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RESEARCH ARTICLE

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Potential value of circulatory microRNA10b gene expression and its target E-cadherin as a prognostic and metastatic prediction marker for breast cancer

Fatma Ibrahim Dwedar[1] | **Reham Said Shams-Eldin**[1] | **Salwa Nayer Mohamed**[2] | **Ayman Farouk Mohammed**[3] | **Salwa Hamdi Gomaa**[4]

[1]Department of Medical Biochemistry, Faculty of Medicine, Alexandra University, Alexandria, Egypt

[2]Department of Cancer management and Research, Medical Research Institute, Alexandria University, Alexandria, Egypt

[3]Department of Clinical and Experimental Surgery, Medical Research Institute, Alexandria University, Alexandria, Egypt

[4]Department of Chemical pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt

Correspondence
Salwa Hamdi Gomaa, Department of Chemical pathology, Medical Research Institute, Alexandria, Egypt.
Email: salwa.hamdi74@yahoo.com

Abstract

Background: Breast cancer (BC) is the leading cause of cancer death in women worldwide. Most BC studies on candidate microRNAs were tissue specimen based. Recently, there has been a focus on the study of cell-free circulating miRNAs as promising biomarkers in (BC) diagnosis and prognosis. Therefore, we aimed to investigate the circulating levels of miR-10b and its target soluble E- cadherin as potentially easily accessible biomarkers for breast cancer.

Methods: Sixty-one breast cancer patients and forty-eight age- and sex-matched healthy volunteers serving as a control group were enrolled in the present study. Serum samples were used to assess miRNA10b expression by TaqMan miRNA assay technique. In addition, soluble E-cadherin expression level in serum was determined using ELISA technique.

Result: Circulating miR-10b expression level and serum sE-cadherin was significantly upregulated in patients with BC compared to controls. Moreover, serum miR-10b displayed progressive up-regulation in advanced stages with higher level in metastatic compared to non-metastatic BC. Additionally, the combined use of both serum miR-10b and sE-cadherin revealed the highest sensitivity and specificity for detection of BC metastasis (92.9% and 97.9% respectively) with an area under curve (AUC) of 0.98, 95% CI (0.958–1.00).

Conclusion: Our data suggest that circulating miR-10b could be utilized as a potential non-invasive serum biomarker for diagnosis and prognosis of breast cancer with better performance to predict BC metastasis achieved on measuring it simultaneously with serum sE-cadherin. Further studies with a large cohort of patients are warranted to validate the serum biomarker for breast cancer management.

KEYWORDS
breast cancer, circulating miR-10b, non-invasive biomarker, soluble E-cadherin, TaqMan miRNA assay

1 | INTRODUCTION

Breast carcinoma is the most common malignancy among women worldwide. It causes more than 0.5 million deaths every year.[1] Despite the advance in the current breast cancer (BC) therapies, metastasis is still the major cause of cancer-related death in most BC patients.[1] Almost 30% of patients diagnosed with early-stage BC may develop distant metastasis months or even years later.[2] So far, metastatic BC is an incurable disease and remains the critical challenge facing oncologists.

The underlying molecular mechanism of BC metastasis is still incompletely understood. MicroRNAs (miRNAs) are a class of short single-stranded cellular RNAs that are approximately between 18 and 25 nucleotides in length.[3] They are important regulators of the expression of protein-coding genes or long non-coding RNAs (lncRNAs),[4] by inhibiting target mRNA translation or by promoting target RNA degradation.[3] Studies have shown that aberrant expression of miRNAs is associated with several types of human cancers, tumor invasiveness, and metastasis.[5,6]

Several miRNAs were found to play a crucial role in tumor metastasis and recurrence, recently called MetastamiRs. Some of these miRNAs have been reported to be dysregulated in metastatic BC.[7]

The miR-10 family of miRNAs consists of two members: miR-10a and miR-10b, which are located at chromosome 17 and 2, respectively.[8] Among all identified miRNAs, miR-10b was the first miRNA to be reported by Ma and his colleagues as a promoter for cancer metastasis.[9] Later on, a number of studies showed that miR-10b is highly expressed in metastatic cancer tissues, including pancreatic cancer,[10] glioblastoma,[11] gastric cancer,[12] and recently metastatic colorectal cancer.[13]

Previous studies on BC showed that miR-10b was over-expressed in metastatic breast cancer tissue promoting tumor cell migration and invasion.[7,14] On contrary, other authors showed miR-10b down-regulation in primary breast tumors compared with normal breast tissue.[15]

However, both in vivo and in vitro studies suggest involvement of miR-10b in BC invasiveness and metastasis and therefore is suggested to be a useful prognostic biomarker.[16,17] A better understanding of the role of circulating miR-10b in metastasis will help in the development of miRNA-based, anti-metastasis targeted therapies.

On the other hand, E-cadherin (E-cad) is one of the members of a family of transmembrane glycoproteins and a calcium-dependent adhesion molecule. The soluble form of E-cadherin (sE-cadherin) is produced by the cleavage of the extracellular domain of the anchored protein (120 kDa), leading to the release of fragments of 80 kDa.[18]

Aberrant expression of E-cadherin have been associated with the development of breast cancer metastases and other cancers.[19] Although in vitro studies have proved an association between reduced E-cadherin expression and tumor invasion, this association has not been confirmed in vivo yet.[20]

It has been postulated that E-cadherin is one of the targets of miR-10b through which it may exert its metastatic effect on breast cancer cell lines.[21,22] However, the exact mechanism of circulating miR-10b involvement in the BC metastasis is still unclear. Based on the proven clinical relevance of miR-10b to BC, the aim of this study was to investigate the circulating levels of miR-10b and its target soluble E-cadherin as a potentially easily accessible biomarkers that could be used in diagnosis, prognosis, and metastasis prediction of breast cancer in Egyptian female patients.

2 | SUBJECTS AND METHODS

Following approval of the Alexandria university committee of medical ethics (approval ID: 0302182), serum samples were obtained from 61 breast cancer patients before surgery at the Department of Surgery at the Medical Research Institute Hospital, Alexandria University. In addition to a group of 48 age and sex-matched healthy volunteers served as a control group. A written informed consent was obtained from every subject, and the study was conducted in compliance with the Helsinki Declaration.

Full history taking, thorough clinical examination and fine needle aspiration cytology (FNAC) or excision biopsy from the breast mass for pathological examination were done to all patients. Laboratory investigations including fasting blood sugar, kidney functions, liver functions, and tumor markers were done. In addition to radiological examination including ultrasound of both breasts, mammography, pelvic-abdominal ultrasound, chest X-ray, and bone scan. Postoperative pathology examination as well as hormonal Estrogen and progesterone receptors (ER & PR), and epidermal growth factor receptor 2(HER-2) were done. Clinical staging was performed according to tumor-node-metastasis classification system (TNM). Serum miR-10b expression and serum E-cadherin levels were estimated for all patients and healthy controls.

2.1 | Assessment of serum miR-10b expression levels

2.1.1 | Sample collection, total RNA extraction, and reverse transcription

The blood samples were collected from all subjects in serum gel separator tubes. Each sample was centrifuged at 3,000 *g* for 10 min to separate serum and then stored at −80°C until RNA extraction. Total RNA containing small RNAs was isolated from 100 μl of serum using miRNeasy kit (cat. no. 217004; Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then, 40 μl of RNase-free water was used for RNA elution. NanoDrop 2000/2000c (Thermo Scientific, Wilmington, DA, USA) was used to check RNA quality and quantity. Complementary DNA (cDNA) was synthesized from RNA samples using TaqMan® MicroRNA Reverse Transcription Kit with miRNA primers specific for miR-10b (ID: 002218) and miR-16 (ID: 000391) (Applied Biosystems, Foster City, CA, USA) following

the manufacturer's protocol. Thermal profile was as follows: 16°C (30 min), 42°C (30 min), followed by 85°C for 5 min and a final hold at 4°C.

2.1.2 | Quantitative real-time PCR (qPCR)

Real-time PCR was performed using Applied Biosystems Step One™ Real-time PCR System, thermal cycler (Block, foster City, CA, USA) using TaqMan MicroRNA Assay reagents purchased from Applied Biosystems, CA. miRNA10b Assay (ID 002218) and miRNA16 Assay (ID 000391) which was used as an internal control.

Amplification was carried out in a final volume of 20 µl including 2× TaqMan Universal Master Mix no AmpErase UNG, 20× TaqMan Assay for miR10b and RT product in a concentration of up to 10 ng per reaction. Thermal cycling conditions were as follow: an initial hold at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s (denaturation step) and 60°C for 1 min (annealing/extension). Comparative cycle threshold (2−ΔΔCt) method[23] was used to calculate miR10b expression in serum samples normalized to miR16 expression[23] and relative to healthy controls.

2.1.3 | Assay for serum levels of soluble E-cadherin

Serum soluble E-cad expression levels were estimated by enzyme-linked immunosorbent assay (ELISA) technique using an immunoassay kit (Miltenyi, Germany) according to the manufacturer's instructions. The standard curve was established with OD as the Y axis and the concentration of standard substance as the X-axis. The level of protein was obtained through the standard curve.

2.2 | Statistical analysis

Data were analyzed using SPSS software package version 20.0 (Armonk, NY-IBM Corp). Qualitative data were compared using chi-square test or Fisher exact test and were expressed as numbers and percent. Normally distributed quantitative data were compared using Student's *t* test and were expressed as mean and SD. Unusually distributed quantitative variables were compared using Mann-Whitney *U* test. Receiver operating characteristic (ROC) curve was used to determine the diagnostic performance of the studied biomarkers. Statistical significance was set at $p < 0.05$.

3 | RESULTS

The study included 61 female patients with BC with a mean age of 54.7 ± 14.1 years and 50 age-matched healthy female volunteers. According to TNM-staging system, most of BC patients ($n = 32$, 52.5%) were at stage II, 11 patients (18.0%) at stage III and 18 patients (29.5%) at stage IV. 18 out of 61 patients (29.5%) had distant metastasis and 14 out of all patients (25.5%) died during the period of the study, Table 1. All demographic and clinical data as regards age, family history, histological grading as well as hormone receptor status and molecular subtypes of BC are shown in Table 1.

3.1 | miR-10b relative expression level and serum sE-cadherin level in breast cancer

The BC patients showed significant up-regulation in miR-10b expression level (3.45 ± 3.0 fold change) compared to that of the control group (1.22 ± 1.40 fold change), ($p = 0.004$), Figure 1. Moreover, miR-10b expression level was significantly higher in the patients with BC metastasis compared to those with non-metastatic BC (5.6 ± 2.5 versus 3.0 ± 2.9 fold change) ($p = 0.008$), Figure 2. As regards serum sE-cadherin level, it was significantly higher in BC patients compared to that of the control group, (1846.3 ± 312.3 ng/ml *vs*. 631.5 ± 63.2 ng/ml, $p < 0.001$), Table 2. Furthermore, it was significantly higher in the patients with BC metastasis compared to patients with non-metastatic BC (2149.4 ± 291.5 ng/ml *vs*. 1719.4 ± 221.6 ng/ml), Table 2. No significant difference was observed between miR-10b expression levels regarding the molecular subtypes of BC, Figure 3.

Significant higher miR-10b expression level and serum sE-cadherin level were observed when patients with HER2 positive were compared to those with HER2 negative ($p = 0.001$, $p = 0.041$, respectively), Figure 4A,B.

On comparing miR-10b level between the different TNM stages in BC patients, a statistically significant difference was detected among stages II, III, and IV ($p < 0.001$). Applying post hoc test revealed a higher miR-10b gene expression in advanced stages (III and IV) as compared to earlier ones (stage II), Figure 5.

3.2 | Correlation analysis of serum miR-10b level and serum sE-cadherin level with the tumor characteristics of breast cancer

Furthermore, the association of the fold change of expression of miR-10b and serum sE-cadherin level with the tumor stage, tumor grade, tumor size, and lymphatic/vascular invasion were assessed. miR-10b expression level demonstrated positive association with serum sE-cadherin level, ($r = 0.683$, $p < 0.001$). In addition, both serum sE-cadherin and circulating level of miR-106b were found closely related to tumor size, tumor grade, and lymph node metastasis, Table 3.

3.3 | Diagnostic performance of serum miR-10band serum sE-cadherin levels

The receiver operating characteristic curve (ROC) analysis showed that miR-10b expression level in serum at a cutoff of >1.4 fold change could discriminate BC patients from control subjects with

TABLE 1 Clinico-pathological characteristics of the studied breast cancer patients (n = 61)

Characteristic	Breast cancer patients (n = 61)	Control group (n = 48)	Test of sig.	p-value
Diagnosis				
Rt Br Ca	45 (73.8%)	–		
Lt Br Ca	16 (26.2%)	–		
Tumor size (cm)				
≤2	5 (8.2%)	–		
>2	56 (91.8%)	–		
Mean ± SD.	3.9 ± 1.6	–		
Median (min–max.)	4 (2–4)	–		
Pathological type				
IDC I	4 (6.6%)	–		
IDC II	34 (55.7%)	–		
IDC III	23 (37.7%)	–		
Vascularitv	59 (96.7%)	–		
Lymphnode status				
Negative	15 (24.6%)	–		
Positive	46 (75.4%)	–		
Histological grading				
I	6 (9.8%)	–		
II	30 (49.2%)	–		
III	25 (41.0%)	–		
ER				
Negative	21 (34.4%)	–		
+	6 (9.8%)	–		
++	16 (26.2%)	–		
+++	18 (29.5%)	–		
PR				
Negative	27 (44.3%)	–		
+	10 (16.4%)	–		
++	14 (23.0%)	–		
+++	10 (16.4%)	–		
HER2				
Negative	33 (54.1%)	–		
+	10 (16.4%)	–		
++	8 (13.1%)	–		
+++	10 (16.4%)	–		
Molecular subtype (receptor classification)				
Luminal A	21 (34.4%)	–		
Luminal B	19 (31.1%)	–		
HER2 enriched	8 (13.1%)	–		
Triple negative	13 (21.3%)	–		
Metastasis				
Absent	43 (70.5%)	–		
Present	18 (29.5%)	–		
TNM stage				
II	40 (65.6%)	–		

(Continues)

TABLE 1 (Continued)

Characteristic	Breast cancer patients (*n* = 61)	Control group (*n* = 48)	Test of sig.	*p*-value
III	8 (13.1%)	–		
IV	13 (21.3%)	–		
Follow up				
Still alive	41 (74.5%)	–		
Died	14 (25.5%)	–		
Age (years)	54.7 ± 14.1	50 ± 17.3	t = 1.563	0.121
Mid arm (cm)	27.95 ± 3.66	28.25 ± 2.67	t = 0.101	0.904
Waist (cm)	114.90 ± 16.80	123.20 ± 11.25	t = 1.988	0.146
Chest (cm)	114.35 ± 14.18	114.60 ± 7.69	t = 1.867	0.164
BMI (kg/m^2)	28.39 ± 1.64	27.75 ± 1.40	t = 0.861	0.428
Family history of breast cancer	2 (3.3%)	0 (0%)	χ^2 = 1.603	^{FE}p = 0.503

Note: Quantitative data were expressed using Mean ± SD.

p: p-value for comparing between the studied groups

Abbreviations: χ^2, Chi-square test; FE, Fisher exact; *t*, Student's *t* test.

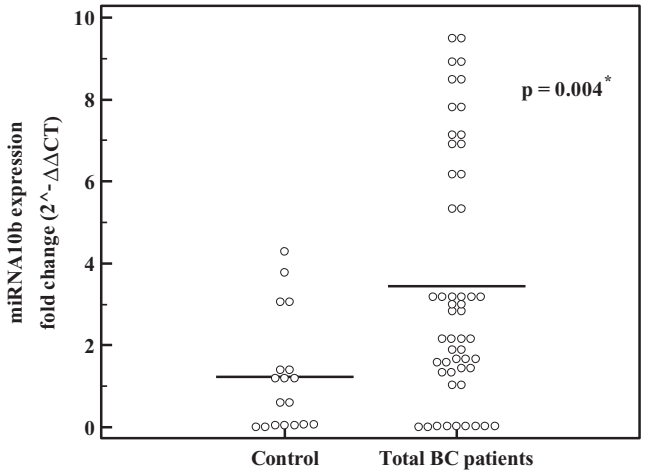

FIGURE 1 Comparison between BC patients and control subjects according to miRNA10b expression level in serum

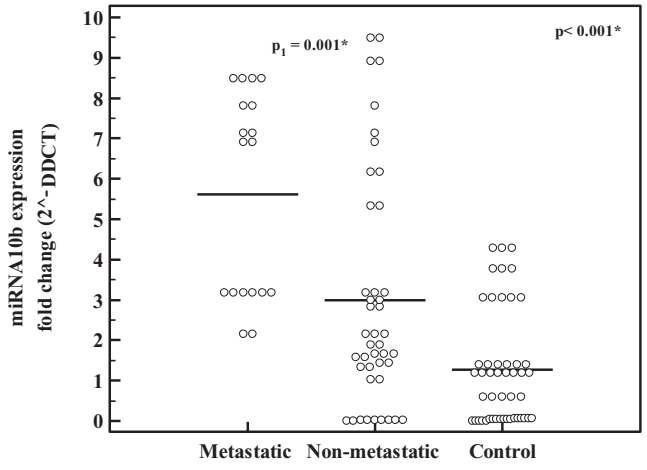

FIGURE 2 Comparison between breast cancer patients (metastatic and non-metastatic) and controls according to miRNA10b expression level in serum

an area under the curve (AUC) 0.75, a sensitivity of 78.69%, a specificity of 77.08%, and a 95% confidence interval (CI) of (0.657–0.845), (p < 0.001), Figure 6A. Moreover, ROC analysis showed that miR-10b expression level in serum at a cutoff of 7.14 fold change has a high ability to distinguish patients with BC metastasis from those without metastasis with an (AUC) 0.98, a sensitivity of 76.9%, a specificity of 97.9% and a 95% (CI) of (0.96–1.0), (p < 0.001), Figure 6B. Furthermore, ROC curve analysis for serum sE-cadherin achieved an AUC of 0.82 at a cutoff of >1,780 ng/ml with a sensitivity of 76.9%, a specificity 70.8% and a 95% CI of (0.72–0.92), Figure 6C. While, combined use of both serum miR-10b and sE-cadherin revealed the highest sensitivity and specificity (92.9% and 97.9%, respectively) with an AUC of 0.98 and a 95% CI of (0.95–1.00), Figure 6D.

Our study concluded that BC with metastasis is clearly accompanied by over expression of serum miR-10b and higher serum sE-cadherin levels compared to BC without metastasis, which are in turn significantly associated with tumor stage and tumor size. This indicates that both miR-10b and sE-cadherin can be involved in the development and progression of breast cancer.

4 | DISCUSSION

Breast cancer is the second most common cause of deaths reported in women worldwide. Ninety percent of cancer-related death is caused by metastasis, and therefore, there is a need to identify breast cancer patients at an early stage.[24] Additionally, the existing diagnostic tools and biomarkers are not sensitive enough to detect every early metastasis in lymph nodes or other organs.[25] MicroRNAs belong to a large group of non-coding RNA molecules that regulate gene expression at post-transcriptional level.[26] Therefore, numerous studies have supported the promising role of candidate microRNAs in breast cancer diagnosis, prognosis and for monitoring response

	Control (n = 48)	Total BC patients (n = 61)	Metastatic cases (n = 18)	Non-metastatic cases (n = 43)
sE-cadherin	$631.5^{c} \pm 63.2$	$1846.3^{\#} \pm 312.3$	$2149.4^{a} \pm 291.5$	$1719.4^{b} \pm 221.6$

Note: Pairwise comparison bet. each 2 groups was done using post hoc test (Tukey) for ANOVA test. Means with common letters are not significant (ie, means with different letters are significant)

Abbreviation: BC, breast cancer.

#For comparing between total cases and control using student's *t* test.

TABLE 2 Comparison between BC patients and controls according to serum soluble E-cadherin expression level

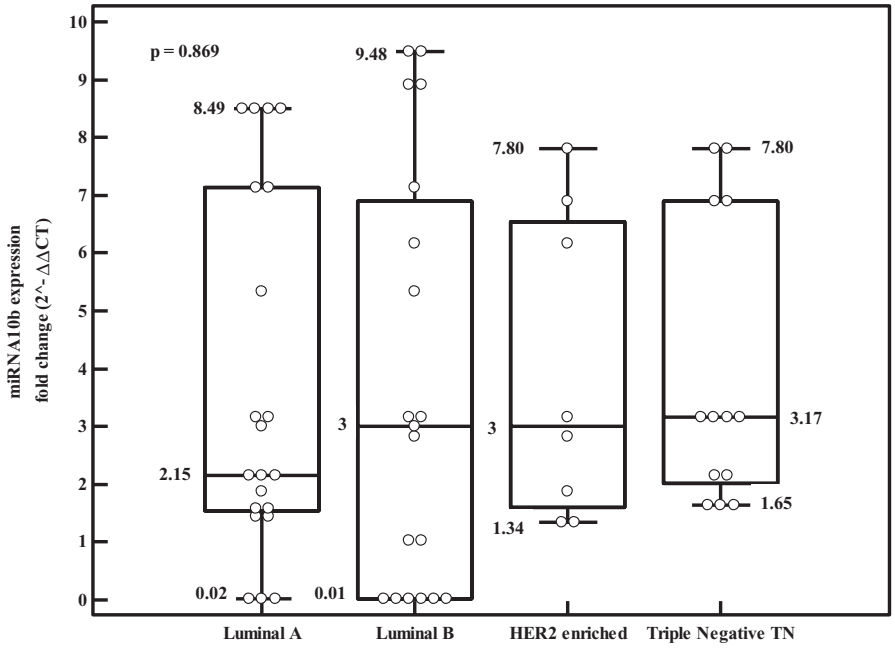

FIGURE 3 Relation between miRNA10b expression with molecular Subtypes (n = 61)

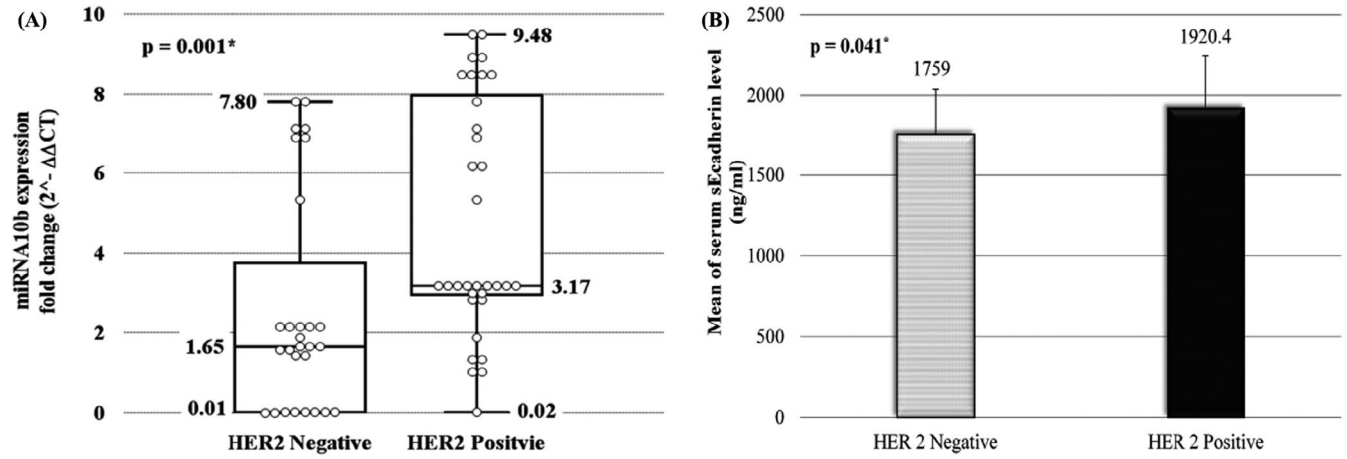

FIGURE 4 (A) Relation between miRNA10b expression and HER2 status (n = 61). (B) Relation between sE-cadherin and HER2 status (n = 61)

to anticancer therapy.[27] Most studies conducted on miRNAs were tissue specimen based. In the previous few years, there has been a focus on the study of cell-free circulating miRNAs as markers for different types of cancers due to the advantages of non-invasiveness, as well as the benefits of stability and possible repeatability of sampling.[28] Among the studied miRNAs is miR-10b, one of the promising candidate microRNAs in BC diagnosis and prognosis and a biomarker of metastasis in a variety of malignancies.[7]

In this work, we found significant higher expression level of circulating miR-10b in breast cancer patients (3.45 ± 3.0 fold change) compared to controls (1.22 ± 1.40 fold change). We analyzed the relation between expression of miR-10b and the clinicopathological

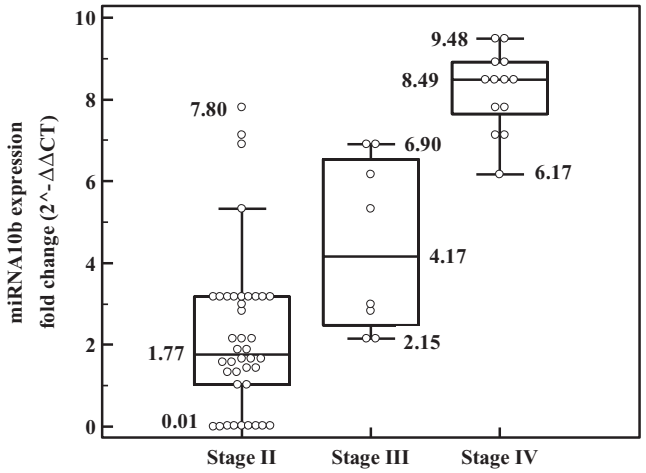

FIGURE 5 Relation between miRNA10b expression level with tumor stage (n = 61)

TABLE 3 Correlation between miRNA10b and tumor characteristics in BC patients (n = 61)

	miRNA10b		E-cadherin	
	r_s	p	r	p
E-cadherin	0.683*	<0.001	–	–
Tumor size	0.537*	<0.001	0.424*	0.001
Lymph node status	0.232	0.072	0.297*	0.020
Tumor grade	0.532*	<0.001	0.637*	<0.001
ER	−0.094	0.473	0.019	0.883
PR	0.028	0.830	0.045	0.729
HER2	−0.061	0.639	−0.174	0.180

Abbreviations: r, Pearson coefficient; r_s, Spearman coefficient.
*Statistically significant at $p \leq 0.05$.

criteria of the studied patients, we found miR-10b expression levels gradually upregulated with the tumor stage with significant higher levels in stage IV compared to earlier stages. There was also a significant relation with the positivity of axillary LNs, tumor size, tumor grade, and HER2 positivity.

Our results agreed with, Khalighfard et al.[29] who noted that the serum expression levels of the oncomiRs such as miR-10b were significantly increased in BC patients compared with the healthy participants and significantly associated with TNM staging. Consistently, Zhang et al.[30] found that the expression of miR-10b in breast cancer tumor tissues gradually increased with different stages of cancer and found maximum level of miR-10b expression at stage IV compared to stage I. Contrariwise, several studies of miR-10b expression on tissue specimens observed decrease in its level in breast cancer cells compared to normal tissue.[9,31,32]

This apparent contradiction in the results of the studies conducted on tissue specimens and those conducted on cell-free blood samples, like our study, could be explained by the fact that breast cancer cells secrete increased amounts of miRNAs compared to normal cells, and this is not accompanied by a parallel increase in intracellular levels, as most disease cell lines show diminished levels of intracellular miRNAs because they discharge miRNAs into extracellular vesicles.[33] Furthermore, Chan et al.[34] revealed that while miR-10b was downregulated in tumor tissues in comparison with normal breast tissue, it showed overexpression in the corresponding serum specimens. They explained that by the presence of a subpopulation within the primary tumor over-expressing miR-10b and responsible for its shedding in the peripheral blood.

The present study revealed, also, a significant higher serum levels of miR-10b in breast cancer patients with metastasis compared with those without metastasis. ROC analysis demonstrated that miR-10b expression level at a cutoff of 7.14-fold change has a high ability to distinguish patients with BC metastasis from those without metastasis with an area under the curve (AUC) of 0.98, a sensitivity of 76.9%, and a specificity of 97.9%.

In concordance with our results, the previous study that examined circulatory miR-10b in breast cancer, performed by Chen et al.,[35] showed that circulating miR-10b was significantly over-expressed in breast cancer patients with lymph node metastasis and could discriminate breast cancer patients with metastasis from those without metastasis with a the sensitivity of 71% and a specificity of 72%. Similarly, Zhao et al.[36] showed that serum miR-10b expression levels were significantly higher in BC patients with bone metastasis than those without metastasis. In addition, Roth et al.[37] confirmed that expression of miR-10b in serum correlated with the presence of overt metastasis.

To understand the role of miR-10b in BC, the identification of critical miR-10b targets is required. A number of conserved targets of miR10b in human genes were identified. Some of these target genes have a strong evidence for their involvement in the development of BC metastasis.[38]

Homeobox D10 (*HOXD10*) is a known target of miR-10b that demonstrated an inverse correlation with miR-10b expression.[9] In addition, it was found that *TWIST-1*, the transcription factor, induces miR-10b expression which in turn directly suppresses the translation of *HOXD10*.[9] Consequently, the suppressed *HOXD-10* represses some genes such as RhoC, alpha-3 integrin and matrix metalloproteinases that are involved in extracellular matrix remodeling, cell migration, and stimulation of the epithelial-mesenchymal transition (EMT) process, which are the key for regulation of cancer metastases.[9] E-cadherin is another target of miR-10b that serves a major role in controlling cell adhesion process and was found to be a tumor suppressor of BC invasion and metastasis.[22]

Our data showed that serum levels of soluble E-cad in breast cancer patients were significantly higher than those of the control group, (p < 0.001). Furthermore, it was significantly higher in the patients with BC metastasis compared to patients with non-metastatic BC. ROC curve for the serum sE-cad to differentiate breast cancer and control group achieved an AUC of 0.82 at a cutoff of >1780 ng/ml. In concordance with our results, Liang et al.[16] found higher serum soluble E-cad levels in BC patients than in healthy controls. Besides, they found a significant correlation between high serum sE-cad

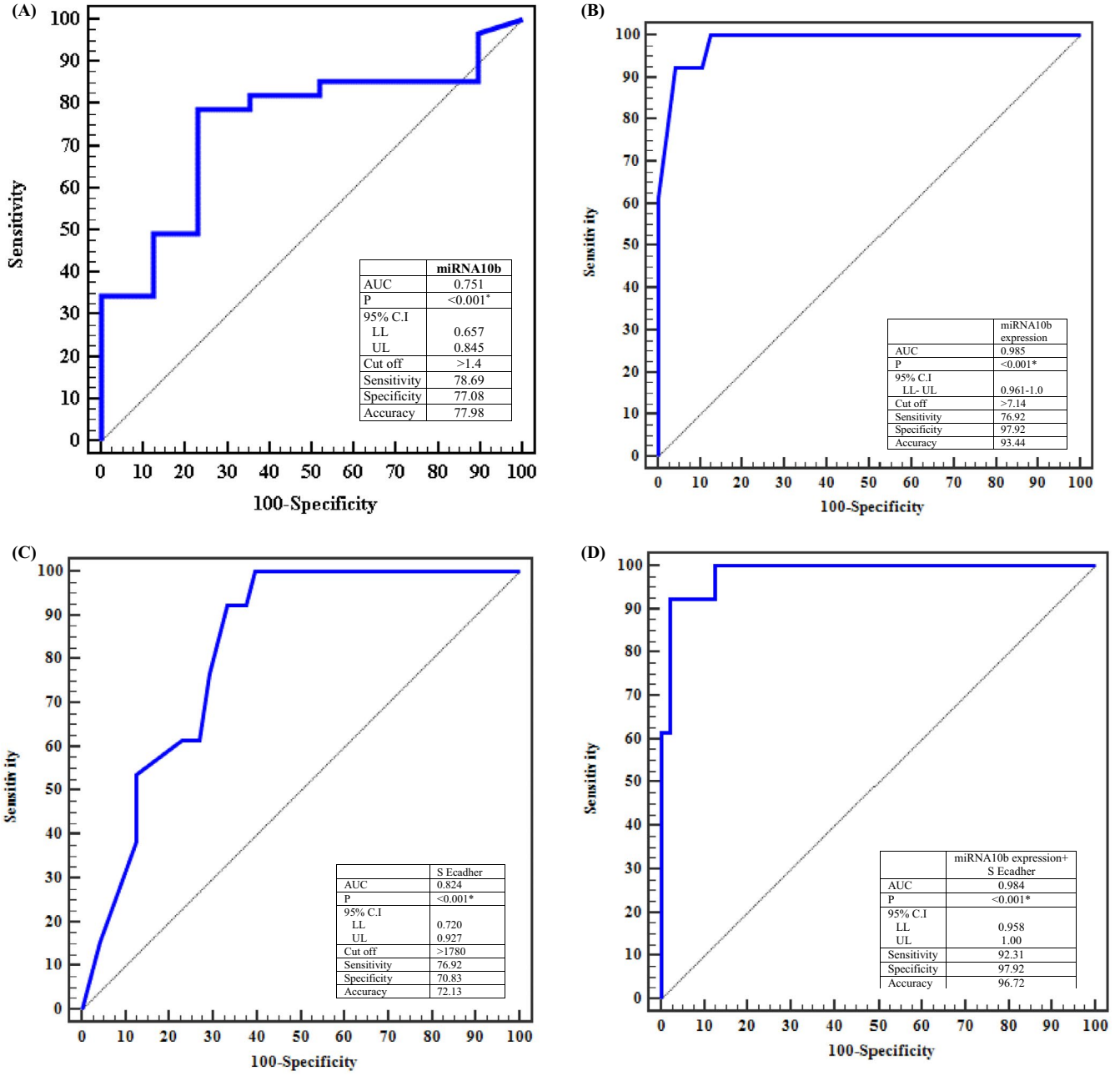

FIGURE 6 (A) ROC curve for miRNA10b expression to diagnose BC patients (n = 61) from Control (n = 48). (B) ROC curve for miRNA10b expression to diagnose metastatic BC patients (stage IV) from non-metastatic BC patients (stages II and III). (C) ROC curve for sE-cadherin to diagnose metastatic BC patients (stage IV) from non-metastatic BC patients (stages I, II, and III). (D) ROC curve for Combined (miRNA10b expression+sE-cadherin) to diagnose metastatic BC patients (stage IV) from non-metastatic BC patients (stages I, II, and III)

level and tumor grade, TNM stage, and lymph node metastasis. In addition, Brouxhon et al.[39] found an association between soluble sE-cad with tumor growth and survival, as well as between its serum levels and the clinical response in cancer patients. Besides, it was found that during carcinogenesis, E-cad, and other adhesion molecules are expected to play an important role in tumor invasion and metastasis.[40,41]

Moreover, when we tried to assess the association between serum miR10b and serum E-cadherin, we found that miR-10b was positively associated with serum E-cadherin level, (r = 0.683, p < 0.001). In addition, both serum sE-cadherin and circulating level of miR-10b were found closely related to tumor size, tumor grade, and lymph node metastasis. ROC analysis revealed that combined use of both serum miR-10b and sE-cadherin revealed the highest sensitivity and specificity (92.9% and 97.9%, respectively) with an AUC of 0.98 for diagnosis of breast cancer metastasis.

To the best of our knowledge, we are the first to study the relationship between circulating miR10b and soluble E-cadherin in serum of Egyptian breast cancer patients. Previous studies were performed in metastatic cells and tissues of breast cancer, in contrast to

our results, they found a significant negative correlation between the levels of these two molecules in clinical samples of breast cancer tissues.[22,42,43] Liu et al.,[22] using metastatic breast cancer cells, have demonstrated that miR-10b regulates E-cadherin expression and that silencing of miR-10b restores E-cadherin expression. Zhang et al.[42] have shown that the expression of E-cadherin mRNA and protein were elevated in cells with miR-10b suppression in non-small cell lung cancer compared with controls. Similarly, Abdelmaksoud-Dammak et al.[43] demonstrated that E-cadherin expression was inversely correlated with miR-10b expression levels in colorectal cancer.

These contradictory findings may be explained by different sample type as we measured serum molecules. In addition, the increase in serum E-cadherin levels in many cancer patients was found to be associated with a concomitant decrease in the level of full-length E-cad expression in tissues, so that it was considered that sE-cadherin originates from the rapid turnover of tumor cells.[40,41] Consequently, it is possible that miR-10 overexpression in breast cancer that occurs through genomic amplification, subsequently causes degradation of its target E-cad mRNA, and subsequently increases its soluble fragment. Furthermore, as previously mentioned, the transcription factor Twist increases the expression of miR10b in BC cells, on the other hand, it is known to directly suppress the E-cad expression in BC tissue. Therefore, Twist may control the E-cad expression, either directly by transcriptional regulation of E-cad, or indirectly by miR-10b up-regulation for E-cad post-transcriptional regulation.[9,44]

In the recent years, the roles of miR-10 in initiation and progression of tumor metastasis established its importance as a therapeutic target in cancer.[45] Further studies on therapeutics based on miR-10b inhibition will help to improve breast cancer management and reduce cancer-related mortality. Interestingly, it was proved recently by Yooe et al.[45] who aimed to develop therapeutics based on miR-10b inhibition that MN-anti-miR10b affects the molecular processes mediated by E-cadherin and greatly inhibited the tumor cells ability to migrate and invade the surrounding tissue through its effect on the transcription factors: *HOXD10* and c-JUN.

Based on our results, we suggest the combined utilization of serum miR10b and sE-cadherin as a serum non-invasive biomarker during diagnosis and prognosis assessments of breast cancer patients. Specifically, we recommend their measurement as a practical tool to predict metastasis in breast cancer patients, which can affect the selection of treatment protocols.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Salwa Hamdi Gomaa https://orcid.org/0000-0002-4447-7498

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