Circulating *miR-34a* and *miR-125b* as Promising non Invasive Biomarkers in Egyptian Locally Advanced Breast Cancer Patients

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**Abstract**

**Background:** Breast cancer (BC) is the second most common cancer worldwide. MicroRNAs are a group of non-coding, single stranded RNAs of ~22 nucleotides, which regulate gene expression at the post-transcriptional level. Circulating miRNAs have been found as potential blood based predictive biomarkers. **Purpose:** we aim to evaluate *miR-34a* and *miR-125b* to predict outcome from neoadjuvant chemotherapy in Egyptian BC patients. **Methodology:** Quantitative assessment of plasma *miR-34a* and *miR-125b* expression was performed by qRT-PCR. Thirty nine newly diagnosed locally advanced BC female patients with 10 age and sex matched healthy volunteers were included in the study. **Results:** We performed ROC curve analysis to evaluate the diagnostic value for the *miR-34a* with AUCs = 0.995, cutoff point of 2.57 sensitivity 97.4%, specificity 100%, PPV 100%, NPV 83.3% and accuracy 97.7%. *miR-125b* had AUC = 0.68 and a cutoff point of 8.69 with sensitivity 66.7%, specificity 70.0%, PPV 90.6%, NPV 41.2% and accuracy 73.5%. *miR-34a* expression were significantly higher in BC patients compared to controls with p value <0.001*. Also, *miR-34a* expression level was significantly higher in patients with progressive disease with P value = 0.03*. However, *miR-125b* expression levels were insignificantly higher in responsive patients with p value = 0.2. **Conclusion:** miRNAs are crucial candidates for novel molecular targeted therapies due to their capability to regulate numerous genes in molecular pathways. Our data suggest that circulating *miR-34a* and *miR-125b* expression levels could be promising highly accurate non-invasive biomarkers in diagnosing BCs. *miR-34a* can predict chemotherapeutic resistance associated with higher expression levels in non-responsive patients. **Keywords:** Breast cancer- circulating microRNA- biomarker- neoadjuvant chemotherapy

**Introduction**

Breast cancer (BC) is the second most common cancer worldwide and is the most common cancer among Egyptian females (Talima et al., 2019). In Egypt, cancer incidence rates at national and regional level was published based upon results of National Cancer Registry Program (NCRP). This registry represented that the commonest sites were liver (23.8%), breast (15.4%), and bladder (6.9%) in both genders, liver (33.6%) & bladder (10.7%) in males, and breast (32.0%) and liver (13.5%) in females (Ibrahim et al., 2014). MicroRNAs (miRNAs) are a group of non-coding, single stranded RNAs of ~ 18-24 nucleotides, which regulate gene expression at the post-transcriptional level (Doench and Sharp, 2004). miRNAs modulate numerous cellular pathways, such as cell proliferation, differentiation and apoptosis and may function as oncogenes or tumor suppressing genes.

Circulating miRNAs have been found as potential blood based biomarkers for cancer detection (Yu et al., 2011). Among the oncogenic miRNAs, *miR-34a* and *miR-125b* which have been reported to be related to breast cancer. *miR-34* gene which located on lp36.23 is a tumour suppressor gene direct downstream component of the p53 network . It was found that *miR-34a* is down-regulated in breast cancer cell lines and tissues, compared with normal cell lines and the adjacent non-tumor tissues, respectively (Li et al., 2012). It was reported that ectopic expression of *miR-34a* inhibits breast cancer cells growth, invasion and migration. It contributes to drug resistance of breast cancer by targeting many oncogenes. It interacts with *BCL-2* and *CCND1* and is reported to be associated with docetaxel resistance. By targeting NOTCH1 and protein kinase D1 (PRKD1), *miR-34a* modulates chemosensitivity of breast cancer cells to adriamycin, and stimulates breast cancer stemness and drug resistance, respectively. *miR
125b is a tumor suppressor in breast tumorigenesis, its overexpression leads to reduced migration and invasion capacities. It can also induce metastasis by targeting STAR related lipid transfer domain containing 13 (STARD13) in MCF 7 and MDA MB 231 breast cancer cells (Li et al., 2017; Tang et al., 2012). Also, upregulation of miR 125b conferred to chemoresistance by targeting B cell lymphoma 2 antagonist killer 1 (Bak1), and it could maintain cancer stem like side population fraction. Finally, it was reported that circulating miR 125b expression was associated with chemotherapeutic resistance of breast cancer (Luo et al., 2017). There are many molecular mechanisms that may contribute to chemotherapeutic resistance in breast cancer patients. So, we aim to evaluate 2 potential biomarkers: miR-34a and miR-125b in diagnosis and to predict outcome from neoadjuvant chemotherapy in locally advanced Egyptian breast cancer females.

Materials and Methods

Study population

The present study included 39 newly diagnosed locally advanced breast cancer female patients. Patients were recruited from the outpatient clinic of Kasr Al-Ainy Center of Clinical Oncology and nuclear medicine, School of Medicine, Cairo University, in the period from October 2017 to February 2018. All the patients have been prepared to receive pre-operative neoadjuvant chemotherapy with an association of anthracycline and taxanes for 8 cycles during 6 months. Ten age- and sex-matched healthy volunteers were included in the study as a control group. The study was approved by the Research ethical Committee of Clinical Oncology department, School of Medicine, Cairo University, and informed consents were obtained from all participants prior to enrollment in the study.

Cell-free total RNA extraction

For the patients and controls, 3 ml peripheral blood sample was collected under complete aseptic conditions for molecular studies. 200 µl EDTA plasma was separated and used for extraction of all RNA molecules from approximately 18 nucleotides (nt) upwards by miRNeasy Serum/Plasma kit (Qiagen, Valencia, CA, USA) according to manufacturer’s instructions. The RNA was eluted in 15 µl RNAse free water, the integrity was tested on the NanoDrop (ND-1000) and finally, RNA stored at −80°C till used in real time PCR reaction. Qiagen offers miScript Serum/Plasma Spike-In Control (a synthetic C. elegans miR-39) which was added to the homogenized plasma samples prior to RNA purification, CT values obtained using the C. elegans miR-39 miScript Primer Assay can be used to calibrate the data sets being analyzed. This calibration can resolve differences in recovery that may occur during the purification procedure and in amplification efficiency.

Quantitative assessment of miRNA-34a and miRNA-125b expression

One hundred ng of total RNA was reverse transcribed using miScript II RT Kit followed by real-time PCR on StepOne Real-Time PCR machine (Applied Biosystems) using an miRNA-specific miScript Primer Assay (forward primer) for {miR16 (reference miR), miR34a and miR125b} and the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix as described by the manufacturer. Samples, validated endogenous controls and interassay controls were used throughout. The relative quantification (RQ) of miRNA gene expression was assessed by $2^{-\Delta\Delta CT}$ method ($\Delta\Delta CT = [(Ct\text{ (miRNA of interest)} - Ct\text{ (reference miR-16 of interest)}) - (Ct\text{ (miRNA of control)} - Ct\text{ (reference miR-16 of control)})]$.

Data analysis

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). Spearman-rho method was used to test correlation between numerical variables. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values. Evaluation of diagnostic value of the miRNAs was done by calculating sensitivity, specificity, positive predictive values (PPV), negative predictive value (NPV) and accuracy. All tests were two-tailed. A p-value < 0.05 was considered significant.

Results

Patients’ characteristics

Thirty nine newly diagnosed locally advanced breast cancer female patients were included in the study. Their age ranged between 29 and 66 years with mean ± SD = 45.4 ± 9 years and median of 45 years. As regards menopausal status, 23/39 (59%) patients were pre-menopausal and 16/39 (41%) patients were post-menopausal. Regarding body mass index (BMI), 7/39 (17.9%) patients were normal, 21/39 (53.8%) patients were obese and 11/39 (28.2%) patients were overweight. Seventeen (43.6%) patients had tumor on left side, 21/39 (53.8%) had tumor on right side and only one patient (2.6%) had tumor on both sides. Regarding, tumor histology, 37/39 (94.9%) patients had invasive ductal carcinoma (IDC), only one patient had invasive lobular carcinoma (ILC) and one had mixed type. Majority of patients were grade II: 36/39 (92.3%) and only 3/39 (7.7%) were grade III. Regarding TNM stage, 9/39 (23.1%) patients were T2, 13/39 (33.3%) patients were T3 and 17/39 (43.6%) patients were T4. Nine patients (23.1%) were N0, 23/39 (59%) patients were N1, 2/39 (5.1%) patients were N2 and 5/39 (12.8%) patients were N3. All patients are non metastatic (M0). Regarding hormone receptor status, estrogen receptor (ER) levels were positive in 29/39 (74.4%) patients and negative in 10/39 (25.6%) patients. Progesterone receptor (PR) levels were positive in 32/39 (82.1%) patients and negative in
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**Table 1. Patients’ Characteristics**

| Patients’ characteristics | No. (%) |
|---------------------------|---------|
| Age (years)               |         |
| Range                     | 29-66   |
| Mean ± SD                 | 45.4 ± 9|
| Median                    | 45      |
| Comorbidities             |         |
| DM                        | 1/39 (2.6%) |
| HTN                       | 3/39 (7.7%) |
| Both                      | 5/39 (12.8%) |
| No                        | 30/39 (76.9%) |
| Body mass index (BMI)     |         |
| Normal                    | 7/39 (18%) |
| Obese                     | 21/39 (53.8%) |
| Overweight                | 11/39 (28.2%) |
| Menopausal status         |         |
| Pre                       | 23/39 (59%) |
| Post                      | 16/39 (41%) |
| Tumor side                |         |
| Left                      | 17/39 (43.6%) |
| Right                     | 21/39 (53.8%) |
| Both                      | 1 (2.6%) |
| Multicentric tumor        |         |
| Yes                       | 8 (20.5%) |
| No                        | 31 (79.5%) |
| Tumor histology           |         |
| IDC                       | 37/39 (94.9%) |
| ILC                       | 1/39 (2.6%) |
| Mixed                     | 1/39 (2.6%) |
| Grade                     |         |
| II                        | 36/39 (92.3%) |
| III                       | 3/39 (7.7%) |
| TNM stage                 |         |
| T2                        | 9/39 (23.1%) |
| T3                        | 13/39 (33.3%) |
| T4                        | 17/39 (43.6%) |
| Lymph nodes               |         |
| N0                        | 9/39 (23.1%) |
| N1                        | 23/39 (59%) |
| N2                        | 2/39 (5.1%) |
| N3                        | 5/39 (12.8%) |
| Metastasis                |         |
| M0                        | 39/39 (100%) |
| Immunohistochemistry      |         |
| ER                        |         |
| Positive                  | 29/39 (74.4%) |
| Negative                  | 10/39 (25.6%) |
| PR                        |         |
| Positive                  | 32/39 (82.1%) |
| Negative                  | 7/39 (17.9%) |

**Table 1. Continued**

| Patients’ characteristics | No. (%) |
|---------------------------|---------|
| HER2neu                   |         |
| Positive                  | 14/39 (35.9%) |
| Negative                  | 25/39 (64.1%) |
| KI67                      |         |
| High                      | 31/39 (79.5%) |
| Low                       | 7/39 (17.9%) |
| Unknown                   | 1/39 (2.6%) |
| Molecular subtypes        |         |
| luminal A                 | 5/39 (12.8%) |
| luminal B                 | 29/39 (74.4%) |
| Her2 positive disease     | 2/39 (5.1%) |
| Triple negative disease   | 3/39 (7.7%) |

Comorbidities: DM, Diabetes mellitus; HTN, Hypertension; ER, Estrogen receptor; PR, Progesterone receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

**Table 1. Continued**

MD: Diabetes mellitus; HTN, Hypertension; ER, Estrogen receptor; PR, Progesterone receptor; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

miR-34a expression in controls and breast cancer patients

In control group, miR-34a expression values ranged between 0.52 and 1.95 with a mean value of 1.13 ± 0.54 and median value of 1.13, while in BC patients, it ranged between 1.54 and 17020.7 with a mean value of 1643.4 ± 3813.6 and median value of 47.3. miR-34a expression levels were significantly higher in BC patients compared to controls with p value <0.001. Correlations of miR-34a expression levels with patients’ characteristics were described in Table 2. ROC (receiver operating characteristic) curve analysis performed to evaluate the diagnostic value for the miR-34a with AUCs (area under the ROC curves) = 0.995. For distinguishing BC patients from normal controls, ROC curve provides a cutoff point for diagnosis of the disease, below it (2.57) were considered as “Negative”, while those with expression level higher than or equal to 2.57 were considered as “Positive”. We also, found that sensitivity was 97.4%, specificity was 100%, PPV was 100%, NPV was 83.3% and accuracy was 97.7%.

miR-125b expression in controls and breast cancer patients

In control group, miR-125b expression values ranged between 0.28 and 260.8 with a mean value of 21.7 ± 52.1 and median value of 4.75. miR-125b expression levels...
were insignificantly higher in BC patients compared to controls with p value = 0.2. Correlations of miR-125b expression levels with patients’ characteristics were described in Table 3. ROC curve analysis performed

Table 2. miR-34a Expression in Breast Cancer Patients

| Items                        | Subgroups        | No. | Mean ± SD     | Median | Range          | P-value |
|------------------------------|------------------|-----|---------------|--------|----------------|---------|
| miR-34a expression           | BC patients      | 39  | 1643.4 ± 3813.6 | 47.3   | 1.54 - 17020.7 | <0.001* |
|                              | Controls         | 10  | 1.13 ± 0.54   | 1.13   | 0.52 - 1.95    |         |
| Menopausal status            | Pre              | 23  | 2232.7±4342.2 | 75.32  | 1.54-17020.7   | 0.12    |
|                              | Post             | 16  | 796.3±2809.5  | 31.78  | 3.19-11307.6   |         |
| BMI                          | Normal           | 7   | 48.48±80.84   | 12.3   | 11.2-229.9     | 0.17    |
|                              | Obese            | 21  | 1291.8±3001.5 | 59.1   | 4.42-11307.6   |         |
|                              | Overweight       | 11  | 3329.6±5663.6 | 75.3   | 5.08-17020.7   |         |
| Multicentric tumor           | Yes              | 8   | 1245±2751.1   | 18.7   | 3.19-7831.1    | 0.29    |
|                              | No               | 31  | 1746.2±4074.7 | 63.3   | 1.54-17020.7   |         |
| Grade                        | II               | 36  | 1554.3±3890.4 | 45.45  | 1.54-17020.7   | 0.23    |
|                              | III              | 3   | 2713.1±3098.5 | 2012.8 | 24.68-6101.7   |         |
| TNM stage:                   | T2               | 9   | 1219.8±2386.1 | 12.7   | 1.54-6101.7    | 0.07    |
| Tumor size                   | T3               | 13  | 3059.9±5491.3 | 229.9  | 5.08-17020.7   |         |
|                              | T4               | 17  | 784.5±2561.95 | 37.7   | 3.19-10550.3   |         |
|                              | N0               | 9   | 3971.1±5788.2 | 95.3   | 1.54-17020.7   | 0.79    |
|                              | N1               | 23  | 1217.1±3118.4 | 63.3   | 4.42-11307.6   |         |
| Lymph nodes                  | N2/N3            | 7   | 51.46±49.04   | 61.6   | 12.25-155.96   |         |
| Immunohistochemistry:        |                  |     |               |        |                |         |
| ER                           | Positive         | 27  | 1346.3±3214.5 | 24.68  | 1.54-11307.6   | 0.02*   |
|                              | Negative         | 12  | 2505.2±5305.6 | 192.94 | 25.9-17020.7   |         |
| PR                           | Positive         | 30  | 1064.2±2643.4 | 31.78  | 1.54-11307.6   | 0.01*   |
|                              | Negative         | 9   | 4291.5±6785.0 | 229.92 | 69.79-17020.7  |         |
| HER2neu                      | Positive         | 13  | 429.7±1232.8  | 21.43  | 1.54-4656.4    | 0.22    |
|                              | Negative         | 26  | 2323.1±4567.7 | 63.34  | 3.19-17020.7   |         |
| KI67                         | High             | 31  | 2060.3±4188.8 | 75.32  | 1.54-17020.7   | 0.03*   |
|                              | Low              | 7   | 28.4±28.5     | 12     | 4.42-69.8      |         |
| Molecular subtypes           | luminal A        | 5   | 24.9±25.2     | 12     | 5.08-6.3       | 0.08    |
|                              | luminal B        | 29  | 1536.6±3249.7 | 43.6   | 1.54-11307     |         |
|                              | Her2 positive disease | 2 | 149.9±113.2 | 149.86 | 69.8-229.9     |         |
|                              | Triple negative disease | 3 | 6369.6±9274.8 | 2012.82 | 75.3-17020.7 |         |

*, Significant at P ≤ 0.05

Figure 1. Correlation of miR34 and miR125b Expression Levels in BC Group
Circulating miR-34a and miR-125b as Promising Non Invasive Biomarkers in Breast Cancer Patients

| miR-125b expression | Subgroups | No. | Mean ± SD | Median | Range     | P-value |
|---------------------|-----------|-----|-----------|--------|-----------|---------|
| BC patients         | 39        | 21.7±52.1 | 4.75 | 0.28-260.8 | 0.2     |
| Controls            | 10        | 1.04±0.35 | 0.92 | 0.7-1.53   |         |
| Menopausal status   | Pre       | 23   | 24.7±55.3 | 6.2  | 0.4-260.8 | 0.05*   |
|                     | Post      | 16   | 17.3±48.5 | 1.96 | 0.28-194.96 |     |
| BMI                 | Normal    | 7    | 6.7±7.1  | 4.9  | 0.42-20.9 | 0.4     |
|                     | Obese     | 21   | 16.2±42.2 | 2.7  | 0.28-194.9 |     |
|                     | Overweight | 11 | 41.7±77.8 | 10.8 | 0.4-260.8 |         |
| Multicentric tumor  | Yes       | 8    | 9.7±13.7 | 3.04 | 0.43-40.7 | 0.9     |
|                     | No        | 31   | 24.75±57.85 | 4.9 | 0.28-260.8 |         |
| Grade               | II        | 36   | 20.7±52.7 | 4.8  | 0.28-260.8 | 0.9     |
|                     | III       | 3    | 32.8±52.6 | 2.5  | 2.37-93.5 |         |
| BMI                  |           |      |           |       |           |         |
| Tumor size          | T2        | 9    | 15.6±30.5 | 2.6  | 0.42-93.5 | 0.4     |
|                     | T3        | 13   | 27.8±52.3 | 10.1 | 0.4-194.9 |         |
|                     | T4        | 17   | 20.2±62.2 | 3.4  | 0.28-260.8 |         |
| Lymph nodes         | N0        | 9    | 19.07±31.51 | 2.7 | 0.4-93.5 | 0.9     |
|                     | N1        | 23   | 27.24±64.91 | 4.9 | 0.28-260.8 |         |
|                     | N2/N3     | 7    | 6.70±5.14 | 5.1  | 2.5-17.5 |         |
| Immunohistochemistry: | ER       | Positive | 27 | 25.3±59.7 | 4.8  | 0.28-260.8 | 0.09   |
|                     | Negative  | 12   | 11.01±14.1 | 6.4  | 0.4-43.02 |         |
|                     | PR        | Positive | 30 | 17.1±37.6 | 4.1  | 0.28-194.9 | 0.6     |
|                     | Negative  | 9    | 42.7±96.3 | 10.1 | 0.4-260.8 |         |
|                     | HER2neu   | Positive | 13 | 8.5±12.3 | 3.2  | 0.28-43.02 | 0.5     |
|                     | Negative  | 26   | 29.03±63.7 | 4.9  | 0.4-260.8 |         |
|                     | KI67      | High   | 31   | 26.4±57.6 | 5.2  | 0.4-260.8 | 0.1     |
|                     | Low       | 7     | 3.44±3.84 | 1.4  | 0.28-10.8 |         |
| Molecular subtypes  | luminal A | Positive | 5  | 2.6±2.2  | 1.4  | 0.43-5.27 | 0.31    |
|                     | luminal B | Positive | 29 | 27.5±59.5 | 4.9  | 0.28-260.8 |         |
|                     | Her2 positive disease | Positive | 2  | 10.5±0.5 | 10.5 | 10.1-10.83 |         |
|                     | Triple negative disease | Positive | 3  | 4.6±5.6  | 2.4  | 0.4-10.98 |         |

*, Significant at P ≤ 0.05

Table 4a. Correlation between miR-34a & miR-125b Expression Levels and Response to Therapy in BC Patients

| MiRNAs       | Subgroups | No. | Mean ± SD | Median | Range     | P-value |
|--------------|-----------|-----|-----------|--------|-----------|---------|
| MiRNA-34a    | CR+PR     | 27  | 1182.6±2849.9 | 25.9 | 1.54-11307.6 |         |
|              | SD+PD     | 12  | 2680.2±5417.6 | 78.3 | 11.20-17020.7 | 0.14   |
| MiRNA-125b   | CR+PR     | 27  | 19.32±40.35 | 5.23  | 0.28-194.9 |         |
|              | SD+PD     | 12  | 26.94±74.06  | 2.85  | 0.40-260.8 | 0.4     |

CR, complete remission; PR, partial remission; SD, stationary disease; PD, progressive disease; *, Significant at P ≤ 0.05

Table 4b. Correlation between miR-34a & miR-125b Expression Levels and Response to Therapy in BC Patients

| MiRNAs       | Subgroups | No. | Mean ± SD | Median | Range     | P-value |
|--------------|-----------|-----|-----------|--------|-----------|---------|
| MiRNA-34a    | CR+PR+SD  | 35  | 984.9±2537.8 | 43.6  | 1.54-11307.6 |         |
|              | PD        | 4   | 7405.4±7867.8 | 6281.6 | 37.7-17020.7 | 0.03*   |
| MiRNA-125b   | CR+PR+SD  | 35  | 16.5±35.9  | 4.9   | 0.28-194.96 |         |
|              | PD        | 4   | 67.1±129.2 | 3.6   | 0.4-260.8 | 0.7     |

CR, complete remission; PR, partial remission; SD, stationary disease; PD, progressive disease; *, Significant at P ≤ 0.05
Correlation between miR-34a and miR-125b expression levels and response to therapy in locally advanced BC patients

Twenty seven (69.2%) patients achieved complete response (CR) or partial response (PR), while 12/39 (30.7%) patients still had stationary disease (SD) or progressive disease (PD). Median miR-34a expression levels in BC patient with SD or PD were higher than the levels in patients with CR or PR. However, it doesn’t reach statistically significant difference with P value = 0.14. Only patients with progressive BC disease had significantly higher miR-34a expression levels with p value= 0.03*. Median levels of miRNA-125b expression in BC patient with CR or PR were insignificantly higher than patients with SD or PD with P value = 0.38 (Table 4a and 4b).

Correlations between miR-34a and miR-125b in BC patients:

There is direct proportional relation between miR-34a and miR-125b expression levels with correlation coefficient (r = 0.58). Statistical analysis shows highly significant statistical correlation between miR-34a and miR-125b expression levels with P value <0.001* (Figure 1).

Discussion

Breast cancer is a heterogeneous disease with many etiological risk factors including genetic and environmental factors. The growing awareness of the molecular pathogenesis of cancer is providing new targets for early diagnosis, disease characterization, patients’ risk stratification, development of predictive biomarkers for monitoring disease progress and therapy effectiveness, etc. Heneghan et al., (2011) reported that miRNAs show great potential as diagnostic and prognostic biomarkers for BC. Although the clinical application of serum miRNAs as a noninvasive strategy is promising, the miRNA signatures should be further investigated in BC patients. In the current study, we analyzed the serum level of two miRNAs which are miR-34a and miR-125b in 39 newly diagnosed locally advanced breast cancer females and 10 age and sex matched healthy volunteers. The miR-34a gene is located at lp36.23, it has been identified as a target of P53 and acted as a tumor suppressor (Misso et al., 2014). Also, Tang et al., (2012) revealed that miR-34a may be involved in regulation of the process of multi-drug resistance (MDR) in BC by targeting BCL-2, CCND1, and NOTCH1. So, miR-34a can serve as an indicator of MDR and prognosis of BC patients. In our study, we found that miR-34a expression levels were significantly higher in BC patients compared to controls with p value <0.001. Our result is in agreement with Roth et al., (2010) who reported that miR-34a could be used for BC diagnosis because BC patients have higher serum miR-34a expression than healthy females, making it as a promising biomarker with another reports revealed an important association between miR-34a and BC risk (OR = 3.12, 95% CI: 1.83–4.39, P < 0.001). While, not in agreement with My (2014) who found lower miR-34a levels in advanced BC cell lines and significantly reduced circulating miR-34a levels in sera of BC patients with lower miR-34a levels in higher stages. These findings may be explained by the difference in sample size and methodical procedures. We performed ROC curve analysis to evaluate the diagnostic value for the miR-34a with AUCs = 0.995 and a cutoff point of 2.57. We found that sensitivity was 97.4%, specificity was 100%, PPV was 100%, NPV was 83.3% and accuracy was 97.7%. Our results were similar to Imani et al., (2017) who reported that miR-34a had more promising accuracy for BC diagnosis with the AUC of the summary receiver operating characteristic (SROC) was 0.80. Accordingly, miR-34a is highly accurate as an independent diagnostic biomarker for BC. We found that miR-34a expression levels in non-responsive BC patients were insignificantly higher than the levels in responsive patients. Only patients with progressive BC disease had significantly higher miR-34a expression levels with p value= 0.03*. However, Li et al., (2017) found that patients with miR-34a low expression had poorer OS and DFS compared to those with high expression, suggesting that low miR-34a expression indicates poor prognosis for breast cancer patients. In our work, we also investigated miR 125b which is a tumor suppressor in breast tumorigenesis. We found that miR-125b expression levels were insignificantly higher in BC patients compared to controls. This is contradictory to Mar-Aguilara et al., (2013) who revealed the expression of miR125b was significantly higher in BC sera than in healthy controls. Also, Wang et al., (2012) found that early stage BC patients had similar miR-125b level to healthy controls, late stage patients had on average 3.5-fold higher mean values of miR-125b than early stage patients and healthy controls with p value < 0.01. However, Han et al., (2013) reported that the serum concentrations of miR-125b showed no difference between BC patients and healthy controls. We performed ROC curve analysis to evaluate the diagnostic value for the miR-125b with AUCs = 0.68 and a cutoff point of 8.69. We found that sensitivity was 66.7%, specificity was 70.0%, PPV was 90.6%, NPV was 41.2% and accuracy was 73.5%.

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Conflicts of interest

Authors declared no conflicts of interest.

Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments (GCP guidelines) or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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