Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 2, 71-77

Study the Expression of miRNA34a-5p Gene among Iraqi Breast Cancer Patients

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Received: September 13, 2023 / Accepted: October 29, 2023 / Published: October 30, 2024

Abstract: MicroRNAs (miRNAs) are short non-coding RNA sequences (approximately 22 nucleotides) that play an important role in gene regulation. This research is to evaluate the serum expression (miR34a-5p) in the Iraqi population as important biomarkers. Circulating serum miRNA34a-5p expression was measured using RT-qPCR in 50 patients women suffering from breast cancer compared to 50 apparently healthy controls. The significant increases in groups (41-50) years old (p=0.016) and over 60 years old (p=0.02) compared to control groups. 28 years old (46%) of these 50 breast cancer women were under 50 years old, and 22 years old (44%) were over 50 years old. According to body mass index, it is a significant difference in the frequency distribution of patients and control subjects according to BMI (P = 0.020). Where high frequency distribution of patients with breast cancer occurs in the obesity group 23 patients (47%) compared with other groups. The results showed the expression of miR34-5p is high1y significant (p=0.004) in the patients compared with contro1groupe.

Keywords: Breast cancer, Body mass index, Gene expression, miRNA, 34-5p.

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Introduction

Cancer is a major public health problem worldwide and is the second 1eading cause of death in the world (1). Breast cancer (BC) is a disease that results from the uncontrolled growth of breast cells. Several subtypes of breast cancer have been identified. Breast cancer types are determined by the types of breast cells that become malignant (2). Cell of the connective tissue, ducts, and lobules (3). Most breast cancers begin in the ducts or lobules (4). miRNAs are 21–25 nucleotides non-coding RNAs which may be related to cell proliferation, differentiation, and apoptosis (5). Modify the level of expression of several genes and proteins by silencing them, typically, miRNAs block translation or cause mRNA degradation by binding to complementary sequence sites in the 3'-untranslated region (UTR) of their intended targets (6). miRs are liberated into the circulation after radiation-induce tumor tissue destruction, so that they can serve as biomarkers to monitor response to radiation therapy (RT) directly and dynamically by analyzing blood samples (7). Over the last few years, miRNAs have gained major attention in breast cancer research due to their imperative role in tumor initiation and progression which has created the new prospect for early cancer diagnosis and therapies (8).The growing number of

research demonstrating the presence of miRNAs in circulating serum/plasma raises the possibility of employing miRNAs as a biomarker for cancer and other disorders (9). Recent research has shown that miR34-5p,is a tumor suppressor (TS): miRNA is involved in cellular proliferation, apoptosis, and is cancer, in such cases it is up-regulated. MiR34-5p regulates gene expression by targeting mRNAs, In addition, to that miR-324-5p is both a circulating miRNA and an intracellular miRNA, indicating that it is frequently present in the cellular microenvironment (10). Specifically, miR-324-5p controls MAPK pathway components to affect cell growth, proliferation, and survival. In particular, miR-34-5p is required for normal levels of cell proliferation because it down regulates RAF and ERK. Its significance in oncogenes is due to the fact that reduced expression stimulates cell growth and proliferation while overexpression suppresses growth of cancer cells (11). The aim of this research is to evaluate the serum expression (miR34a-5p) in the Iraqi population as important biomarkers

Materials and methods

Participants were enrolled in the study between November 2022 and March 2023 at the genetic engineering and biotechnology institute Baghdad University. Breast cancer patients who sought care at Baghdad's Al-Andalus Specialist Oncology Hospital or the Center for Cancer Treatment at Al-Fallujah Hospital provided the samples.

Fifty blood samples from female patients with pathologically proven breast cancer from Iraq were collected, and fifty blood sample of controls female were also collected. Patients and apparently healthy controls each had 5 mL of venous blood drawn; those with a diagnosis of BC had their blood deposited in gel tubes and centrifuged for 15 minutes at room temperature to separate their serum for molecular testing.

Protocol of microRNA Extraction According to the TRIzol™ Reagent extraction methodology, total RNA was isolated from the sample; 0.2 m1 of chloroform was added to the aqueous phase containing the RNA; 0.5 m1 of isopropanol was used to precipitate the RNA as a white gel-like pellet; and 0.5 mL of 70% ethanol was used to wash the pellet. To conclude, Pellet was rehydrated in 50 1 of nuclease-free water and then heated in a water bath at 55-60 °C for 10 to 15 minutes.

Revers transcription for complementary DNA (c DNA) synthesis

Four microliters of RNA material was combined with one microliter of miRlet-7-5p stem-loop RT primers. In this investigation, primers were designed and synthesized using the NCBI Gene Bank data base and miRBASE. The primers used in this study were selected from those in the Macrogene (South Korea) primer set (Table 1). The bonds in the hairpin loop structures seen in (Table 2) were broken by incubating the mixture for 5 minutes at 70iC and then 10 minutes at 4iC.

Table (1): Primer Sequence for miR34a-5p Gene Expression

Primer Name	Sequence
MiR34-5P R	5̀ - GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACACAACC-3̀
MiR34-5P F	5̀- GGTTTTTTTTGGCAGTGTCTTAGCT -3̀
Universal Reverse	5̀ - GTGCAGGGTCCGAGGT -3̀
MiR-16-1 R	5̀-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCGCCAAT-3̀
MiR-16 -1 F	5̀- GGTTTTTTTTAGCAGCACGTAAAT -3̀

Table (2): Thermal Cycler Program for First reaction

Steps	°C	min:sec	Cycle
Priming annealing	70	05:00	1
Hold	4	10:00	
Cool in ice and spin			

Quantitative real-time polymerase chain reaction (RT-q PCR)

RT-qPCR was performed using SYBR Green PCR Kit (Synthol /Russia) following the manufacturer's instructions. The quantitative reaction was performed as 10 µL total reaction volume, containing 5 µL of SYBR Green Master Mix, 0.5 µL of specific miRNA primer, forward and reverse, 2 µL of RNase-free water and 2 µL of cDNA, and performed using a Real-Time PCR system (Synthol /Russia). The cycling conditions were set as follow: 95i◦C for 1 min (1 cycle): 45 cycles of 95i◦C for 20is, 55i◦C for 20is and 72 ◦C for 20s. MiRNA expression levels were presented in terms of fold change normalized by the house keeping gene *miR-16-1* using the formula $2^{-\Delta\Delta CT}$.

Statistical analysis

Analysis of data was carried out using International Business Machine Statistical package for the Social Sciences (IBM SPSS– version 28).The results were analyzed statistically, and the values were expressed as Mean ± S.E.T-test test was used to significant compare between two samples. Chi-square test was used to significant compare between percentages. Statistica1 significance was considered whenever the P value was equa1 or less than 0.05.

Results and discussion

This study involved 100 females, who were enrolled in the study; consist of 50 females with BC of diagnosis during chemotherapy, along with 50 healthy females to serve as a control group. All BC samples were divided into five groups according to age, these groups ranged from ≤ 30 year old to upper than 60 year. The age distribution of females involved in control group showed a mean of about 49.1 ±10.6 years. The mean of female's age with BC was 35.9 ±11.9 years, that significant increase in grouped (41-50) (p=0.016) and significant in grouped upper than 60 (p=0.02) as compared to control groups. Among these 50 Breast cancer females 28 (46%) were aged less than 50 years, and 22 (44 %) were upper than 50 year as shown in (Figure 1).

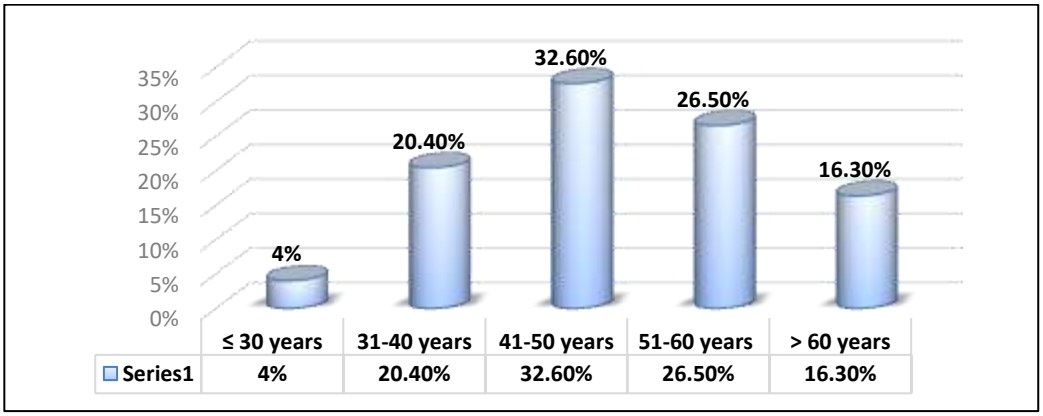

Figure (1): Distribution of patients according to age groups.

The results demonstrated a substantial as compared to the control group, with 28 (55.1%) of the tota1 patients having a favorable fami1y history whereas the remaining 22 (44.9%) did not have a family history as shown in (Figure 2).

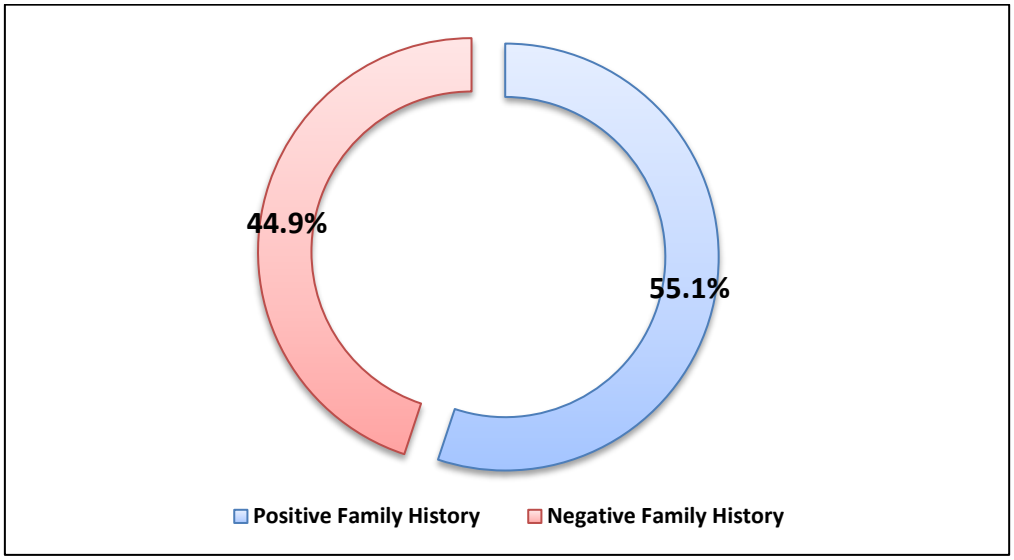

Figure (2): Distribution of patients according to family history.

According to the body mass index (BMI): the present study indicate significant difference in the frequency distribution of patients and control subjects according to BMI (P = 0.020). The high frequency distribution of patients with breast cancer occurs in the obesity group 23 (47%) in compared with other groups.as shown in (Figure3).

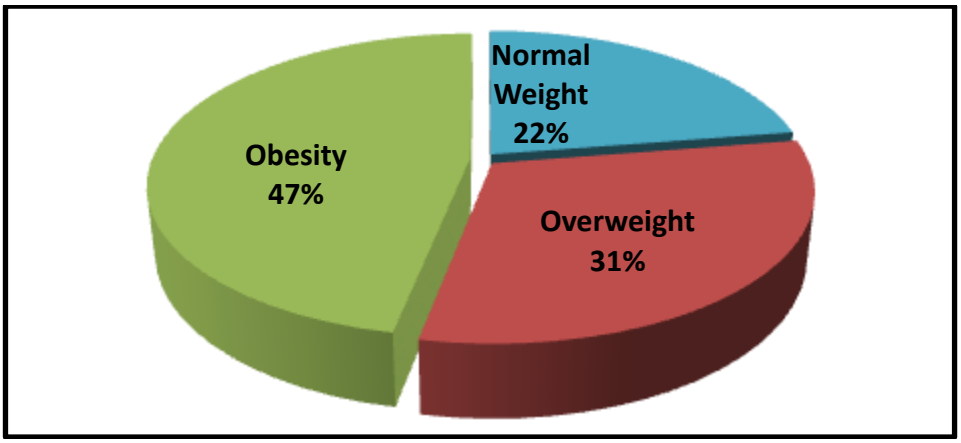

Figure (3): Distribution of patients according to BMI.

The molecular experiment of miR34a5p. Expression was performed to detect the amplification plots of miR34a-5p and miR-16 (reference gene) to find the threshold cycle (Ct) value for each. The Curves of miR34a-5p are shown in (Figure 4).

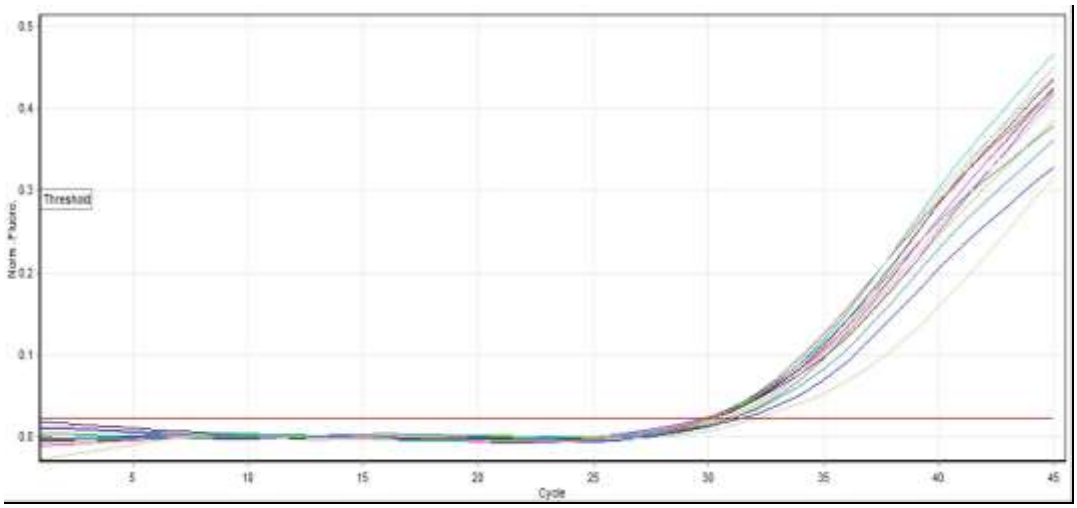

Figure (4): *miR34a-5p* dissociation curves by q PCR Samples included all study groups.

The comparison of miR34-5p level between breast cancer patients and apparently healthy control subjects has been carried out. Mean levels of miR34-5p were 1.73 ± 0.30 and 0.79 ± 0.08, in patients with breast cancer and apparently healthy control respectively; the level was significant higher than in patients with breast cancer in comparison with apparently healthy control (P= 0.004).As shown in (Table 3).

Table (3): Comparison between serum miR34a-5p expression between apparently healthy control and breast cancer groups.

Cases	Mean of fold	P-value
Patients	1.73 ± 0.30	0.004 (HS)
Control	0.79 ± 0.08	
(P≤0.01) Highly Significant		

Breast cancer is affected by many risk factors, such as older age, obesity and hormonal factors. The findings of this study confirmed that breast cancer can appear at any age, however it is most prevalent among middle-aged women. These results agree with the previous Iraqi studies such as (AL-Naqqash *et al*. (14). which separated patients into two categories based on their age (<50 and >50): and discovered that older women were at a higher risk of developing breast cancer The current findings confirmed the importance of screening and early detection of breast cancer, as well as the fact that breast cancer risk is not age-specific but is highest in middle-aged women. A recent Iraqi study about breast cancer done by Alwan *et al*. (12). agreed with current study, the study revealed that the high frequent age groups were (35-49): (50-64) 42.4%, 42.2% respectively(12). Moreover, another study that was done by (AL-Bedairy *et.al* (13) that the breast cancer incidence increased between 51-60 years (38.89%). Al-Naqqash *et.al*. (14) show the age developed give the cells more time to mutate to grow into cancer or older age have, cigarette smoke, chemicals and other cancer-causing (14). The present study showed that patients with positive family history of breast cancer 27 (55.1%) of cases compared with 3 (6.3%) of apparently healthy control women have positive family history, and the differences were highly significant. This finding supports the genetic bases of the disease, it

demonstrated the role genetics play in the development of breast cancer in Iraqi women (since there is a high rate of consanguineous marriages in the country). One of the main risk factors of breast cancer was family history(15). The present result higher than the results of AL-Saqabi and Aziz (15). Alwan *et al*. (12), who found that 19 (38 %) of total patients had a positive family history of breast cancer, revealed that positive family history was found in 43.7 % of cases in a 204 Iraqi patients (16). Another study done by (Abbas and Aziz) found that 9 (18.00%) and (10.4%) of breast cancer patients have a positive family history (17) . These data suggest that the percentage of patients with a family history of breast cancer could depend on the sample size and population. It has been emphasized that women with such genetic susceptibility have a much higher risk of developing cancer than the general population, accordingly recommending prompt thorough screening (18).(Abbas and Aziz):showed that 44% of BC were obese in compared with only 14.81% obesity in the control group. The present findings show that the obesity related to high risk of breast cancer (17). The previous studies that were done by (liu *et.al*) found that there is a complex relationship between BMI and cancer risk, possible breast cancer risk, and general metabolic health (19). The present study disagree with (Montgomery *et al*.): who showed high BMI it was not a risk factor; (P = 0.159). In present study, breast cancer group demonstrated extremely higher miR34-5p expression (up regulation) when compared with control group(20). (Maroni *et al.* (20) observed that miR-34a-5p was highly expressed in ductal breast carcinoma without and with bone-metastasis outcome, while being practically absent in normal mammary gland (21). Excessive cell proliferation is an essential element for tumor growth and development. Previous reports illuminated that miR-34 over-expression could repress migration and invasion of non-small-cell lung cancer cells(22) .investigated that miR-34b/c-5p was associated with breast cancer cell proliferation and apoptosis. but the current results are different from the result of (Dong *et al*.): who showed an obvious decrease of miR-34b-5p expression in breast cancer patients compared with the healthy control ($P<0.01$) (23).

Conclusion

In this work, it was found that miR34a-5p expression levels in breast cancer patients were significantly higher than the apparently healthy controls, therefore, it is correlated with breast cancer, also the results of this study show that the obesity related to high risk of breast cancer.

References

1. Siegel, R.L.; Miller, K.D.; Fuchs, H.E. and Jemal, A. (2022). Cancer statistics. A Cancer Journal for Clinicians. 2022; 72 (1): 7-33.
2. Miller, K.D.; Kramer, J.L. and Newman, L.A. Breast Cancer Statistics , 2022. 2022;(2019).
3. Thomas, M.; Downs, E.; Abraham, J. and Kruse, M. (2019). Seminars in Oncology Invasive lobular breast cancer : A review of pathogenesis , diagnosis , management , and future directions of early stage disease. Seminars in Oncology, 46(2): 121-132.
4. Olukayode, E. V.; Olajide, K. A. and Preye, M. G. (2023). The study of mutations of CDK4 and TP53 genes in selected breast lesions. Sokoto Journal of Medical Laboratory Science, 8(1): 65-77.
5. Badr, E.A.E.; Ali Assar, M.F.; Gohar, S.F.; Badr, M.H.; Hathout, R.M. and El-kousy, S.M. (2018). The clinical impact of miRNA34a and P53 gene expression in colon cancer. Biochemistry and Biophysics Reports, 16(10): 88-95.
6. Hironaka-Mitsuhashi, A.; Takayama, S.; Jimbo, K.; Suto, A.; Shimomura, A. and Ochiya, T. (2022). Clinical application of MicroRNAs in breast cancer

treatment. Archives of Breast Cancer, 9(1): 20-31.

7. To, N. H.; Nguyen, H. Q.; Thiolat, A.; Liu, B.; Cohen, J.; Radosevic-Robin, N.*, et al.* (2022). Radiation therapy for triple-negative breast cancer: emerging role of microRNAs as biomarkers and radiosensitivity modifiers. A systematic review. Breast Cancer Research and Treatment, 193(2): 265–279.
8. Mumtaz, S.; Saeed, R. F.; Qazi, A. S.; Akhtar, N. and Awan, U. A. (2023). The Emerging Role of microRNAs as Novel Diagnostic and Therapeutic Tools For Breast Cancer: a systematic review. Life and Science, 4(2): 11-11.
9. AL-Aflwky, A. K. H. and Shaheed, O. M. (2022). The Role of the Let-7 microRNA Family as Biomarker Prognostic Indicator Against Breast Cancer. Journal of Pharmaceutical Negative Results, 1253-1260.
10. Wang, W.; Hao, L. P.; Song, H.; Chu, X. Y. and Wang, R. (2022). The Potential Roles of Exosomal Non-Coding RNAs in Hepatocellular Carcinoma. Frontiers in Oncology, 12(2):1-14.
11. Liu, S. M.; Lu, J.; Lee, H. C.; Chung, F. H. and Ma, N. (2014). miR-524-5p suppresses the growth of oncogenic BRAF melanoma by targeting BRAF and ERK2. Oncotarget, *5*(19): 9444–9459.
12. Alwan, N. A.; Tawfeeq, F. N. and Mallah, N. A. (2019). Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. Journal of Contemporary Medical Sciences, *5*(1).
13. AL-Bedairy, I. H.; AlFaisal, A. H. M.; AL-Gazali, H.R. and AL, H. (2020). Molecular Subtypes by Immunohistochemical for Iraqi Women with Breast Cancer. Iraqi Journal of Biotechnology, 19(1):18-27.
14. Al-Naqqash, M. A.; Radhi, S. M.; Kareem, T. F. and Fawzi, H. A. (2019). Young age Iraqi Women with Breast Cancer: an overview of the correlation among their clinical and pathological profile. *Medical Science*, *23*(95): 6-11.
15. AL-saqabi, A. N. and Jaber, I. H. A. M. M. (2022). Demographic Study of Age, Family History, Stages, Grade and Expression of miRNA-195-5p in Sample of Iraqi Breast Cancer Patients. Iraqi Journal of Biotechnology, *21*(2): 465-476.
16. Abbas, R.A. and Aziz, I.H. (2023). Role of miRNA-21-5p Gene Expression as a Tumor Marker and the Progression of Clinic-Pathological Characters in Breast Cancer Iraqi Female Patients, Iraqi Journal of Biotechnology, 22(1): 8-18.
17. Hemminki, K.; Li, X.; Försti, A. and Eng, C. (2023). Are population level familial risks and germline genetics meeting each other? Hereditary Cancer in Clinical Practice, 21(1): 1-7.
18. Liu, B.; Giffney, H. E.; Arthur, R. S.; Rohan, T. E. and Dannenberg, A. J. (2021). Cancer risk in normal weight individuals with metabolic obesity: a narrative review. Cancer Prevention Research, 14(5): 509-520.
19. Montgomery, A.; van Eeden, L.; Boo, M.; Maul, K.; de Silva, K.; Klasan, A., *et al*. (2023). Does Closed Incision Negative Pressure Wound Therapy Reduce Surgical Site Infection in Endometrial Carcinoma Patients Undergoing Laparotomy? A Multicentre Retrospective Cohort Study. Clinical and Experimental Obstetrics and Gynecology, *50*(5): 102.
20. Maroni, P.; Puglisi, R.; Mattia, G.; Carè, A.; Matteucci, E.; Bendinelli, P., *et al*. (2017). In bone metastasis miR-34a-5p absence inversely correlates with Met expression, while Met oncogene is unaffected by miR-34a-5p in non-metastatic and metastatic breast carcinomas. Carcinogenesis, *38*(5): 492-503.
21. Zhuang, X. F.; Zhao, L. X.; Guo, S. P.; Wei, S.; Zhai, J. F. and Zhou, Q. H. (2019). miR-34b inhibits the migration/invasion and promotes apoptosis of non-small-cell lung cancer cells by YAF2. European Review for Medical and Pharmacological Sciences, 23(5): 2038-2046.
22. Dong, L.; Chen, F.; Fan, Y. and Long, J. (2020). MiR-34b-5p inhibits cell proliferation, migration and invasion through targeting ARHGAP1 in breast cancer. American Journal of Translational Research, 12(1): 269-280.