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ORIGINAL ARTICLE

Measuring The Level of MIR-148A in Iraqi Women Diagnosed with Breast Cancer

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ABSTRACT

Background: Breast cancer is the most common type of cancer in Iraq, and the incidence of it has increased among Iraqi women in the past two decades, as it represents one of the most important threats to women's health.
Aim: this study aimed to investigate the miR-148a gene expression as a biomarker in different age groups of Iraqi women with breast cancer, as well as its association with patient characteristics such as body mass index, disease stage, and tumor location.
Method: Fifty women diagnosed with breast cancer were enrolled in this study in addition to 25 healthy individuals as a control group. The fold expression (FE) of miRNA-148a was detected by quantitative polymerase chain reaction (qPCR) techniques.
Results: The results showed the highest significant difference in the gene expression of miR-148a in breast cancer patients in the age group (50) compared to the control group, and the difference was significant in the age group (<40) compared to the control group, highly significant differences was found in the age group (40-49) compared with the control group. The results also showed no significant differences in the fold level of gene expression for miR-148a in the grades (I, II, and III) of breast cancer patients, and that the highest level was recorded in the second grade, and the highest level was recorded in the grade.
Keywords: Breast Cancer, miR-148a, BMI

INTRODUCTION

Breast cancer is the most type of cancer that affects the breast tissue in women causing abnormal and uncontrolled cells proliferation [1], [2] The Iraqi Ministry of Health (2019) reported that the total number of cancer deaths during 2019 was 10,957, with a mortality rate from 10.28/100,000. The 15.99% incidence of bronchial and lung cancer was 4.48/100,000 F, while the highest mortality was for females with breast cancer (22.58%, 6.22/100,000 F).

microRNAs (miRNAs/miRs) are a group of single small non-coding RNAs in eukaryotes with a length of 21–23 nucleotides [3]. miRNAs transcript inhibit the gene expression by binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs [4]. It mediates a wide range of cancers, including lung, breast, stomach, colorectal, liver, prostate, and ovarian cancers [5]. Increasing gene expression levels for miRNA molecules have been reported in breast cancer such as miR-328 [6] and miRNA-370 [7]. The miR-148a has many biological functions, including cellular development and differentiation, abnormal expression of miR-148a has a role in the development of various tumors [8], by regulating different types of target pathways and genes that contribute to tumor proliferation, invasion, and metastasis [9] and also it has a role in preventing of tumor invasion by regulating specific target genes such as matrix metalloproteinase-7 (MMP-7) [10]. The miR-148 family also controls HLA-G expression at the post-transcriptional level [11] and may serve as prognostic markers, as a therapeutic strategy for tumors During its tumor-suppressive activity [12].

In this study, the miR-148a gene expression in breast cancer females and healthy women was determined, and its association with patient characteristics was investigated.

METHODS

Search Strategy: Blood was collected from 50 Iraqi patients (newly diagnosed with breast cancer), from the Tumor Teaching Hospital in the Medical City in Baghdad and Al-Alawiyeh Hospital during the period from March to September 2021. The patient's ages ranged (30-65) years, with an average age of 48 years, Ethical permission was obtained from all participants in this study. Patients were selected and diagnosed under the supervision of the medical advisory staff and the pathology committee at both Hospitals. The cases were diagnosed by mammography and histological findings. It was found that the lesion was early in the patients and none of the patients had received chemotherapy, radiotherapy, or mastectomy before blood collection. Blood was also collected 25 from apparently healthy women as a control group with an age range (30-67) years with an average age of 49 years. The expression level of the miR-148a gene was detected by RT-qPCR technique using the master mix miScript SYBR Green PCR (QIAgen) kit (Cat No. /ID: 218073), the relative amount of miR-148a was normalized against U6 small nuclear (sn) RNA (as an internal control). The primers that used for qRT-PCR reactions was MiR-148a F (5`-TC AGTGCACTACAGAACTTTGT-3`) and MiR-148a R (5`-GCGAGCACAGAATTAATA CAC-3`) for U6 -F (5`-ATTGGAACGATACAGAG AAGATT-3`) and U6-R (5`-GGAACG CTTCACGAATTTG-3`) respectively. All primers were purchased from (Alpha DNA Company, Canada). The total volume of the qRT-PCR reaction was 20 µl containing 10µl master mix,1 µl for each forward and reverse primers, 6 µl Nucleas free water, and 2 µl of cDNA template. The PCR condition was 94°C for 30 minutes, followed by 35 cycles at 94°C for 5 seconds for denaturation then 15 seconds at 52°C for annealing, and finally 20 seconds at 72°C. The threshold cycle (Ct) is described as the cycle number at which the fluorescence level will pass into the assumed threshold. To analyze miR-148a expression, the fold-change of gene expression between patient and control was calculated by the 2-ΔΔCt method, in which ΔΔCt = (CtmiR-148a - CtU6 snRNA) mean tumor-(CtmiR-148a – CtU6 snRNA) means normal. The relative expression levels of miRNAs in the patient compared to the control group were selected and calculated using the method of 2-ΔΔCt [13].

RESULTS

The results in Table (1) showed the highest significant difference in fold expression of miR-148a in breast cancer patients (2.257±0.182) in the age group (≥ 50) compared with the (1.292±0.149) in the control group (P≤0.001), The difference was also significant in the age group (<40), it where (2.364±0.572) compared with the (0.769±0.051) in the control group, (P≤0.05), also the difference was highly significant in the age group (40-49), it where (1.967±0.151) compared with the control group (1.076±0.150), (P≤0.01). However, no significant difference in the fold of gene expression for miR-148a between all age groups of patients (<40, 40-49, 50). A high significant difference in the folded of expression for miR-148a in breast cancer patients, (2.026 ± 0.177) in relation to body mass index (BMI ≥30) compared to the control group (1.136 ± 0.126) in breast cancer patients (P≤0.001), Table (2). The difference was also significant in the (BMI 18.5-24.9), the fold expression was (1.765 ± 0.212) compared to the control group (0.644 ± 0.017), (P≤0.05). A significant difference appeared in the fold expression of miR-148a in the (BMI 25-29.9)

group, it was (2.438 ± 0.226) compared to the control group (1.149 ± 0.147), (P≤0.01). However, no significant differences in the level between the different body masses (18.5-24.9, 25-29.9, ≥30), but the highest value was recorded in the BMI (25-29.9) and (≥30),

Table 1: The association of the fold of miR-148a gene expression with age in the patients and control groups

Age	FE of miR-148a in Patients Group (No. 50) $2^{-\Delta\Delta Ct}$ Pa./$2^{-\Delta\Delta Ct}$Co.			FE of miR-148a in Control Group (No. 25) $2^{-\Delta\Delta Ct}$ Co./$2^{-\Delta\Delta Ct}$Co.			T-test	P-value
	No	%	Mean ± SE	No.	%	Mean ± SE		
<40	7	14	2.364±0.572	5	20.	0.769±0.051	2.320	0.043*
40-49	26	52	1.967±0.151	10	40	1.076±0.150	3.400	0.002**
≥50	17	34	2.257±0.182	10	40	1.292±0.149	3.647	0.001***
F-test			0.838			2.487		
P-value			0.439NS			0.106NS		

F.E: Fold Expression, NS: Non significant, Pa.: patient, Co.: control
*: significant (p≤0.05), **: significant (p≤0.01), ***: significant (p≤0.001)

Table 2: The association between a fold of expression miR-148a with BMI in patients and control groups

BMI(Kg/m2)	FE of miR-148a in Patients Group (No. 50) $2^{-\Delta\Delta Ct}$ Pa./$2^{-\Delta\Delta Ct}$Co.			FE of miR-148a in Control Group (No. 25) $2^{-\Delta\Delta Ct}$ Co./$2^{-\Delta\Delta Ct}$Co.			T-test	P-value
	No.	%	Mean ± SE	No.	%	Mean ± SE		
Normal (18.5-24.9)	7	14%	1.765±0.212	5	20%	0.644±0.017	2.688	0.031*
Overweight (25-29.9)	16	32%	2.438±0.226	10	40%	1.149±0.147	3.469	0.001**
Obese(≥30)	27	54%	2.026±0.177	10	40%	1.136±0.126	4.093	0.000***
F-test			1.780			1.095		
P-value			0.180 NS			0.352NS		

FE: Fold Expression, NS: Non significant, Pa.: patient, Co.: Control
*: significant (p≤0.05), **: significant (p≤0.01), ***: significant (p≤0.001)

Table 3: The association between a fold of expression miR-148a with tumor site in patients

Tumor Site	FE of miR-148a in Patients Group (No. 50) $2^{-\Delta\Delta Ct}$ Pa./$2^{-\Delta\Delta Ct}$Co.		
	No.	%	Mean ± SE
Right	15	30	1.850± 0.285
Left	27	54	2.467± 0.110
Bilateral	8	16	1.467± 0.318
F-test			5.844
P-value			0.005**

F.E: Fold Expression,
**: Significant (p≤0.01)

Table 4: The association between a fold of expression miR-148a with Grad and Stage of tumor in patients

	FE of miR-148a in Patients Group (No. 50) $2^{-\Delta\Delta Ct}$ pa./$2^{-\Delta\Delta Ct}$co.			
Grad		No.	%	Mean ± SE
	I	2	4%	2.009±0.000
	II	46	92%	2.142±0.137
	III	2	4%	1.761±0.000
	F-test			0.0185
	P-value			0.832NS
Stage	I	6	12%	2.258±0.052
	II	23	46%	2.062± 0.204
	III	21	42%	2.148± 0.205
	F-test			0.126
	P-value			0.882NS

FE: Fold Expression, NS: Non significant

The results of Table (3) showed a highly significant difference in the fold of expression miR-148a depending on tumor sites (right, left, bilateral). A high level of FE miR-148a was recorded on the left side (2.467± 0.110), while the tumor scored the lowest on the bilateral side of the breast reached (1.467± 0.318), and the expression (1.850± 0.285) was in those with the left side (p ≤ 0.01).

Table (4) indicates no significant differences in the fold of gene expression for miR-148a depending on tumor grades (I, II, III) of breast cancer patients, the results showed a high level (2.142 ± 0.137) was recorded in grade II. , and the fold expression level was (2.009. ± 0.000), (1.761 ± 0.000) in the grad (I, III) respectively, no significant differences depend on tumor stage (I, II, III), the high level (2.258 ± 0.052) of FE for miR-148a was scored in the first stage while FE expression was (2.062 ± 0.204, 2.148 ± 0.205) in stage (II, III) respectively.

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DISCUSSION

The result in Table (1) did disagree with [14] where it was found that the expression of miR-148a was downregulated in cancer patients, and a significant difference was found in its expression compared to healthy people, and miR-148a downregulation was also observed in other cancers such as gastric, breast, squamous cell carcinomas and esophagus [15]. However, the results of the current study agreed with another study, the miR-148a was upregulated in OSCC oral squamous cell carcinoma cell lines, SCC-9 compared to normal cells. Overexpression of miR-148a suppressed the ability of SCC-9 cells to migrate and invade. It also worked to induce programmed death of colorectal cancer cells by identifying Bcl-2 as the direct target [16], and elevated miR-148a expression has been shown in breast cancer patients. Overexpression of miR-148a impairs the lifespan of breast cancer cells [17]. It also prevents migration and invasion in hepatocellular carcinoma [18]. The increase in miR-148a expression in the current study may be considered as oncogenes or tumor suppressors depending on their interaction with specific targets, the miR-148a inhibits the gene expression of HLA-G as its target gene, and thus prevent the inhibition of the work of natural killer cells, this is a defensive way for the body to reduce the severity and progression of the disease. HLA-G interacts with miR-148a to suppress oral squamous cell carcinoma leading to inhibiting the migration and invasion and thus, miR-148a/HLA-G interaction may be a therapeutic strategy for OSCC oral squamous cell carcinoma [19]. That the anticancer effect mediated by miR-148a depends on the inhibition of STAT3 and Akt that regulate pathways contributing to cell proliferation and migration. miR-148a acts as a tumor suppressor in human gastric cancer by targeting the cholecystokinin B receptor CCK-BR through the inactivation of STAT3 and Akt [20] importantly, a low level of the miR-148a gene expression in breast cancer, it plays a role in tumor angiogenesis and growth [15], [21] miRNAs can control cancer development, invasion, and migration directly and indirectly through the

regulation of specific Matrix Metallo Proteinases MMPs [22], The current results in the table (2) The present results disagree with the study that confirmed the lower level of miR-148a expression in breast cancer compared to adjacent tissues [23], in contrast, the current results showed an elevated miR-148a fold expression level in patients compared to control group. This is due to the high percentage of adipose tissue with an increase in body mass index, which leads to this decreased level of adiponectin and a negative correlation between adipokines and miR-148a, and thus miR-148a level will rise [24] and increased expression of miR-148a lead to differentiation of primary adipocytes into mature adipocytes leading to their overexpression. Expression of miR-148a also increases after causing exogenous B-cell activation. Regulating miR-148a gene expression thus, it promotes the differentiation of activated B cells into plasma cells and supports their survival by inhibiting the transcription factors MITF and BACH2 and pro-apoptotic factors BIM and PTEN miR-148a has many biological functions Including cellular differentiation contributes to the formation of osteoclasts and transforms unicorns into osteoclasts through transcription factor inhibition V-maf engine. Also, enhanced expression of miR-148a leads to induction of hepatocyte differentiation and maturation by inhibiting DNA (cytosine-5)-methyltransferase 1 (DNMT1) [25]. The results of the current study in the table (3) agreed with one of the studies that demonstrated elevated miR-370 expression in women with left breast cancer [7], but it does disagree with another study that the gene expression level of miR-148a was lower in cancer patients compared to healthy people who had a significant difference, and there is no relationship between tumor site and expression of miR-148a, the results may have been affected by the small sample size [14]. The results obtained in Table (4) confirm that miR-148a expression was high in women with breast cancer, and this was in agreement with many previous studies where overexpression of miR-148a in breast cancer cell lines with high metastatic potential inhibited breast cancer tumorigenesis in vivo, by decreasing tumor cell extravasation [26], as the miR-148a suppressor gene was negatively observed, tumor grade and lymph node metastasis [9], [21] have also been negatively associated with the TNM stage [25], [27].

It was also observed that patients with elevated miR-148 had longer survival [28] high expression of miR-148a prevents immune escape in breast cancer by controlling HLA-G expression, so HLA-G plays an important role in immune regulation [25] working miR-148a inhibits the migration and invasion of breast cancer cells By affecting WNT-1 and suppressing Wnt/β-catenin. Mechanisms where there is a negative relationship between expression of miR-148 and WNT-1 in cancer tissues [28], on this basis, low expression of miR-148a in breast cancer has been associated with poor prognosis Its decrease was also associated with higher tumor grade and tumor recurrence in bladder cancer tissues, and its expression level was positively correlated with survival rate [17], [29] a decrease in it may cause a decrease in the disease-free survival rate [30], [31] Some studies have also been recorded The expression level of miR-148a was decreased in cancer patients compared to the control group, and this was related to the stage of malignancy, as well as the high grade and progression of the tumor. Its expression was also low in women with ovarian cancer and was related to tumor grade and stage of nodular metastasis [32]. The decreased expression was observed to be caused by hypermethylation of miR-148a in the promoter region of triple-negative breast cancer (TNBC) [25]

U. Ihsan, S. A. Gilani, M. K. Akhter et al

CONCLUSION

The current study revealed an increase in miR-148a gene expression in women with breast cancer, where a significant association was found between FE miR-148a and body mass index (BMI), and its expression increased in women with left breast cancer, which may be a new biomarker in breast cancer diagnosis.

Conflict of interest: Nil

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