailed lower expression levels of both miRNA-148a and miRNA-30c in cancer patients than in controls and the lower expressions of both miRNAs were related to advanced tumor stages and presence of metastasis. Also, miRNA-148a and miRNA-30c had good sensitivity and specificity to recognize breast cancer patients and presence of metastasis.

In consistent with our finding, previous studies conducted on tissue specimens, reported a decreased expression of miR-148a in breast cancer tissue [13,25]. Additionally, **Jiang et al.,** [12] reported similar results of down regulation of miR-148a in both breast cancer tissue and confirmed by similar outcomes on cell lines. They also correlated the lower expression levels to the presence of nodal metastasis, which come on line with our finding of lower miR-148a expression level in advanced lymph node involvement stage. Moreover, our results reveled the decreased expressions of miR-148a were related to advanced tumors stage and presence of metastasis.

Breast cancer metastasis is a multi-factorial pathology. Various mechanisms like TGF-β, WNT, NFκB and JAK-STAT signaling mechanisms, are supposed to participate in tumor progression and development of metastasis [26]. **MacDonald and colleagues** [27] detailed that Wnt/β-catenin pathway have a critical act in controlling cellular proliferation and differentiation. Also **Jiang et al.,** [12] stated that miR-148a might suppress the migration and invasion of malignant breast cells via affecting WNT-1 and repression of Wnt/β-catenin mechanisms. They likewise verified a negative relation between miR-148 and WNT-1 expression in cancer tissues.

Xu et al., [28] stated that, miR-148a acts as a tumor suppressor and inhibits the extravasation of malignant cells through affecting some genes such as WNT1. They also reported down regulation of miR-148a in higher-grade tumor and presence of metastases, which in agreement

Table 3
Agreement (sensitivity, specificity) for CEA, CA15.3, Mir-148a and Mir-30c to differentiate between different groups.

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Patients with breast cancer (n = 75) vs. control (n = 55)								
Mir-148a	0.995	<0.001*	0.988–1.002	≤0.1	94.67	90.91	93.4	92.6
Mir-30c	0.998	<0.001*	0.994–1.002	≤20.6	97.33	96.36	97.3	96.4
CEA	0.751	<0.001*	0.667–0.836	>2.5	70.67	63.64	72.6	61.4
CA15–3	0.930	<0.001*	0.883–0.976	>21.3	88.0	81.82	86.8	83.3
Patients with metastasis (n = 22) vs.. non-metastatic (n = 53)								
Mir-148a	0.892	<0.001*	0.817–0.966	≤0.02	81.82	79.25	62.1	91.3
Mir-30c	0.970	<0.001*	0.929–1.011	≤1.05	95.45	94.34	87.5	98.0

AUC: Area Under a Curve p value: Probability value.
CI: Confidence Intervals.
NPV: Negative predictive value PPV: Positive predictive value.

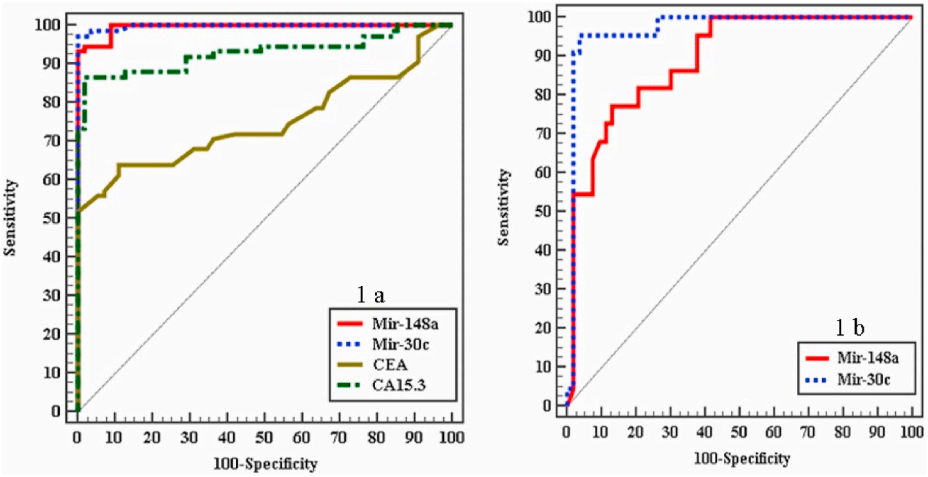

Fig. 1. (1 a): ROC curve for CEA, CA15.3, Mir-148a and Mir-30c to discriminate patients with breast cancer (n = 75) vs. control (n = 55) (1 b): ROC curve for Mir-148a and Mir-30c to discriminate patient with metastasis in breast cancer group (22/53).

Table 4
Correlation between Mir-148a with Mir-30c and laboratory finding in cases group (n = 75).

	Mir-148a		Mir-30c	
	r_s	p	r_s	p
ALT (U/L)	0.010	0.933	0.208	0.073
AST (U/L)	-0.047	0.687	0.165	0.157
ALP	-0.053	0.655	0.122	0.299
Urea (mg/dl)	-0.062	0.597	0.102	0.385
Creatinine (mg/dl)	-0.149	0.202	-0.135	0.249
Hb (g/dl)	0.346	0.002*	0.263	0.022*
WBCs (×10³/μl)	-0.038	0.746	0.074	0.530
Platelets (×10³/μl)	0.368	0.001*	0.186	0.110
CEA	-0.594	<0.001*	-0.765	<0.001*
CA15-3	-0.736	<0.001*	-0.749	<0.001*
Mir-30c	0.821	<0.001*		

r_s: Spearman coefficient

ALT: Alanine transaminase
AST: Aspartate transaminase
ALP: Alkaline phosphatase
Hb : Hemoglobin
WBC: White blood cells
CEA : Carcinoemberionic antigen
CA15-3: Carbohydrate antigen 15-3

with our finding.

Induction of miR-148a expression in breast cancer cell line was reported to inhibit cellular proliferation and migration [29] Furthermore, over expression of miR-148a led to inhibition of breast cancer cell proliferation with an elevation in apoptosis through targeting B-cell lymphoma, that previously had established in breast cancer cells [25]. In addition, elevated miR-148a level suppresses migration and invasion in hepatocellular carcinoma and, knocking down miR-148a has the reverse effect [30].

Additionally, our current analysis demonstrated decreased circulatory expression levels of miR-30c in breast cancer patients. In agreement with our finding miRNA-30c levels were found to be down regulated in multi-drug resistant breast cancer cell lines versus parent cells [31]. Recently, these findings were confirmed also by **Pei and colleagues** on

Table 5
Relation between Mir-148a, Mir-30c and different variables in cases group (n = 75).

	N	Mir-148a		Mir-30c	
		Median (Min. – Max.)	Mean ± SD.	Median (Min. – Max.)	Mean ± SD.
Pathological subtype					
IDC	**65**	0.04(0.0 – 0.17)	0.05 ± 0.04	1.890.02 – 24.87	3.62 ± 4.49
ILC	**4**	0.01(0.0 – 0.08)	0.03 ± 0.04	2.060.02 – 4.80	2.24 ± 2.57
Mixed IDC & ILC	**1**[#]	0.01[#]			0.26[#]
Other	**5**	0.03(0.02 – 0.08)	0.05 ± 0.03	5.724.94 – 10.45	6.53 ± 2.22
H(p)		2.538(0.281)		6.409*(0.041*)	
Pathological stage					
Stage 1	**6**	0.08(0.02 – 0.10)	0.07 ± 0.03	6.691.05 – 7.96	5.95 ± 2.57
Stage 2	**30**	0.07(0.01 – 0.17)	0.07 ± 0.04	5.251.09 – 24.87	6.40 ± 5.22
Stage 3	**26**	0.03(0.0 – 0.13)	0.03 ± 0.03	1.480.02 – 6.90	1.77 ± 1.66
Stage 4	**13**	0.01(0.0 – 0.02)	0.01 ± 0.0	0.090.02 – 1.0	0.28 ± 0.36
H(p)		34.472*(<0.001*)		46.138*(<0.001*)	
Metastasis status					
No	**53**	0.06(0.0 – 0.17)	0.06 ± 0.04	4.530.02 – 24.87	5.06 ± 4.49
Yes	**22**	0.01(0.0 – 0.04)	0.01 ± 0.01	0.210.02 – 1.89	0.41 ± 0.48
U(p)		126.0*(<0.001*)		35.0*(<0.001*)	
Type of metastasis					
Oligo metastasis (1–3)	**8**	0.020.01 – 0.04)	0.02 ± 0.01	0.540.02 – 1.89	0.65 ± 0.60
Widespread	**14**	0.01(0.0 – 0.02)	0.01 ± 0.0	0.140.02 – 1.0	0.28 ± 0.34
U(p)		15.0(0.004*)		30.0 (0.082)	
Grade					
Grade I	**3**	0.03(0.03 – 0.08)	0.05 ± 0.03	5.644.94 – 10.45	7.01 ± 3.0
Grade II	**57**	0.04(0.0 – 0.17)	0.05 ± 0.04	3.510.03 – 24.87	4.28 ± 4.60
Grade III	**15**	0.02(0.0 – 0.06)	0.03 ± 0.02	0.260.02 – 2.65	0.82 ± 0.96
H(p)		5.768(0.056)		16.928*(<0.001*)	
PT status					
T1	**9**	0.07(0.01 – 0.10)	0.05 ± 0.03	5.721.05 – 7.96	4.62 ± 2.89
T2	**39**	0.05(0.01 – 0.17)	0.06 ± 0.04	4.800.18 – 24.87	5.22 ± 5.17
T3	**16**	0.01(0.0 – 0.09)	0.02 ± 0.02	0.080.02 – 1.89	0.65 ± 0.77
T4	**11**	0.02(0.01 – 0.070)	0.03 ± 0.02	1.600.26 – 4.95	1.99 ± 1.41
H(p)		18.891*(<0.001*)		25.905*(<0.001*)	
PN status					
N0	**23**	0.08(0.02 – 0.17)	0.08 ± 0.04	5.901.05 – 24.87	7.62 ± 5.43
N1	**30**	0.02(0.0 – 0.12)	0.03 ± 0.03	1.840.02 – 9.85	2.51 ± 2.29
N2	**10**	0.02(0.01 – 0.06)	0.02 ± 0.02	1.390.80 – 2.65	1.41 ± 0.56
N3	**12**	0.01(0.0 – 0.13)	0.03 ± 0.04	0.150.02 – 6.90	1.06 ± 2.24
H(p)		27.141*(<0.001*)		36.321*(<0.001*)	
ER					
Negative	**10**	0.02(0.0 – 0.07)	0.03 ± 0.03	2.270.02 – 6.80	2.86 ± 2.48
Positive	**65**	0.03(0.0 – 0.17)	0.05 ± 0.04	1.890.02 – 24.87	3.83 ± 4.55
U(p)		252.0(0.255)		0.308(0.791)	
PR					
Negative	**12**	0.02(0.0 – 0.07)	0.03 ± 0.02	1.890.02 – 6.80	2.63 ± 2.31
Positive	**63**	0.04(0.0 – 0.17)	0.05 ± 0.04	2.650.02 – 24.87	3.90 ± 4.61
U(p)		300.0(0.260)		347.0(0.654)	
HER2 neu					
Negative (0, +1)	**52**	0.06(0.0 – 0.17)	0.05 ± 0.04	3.660.02 – 24.87	4.46 ± 4.85
Positive (+3)	**23**	0.02(0.0 – 0.13)	0.03 ± 0.03	1.050.02 – 6.90	1.97 ± 2.02
U(p)		437.0 (0.064)		398.0*(0.022*)	

H: H for Kruskal Wallis test

#: Excluded from the comparison due to small number of case (n = 1)

IDC: Invasive duct carcinoma ILC: Intralobular carcinoma

PT status: Tumor status PN status: Nodal status

ER: Estrogen receptor PR: Progesterone receptor

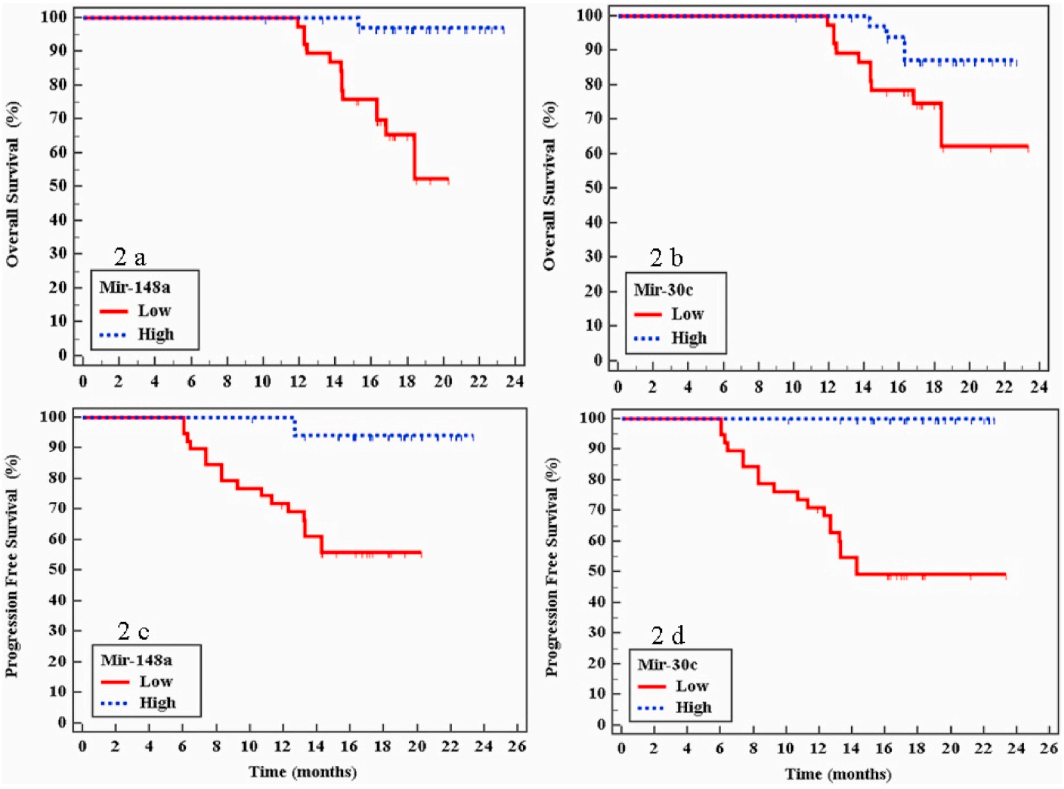

Fig. 2. (2 a): Kaplan-Meier survival curve for Overall Survival according to Mir-148a in breast cancer group (2 b): Kaplan-Meier survival curve for Overall Survival according to Mir-30c in breast cancer group (2 c): Kaplan-Meier survival curve for Progression Free Survival according to Mir-148a in breast cancer group (2 d): Kaplan-Meier survival curve for Progression Free Survival according to Mir-30c in breast cancer group.

their analysis on breast cancer tissues [32]. They determined decreased expression of miR-30c-5p in breast cancer tissues, moreover they reported an inverse relationship of miR-30c-5p with coactosin-like protein 1 (COTL1) in these tissues. Also, miR-30c-5p down regulation induced the expression of COTL1 resulting in enhanced cellular migration.

The miR-30c has been shown to inversely control triple helix repeat containing-1(CTHRC1) which is more expressed in breast cancer cell and related to progressive tumor and poor prognosis and was assumed to enhance cellular proliferation and migration with repressed apoptosis [33]. In addition, miR-30c was reported to regulate regulates cell cycle progression via affecting NF-kB signaling pathway and its elevated expression is associated with decreased cell viability and enhanced apoptosis [34]. All explains our finding of decreased circulatory expression levels of miR-30c in breast cancer patients and their relation to advanced tumors stage and presence of metastasis.

Additionally in this study, we reported a significant value of miRNA-30c and miRNA-148a as sensitive and specific markers for recognition of breast cancer patients and presence of metastasis and based on Kaplan-Meier survival curve analysis, the lower expression was linked to lower progression free survival. The lower miRNA-148a level was associated with lower overall survival as well.

Similarly, **Rodríguez-González et al.,** [18] reported that over expression of miR-30c was associated with better response to tamoxifen therapeutics and longer progression free survival and miRNA-30c was considered as an independent predictor in breast cancer. In addition, miRNA-30c was allied to poor prognosis in breast cancer [35].

Nanotechnology has shown a great deal of possibilities in cancer diagnosis throughout the last years due to its enhanced pharmacokinetic and pharmacodynamics properties [36].Recently, miRNAs in body fluids are considered as potential noninvasive biomarkers of various diseases such as breast cancer [37].

Lower level of miR-148a was found in women with ovarian cancer and was related to tumor grade, stage and nodal metastasis. Further, patients with elevated level of miR-148a had longer survival [38]. In bladder cancer tissues, decreased miR-148a expression was related to high tumor grade and tumor recurrence with positive association between its expression level and survival time [39].

5. Conclusion

From our results, we conclude that both miR-148a and miRNA-30c expressions were down regulated in female patients with breast cancer and might be considered as potential blood biomarkers. Both also might have rule in disease treatment and selection of therapeutic targets. Future studies are needed to improve their role in predicting response to treatment and prognosis.

Authors' contributions

Nesreen G. Elhelbawy (the corresponding author), Nesreen G. Elhelbawy and Eman A Fouda performed the laboratory investigations and the molecular analysis beside to selecting the study design. Ibrahim F Zaid was a major contributor in writing the manuscript. Suzy F Gohar and Aya A Khalifa collected the samples and analyzed and interpreted the results. All authors shared in writing and revision of the manuscript and approved the final copy.

Funding

None.

Declaration of competing interest

All authors declare no conflicts of interest.

Acknowledgements

We appreciate all participants who contributed in this study.

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