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ORIGINAL ARTICLE

Relationship between miR-29a Gene Expression and Progesterone Levels in Iraqi Patients with Breast Cancer

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ABSTRACT

Background*: In Iraq and around the world, Breast Cancer (BC) is the most frequent disease that affects women. About 22 nucleotides, short and non coding RNA molecules known as microRNAs (miRNAs) are essential for regulating genes and a range of cellular functions, including differentiation, proliferation, and death. The studies have shown that progestogens may bind to both nuclear and membrane receptors, which can stimulate the development of tumors in the breast and increase of cells causing breast cancer. The study was aimed to assess the miRNA-29 expression and the progesterone hormone levels and their association at the patients diagnosed with the breast cancer.* ***Methodology****: The number of samples was 74 blood samples, collected from breast cancer patients who were 54 females and apparently healthy subjects (20). The total RNA was extracted from whole blood to synthesis the complementary DNA(cDNA) and level of miR-29a expression was determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR) method. Serum levels of the progesterone hormone were measured by Enzyme linked lmmunosorbent assay (ELISA) method.* ***Results****: The Progesterone hormone level was significantly decreased in breast cancer patients having a mean equal to (13.27±1.6 ng/ml) in comparison to (20.99±0.8 ng/ml) for the healthy control group ($p<0.01$) while miR-29a expression was significantly increased in the breast cancer patients (1.2±0.28) in comparison with healthy subjects (0.006± 0.0001) with p value (0.0021).* ***Conclusion****: As stated by the findings, the miR-29a expression can be used as diagnostic marker for the breast cancer examination in the laboratory investigations .miR-29 was significantly correlated with progesterone levels in breast cancer. Further studies are needed to this correlation .*

INTRODUCTION

Breast cancer (BC) is currently the most prevalent cancer affecting women worldwide, which exhibits significant metastasis, disease heterogeneity and treatment resistance. This cancer continues to be the primary cause of cancer-related death in women[1,2]. Breast tissue cells can cause BC to develop, which is caused by a presence of malignant cells. These cells are known by their capacity to locally infiltrate normal tissues, and for their uncontrollably dividing cells, which result in aberrant development known as in situ carcinoma[3]. Given that breast cancer accounts for the largest proportion of malignant tumors in women, It's the most common cancer form among Iraqi women[4-6] . Iraq's rising breast cancer prevalence is a significant cause of health issues. Greater than even lung cancer, the breast cancer is the most prevalent kind of carcinogenesis in women, making up around one-third of all female malignancies reported, in accordance to the most recent Iraqi Cancer Registry (ICR)[7]. Therefore, An essential factor in improving the prognosis of individuals with BC is early and accurate diagnosis[8].

Short and non coding RNA molecules called microRNAs (miRNAs) have around 22 nucleotides and are important for controlling genes and a range of cellular processes, including differentiation, proliferation, and death[9,10]. As has been demonstrated that miRNAs cause cancer by either overexpressing oncogenic miRNAs (oncomiRs) or lowering the expression of tumor suppressor miRNAs, as evidenced by published research[11]. Several microRNAs (miRNAs or miR) may modulate human BC, according to several studies. The little non-coding RNA molecules known as miRNAs open up many options for early detection of cancer discoveries by researchers[12].

As more data point to the importance of microRNAs in regulating the development of cancer, the breast cancer cells had far greater quantities of miR-29a than non-tumor tissues did. Furthermore, in individuals with breast cancer, the increased expression of miR-29a was strongly connected with the spread of tumors and a reduced overall survival rate[13]. There are known to be

three matured members of the humankind miR-29 family of microRNAs: miR-29a, miR-29b, and miR-29c. There are two genes clusters that encode miR-29s. The miR-29 genes' promoter regions include binding sites for a number of transcriptional factors. The members of the miR-29 family target mostly overlapping groups of genes and have a similar sequence of the seed region[14].

Progesterone, significant gonadal hormone that is created in corpus luteum of the ovaries and also by the placenta during pregnancy. It is an endogenous 21-carbon steroid hormone that is synthesized from cholesterol via pregnenolone[15]. This ovarian steroid and its nuclear receptor, known as progesterone receptor (PR), are crucial for controlling the mammary gland's cell division and proliferation. Furthermore, data from experiments and clinical settings demonstrates their crucial function in regulating the evolution of mammary gland tumors and breast cancer's progression. The principal function of PR when attached to its ligand is that of a transcription factor, controlling target gene networks' expression[16]. Pregnancy-related hormone progesterone (Pg) inhibits myometrial contractility to maintain pregnancy and becomes ready for the endometrium to receive the fertilized zygote. Normal mammary gland growth, function and menstruation regulation depend on progesterone. progesterone could attach to both nuclear and membrane receptors, which can stimulate the development of breast tumors and growth of cells that cause breast cancer[17]. The hormone functions via its receptor(PR), which is expressed in a portion of breast epithelial cells, and called sensor cells. Different signaling outcomes are elicited in specific cells by this receptor, resulting in diverse cell-intrinsic and paracrine signaling that involves various mediators for various intercellular interactions[18].

The current study objectives were to assess progesterone levels and miRNA-29a gene expression in the breast cancer patients relative to healthy controls, examine the correlation between these variables and look into the potential of miRNA 29 as a diagnostic biomarker for the detection of the breast cancer.

METHODOLOGY

This study was carried out in the National Hospital for Oncology and Hematology (NHOH) in Al-Najaf province and laboratory of Molecular Biology in the Department of Biology/ Faculty of Science – University of Kufa, from January 2023 to August 2024. This Study included 54 patients suffering from breast cancer and 20 apparently healthy group. The blood samples were collected in EDTA tubes used for RNA extraction by utilizing Trizol reagent (Ambion, USA) following the manufacturer's instructions. The plasma was utilized fresh for the RNA extraction and PCR test after 1 milliliter of blood was drawn, put in anticoagulant EDTA tubes, and centrifuged for five minutes at 5000 rpm.While blood collected in gel tubes was centrifuged at 5000 rpm for 5 minutes; serum was used for the ELIZA method to measure progesterone level[19,20]. The gene expression of microRNA 29a was established by the real time PCR in the BC patients and healthy control. The reverse transcription quantitative polymerase chain reaction (RT-qPCR) was completed with the primers shown in table 1[21].The level of progesterone hormone was determined by using ELIZA methods according to the manufacturer's instructions.

Table 1: The sequence of miR-29 gene

Sequence of *miR-29a*	
Forward primer	5’TCGTATCCAGTGCGTGTCGTGGAGTCGGC 3’
Reverse primer	5’ AATTGCACTGGATACGACTAACCGA 3’

Statistical analysis

Statistical analyses of all results were carried out by using Statistical Package for the Social Sciences (SPSS) version 5 software statistical package using t-test and Chi-square test (with P value at level of significance less than 0.05) in order to compare values of the results between groups. The result values were stated as mean ± SE 22.

RESULTS

During the study period, 54 females with breast cancer were seen at the National Hospital for Oncology and Hematology (NHOH) in Al-Najaf. Their ages ranged from 22 to 82 years. According to the statistical results the micRNA 29a gene expression raised in the breast cancer patients than healthy subjects (1.2±0.28) and (0.006± 0.0001) respectively. There was an important association between the gene expression of micRNA 29a and incidence of the breast cancer with p value (0.0021). The results showed that there is an association between the intensity of microRNA gene expression and BC, as gene expression was significantly increased in all breast cancer patients based on the value of the delta-delta CT square, which represents the quantity of fold gene expression.2^-(ΔΔCt) as shown in figure (1) and table (2).

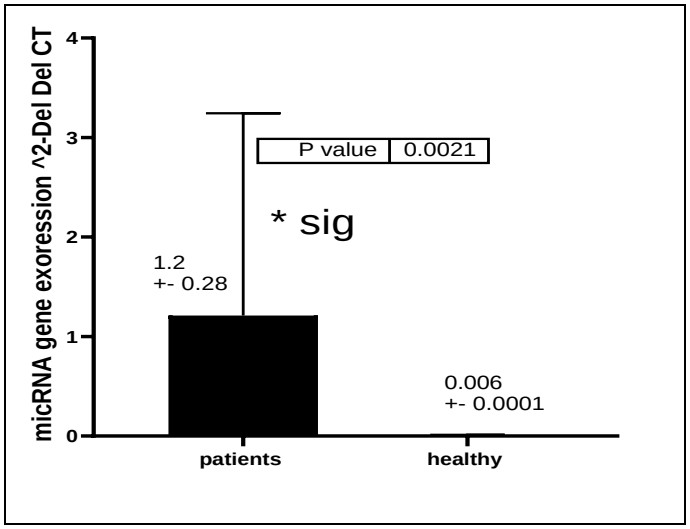

Fig. 1: Expression of microRNA29a gene in breast cancer patients and healthy subjects. (*): indicates significant difference at the level of ($p<0.05$).

The statistical findings indicated that there was non significant diference between age groups with the folding of microRNA 29a gene expression according to what was stated in the figure (2) that showed that as age get older the gene expression of micRNA-29a increased with a non significant value as in age group (40-49) year with p value 0.6482.

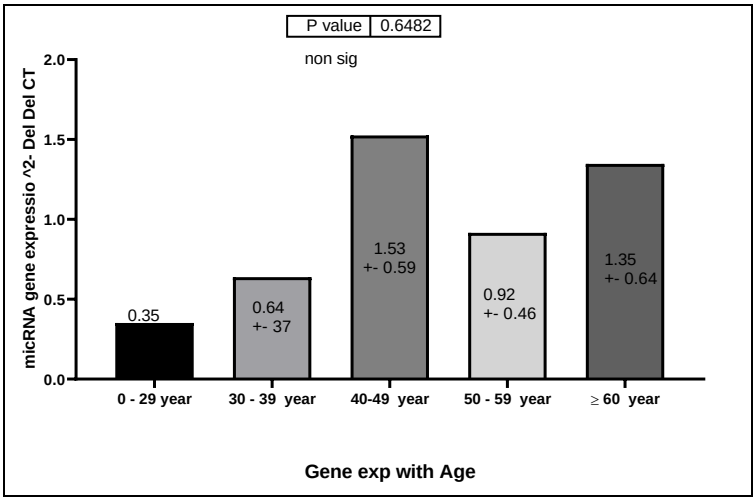

Fig. 2: Age and microRNA gene expression.

Table 2: Correlation between microRNA 29a and levels of progesterone hormone in the age and side of breast cancer patients

		Right breast					Left breast				
Age groups		0 -29	30– 39	40 - 49	50-59	≥ 60	0 -29	30 – 39	40- 49	50 - 59	≥ 60
MicRNA gene expression 2^-(ΔΔCt)	Mean	0.14	2.378	1.283	1.283	3.205	0.09000	0.04000	1.371	0.1567	0.3233*
	SD	0.1414	3.082	1.941	2.012	2.873	0.07071	0.04243	2.690	0.1823	0.5871
	SE	± 0.1	±1.54	0.73±	0.77	±1.17	0.05±	0.03±	1.017±	±0.07	±0.24
Progesterone Hormone level ng/ml	Mean	10.07	10.03	14.08	9.702	12.58	13.22	11.78	23.84	10.58	11.04
	SD	2.735	2.725	4.137	2.838	5.522	7.196	1.731	29.95	3.117	2.208
	SE	±1.93	±1.36	1.56±	±1.07	±2.25	5.09±	±1.22	11.32±	1.272±	0.90 ±

* P value=0.0368 (significant between ≥60 year groups Right& left breast)

** P value=0.0290 (significant between ≥ 60-year groups Right & left breast)

The statistical results also showed that there was a prominent relationship ($p<0.05$) between age groups and the concentration of progesterone hormone (fig 3).

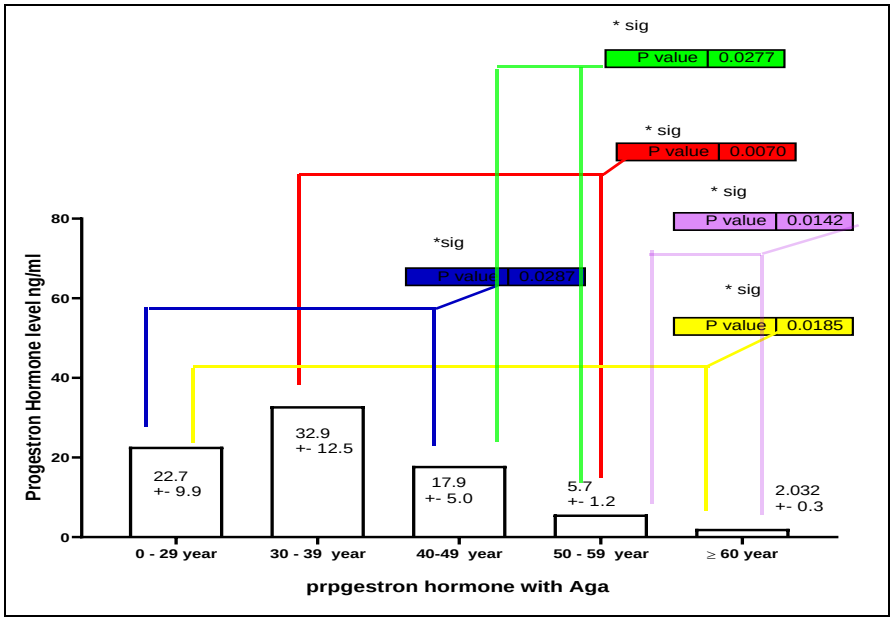

Fig. 3: Progesterone hormone levels and age of breast cancer patients. (*): Significant difference at level of ($p< 0.05$).

Fig 4 shows a significant relationship with the concentration of the hormone progesterone between cases with breast cancer and healthy people. Women with breast cancer had a significant decrease ($p<0.05$) in the concentration of the hormone progesterone. In healthy individuals, the average concentration was 20.99±0.8 ng/ml, while for females with breast cancer, the average concentration was 13.27±1.6 ng/ml, with a significant p value 0.0008.

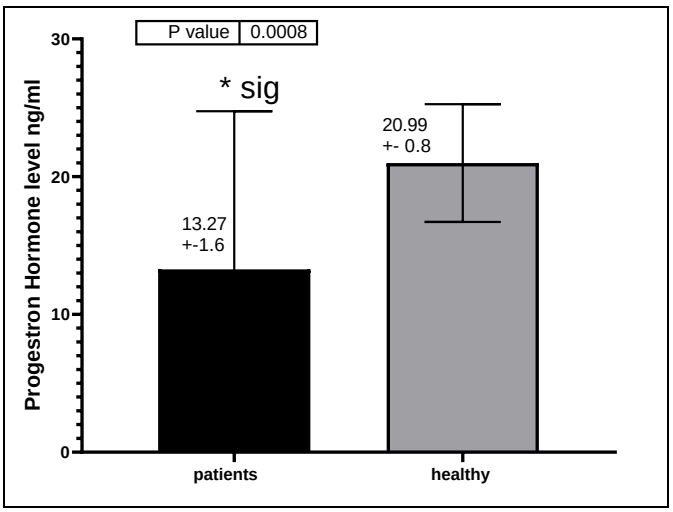

Fig. 4: Progesterone hormone levels in breast cancer patients and healthy individuals. (*): means significant difference at level of ($p<0.05$).

DISCUSSION

According to our current results, this study showed a notable connection between micRNA 29a gene expression and progesterone hormone. The study showed that miR 29 expression was significantly increased in BC patients compared to healthy control. In contrast, serum levels of progesterone hormone showed a noteworthy decrease in BC patients than healthy control.

Our results agree with the study of Rasheed[23] which discovered that the gene expression levels of mir29a in patients revealed a significant increase than in control (9.50± 1.30 and 3.47± 0.70, respectively).The results we obtained are in agreement with a previous study[24] showing that miR-29a was significantly increased in

patients with breast cancer (P<0.05). While the current results were not consistent with a study reported that the gene expression of miR-29a was significantly reduced in the patients in contrast to healthy individuals[25].These findings agree with a study showed that miR-29a expression in the breast cancer is connected to the concentration of the hormone progesterone[26].

miRNA miR-29a was verified by RT-PCR, showing a much lower expression level within the blood of breast tumor patients when compared with healthy controls (p=0.001)[27]. Aberrant microRNA expression contributes to breast carcinoma and other diseases[28-31]. MiRNAs in the bloodstream are viable candidates for non-invasive breast carcinoma biomarkers because they are simple to identify with long-term stability[32].

The present study indicates that expression of mir-29a is linked to breast cancer incidence and significantly increases in gene expression was observed at all the patients with breast cancer. In addition, this miRNA probably act as tumor suppressor miRNA in BC. Amirian et al[33] proved that although data on miR-29's functions in other malignancies are contradictory, it has mostly been identified as a tumor suppressor miRNA in breast carcinoma. As a tumor suppressor, miR-29 is well-known to have a significant impact on the development of cancer. But in other circumstances, it could also function as an carcinogen or oncogene[34].

The present study is in line with a previous study[35] that showed a significant differences between the concentration of progesterone in control and breast cancer patient groups (p<0.05). Tadesse et al[36] discovered that the mean serum progesterone levels in individuals with breast cancer were considerably lower when compared to healthy controls (0.4±0.5 and 1.68±3.3) (p<0.05) respectively and these results are close to the current findings.

Our findings showed that progesterone levels decreased in the patients than control and this decrease might be because of age of patients since most of them was in the premenopausal and postmenopausal state also due to the cancer treatment as demonstrated by Khan et al[37] that showed that serum progesterone levels show significant difference (p<0.05) between female breast cancer patient and healthy control. Chemotherapy treatments might lead in increasing or decreasing level of progesterone (4.6±5.6) (6.63±8.5) for patients and control respectively. Reproductive hormones and receptors play essential roles in breast cancer development and progression[38]. Reproductive hormones are among the variables that have been connected to a woman's likelihood of getting BC.

During menstrual cycles, Estradiol has very minimal impact on normal breast epithelium, while progesterone has a significant proliferative effect. Proliferation-related DNA replication mistakes have been closely connected to the genesis of breast carcinoma, and the mutational epithelial load may be affected by Progesterone's recurrent excitation of the breast tissue throughout each menstrual cycle. Studies revealed that breast cancers typically require several decades to form before being discovered because long-lasting cells, like stem cells, found in the breast tissue, may bring mutations into the future for a long time[39-41]. Therefore, given the limitations and poor diagnostic precision of screening tests, make developing a reliable method of diagnosis is critical.

CONCLUSION

Finally, the research revealed that progesterone levels in the patients with BC were correlated with the microRNA-29a gene expression. The statistical analysis the miR-29a expression can be used as diagnostic indicator to breast carcinoma detection along with laboratory examinations. Additional studies are required to hypothesize the findings.

Ethical Approval Declaration
The procedures followed in this study were in accordance with the regulations of the relevant clinical research ethics committee. Ethical approval was obtained from the ethical committee of the Ministry of Health and Environment in Iraq. In addition, each participant provided written consent following a concise overview of the project.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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