RESEARCH PAPER

Micro-RNA 34a: Predictive Non-Invasive Biomarker for Detecting Neoadjuvant Chemotherapy Response in Egyptian Females with Breast Cancer

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ABSTRACT

Background: Breast cancer is the most prevalent malignancy among women globally.
Neoadjuvant chemotherapy (NACT) improves surgical outcomes, yet reliable non-invasive biomarkers to predict treatment response remain limited. MicroRNAs (miRNAs) have emerged as promising molecular markers. MiR-34a, a tumor suppressor miRNA, has been linked to treatment response in breast cancer.
Aim: To evaluate the potential utilization of circulating microRNA-34a (miR-34a) as a non-invasive biomarker for predicting NAC response in Egyptian females with breast cancer.
Methods: This observational cross-sectional study included 40 pathologically confirmed breast cancer patients indicated for NAC at Cairo University Hospitals (June 2023–October 2024). Cases were indicated for 6 cycles of taxane-based NAC. Blood samples have been gathered at baseline (C0) and after two cycles (C2). Serum miR-34a expression has been determined using quantitative real-time PCR and normalized to miR-16. Clinical and pathological responses were assessed using RECIST criteria and post-surgical histopathology.
Results: A complete response was achieved in 12/40 (30%) patients. Overall, miR-34a expression showed no significant change between C0 and C2 ($p>0.05$). However, subgroup analyses revealed significant associations. ER-negative complete responders had higher baseline miR-34a expression compared with non-responders ($p=0.014$). Among PR-negative patients, complete responders showed significantly higher baseline miR-34a than non-responders ($p=0.028$). In the Luminal B subtype, complete responders demonstrated significant downregulation of miR-34a after therapy ($p=0.046$).
Conclusion: Dynamic changes in circulating miR-34a, particularly in ER-negative, PR-negative, and Luminal B subgroups, can act as a potential non-invasive biomarker for predicting NAC response in Egyptian BC patients.
Keywords: *Breast cancer; Neoadjuvant chemotherapy; Biomarker; MicroRNA-34a*
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INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed tumor and the primary etiology of death from cancer in females globally (1). Risk factors for breast cancer are well known, such as old age, females, positive family history, black women, several genetic mutations such as BRCA1 and BRCA2, and other preventable lifestyle factors such as using hormonal therapy, smoking, alcohol consumption, and sedentary life (2).

Neoadjuvant chemotherapy has been utilized in the treatment of breast cancer prior to surgery. Apart from decreasing tumor size and eliminating micro metastases, neoadjuvant chemotherapy can enhance the rates of breast-conserving surgery and facilitate surgery for inoperable advanced BC (3).

No efficient method exists for expecting a chemotherapy response. Subsequently, it is essential to recognize noninvasive and specific markers for assessing the advantages of Neoadjuvant chemotherapy in the early stages of treatment. The utilization of specific markers can enhance individualization of management and prevent needless treatments (4).

MicroRNAs are small, single-stranded, non-coding RNAs of about eighteen–twenty-two nucleotides. In human cells, there are more than 2300 different microRNAs with time- and tissue-dependent expression patterns (5).

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MiR 34a was found to have tumor suppressor as well as proto-oncogenic function through different mechanisms that regulate cell cycle (6,7). Several studies indicated that the expression level of MiR34a fluctuates during neoadjuvant chemotherapy administration from its baseline expression level, which correlates to treatment response, and this fluctuation supports the idea of using MiR34a as a predictive biomarker for treatment response. (7, 8, 9).

The goal of the current work was to assess the potential use of microRNA-34a as a non-invasive biomarker for detecting neoadjuvant chemotherapy response in Egyptian females with BC.

PATIENTS AND METHODS

This observational cross-sectional research was carried out on a total number of 40 BC cases attending the medical oncology department clinic at the Faculty of Medicine, Cairo University, in the period from June 2023 to October 2024.

Inclusion criteria: Newly diagnosed, pathologically proven breast cancer female patients; BC cases indicated for NACT and age > 18 years' old

Exclusion criteria: Metastatic BC, cases of those who refuse to participate in the study, and patients who are younger than 21 years old.

Ethical consideration

The research has been approved by the investigation ethics committee at the Faculty of Medicine, Cairo University (MD-224-2023) on 13-3-2024. Written consents were obtained from the participants before the commencement of the study.

Sample size calculation:

Using Epi-info™ v7.2, with the proposed power of the study at 95% and a two-sided confidence interval of 95%, with a ratio between responders and non-responders of 1.8, the percentages of decreased miRNA-34a expression among responders and non-responders are 87.3% and 29%, respectively (http://dx.doi.org/10.1016/j.biopha.2017.01.133); the required number of participants is 36 patients. However, as the dropout rate is not expected to exceed 10% throughout the duration of the research, the net number of participants required for the research is 46 patients **(4).**

Methods

All patients in this research have been subjected to the following: Complete clinical examination, full history taking, laboratory workup

Clinical and Laboratory Assessment

All patients underwent:

History and Examination: complete medical history and full clinical evaluation.

Imaging: mammography and/or breast MRI; CT scans of the abdomen, chest, as well as pelvis; bone scans in lymph node–positive patients; and echocardiography prior to chemotherapy.

Laboratory Investigations: complete blood count, kidney and liver function tests (urea, creatinine, AST, ALT, bilirubin, albumin, total protein).

Pathological assessment: A core biopsy will include the histological type and grade, immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), HER2/neu, Ki-67, and the determination of molecular subtype.

Staging: TNM classification according to standard guidelines.

Treatment and Response Evaluation

All cases received 6 cycles of taxane-based neoadjuvant chemotherapy (one cycle every 21 days).

Response to Therapy was assessed:

Clinical Response: After completion of 6 cycles using RECIST criteria, classifying patients into partial response (PR), complete response (CR), progressive disease (PD), or stable disease (SD), cases with CR or PR were considered responders, while SD and PD have been classified as non-responders.

Pathological response: After surgery, tumors were classified as complete pathological response (pCR) or non-pCR.

MicroRNA Analysis

Sample Collection and Processing:

Venous blood samples (3 mL) have been collected before starting chemotherapy (baseline, C0) and after two cycles (C2). Samples have been allowed to clot for thirty minutes and centrifuged at 3000 rpm for ten minutes, and the supernatant was recentrifuged at 15,000 rpm for ten minutes to remove debris. Serum was stored at −80°C until RNA extraction.

RNA Extraction: Total RNA, including small RNAs, has been isolated from serum utilizing the Qiagen miRNeasy Serum/Plasma Kit regarding the manufacturer's protocol.

Reverse Transcription and Quantitative PCR: cDNA has been synthesized from purified RNA utilizing the miRCURY LNA miRNA RT Kit. Quantitative real-time PCR (qRT-PCR) has been carried out with miRCURY LNA miRNA PCR assays and SYBR Green chemistry on a Rotor-Gene Q cycler (Qiagen). miR-34a expression was measured and normalized to miR-16, which served as an internal control. Relative expression levels have been calculated utilizing the comparative cycle threshold (ΔΔCT) method.

RESULTS

Table (1) The frequency of clinical characteristics of the patient's subgroups

	Complete responders n=12/40(30%)	**Non-Complete responders n=28/40(70%)**	**P value**
Age Mean±SD**(years)**	53.58± 10.78	53.89± 14.54	0.941*
Previous history of breast cancer Positive Negative	1/12 (8.3%) 11/12 (91.7%)	2/28 (7.1%) 26/28 (92.9%)	1.000
Family history of breast cancer Positive Negative	2/12 (16.7%) 10/12 (83.3%)	6/28 (21.4%) 22/28 (78.6%)	1.000
History of menopause **Pre-menopause** **Pos-menopause**	6/12 (50%) 6/12 (50%)	15/28 (53.6%) 13/28 (46.45%)	0.836

Data are represented as frequency and percentage or mean ± SD. A p-value below 0.05 is significant.

Table 1 illustrates that non-complete responders and complete responders have been comparable regarding age (53.58 ± 10.78 vs. 53.89 ± 14.54 years, p=0.941). Previous history of BC, family history of BC, and menopausal status show statistically insignificant variance between the both groups (all p-value below 0.05).

Table (2) Frequency of molecular types among the patient sub groups:

	Complete responders n=12 (30%)	**Non -Complete responders n=28(70%)**	**P value**
Luminal a	1/12 (8.3%)	2/28 (7.1%)	**0.172**
Luminal b	7 /12 (58.3 %)	20/28 (71.4%)	
Triple negative	2/12 (16.7%)	6/28 (21.4%)	
HER2 positive	2/12 (16.7%)	0/28 (0%)	

Table 2 illustrates that complete responders and non-complete responders showed no statistically significant in molecular msubtypes (p=0.172). Among complete responders, Luminal B was most frequent (58.3%), followed by triple negative and HER2 positive (16.7% each), and Luminal A (8.3%). Among non-complete responders, Luminal B was also most frequent (71.4%), followed by triple negative (21.4%) and Luminal A (7.1%), with no HER2-positive cases observed.

Table (3) Frequency of status of theranostic and proliferation receptors biomarkers among patient's subgroups

	Complete responders n=12 (30%)	**Non -Complete responders n=28(70%)**	**P value**
ER positive Negative	8/12 (6.7%) 4/12 (33.3%)	19/28 (67.9%) 9/28 (32.1%	1
PR Positive Negative	6 /12 (58.3 %) 6 /12 (58.3 %)	20/28 (71.4%) 8/28 (28.6%)	0.281
HER2NEU Positive negative	6 /12 (58.3 %) 6 /12 (58.3 %)	6/28 (21.4%) 22/28 (78.6%)	0.130
Ki 67 Positive Negative	12/12 (100%) 0/12 (0%)	23/28 (82.1%) 5/12 (17.9%)	0.298

Data are represented as frequency and percentage. ER: estrogen receptor PR: progesterone receptor HER2/neu: human epidermal growth factor 2 neu KI67: A KI67 protein p-value below 0.05 is significant.

Table 3 shows that ER, PR, HER2, and Ki-67 status illustrated insignificant variances among complete and non-complete responders, although HER2 positivity was more frequent in complete responders.

Table (4) median MIir34a relative expression before start of therapy (C0) and after second cycle therapy (C2) among whole patient group and subgroups

	Mir34a before therapy (C0)		**Mir34a after therapy (C2)**		**P value**
	Median	**Range**	**Median**	**Range**	
Whole patients' group n=40(100%)	**1.07**	**0.003-200.8535**	**0.42**	**0.00003-136.2394**	**0.232**
Complete responders n=12 (30%)	3.15	0 - 99.73	0.73	0.15-6.26	1.82 0.232
Non -Complete responders n=28(70%)	0.91	0.15-3.43	0.38	0.05-4.89	0.600 0.600

Data are represented as median and range

Table 4 illustrates that there was a statistically insignificant change in miR-34a expression before and after therapy in the whole patient group, complete responders, or non-complete responders (p-value below 0.05).

Table (5): Comparison of the relative expression of MiR 34a dynamic changebefore and after second cycle of therapy as regards toEstrogen Receptors (ER) status in complete responders (n=12) Vs non-complete responders(n=28).

	MiR34a before therapy (C0)		**MiR34a after C2**		**P* value**
	Median	IQR	Median	IQR	
ER positive patients Completeresponders(n=8/12)	**1.63**	**0.1 - 3.59**	**0.29**	**0.08 - 1.1**	**0.050**
Non completeresponders(n=19/28)	1.38	0.21 -7.01	0.15	0.04 - 4.96	0.243
P value	0.396		0.873		
ER negative patients Complete responders(n=4/12)	**12.06**	**4.6- 57.87**	6.26	1.72 - 73.15	1.0
Non complete responders(n=9/28)	**0.13**	**0.21 -7.01**	1.21	0.38 - 2.6	0.260
P value	**0.014**		0.165		

Data are represented as median & IQR. IQR= interquartile range (range between 25-75th percentiles), p value is considered significant if < 0.05, P* is for comparison between before and after therapy

Table 5 shows that, in ER-positive patients, MiR34a expression decreased after therapy in both complete and non-complete responders without significant variances. In ER-negative patients, complete responders had significantly higher baseline MiR-34a compared with non-complete responders, but this difference was not maintained after therapy.

Table (6): Comparison of the relative expression of MiR 34a dynamic change before therapy as regards to progesterone Receptors (PR) status in complete responders Vs non-complete responders

	MiR34a before therapy (C0)		**P value**
	Median	IQR	
PR negative complete responders (n=6/12)	**9.55**	**(1.16 - 66.26)**	
PR negative non-complete responders (n=8/28)	**0.42**	**(0.06 - 1.88)**	**0.028**

Data are represented as median & IQR. IQR= interquartile range (range between 25-75th percentiles), p value is considered significant if < 0.05, P* is for comparison between before and after therapy

Table 6 shows that, among PR-negative patients, complete responders had significantly higher baseline MiR34a expression (median 9.55, IQR 1.16–66.26) compared with non-complete responders (median 0.42, IQR 0.06–1.88) (p=0.028).

Table (7): comparison of MiR34a relative expression among complete responders Versus non-complete responders before & after therapy (C0 VsC2) as regards to Luminal b molecular type

	MiR34a before therapy (C0)		MiR34a after therapy (C2)		P* value
	Median	Range	Median	Range	
Luminal bComplete responders (n=7/12)	**3.1**	**0.12 - 1.73**	**0.41**	**0.12 - 1.73**	**0.046**
Luminal b non- Complete responders (n=20/28)	**1.18**	**0.21 - 6.04**	**0.27**	**0.05 - 4.98**	**0.391**

Data are represented as median & IQR. IQR= interquartile range (range between 25-75th percentiles), p value is considered significant if < 0.05, P* is for comparison between before and after therapy

Table 7 shows that, in the Luminal B subgroup, complete responders showed a significant decrease in MiR34a expression after therapy (median 3.1 → 0.41, p=0.046), while non-complete responders showed a non-significant decrease (median 1.18 → 0.27, p=0.391).

DISCUSSION

According to our results, non-complete responders and complete responders have been comparable regarding age (53.58 ± 10.78 vs. 53.89 ± 14.54 years, p=0.941). Previous history of BC, family history of breast cancer, and menopausal status show statistically insignificant variance between the both groups (all p-value below 0.05). Also, non-complete responders and complete responders showed no statistically significant in molecular subtypes (p=0.172). Among complete responders, Luminal B was most frequent (58.3%), followed by triple negative and HER2 positive (16.7% each), and Luminal A (8.3%). Among non-complete responders, Luminal B was also most frequent (71.4%), followed by triple negative (21.4%) and Luminal A (7.1%), with no HER2-positive cases observed.

This was in contrast to the results of **Zhang et al (10)** who reported that the relative expression of MiR 34a in luminal B BC remained relatively stable after therapy in responders, while in non-responders it was significantly upregulated when compared to its baseline levels in a study done on 37 Chinese luminal B breast cancer Chinese patients who were indicated for neoadjuvant chemotherapy (treated by taxanes and anthracycline).

According to our results, ER, PR, HER2, and Ki-67 status illustrated insignificant variances among complete and non-complete responders, although HER2 positivity was more frequent in complete responders.

This was in contrast with **kassem et al**. (11), who reported that the relative expression of MiR 34a at baseline of 39 Egyptian breast cancer patients was significantly up-regulated in cases with ER-positive status compared to cases with ER-negative status.

Bonetti et al., (12) explained that the expression level of MiR 34a is reduced in all hormone-positive subtypes of breast cancer, and this may explain the aggressive behavior of these subtypes.

Regarding our outcomes, there was statistically insignificant change in Mir34a expression before and after therapy in the whole patient group, complete responders, or non-complete responders (p>0.05).

This was in contrast to the results of **Liu et al., (4)** who reported a significant reduction in MiR 34a relative expression in breast cancer stage II/III responders after the second cycle of neoadjuvant chemotherapy (treated by anthracyclines, cyclophosphamides, and docetaxel) if compared to its baseline expression with P value =0.016.

According to our results, in ER-positive patients, MiR34a expression decreased after therapy in both non-complete and complete responders without significant variances. In ER-negative patients, complete responders had significantly higher baseline MiR34a compared with non-complete responders, but this difference was not maintained after therapy.

In contrast to the present study, **shaban NZ et al (13)** in research on 159 BC Egyptian cases, found that the relative expression of MiR 34 a has been upregulated in all patients following administration of neoadjuvant chemotherapy compared to its baseline expression (p-value < 0.001).

The same results were concluded by **Chen et al, (14)** in a study conducted on formalin-fixed, paraffin-embedded tumor tissue specimens of 55 Chinese breast cancer patients to assess the change in relative expression of MiR 34a in surgically resected specimens than pre-neoadjuvant chemotherapy biopsies, as they found that the relative expression of MiR 34a has been significantly upregulated in surgically resected specimens following neoadjuvant chemotherapy**.** According to our results, among PR-negative patients, complete responders had significantly higher baseline MiR34a expression (median 9.55, IQR 1.16–66.26) compared with non-complete responders (median 0.42, IQR 0.06–1.88) (p=0.028).

In contrast to the outcomes of this research, **Frères et al, (15)** reported that the relative expression of MiR 34a has been significantly upregulated after administration of neoadjuvant chemotherapy if compared to baseline expression level in twenty-five newly diagnosed French breast cancer patients indicated for neoadjuvant chemotherapy (treated by anthracyclines, cyclophosphamides, and docetaxel).

Todorova et al (16) concluded in a study conducted on 20 American breast cancer patients indicated for neoadjuvant chemotherapy (anthracyclines, cyclophosphamides, and

docetaxel) that the relative expression of MiR 34a that was done by next-generation sequencing was significantly upregulated after administration of neoadjuvant chemotherapy in non-complete pathological responders. According to our results, in the Luminal B subgroup, complete responders showed a significant decrease in MiR34a expression after therapy (median 3.1 → 0.41, p=0.046), while non-complete responders showed a non-significant decrease (median 1.18 → 0.27, p=0.391).

This was in agreement with **Liu et al**., (4) who reported no statistical significance difference as regards to the molecular subtypes found in the baseline relative expression level of MiR 34a among the responding and non-responding in their study, which was done on 83 HER+ 2Chinese breast cancer patients indicated for a neoadjuvant chemotherapy combination.

CONCLUSION

Dynamic changes in MiR34a detected in ER-positive responders and luminal B complete pathological responders after the second cycle of NAC administration in breast cancer patients compared to its baseline expression may reflect its usefulness as a predictive biomarker to NAC response.

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