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Reviewer

mir31and mir214 as a Potential Biomarker: Evaluating Its Expression and Progesterone Levels in Breast cancer

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ABSTRACT
Breast cancer remains the most frequently diagnosed malignancy among women globally. (miRs) are small non-coding RNAs that critically regulate gene expression and are often dysregulated in various malignancies, serving as potential biomarkers for cancer progression. Progesterone, an endogenous steroid hormone, acts as a key regulator in mammary gland development and can significantly influence breast cancer risk and cyclical tumor cell proliferation. This study aimed to evaluate the expression levels of *mir31* and *mir214* as potential blood-based biomarkers in Iraqi women with breast cancer, and to assess their correlation with serum progesterone levels across different age groups and clinical stages. To achieve this, a case-control study was conducted involving 100 participants, including 50 apparently healthy controls and 50 breast cancer patients (clinical stages I–III, pre-metastasis, both treated and untreated). Circulating miRs were extracted, and the relative expression fold changes of *mir31* and *mir214* were quantified via Quantitative Real-Time PCR (qRT-PCR) using a SYBR Green assay, normalized against the *miRU6* small nuclear RNA housekeeping gene, while serum progesterone levels were determined using the Cobas e 411 automated immunoassay system with statistical analyses performed via IBM SPSS Statistics version 26. The results demonstrated that the highest distribution of breast cancer patients was within the 51–60 years age category (48.00%), showing a statistically significant difference compared to other age groups ($p = 0.01$). Significant differences were also observed across clinical stages, with mean values reflecting advancement from stage I to stages II and III ($p = 0.001$). Serum progesterone levels showed age-dependent fluctuations, with significant decreases in the 41–50 and 61–70 age brackets compared to healthy controls ($p = 0.05$). Notably, correlation analysis revealed a significant positive relationship between the expression fold changes of *mir31* and *mir214* in association with progesterone hormone levels ($r = 0.284$, $p = 0.026$). These findings suggest that the dysregulated expression of circulating *mir31* and *mir214*, alongside their positive correlation with serum progesterone levels, underscores their potential utility as non-invasive blood-based biomarkers for monitoring breast cancer progression and hormonal alterations in Iraqi patients.

KEYWORDS: ***mir31, mir214*, Potential, Biomarker**

1-INTRODUCTION

1- *miRs* *:* Are non-coding RNAs composed of 18 to 25 nucleotides that regulate the expression of eukaryotic genes. Ambros and Ruvkun's lab found the first miRs in 1993 [1]. These genes bind to (*3'UTRs*) of the target messenger RNA and regulate the post-transcriptional stage of gene expression. Abnormal genes expression has been associated with several of illness conditions, and it plays crucial roles in the initiation, progression, and pathogenesis of cancer because they influence gene expression and disease development; including malignancies, diabetes, cardiovascular disease, Alzheimer's disease, and autoimmune diseases [2,3]. Biogenesis of miRs are intragenic and derived from introns, which had a cap structure at the 5' end and 3' end was polyadenylated. Biogenesis starts in the nucleus, where (pri- miRNA) is transcribed by RNA polymerases II and III into hairpin-containing (pri- miRNA) [4]. Pri-miRNAs are subsequently cleaved in the nucleus by the protein DROSHA/DGCR8, which binds to the hairpin structure and cuts it to create pre – miRNA [5]. The pre-miRNA is then exported from the nucleus via XPO5 and enters the cytoplasm where it is further processed by DICER resulting in an miRNA duplex with the hairpin removed. This duplex can then be loaded into AGO, which removes the reverse complement of the miRNA and can bind to target messenger RNAs and recruit the miRISC components to facilitate degradation [6]. Typically, the

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strand that is put into Ago2 is considered the guiding strand. The passenger strand is the unloaded strand (the reverse complement of miR). The guide strand may attach to target mRNA and attract the minimum miRNA-induced silencing complex (miRISC) components to promote degradation or inhibition; however, proteins will not be produced [7], miR_S biosynthesis is seen in the figure (1) .

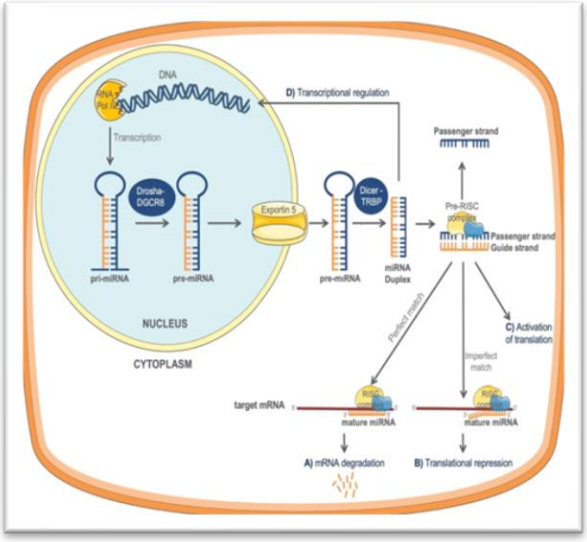

Figure (1): The biosynthesis of microRNAs and their mechanisms of action [5]

***1.2- mir31* gene: The** *mir31* gene is found on chromosome 9 on the short arm, region 2, band 1, and sub band 3 (9p21.3) and is 71 bases in length. The image of the gene is shown according to the gene card site (figure 2). It is found in the cytosol, the nucleus, the mitochondria, the plasma membrane, and the endoplasmic reticulum. *mir31* expression levels are changed in several cancers.

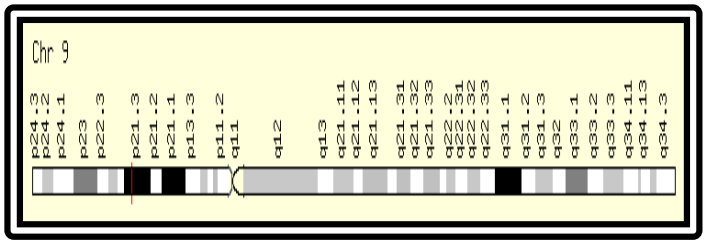

Figure (2): genomic location of *mir31* on chromosome

1.3 mir214: The human *mir214* gene is found on chromosome (no.1) on the long arm, region (2), band (4), and sub band (3), with a size of 110 bases (1q24.3). The image of the gene is shown according to the gene card site (figure 3). It resides in the nucleus, the extracellular space, the cytosol, the mitochondrion, the endosome, the cytoskeleton, the golgi apparatus, the lysosome, the peroxisome, the plasma membrane, and the endoplasmic reticulum.

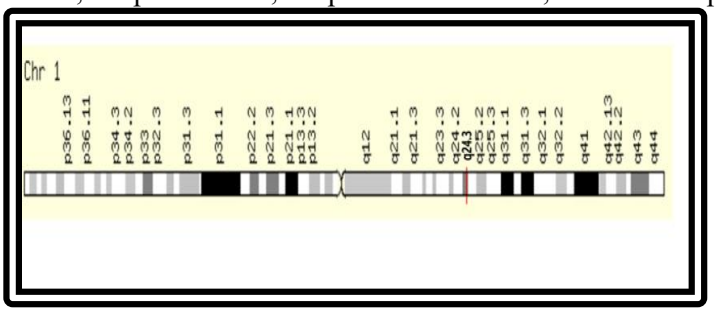

Figure (3): genomic location of *mir214* on chromosome.

2- cancer disease

Cancer is one of the world's top causes of mortality and one of the deadliest illnesses. Cancer is the uncontrolled proliferation of aberrant cells in any part of the body. These unusual cells are cancer cells (malignant cells) or (tumor cells). Cancer cells migrate through the blood and lymphatic systems, located in other organs where they may continue their uncontrolled development cycle, and infect healthy bodily tissues [9]. Cancers may be caused by anything that might cause normal body cells to grow abnormally. Some cancer causes are unknown, while others have been linked to human genes; approximately 5-10 percent of cancer is believed to be caused by hereditary factors, while the remaining 90-95 percent is either caused by or due to environmental factors **(Heredity, Ionizing radiation, Chemical substances, Dietary factors, Estrogen, The smoking, Viruses, Stress ,Age)**

2.1 Breast cancer: is the most often diagnosed malignancy among women globally. Males and females may get breast cancer, however male breast cancer accounts for less than 1% of all breast cancer cases. The aberrant proliferation of breast cancer cells and the formation of tumors or lumps accounted for one in four instances of cancer in females. Typically, these cells block the ducts (the milk carrier of the nipple tubes known as the lobules) [9]. There are two forms of breast cancer exist :

1) **Non – invasive or** Ductal carcinoma in situ (DCIS) Stage (0): Breast cancer cells remain inside the milk ducts or lobules. They do not transfer into normal tissues or develop beyond breast cells. Occasionally, they are referred to as carcinoma in situ or pre-cancers [11].

2 – **Invasive breast cancer stage (I –IV)**: During these phases, cancerous cells exit the ducts or lobules and move to the surrounding tissue, lymph nodes, and other organs [12].

3- **The role of *miRs* in human cancers** *miRs* are a class of short non-coding RNA*S* that modulate several biological processes, including carcinogenesis. In 2005, the first studies of the mechanistic function of *miRs* in cancer were published by Steven Johnson and coworkers in the laboratory of Frank Slack . Loss of one *miR* may result in carcinogenesis [13]. *miR*$_S$ have been discovered to be significantly dysregulated, with the biological causes including abnormal expression levels of mature or precursor *miR* transcripts, chromosomal abnormalities (amplification, deletion, or translocation, hyper methylation, and, hypomethylation), genomic mutations, and changes in *mirR* biogenesis, in comparison to those in the corresponding normal tissues. One *miR* may have a big impact; it may function as either oncogenes or tumor suppressors under conditions. The dysregulated *miRs* have been shown to affect the hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, activating invasion and metastasis, and inducing angiogenesis. Regulatory mechanisms of oncogenic and tumor suppressor miRs in tumorigenic events. Increased expression of oncogenic miRs in cancerous cells inhibits tumor suppressor genes. Decreased expression of tumor suppressor miRs potentially enhances the expression of oncogenes. Consequently, both oncogenic and tumor suppressor miRs lead to tumor development by stimulating cell proliferation, anti-apoptotic response, replicative immortality, invasion, metastasis and angiogenesis (figure 4) [14].

3. Progesterone hormone an endogenous steroid hormone, is a 21-carbon steroid that exerts its primary physiological functions by binding to progesterone receptors A and B (PR-A and PR-B), which initiate transcription of targeted genes, resulting in the conversion of proliferative endometrium in an estrogen-primed uterus. Primarily produced by the corpus luteum in the early postovulatory phase of the ovary. Progesterone's physiological significance during menstruation limited to the peri- and post-ovulatory stages of the menstrual cycle (when an egg is produced from the ovary) as well as pregnancy support in the female body. Progesterone is mainly involved in the progression of pubertal breast development through a paracrine mechanism, and for mammary gland cell growth. The loss of ovarian follicles grows exponentially with age and accelerates as a woman approaches menopause [15]. In breast cancer, progesterone hormone is a risk factor for breast cancer, greater exposure to progesterone increases breast cancer risk and leads to the development of progesterone's biological actions are a) hormone receptor-positive nuclear receptors and b) hormone receptor-negative nuclear receptors and it promotes pre-neoplastic progression via stimulation of cyclical proliferation of mammary stem cell pools or occult tumor initiating cells in the mature breast epithelium [16,17].

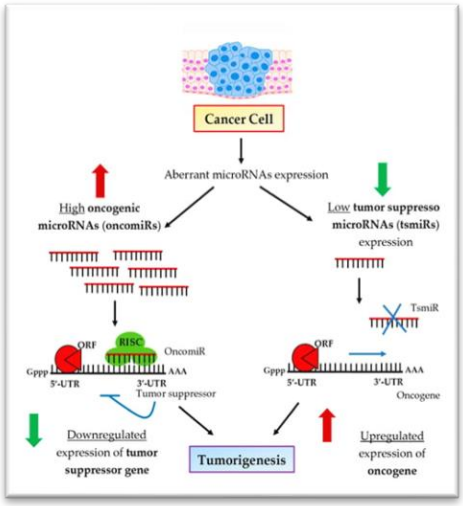

Figure (4) Roles of miR_S in cancer cells [14].

MATERIALS AND METHODS

Samples collection

The total number of participants in the study was 100 individuals**,** study groups included the following: - ***Group 1:*** Fifty samples of apparently healthy individuals of women, aged between (20-70 years) were obtained for controls. ***Group 2:*** Fifty patients's samples of women diagnosed with breast cancer, divided into two groups with and without treatments, aged between (30-70 years) and stages (I, II, and, III), patients were excluded at metastasis stage. The samples were collected at Al-Amel National Hospital for Cancer Treatment in Baghdad/Iraq, their clinical information was obtained from their hospital files and case-sheet records.

miRs Extraction from Blood Samples: From each participant, (1.5-2 ml) of whole blood was needed to be collected from the venous blood directly into an EDTA containing tube**,** this procedure was done under aseptic conditions [18]. The miR extraction from whole blood of patients and apparently healthy by using protocol in *EasyPure*® Blood Genomic miRNA Kit (Transgen, China). The steps for miR extraction method from whole blood samples as shown in figure (5).

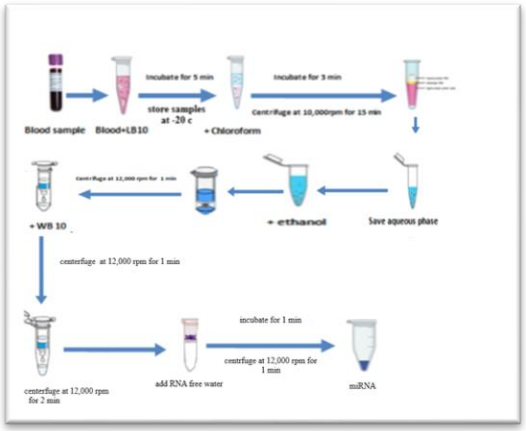

Figure (5): The extraction miRNA steps from whole blood sample [Modified].

Progesterone hormone: -Blood samples collected in gel tubes 2ml to obtain serum to determined progesterone , this test applied by cobas e 411 , test number 1380 [19]. This system intended to use immunoassay for the vitro quantitation of progesterone in human serum and plasma.

***TransStart*® Top Green qPCR SuperMix for gene expression**

This procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions (Transgene, China) [20].

Procedure: -

1. The master mix was allowed to thaw at room temperature 25°C.
2. The volumes of components needed to prepare the required number of reactions were calculated according to the table (1)
3. **Table (1): Reaction Components and volume for gene expression.**

No .	Component	Volume (20µl)
1	Master mix SYPER Green	10
2	Forward primer	1
3	Reverse primer	1
4	cDNA	3
5	Nuclease free water (N.F.W)	5

3.The tube where all the component is collected was briefly centrifuged to spin down the contents and eliminate any air bubbles

4.The tube was set inside the qRTPCR well and the program was run.

5.The qRTPC program conditions were designed for this study as shown in table (2)

Quantitative Real Time PCR (qRT–PCR):

The expression levels of (*mir31, mir214, and miRU6*) genes were estimated by the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, a sensitive technique for the quantifying of steady-state miRNA levels. To confirm the expression of the target gene, a quantitative real-time qRT-PCR , SYBR Green assay was used. *mir31, mir 214, and miRU6* genes stored at -20°C. Table (2) shows the primer sequences for (*mir31, mir214, and miR U6*). The Quantitative Real Time PCR (qRT–PCR) was carried out using the QIAGEN Rotor gene, (Germany).

Table (2) the primer sequences for (*mir31, mir214, and miR U6*).

Primers	Sequence (5`→3` direction)	Ref.
miRNAs		
mir31	GGCAAGATGCTGGCATAGCTG	21
mir214	ACAGCAGGCACAGACAGGCAG	22
F.P. miRU6(housekeeping gene)	AGAGAAGATTAGCATGGCCCCT	22
Univ.miRNA-qPCR-R	CAGTGCAGGGTCCGAGGT	22
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC	23

Housekeeping Gene Amplification: The housekeeping gene was used as an internal control to be used in calculating the ΔCT value. A qPCR reaction of amplification of housekeeping genes (*miRU6*) for (*mir-31)* genes were done with the thermal profile shown in table (3), (4) and, (5), respectively. Thermal profiles of genes amplification taken directly from the qPCR machine as shows in figures (6), (7), and (8), respectively.

Table (3): Thermal profile of *mir31* gene expression.

Step	Temperature/ºc	Duration /sec	Cycles
Enzyme activation	95	60	1
Denature	95	5	
Anneal	62	15	40
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

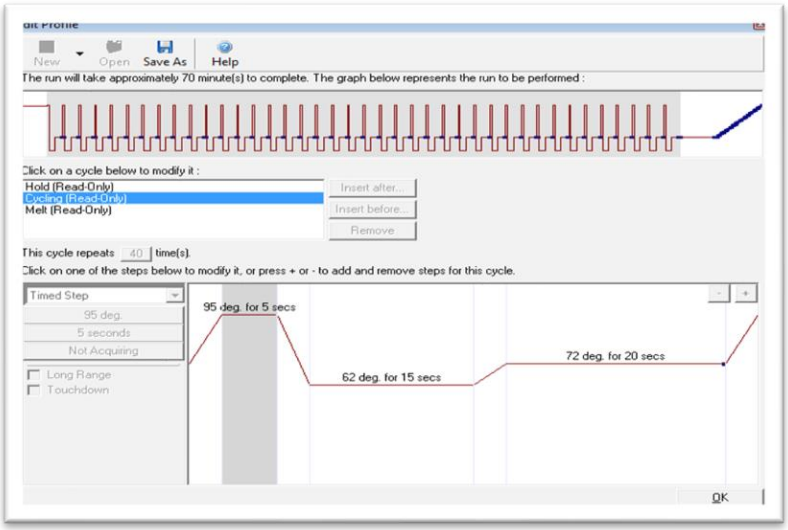

Figure (6): Thermal profile of amplification of *mir31*. The photograph was taken directly from qPCR machine.

Table (4): Thermal profile of *mir214* gene expression.

Steps	Temperature/ºc	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	35
Anneal	64	15	
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

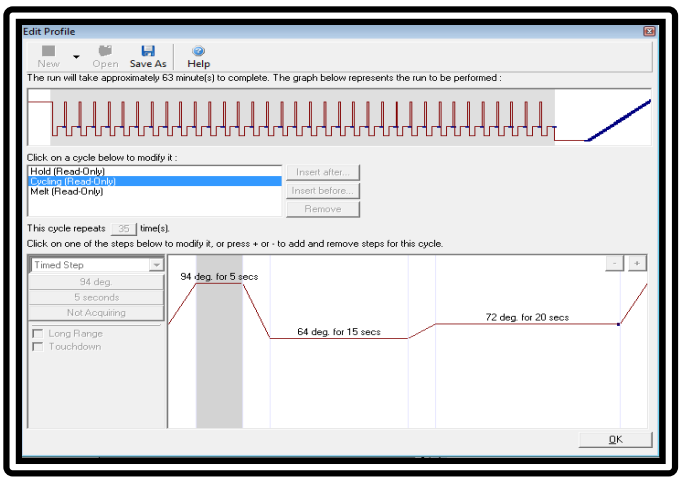

Figure (7): Thermal profile of amplification of *mir214*. The photograph was taken directly from qPCR machine.

Table (5): Thermal profile of *miRU6* gene expression.

Steps	Temperature/°c	Duration /sec	Cycles
Enzyme activation	94	30	1
Denature	94	5	35
Anneal	64	15	
Extension	72	20	
Dissociation	55 °C-95 °		1

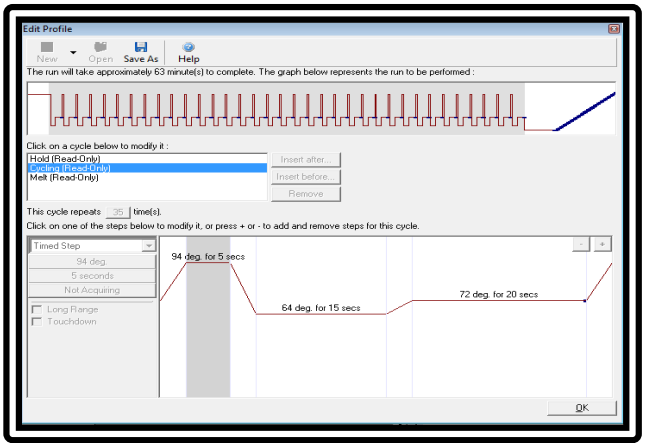

Figure (8): The thermal profile of *miRU6*gene expression. The image was taken directly from the qPCR machine

cDNA Reverse Transcription

Complementary DNA (cDNA) reverse transcription was performed on the second day following RNA extraction. A unified primer reaction was utilized to synthesize cDNA for the target microRNAs (mir31 and mir214) and the housekeeping gene (miR-U6). The efficiency of the cDNA concentration was subsequently confirmed via qPCR. Optimal primer annealing temperatures (Ta) were determined based on the melting temperature (Tm) of each specific primer sequence using the following formulas:

Melting temperature ™ = 2(A+T)+4(G+C)

Annealing Temperature (Ta)= Tm – (2to5)°C

The calculated annealing temperatures for the microRNAs are compiled in Table 6, where the lowest estimated temperature was selected to ensure optimal primer binding.

Table (6): Concentration and Annealing temperature of the miRs (*31,214, and, U6*)

Genes	Primer concentration / picoml	Annealing temperature / C^{o}
Mir31	10	62
Mir 214	10	64
MirRU6	10	64

Quantitative Real-Time PCR (RT-qPCR)

The quantitative expression of the target miRs (*mir31* and *mir214*) was determined via RT-qPCR using relative quantification. Normalization was performed against the *miR-U6* housekeeping gene to calculate Δ Ct values.

Real-time quantification was achieved using SYBR Green fluorescent dye, where the recorded threshold cycle (Ct) value is inversely proportional to the initial template copy number; lower Ct values indicate higher gene expression, whereas higher Ct values reflect lower expression levels.

miRU6 Expression Normalization

The threshold cycle (Ct) values and dissociation curves for the *miR-U6* housekeeping gene were analyzed to verify its stability as an internal control (Figures 9 and 10). The calculated relative expression fold changes for *miR-U6* in the breast cancer group and the healthy control group were 0.946 and 1.000, respectively.

The absolute 2^{-Ct} value was $1.886 \times 10^{-7}$for the breast cancer group and 1.994×10^{-7} for the healthy controls. Due to these minimal, non-significant variations in expression levels between the study groups, *miRU6* served as a highly suitable reference gene for normalizing target miRNA data via the $2^{\Delta Ct}$ method.

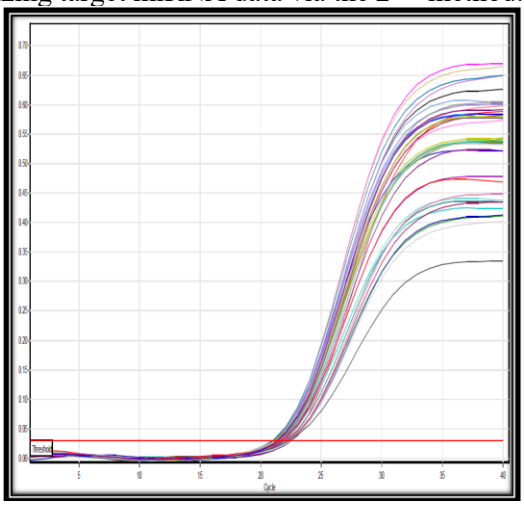

Figure 9): *miRU6* gene amplification was plotted using qPCR samples that covered all research groups. The photograph was taken directly from Qiagen Rotor gene qPCR machine.

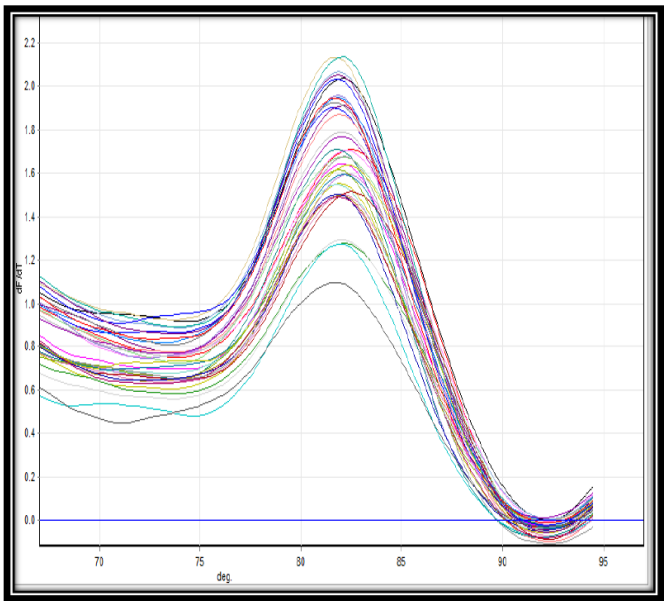

Figure (10): *miRU6* gene dissociation curves using qPCR samples that covered all research groups. The images were captured using the Qiagen Rotor Gene qPCR apparatus.

Quantification of *mir31* Expression via RT-qPCR

The relative expression of *mi31* was quantified in duplicate for each sample across the breast cancer and healthy control groups. Amplification and dissociation curves were systematically plotted for each individual run to confirm reaction specificity. The representative RT-qPCR profiles for *mir31* expression in both the breast cancer patients and the healthy control group are illustrated in Figures 11 and 12

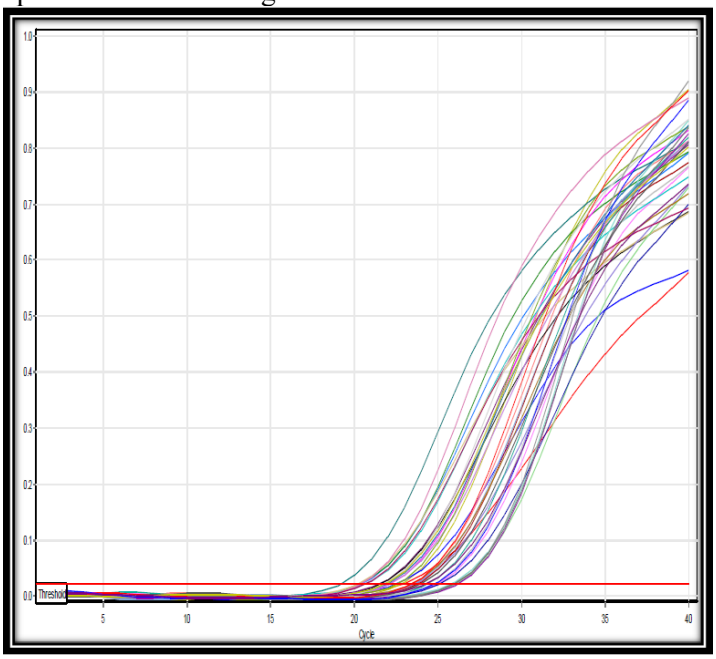

Figure (11): *mir31* amplification plots by qPCR. Samples included healthy study groups. The photograph was taken directly from Qiagen Rotor gene qPCR machine.

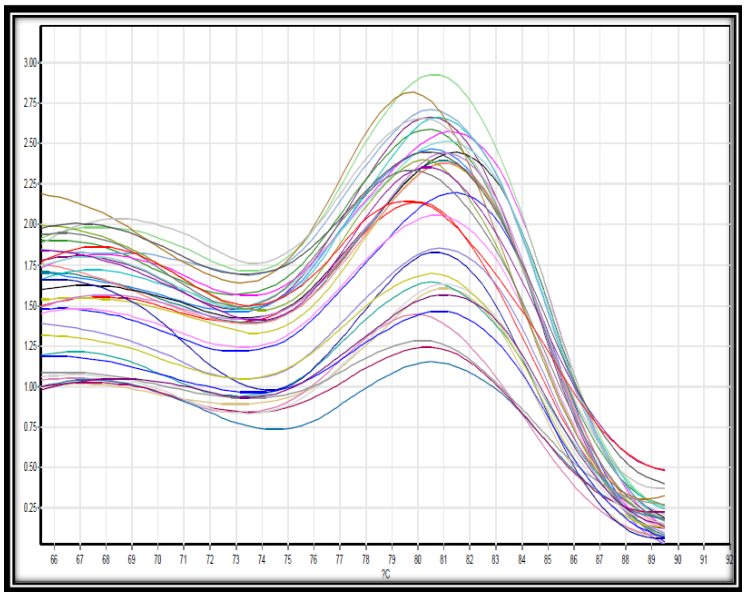

Figure (12): *mir31* dissociation curves by qPCR Samples included healthy study group. The photograph was taken directly from Qiagen Rotor gene qPCR machine.

Quantification of *mir214* Expression via RT-qPCR

The relative expression of *mir214* was evaluated in triplicate for each sample across the breast cancer group and the healthy control group. Amplification and dissociation curves were systematically generated for each individual run to ensure amplification specificity. The representative RT-qPCR analysis profiles for *mir214* in both the breast cancer patients and healthy individuals are presented in Figures 13 and 14.

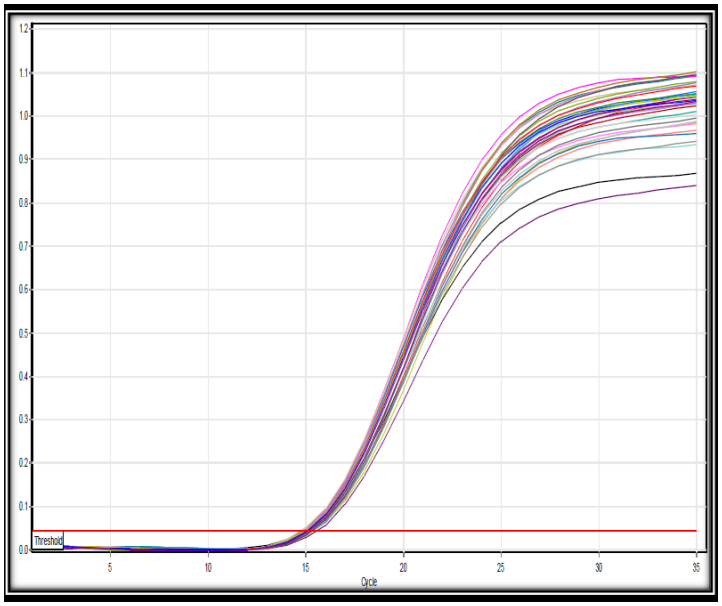

Figure (13): *mir214* amplification plots by qPCR. Samples included healthy study groups. The photograph was taken directly from Qiagen Rotor gene qPCR machine.

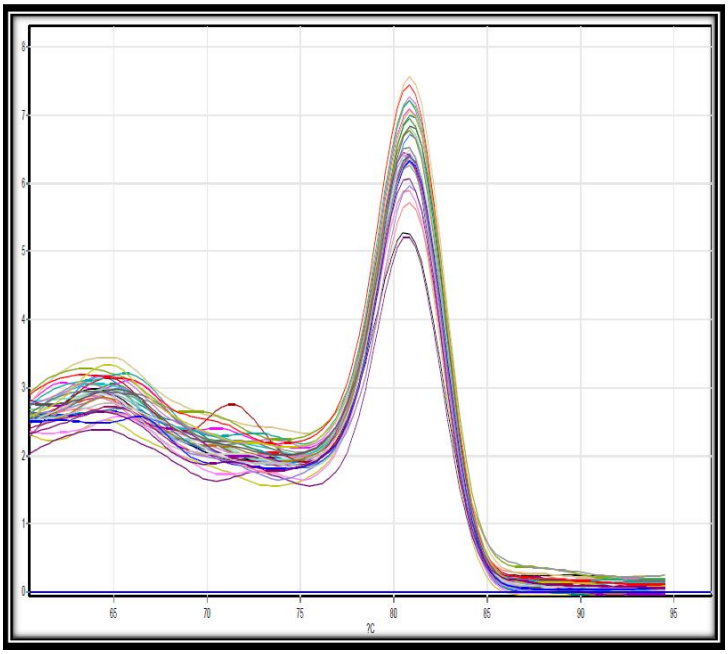

Figure (14): *mir214* dissociation curves by qPCR Samples included healthy study group. The photograph was taken directly from Qiagen Rotor gene qPCR machine.

Relative Quantification of miRNA Expression (Fold Change)

The relative expression levels of the target microRNAs (***mir31*** *and* ***mir214***) were calculated using the comparative Ct ($2^{\wedge\Delta\Delta\ Ct}$) method described by Livak and Schmittgen (2001). The cycle threshold (Ct) values for each target gene were normalized against the internal reference gene (***miRU6***) to obtain ΔCt values for both the control and patient groups as follows:

ΔCt(patients)=Ct(target miR)-Ct(*miR-U6*)

ΔCt(controls)= Ct(target miR)-Ct(*miR-U6*)

The final relative fold change in gene expression was determined using the formula:

Fold Change = $2^{-\Delta\Delta ct}$

Where $\Delta\Delta C_t$ (patients) – ΔC_t (controls)

A fold change value greater than 1.0 indicates up-regulation, a value between 0 and 1.0 indicates down-regulation, and a value of 1.0 indicates no change in gene expression relative to the control group. [23].

Statistical Analysis

The Statistical Analysis IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA and T-test was used to significantly compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study [25,26].

RESULTS

Distributions of age in breast cancer group

In this study, fifty samples were classified into four age categories: (30-40 years, 6.00%), (41-50 years, 24.00%), (51-60 years ,48.00%) and (61-70 years,22.00%) . The comparison of these groups revealed a statistically significant difference (*p.* value = 0.01 <0.05). When breast cancer and apparently healthy women are compared. as shown in table (7).

Table (7): Distributions of control and breast cancer patients on the basis of age groups.

Age categories	Control No.	Percentage	Breast cancer(No.)	percentage	*P*. value	
30-40 y	3	6.00%	3	6.00%	0.001	S.
41-50 y	12	24.00%	12	24.00%		
51-60 y	24	48.00%	24	48.00%		
61-70 y	11	22.00%	11	22.00%		
Total	50	100.00%	50	100.00%		

S. = Significant difference (*p*. value ≤ 0.05)

The comparison between breast cancer's stages.

Stages (I,II, and III) of breast cancer showed significant differences (ANOVA, *p*. value = 0.001<0.05) with breast cancer data as mean ± SEM (39.00 ± 1.64 a) , (55.66±1.32b) and (56.78 ± 1.73 b), respectively, based on their stages. As shown in table (8).

Table (8): Demographic and clinical characteristics of the study groups (Values are expressed as Mean±SEM)

Groups	No.	Stages	Mean ± SEM	*P*. value	
Breast cancer	3	I	39.00 ± 0.64 a	0.001	S.
	24	II	55.66 ± 1.32 b		
	23	III	56.78 ± 1.73 b		

- Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.
- S. = Significant difference (*p*.value ≤ 0.05)

Biochemistry tests

Progesterone levels in breast cancer.

The correlation of serum progesterone levels in breast cancer according to the patient's age.

Patients with breast cancer are classified into four age categories (in years) showed (9). Patients in (30-40y), had normal levels and showed no significant differences when compared with control, as mean± SEM(1.00± 0.56), (0.43± 0.16), respectively, (*p*. value= 0.25>0.05). furthermore, progesterone levels revealed high with no significant differences when compared with control in patients had (51-60), as mean± SEM (1.00± 0.36), (3.37± 1.05), respectively (*p*.value=0.06 >0.05). Progesterone hormone was normal levels in (41-50y), and, (61-70) were significant differences as mean ±SEM (1.00± 0.36), (0.28± 0.01), (1.00± 0.04), (1.00± 0.24), and, (0.33± 0.04), respectively, (*p*. values= 0.05, and, 0.02<0.05), when patient's ages were compared with the ages of apparently healthy.

Progesterone hormone in normal level was (0.27-1.72 ng/ml), it begins declining in late 20s decrease significantly after age 30. Progesterone levels begin to rise after ovulation through the end of the menstrual cycle. Symptoms of high progesterone are similar to premenstrual syndrome and can include anxiety and agitation, bloating, breast swelling and tenderness, depression, fatigue, and weight gain. high progesterone can also be the result of ovarian cysts, However, problems with your adrenal glands (such as congenital adrenal hyperplasia) and ovarian cancer. When progesterone is production is lower lead to fewer ovulations. Based on the results of a French cohort showed that progesterone levels do not correlate with breast cancer in women aged (41-50 years) which was the opposite

of this data. The contradictory results concerning the proliferative effect of progesterone may be associated with a different local metabolism in normal compared to malignant breast cells, the statistical test type, and clinical data differences, especially nations of the patients [27]. interestingly, a study revealed that low levels of progesterone in women under 40 years are consistent and responsible for the reduction in breast cancer incidence in these women [28]. However, a study by Britton *et al*. revealed that elevated progesterone levels were associated with a 16% increase in the risk of breast cancer , especially in women within >60 years [29].

Table (9): The relationship between serum progesterone levels in control and breast cancer patients according to their ages.

Age categories (year)	Progesterone		*P*. value	
	Control (N=50)	Breast cancer (N=50)		
30-40 y	N=3	N=3	0.25	N.S.
Mean± SEM	1.00± 0.56	0.43± 0.16		
41-50 y	N=12	N=12	0.05	S
Mean± SEM	1.00± 0.36	0.28± 0.01		
51-60y	N=24	N=24	0.06	N.S.
Mean± SEM	1.00± 0.04	3.37± 1.05		
61-70y	N=11	N=11	0.02	S
Mean± SEM	1.00± 0.24	0.33± 0.04		

S. = Significant difference (p. value ≤ 0.05) , N.S = Non significant difference (p.value > 0.05)

Correlation Between miRs Expression and Serum Progesterone Levels

To evaluate the potential of these miRs as biomarkers integrated with hormonal status, Pearson's correlation analysis was performed (**Table 10**). The statistical analysis demonstrated a significant negative correlation between ***mir31*** expression and serum progesterone levels (r = -0.315, P = 0.026), indicating that lower expression of ***mir31*** is statistically associated with hormonal fluctuations during breast malignancy progression. On the other hand, a robust and significant positive correlation was observed between ***mir214*** expression and total serum progesterone levels (r = 0.412, P = 0.003). This strong positive association supports the primary conclusion of this study, indicating a synchronized interplay between ***mir214*** up-regulation and progesterone pathway alterations in breast cancer pathophysiology. Furthermore, ***mir31*** demonstrated a positive internal correlation with ***mir214*** (r = 0.284, P = 0.026) in relation to progesterone hormone levels. Table (10).

T he correlation between expression fold of *miRs* and progesterone levels in breast cancer.

Parameters	***mir31* (Fold Change)**	***mir214* (Fold Change)**	**Serum Progesterone (ng/mL)**
***mir31* (Fold Change)**	1.000	—	—
***mir214* (Fold Change)**	r = -0.14 *P* = 0.325	1.000	—
Serum Progesterone (ng/mL)	r = -0.315 *P* = 0.026*	r = 0.412 *P* = 0.003**	1.000

** Correlation is significant at the 0.05 level (2-tailed). Correlation is significant at the 0.01 level (2-tailed).*

DISCUSSION

The results of the present study demonstrate a profound correlation between advancing age and the increased incidence of breast cancer, reaching a peak in the 51–60 years age group (48.00%). From a molecular biology perspective, these findings can be explained by the cumulative acquisition of somatic genetic mutations and the

gradual decline of DNA repair mechanisms that accompany cellular senescence. Prolonged lifetime exposure to ovarian hormones—estrogen and progesterone—stimulates continuous proliferation of the mammary epithelial cells, significantly increasing the probability of stochastic genetic errors. These errors eventually bypass cellular surveillance, leading to malignant transformation. The statistical significance observed (P = 0.01) reinforces the theory that age is not merely a chronological factor but a biological marker for the accumulation of genomic damage and environmental stressors, aligning with local and global research that identifies women over 40 as the high-risk demographic for invasive carcinomas.

Regarding clinical staging, the predominance of patients in Stages II and III (92% of the sample) highlights a critical shift in the biological behavior of the tumors. Molecularly, the progression from the localized Stage I to the more advanced Stages II and III is characterized by heightened genomic instability and the loss of control over cell cycle checkpoints. The higher mean ages observed in these advanced stages suggest that the tumors had sufficient time to activate the Epithelial-Mesenchymal Transition (EMT). This molecular process allows cancer cells to lose their adhesion, gain motility, and invade surrounding tissues. The scarcity of Stage I cases in this study compared to Western populations may be attributed to a combination of aggressive tumor biology and the absence of early molecular screening, which allows the disease to evolve into a more complex clinical state before detection.

The most intricate finding lies in the downstream molecular crosstalk between progesterone levels and the differential expression profiles of the investigated miRs. Progesterone acts as a master regulator of the RANKL (Receptor Activator of NF-κB Ligand) pathway, which is the primary mediator of progesterone-driven cell proliferation. Interestingly, the statistical correlation analysis in this study revealed a distinct divergent expression pattern between *mir31* and *mir214* in relation to progesterone hormone levels.

Specifically, *mir31* demonstrated a significant negative correlation with progesterone levels ($r = -0.315$, $P = 0.026$). This inverse relationship highlights its potential role as a down-regulated biomarker in response to elevated hormonal signaling, suggesting that progesterone may exert an inhibitory effect on the upstream promoters of *mir31*, or that the pathological decline of mir31 accelerates aberrant cellular pathways. Conversely, *mir214* exhibited a robust, statistically significant positive correlation with progesterone ($r = 0.412$, $P < 0.01$). This strong positive axis reinforces the hypothesis that *mir214* acts as a hormonally responsive non-coding RNA whose transcription is directly or indirectly stimulated by progesterone receptor activation.

Importantly, the cross-correlation between mir31 and mir214 expression profiles themselves was found to be statistically non-significant ($r = -0.142$, $P = 0.325$). This critical finding implies that although both miRs are significantly linked to progesterone fluctuations, they operate as independent molecular rheostats that modulate the breast tissue's response to hormonal signals through parallel, non-overlapping pathways. In a malignant environment, the distinct dysregulation of these two miRs can synergistically amplify the proliferative signals of the RANKL pathway, even when systemic progesterone levels begin to decline post-menopause.

Furthermore, the significant differences in progesterone levels within the 41–50 and 61–70 age groups compared to controls point toward a disrupted endocrine-molecular axis. While progesterone typically declines after age 30, the presence of malignant cells can alter local steroid metabolism, sometimes leading to an "intracrine" effect where the tumor produces or concentrates hormones locally to sustain its growth. The contradictions between these findings and certain international studies—where low progesterone is sometimes seen as protective—underscore the influence of genetic polymorphism and ethnic variations in hormone receptor sensitivity. Ultimately, the results suggest that breast cancer in Iraqi patients is driven by a complex interplay of late-stage genetic evolution and a specific molecular environment where distinct miRs and hormones synergistically promote tumor progression [30,31].

CONCLUSION

advancing chronological age, advanced histopathological staging, and specific microRNA dysregulation to breast cancer progression in Iraqi patients. Chronologically, the study identifies women aged **51–60 years** as the critical peak high-risk demographic, accounting for **48.00%** of the patient cohort ($p = 0.01$). This biological vulnerability is strictly coupled with a profound delay in clinical detection, as evidenced by a statistically significant predominance ($p = 0.001$) of patients presenting in advanced clinical stages—specifically **Stage II (48.00%)** and **Stage III (46.00%)**—with elevated mean age trends characterizing these invasive phases.

At the epigenetic level, this oncogenic progression is modulated by distinct expression dynamics of circulating non-coding RNAs integrated within the host's endocrine microenvironment. The study confirms that ***mir31*** and ***mir214*** exhibit statistically significant, divergent correlation profiles in response to total serum progesterone fluctuations. Specifically, ***mir31*** expression is characterized by a significant inverse relationship ($r = -0.315$, $p = 0.026$), whereas ***mir214*** displays a robust positive co-regulation ($r = 0.412$, $p = 0.003$) relative to systemic progesterone alterations. Crucially, the lack of a direct, significant cross-correlation between the two microRNAs themselves ($r = -0.142$, $p = 0.325$) confirms that they function as separate upstream molecular rheostats.

Operating through parallel, independent biochemical cascades rather than a synchronized axis, their dual dysregulation synergistically amplifies downstream proliferative signals, such as the progesterone-driven RANKL pathway, even amidst post-menopausal hormone variations. Ultimately, these unique molecular signatures underscore the multi-faceted nature of breast malignancy in Iraqi cohorts, providing a robust, data-validated foundation for utilizing ***mir31*** and ***mir214*** as independent, blood-based diagnostic biomarkers for molecular stratification and targeted therapeutic interventions.

REFERENCES

[1] Kuang, J., Yan, X., Genders, A. J., Granata, C., & Bishop, D. J. (2018). An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. *PLOS ONE*, *13*(5), e0196438. https://doi.org/10.1371/journal.pone.0196438

[2] Xiao, Y., Humphries, S., Yang, Q., & Wang, Z. (2019). MiR-205 dysregulations in breast cancer: The complexity and opportunities. *Non-Coding RNA*, *5*(4), 53. https://doi.org/10.3390/ncrna5040053

[3] O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology*, *9*, 402. https://doi.org/10.3389/fendo.2018.00402

[4] Mansoori, B., Baradaran, B., Nazari, A., Gaballu, F. A., Cho, W. C.-S., & Mansoori, B. (2022). MicroRNAs in the cancer cell-to-cell communication: An insight into biological vehicles. *Biomedicine & Pharmacotherapy*, *153*, 113449. https://doi.org/10.1016/j.biopha.2022.113449

[5] Caus, M., Eritja, À., & Bozic, M. (2021). Role of microRNAs in obesity-related kidney disease. *International Journal of Molecular Sciences*, *22*(21), 11416. https://doi.org/10.3390/ijms222111416

[6] Al-Alem, L. F., Baker, A. T., Pandya, U. M., Eisenhauer, E. L., & Rueda, B. R. (2019). Understanding and targeting apoptotic pathways in ovarian cancer. *Cancers*, *11*(11), 1631. https://doi.org/10.3390/cancers11111631

[7] Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discovery*, *12*(1), 31–46. https://doi.org/10.1158/2159-8290.cd-21-1059

[8] Pietrykowska, H., et al. (2022). Biogenesis, conservation, and function of miRNA in liverworts. *Journal of Experimental Botany*, *73*(13), 4528–4545. https://doi.org/10.1093/jxb/erac098

[9] Carlo, M., et al. (2018). Prevalence of germline mutations in cancer susceptibility genes in patients with advanced renal cell carcinoma. *JAMA Oncology*, *4*(9), 1228. https://doi.org/10.1001/jamaoncol.2018.1986

[10] Ghoncheh, M., Pournamdar, Z., & Salehiniya, H. (2016). Incidence and mortality and epidemiology of breast cancer in the world. *Asian Pacific Journal of Cancer Prevention*, *17*(Suppl 3), 43–46. https://doi.org/10.7314/apjcp.2016.17.s3.43

[11] Akram, M., Iqbal, M., Daniyal, M., & Khan, A. U. (2017). Awareness and current knowledge of breast cancer. *Biological Research*, *50*(1), 33. https://doi.org/10.1186/s40659-017-0140-9

[12] Shepherdson, J. L., Zheng, H., Amarillo, I. E., McAlinden, A., & Shinawi, M. (2021). Delineation of the 1q24.3 microdeletion syndrome provides further evidence for the potential role of non-coding RNAs in regulating the skeletal phenotype. *Bone*, *142*, 115705. https://doi.org/10.1016/j.bone.2020.115705

[13] Wu, K.-J. (2020). The role of miRNA biogenesis and DDX17 in tumorigenesis and cancer stemness. *Biomedical Journal*, *43*(2), 107–114. https://doi.org/10.1016/j.bj.2020.03.001

[14] Loh, H.-Y., Norman, B. P., Lai, K.-S., Rahman, N. M. A. N. A., Alitheen, N. B. M., & Osman, M. A. (2019). The regulatory role of microRNAs in breast cancer. *International Journal of Molecular Sciences*, *20*(19), 4940. https://doi.org/10.3390/ijms20194940

[15] Diep, C. H., Daniel, A. R., Mauro, L. J., Knutson, T. P., & Lange, C. A. (2015). Progesterone action in breast, uterine, and ovarian cancers. *Journal of Molecular Endocrinology*, *54*(2), R31–R53. https://doi.org/10.1530/jme-14-0252

[16] Trabert, B., Sherman, M. E., Kannan, N., & Stanczyk, F. Z. (2019). Progesterone and breast cancer. *Endocrine Reviews*, *41*(2), 320–344. https://doi.org/10.1210/endrev/bnz001

[17] Pedroza, D. A., Subramani, R., & Lakshmanaswamy, R. (2020). Classical and non-classical progesterone signaling in breast cancers. *Cancers*, *12*(9), 2440. https://doi.org/10.3390/cancers12092440

[18] Wang, J., Yan, Y., Song, L., Liu, L., & Liu, B. (2017). The association of plasma miR-155 and VCAM-1 levels with coronary collateral circulation. *Biomarkers in Medicine*, *11*(2), 125–131. https://doi.org/10.2217/bmm-2016-0282

[19] Chen, J., et al. (2018). KLHL22 activates amino-acid-dependent mTORC1 signalling to promote tumorigenesis and ageing. *Nature*, *557*(7706), 585–589. https://doi.org/10.1038/s41586-018-0128-9

[20] Sun, X., et al. (2017). Activation of secondary cell wall biosynthesis by miR319-targeted TCP4 transcription factor. *Plant Biotechnology Journal*, *15*(10), 1284–1294. https://doi.org/10.1111/pbi.12715

[21] Xu, R.-S., et al. (2012). The tumor suppressor gene RhoBTB1 is a novel target of miR-31 in human colon cancer. *International Journal of Oncology*, *42*(2), 676–682. https://doi.org/10.3892/ijo.2012.1746

[22] Chu, Q., Sun, Y., Cui, J., & Xu, T. (2017). Inducible microRNA-214 contributes to the suppression of NF-κB-mediated inflammatory response via targeting myd88 gene in fish. *Journal of Biological Chemistry*, *292*(13), 5282–5290. https://doi.org/10.1074/jbc.m117.777078

[23] Younus, N., Altaee, M., & Sharba, Z. (2021). Correlation of microRNAs-122a gene expression with diabetic for Iraqi patients. *Journal of Applied Sciences and Nanotechnology*, *1*(3), 64–72. https://doi.org/10.53293/jasn.2021.3789.1043

[24] Stephenson, F. H. (2016). Real-Time PCR. *Calculations for Molecular Biology and Biotechnology*, 215–320. https://doi.org/10.1016/b978-0-12-802211-5.00009-6

[25] Lopatofsky, E. (2009). A review of 'Developing Multicultural Educators (2nd ed.)'. *Educational Studies*, *45*(1), 84–89. https://doi.org/10.1080/00131940802546902

[26] Forthofer, R. N., & Lee, E. S. (2014). *Introduction to biostatistics: A guide to design, analysis, and discovery*. Elsevier.

[27] Trabert, B., et al. (2020). Association of circulating progesterone with breast cancer risk among postmenopausal women. *JAMA Network Open*, *3*(4), e203645. https://doi.org/10.1001/jamanetworkopen.2020.3645

[28] Kuhl, H., & Schneider, H. P. G. (2013). Progesterone – promoter or inhibitor of breast cancer. *Climacteric*, *16*(sup1), 54–68. https://doi.org/10.3109/13697137.2013.768806

[29] Dowsett, M., & Folkerd, E. (2014). Reduced progesterone levels explain the reduced risk of breast cancer in obese premenopausal women: a new hypothesis. *Breast Cancer Research and Treatment*, *149*(1), 1–4. https://doi.org/10.1007/s10549-014-3211-4

[30] Ming, J., Cronin, S. J. F., & Penninger, J. M. (2020). Targeting the RANKL/RANK/OPG axis for cancer therapy. *Frontiers in Oncology*, *10密*, 1283. https://doi.org/10.3389/fonc.2020.01283

[31] Trabert, B., Sherman, M. E., Kannan, N., & Stanczyk, F. Z. (2020). Association of circulating progesterone with breast cancer risk among postmenopausal women. *JAMA Network Open*, *3*(4), e203645. https://doi.org/10.1001/jamanetworkopen.2020.3645