LET-7B MICRORNA AND SIRTUIN-1 MRNA GENE EXPRESSION AS POTENTIAL BIOMARKERS FOR BREAST CANCER EGYPTIAN PATIENTS

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ABSTRACT:

Background and objectives: *The most frequently detected life-threatening malignancy in women is breast cancer. We aimed to evaluate Sirtuin-1 mRNA and let-7b microRNA gene expression in breast cancer patients in Egypt and to relate them with clinicopathological features.*

Subjects & methods: *The study recruited 225 female participants divided into the following groups:* ***Group I:*** *75 patients histopathologically proven breast cancer,* ***Group II:*** *75 patients histopathologically proven benign breast tumor; and* ***Group III:*** *75 age matched healthy controls, from general surgery department at Menoufia University Hospital in Egypt. RT-qPCR was used to assess the expression levels of Sirtuin-1 mRNA and let-7b microRNA, while CA 15-3 was assessed by ELISA.*

Results: *Let-7b microRNA was downregulated in breast cancer patients on comparison with benign breast tumors and control group (P< 0.001), and it was downregulated in patients with benign breast tumors compared to the control group (P <0.001). Sirtuin-1 mRNA was upregulated in the breast cancer patients compared to the benign breast tumor patients (p < 0.001) and control (p < 0.001) groups. Let-7b microRNA expression level was inversely correlated with tumor size (P=0.001; r =0.377) and affected lymph nodes (P<0.001; r=00.569). Moreover, Sirtuin-1 mRNA expression level was directly correlated with tumor size (P<0.001; r=0.438).*

Conclusion: *Let-7b microRNA and Sirtuin-1 mRNA can be targeted for treatment and utilized as potential biomarkers for the earlier detection of breast cancer patients.*

Keywords*: Let-7b, Sirtuin-1, microRNA, Breast cancer, CA 15-3*

INTRODUCTION:

Breast cancer is a serious health risk for women around the world. According to GLOBOCAN 2018, in the most majority of nations, breast cancer is the most frequently diagnosed cancer according to a profile of the most frequently diagnosed malignancies worldwide [1]. In 2020, there were 22,038 new cases of breast cancer in Egyptian women, with 32.4% of all cancer cases in terms of morbidity with 10.3% mortality rate of those cases[2].

Many studies have demonstrated that one of the most accurate and widely applied tumor markers in breast cancer is Carbohydrate antigen 15-3 (CA 15-3). CA 15-3 is mucin like glycoprotein with a large mass [3]. In primary breast cancer, CA15-3 is used to predict prognosis following treatment; in combination with alkaline

phosphatase, it predicts metastases and recurrence of breast cancer [4]. Most of patients with advanced breast cancer have demonstrated an increase in (CA 15-3) serum level; Early stage breast cancer or localized malignancy only minimally raise CA15-3 level [5].

Sirtuin-1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase that controls how stressed-out cells respond, regulates cell metabolism, apoptosis and DNA damage repair[6]. SIRT1 takes role in the development, progression and drug resistance of tumors by controlling key aspects of carcinogenesis, including cell proliferation, angiogenesis, senescence, and programmed cell death[7]. SIRT1's roles in breast cancer are debatable; nevertheless, a poor breast cancer prognosis is linked to SIRT1 mRNA overexpression[8]. While Moore et al.[9] showed that SIRT1 mRNA lowers breast cancer cell growth and suppresses the transcriptional response to the estrogen receptor.

The family of microRNAs let-7 includes let-7b. This family's members are involved in several major physiological and pathological processes, such as development, differentiation, and carcinogenesis. From C. elegans to humans, they are widely homologous in sequence and function[10]. Functionally, let-7 microRNAs family restricts the growth of a variety of aggressive malignancies by preventing the expression of oncogenes and important mitogenic pathway regulators[11].

Indeed, several different malignancies showed decreased let-7 family member expression[12]. Additionally, hepatocellular carcinoma mice models, in vitro lung, and breast cancer cell proliferation have been found to be successfully suppressed by the restoration of let-7b microRNA expression[13]. Significantly, low levels of let-7b microRNA expression were linked to higher tumor aggressiveness and proliferation. Low levels of let-7b microRNA expression were also inversely correlated with tumor lymph node metastasis. Moreover, breast cancer patients with downregulated let-7b microRNA had bad prognosis, suggesting that let-7b microRNA may function as a tumor suppressor miRNA during breast cancer development and progression[10].

Wang et al.[14] identified the relationship between let-7b microRNA and SIRT1, they showed that let-7b's targeting sites were present in the 3′UTR of SIRT1 mRNA and that let-7b significantly suppressed SIRT1 expression. Let-7b microRNA inhibitor clearly increased SIRT1 expression at both the mRNA and protein levels. These findings suggest that let-7b microRNA targets SIRT1 mRNA. Our objective was to evaluate Sirtuin-1 mRNA and let-7b microRNA expression in breast cancer patients in Egypt and to relate them with clinicopathological features.

AIM OF THE STUDY:

We aimed to evaluate Sirtuin-1 mRNA and let-7b microRNA gene expression in breast cancer patients in Egypt and to relate them with clinicopathological features.

PATIENTS AND METHODS:

Subjects:

In this case-control study, 225 females recruited from the department of general surgery at Menoufia University Hospital in Egypt, in the period from August 2021 and December 2022. Three groups of participants included: **Group I** 75 patients with recently diagnosed histopathologically confirmed breast cancer (tissue samples were determined to have invasive ductal carcinoma), **Group II** 75 patients with newly diagnosed benign breast tumors who have had histopathological proof; and **Group III** 75 ages matched healthy controls. Patients with serious comorbidities including failure of the heart, liver, or kidneys were not

included. For staging workup, chest X-rays and pelviabdominal ultrasonography, chest, abdomen, and pelvic CT scans, contrast studies, and bone scans were used. The classification of Tumor Node Metastasis (TNM) determined the stage of the tumor [15] and The Bloom-Richardson system's Nottingham modification criteria were used for grading[16]. The molecular subtypes of breast cancer were established on the status of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her2/neu), and Ki 67 [17]. Each participant provided a written consent that the ethics committee had examined and approved it at Faculty of Medicine, Menoufia University. Every study subject underwent a complete evaluation of the patient's medical history, clinical examination, and laboratory tests.

Laboratory procedures:

Laboratory procedures were done at clinical pathology department, Faculty of medicine, Menoufia University. Venipuncture ® was used to withdraw 5 mL of venous blood, put in a plain tube, and then coagulated at room temperature, and then centrifuged for 10 min at 4000 r.p.m. The collected serum used for analysis of the Cancer antigen 15-3 (CA 15-3) by Enzyme-linked immunosorbent assay (ELISA) utilizing a kit provided by Chemux BioScience, Inc., USA.

A fresh sample of the tumor mass was taken for RNA extraction in an Eppendorf tube with RNALater (Qiagen) (10 µl reagent for each 1 mg tissue) and kept at -80 ºC for let-7b microRNA and SIRT1 mRNA assay.

Expression assay for let-7b microRNA and SIRT1 mRNA:

Total RNA including microRNA was extracted from tissues using the Qiagen® MiRNeasy® RNA extraction Kit (Qiagen, Valencia, USA) as per instructions provided by the manufacturer. Using a NanoDrop®-1000 spectrophoto-meter, Purified RNA samples' concentrations and purity were evaluated. (NanoDrop® Technologies, Inc. Wilmington, USA). . Purified microRNA was kept at -80 °C. The Qiagen® miScript II RT Kit (Qiagen, Valencia, USA) was used to perform reverse transcription. Each 20-µl reaction tube contained 10 µl of template RNA, 2 µl of miScript Reverse Transcriptase Mix, 2 µl of RNase-free water, 4 µl of 5×miScript HiSpec Buffer, and 2 µl of 10×miScript Nuclease Mix. Reverse transcription was carried out at 37 °C on an Applied Biosystems 2720 thermal cycler for 60 minutes and 95°C for 5 minutes (Bioline, Singapore, USA). The cDNA obtained was kept at -20 C. Real-time quantitative polymerase chain reaction (RT-qPCR) was employed to quantify the transcript levels after the cDNA product had been diluted to 5 ng/µl. Each Primer (25 nmole) was dissolved in 250 µl RNasefree water to produce a solution with the desired concentration of 100 µmol/L.

For the let-7b microRNA expression assay, RT-qPCR was carried out using the miScript SYBR Green® PCR Kit (Qiagen, Valencia, USA).. 12.5 µl of 2x QuantiTect SYBR Green PCR Master Mix, 2.5 µl of 10x miScript Universal Primer based on miRBase database let-7b sequences with forward primer: 5′- CUAUACAACCUACUGCCU UCCC-3′ and reverse primer: 5′- GAAG GCAGUAGGUUGUAUAGUU-3′, 2.5 µl of template cDNA, and 2.5 µl of RNase-free water in a total volume of 20 µl made up the reaction mixture. The following steps were programmed to run 40 cycles on the Applied Biosystems®7500 real-time thermal cycler (Applied Biosystems, Foster City, CA, USA). The initial heat activation step at 95°C was run for 2 min, followed by denaturation at 95°C for 10 s, annealing at 56°C for 30 s, and extension at 70°C for 30 s. An endogenous reference control was U6 snRNA with the following primers: forward 5′-CTCGCTTCGGCAGCACAT-3′ and reverse 5′-TTTGCGTGTCATCCTTGCG-3′.

The Applied Biosystems®7500 software

version 2.0.1 was used to calculate relative quantitative expression levels using the comparative 2−ΔΔCt analysis method.

For SIRT1 mRNA expression assay, using real-time PCR, the following primers were used. Forward primer 5′- TAGACACGCTGGAACAGGTTGC -3′ and reverse primer 5′- CTCCTCGTACAG CTTCACAGTC -3′. GAPDH with the following primers were utilized as an endogenous reference gene: forward primer 5′- GAAGG TGAAGGTCGGAGTC -3′ and reverse primer 5′- GAAGATGGTGAT GGGATTTC -3′. The following conditions were used for the PCR process: A total of 20 µl, 10 µl of SYBR Green 2×QuantiTect PCR Master Mix, 3 µl of cDNA, 1 µl of forward primer, 1 µl of reverse primer, and 5 µl of RNase-free H2O were used in each reaction. After incubating this combination for 3 minutes at 94°C before going through 40 cycles of 94°C denaturation for 30 seconds, 55°C for 40 seconds for annealing, and 72°C for 30 seconds for extension. The final extension stage involved incubating the mixture at 72°C for 10 minutes. Data analysis was performed using Applied Biosystems®7500 software, version 2.0.1. The relative level of gene expression was calculated using the comparative ΔΔCt method. SIRT1 mRNA levels were evaluated with those of a housekeeping gene (GAPDH). To prove the specificity of the amplification and the absence of primer dimers, the melting curve was used.

Statistical analysis

To analyze our data we used the international Business Machine Statistical Package for the Social Sciences (SPSS Inc. Released 2011; IBM SPSS statistics for windows, v.20.0, Armonk, NY: IBM Corp). Quantitative data were displayed by means, standard deviations, medians, and interquartile ranges (IQRs), while qualitative data were displayed by frequencies and percentages. We used the Mann-Whitney U test to compare pairs of groups of non-normally distributed quantitative data, while the chi-square test was employed to evaluate the relationship between qualitative variables. Association between two nominal variables when a predicted cell count of 25% < 5 was evaluated using the Fisher exact test. We utilized regression analysis models to find independent predictors for breast cancer, and the Spearman correlation test was performed to assess correlations and P-values of 0.05 or less were regarded as statistically significant.

Ethical Consideration:

This study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, having been approved by the Research Ethics Committee at the Faculty of Medicine, Menoufia University (Code 1/2023CPATH8).

RESULTS:

Age differences among the patients in the three groups were not statistically significant. Demographic, clinical and biochemical analyses are outlined in Table 1. The tumor characteristic in breast cancer patients are outlined in Table 2.

Let-7b microRNA expression level was inversely correlated with SIRT1 mRNA expression level (P<0.001; r=0.420) and inversely correlated with tumor size (P=0.001; r =0.377) and affected LN (P<0.001; r=00.569). Moreover, SIRT1 mRNA expression level was directly correlated with tumor size level (P<0.001; r=0.438) and affected LN (P=0.012; r=0.289). (Table 3)

Let-7b microRNA expression level was significantly related to tumor grade, tumor stage and lymph node stage. Moreover, SIRT1 mRNA expression level was also significantly related to tumor grade. (Table 4)

To identify independent determinants for the parameters affecting breast cancer patients from control group, univariate logistic regression analyses were performed let-7b microRNA expression level, SIRT1 mRNA expression level and CA 15-3 levels were independent determinants. (Table 5)

The ROC analysis of expression levels of let-7b microRNA, SIRT1 mRNA and CA-15-3 serum level was used to assess their diagnostic performance. To discriminate breast cancer patients from benign breast tumor, let-7b microRNA expression level was the best with a cut-off value ≤ 2.258 (AUC = 0.988, P < 0.001), the sensitivity of 96 %, and specificity of 92%. However, SIRT1 mRNA expression level reported a sensitivity of 92.22% and specificity of 90.67% (AUC = 0.98, cut-off value > 6.932, P < 0.001). CA-15-3 serum level had the worst achievement with a sensitivity of 90.67% and specificity of 88% (AUC = 0.949, cut-off value > 22.84, P < 0.001). (Tables 6, Figure 1)

To discriminate breast cancer patients from control group, let-7b microRNA expression level was the best with a cut-off value ≤ 2.373 (AUC = 0.995, P < 0.001), the sensitivity of 98.67 %, and specificity of 97.33%. However, SIRT1 mRNA expression level reported a sensitivity of 97.33% and specificity of 96% (AUC = 0.997, cut-off value > 6.158, P < 0.001). CA-15-3 serum level had the worst achievement with a sensitivity of 90.67% and specificity of 88% (AUC = 0.962, cut-off value > 23.5, P < 0.001). (Tables 6, Figure 2)

To discriminate benign breast tumor from control group, let-7b microRNA expression level was the best with a cut-off value ≤ 6.995 (AUC = 0.965, P < 0.001), the sensitivity of 98.67 %, and specificity of 96%. However, SIRT1 mRNA expression level reported a sensitivity of 98.67% and specificity of 887% (AUC = 0.92, cut-off value > 3.026, P < 0.001). CA-15-3 serum level was non-significant with P= 0.16). (Tables 6, Figure 3)

Table (1): Demographic, clinical, and laboratory data of the studied participants

	Group I (Breast cancer patients) (n = 75)	Group 2 (Benign tumor patients) (n = 75)	Group 3 (Control) (n = 75)	P	Sig. bet. grps.
Age (years)					
Mean ± SD.	48.7 ± 9.71	50 ± 9.46	49 ± 10.5	0.675	
Menarche age					
Mean ± SD.	11 ± 2.31	11.5 ± 1.28	11.6 ± 0.99	0.355	
BMI (kg/m^2) Mean ± SD.	37.3 ± 4.54	26.9 ± 2.17	27.7 ± 2.37	<0.001*	p_1<0.001* p_2<0.001* p_3=0.276
Family history Negative Positive	 66 (88%) 9 (12%)	 69 (92%) 6 (8%)	 72 (96%) 3 (4%)	 0.196	
Menstrual status Pre-menopausal Post-menopausal	 33 (44%) 42 (56%)	 15 (20%) 60 (80%)	 33 (44%) 42 (56%)	0.002*	
Tumor characteristics: Side Right Left	 39 (52%) 36 (48%)	 33 (44%) 42 (56%)	 – –	0.327	
Tumor size(cm) Mean ± SD.	 3.71 ± 1.51	 2.39 ± 0.56	 –	<0.001*	
CA 15-3(IU/ ml)				<0.001*	

Mean ± SD.	34 ± 8.13	14.6 ± 6.65	13.4 ± 6.61		p_1<0.001* p_2<0.001* p_3=0.304
Let-7b Mean ± SD.	1.14 ± 0.60	4.12 ± 1.63	16 ± 8.54	<0.001*	p_1<0.001* p_2<0.001* p_3<0.001*
SIRT1 Mean ± SD.	15.9 ± 5.61	5.11 ± 1.38	1.68 ± 1.80	<0.001*	p_1<0.001* p_2<0.001* p_3<0.001*

*: Statistically significant at $p \leq 0.05$ CA-15-3: carbohydrate antigen
p1: p value for comparing between breast cancer patients and benign tumor patient
p2: p value for comparing between breast cancer patients and control group
p3: p value for comparing between benign tumor patient and control group

Table (2): Tumor characteristics in the breast cancer patients group (n = 75)

No. (%)	
Total lymph node	
Median (Min. – Max.)	23 (15 – 32)
Affected LN	
Median (Min. – Max.)	10 (0 – 23)
Carcioma insitu	6 (8%)
Multicentric	15 (20%)
Tumor grade	
I	6 (8%)
II	57 (76%)
III	12 (16%)
Tumor stage	
T1	9 (12%)
T2	54 (72%)
T3	9 (12%)
T4b	3 (4%)
Nodal stage	
N0	12 (16%)
N1	12 (16%)
N2	21 (28%)
N3	30 (40%)
Distant metastasis	
No metastasis	66 (88%)
Metastasis	9 (12%)
Bone metastasis	3 (4%)
Bone + Lung metastasis	6 (8%)
Final Stage	
IA	3 (4%)
IB	3 (4%)
IIA	9 (12%)
IIB	9 (12%)
IIIA	18 (24%)
IIIC	24 (32%)
IV	9 (12%)
ER	51 (68.0%)
PR	51 (68.0%)
Pathology	
IDC	75 (100%)
ILC	0 (0%)
Her2neo	24 (32%)
Molecular Subtype	

Triple negative	15 (20%)
Her2/neo +	9 (12%)
Luminal	51 (68%)

IDC: invasive duct carcinoma, ILC: intralobular carcinoma, ER: estrogen receptor, PR: progesterone receptor

Table (3): The correlation of Mir- Let-7b and SIRT1 with clinical data in breast cancer group

	Mir- Let-7b		mRNA SIRT 1	
	r_s	P	r_s	p
SIRT1	-0.420	<0.001*		
Age (years)	0.110	0.347	0.218	0.060
BMI (kg/m2)	-0.134	0.251	0.055	0.640
Tumor size(cm)	-0.377	0.001*	0.438	<0.001*
Total lymph node	0.286	0.013*	0.092	0.435
Affected LN	-0.569	<0.001*	0.289	0.012*
CA 15-3(IU/ ml)	0.020	0.862	0.211	0.069

r_s: Spearman coefficient *: Statistically significant at $p \leq 0.05$

Table (4): The Relation between Mir- let-7b and mRNA SIRT 1 with clinical data in breast cancer group

		Mir- let-7b		mRNA SIRT 1	
	No.	Median (IQR)	P	Median (IQR)	P
Grade					
I	6	1.82 (1.50 – 1.92)	p = 0.007*	19.97 (19.91 – 23.44)	p = 0.021*
II	57	0.83 (0.62 – 1.43)		15.27 (11.18 – 19.27)	
III	12	1.40 (1.04 – 1.63)		17.66 (10.63 – 19.20)	
Final Stage					
IA + IB	6	1.07 (0.63 – 1.44)	p = 0.006*	16.84 (13.74 – 19.94)	p = 0.484
IIA + IIB	18	1.80 (1.60 – 1.89)		18.64 (10.16 – 21.59)	
IIIA + IIIC	42	0.83 (0.54 – 1.33)		16.11 (11.18 – 19.27)	
IV	9	0.87 (0.77 – 1.38)		15.31 (10.49 – 18.48)	
ER					
Negative	24	0.94 (0.52 – 1.63)	p = 0.460	12.90 (9.40 – 18.50)	p = 0.080
Positive	51	0.93 (0.78 – 1.63)		17.20 (13.25 – 19.92)	
PR					
Negative	24	0.94 (0.52 – 1.63)	p = 0.460	12.90 (9.40 – 18.50)	p = 0.080
Positive	51	0.93 (0.78 – 1.63)		17.20 (13.25 – 19.92)	
HER2neu					
Negative	51	0.86 (0.56 – 1.47)	p = 0.066	16.04 (11.35 – 19.75)	p = 0.633
Positive	24	1.38 (0.83 – 1.73)		18.48 (12.16 – 19.83)	
Molecular type					
Triple negative	15	0.57 (0.47 – 1.41)	p = 0.188	11.53 (9.40 – 16.61)	p = 0.093
Her2 Positive	9	1.15 (0.89 – 1.60)		18.32 (14.21 – 19.56)	
Luminal	51	0.93 (0.78 – 1.63)		17.20 (13.25 – 19.92)	
T stage					
T1	9	0.63 (0.62 – 1.41)	p = 0.494	13.98 (13.71 – 19.80)	p = 0.952
T2	54	0.93 (0.71 – 1.75)		16.29 (10.97 – 19.89)	
T3	9	0.90 (0.80 – 1.91)		16.18 (10.49 – 17.77)	
T4b	3	1.38 (1.35 – 1.40)		18.48 (13.16 – 18.66)	
N stage					
N0	12	1.60 (1.42 – 1.82)	p = 0.024*	19.64 (10.16 – 19.96)	p = 0.473
N1	12	1.77 (0.86 – 1.87)		17.66 (14.07 – 23.37)	
N2	21	0.93 (0.57 – 1.33)		16.53 (10.82 – 18.68)	

N3	30	0.80 (0.54 – 1.40)		15.29 (11.59 – 19.27)	
M stage					
M0	66	0.93 (0.63 – 1.73)	p = 0.613	16.65 (11.53 – 19.91)	p = 0.275
M1	9	0.87 (0.77 – 1.38)		15.31 (10.49 – 18.48)	

*: Statistically significant at $p \leq 0.05$

Table (5): Univariate logistic regression analysis for the parameters affecting breast cancer patients from control group (n = 75 vs. 75)

	Univariate	
	P	OR (LL – UL 95%C.I)
Age (years)	0.865	0.997 (0.966 – 1.029)
Menarche age	0.079	0.807 (0.635 – 1.025)
BMI (kg/m^2)	0.956	–
Presence of Family history	0.085	3.273 (0.850 – 12.607)
Menstrual status [Post-menopausal]	1.000	1.000 (0.525 – 1.906)
CA 15-3(IU/ ml)	<0.001*	1.317 (1.212 – 1.430)
Mir-let-7b	0.027*	0.046 (0.003 – 0.699)
mRNA SIRT 1	0.015*	5.057 (1.361 – 18.786)

OR: Odd`s ratio C.I: Confidence interval L L: Lower limit UL: Upper Limit
*: Statistically significant at $p \leq 0.05$

Table (6): Diagnostic Performance of Mir- let-7b, mRNA SIRT 1 and CA-15-3 for discriminating between different groups

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Patients with breast cancer from benign tumor patients								
Mir- Let-7b	0.988	<0.001*	0.974 – 1.000	≤2.258	96.0	92.0	92.3	95.8
mRNA SIRT 1	0.980	<0.001*	0.964 – 0.997	>6.932	92.00	90.67	90.8	91.9
CA 15-3(IU/ ml)	0.949	<0.001*	0.916 – 0.983	>22.84	90.67	88.0	88.3	90.4
Patients with breast cancer from control								
Mir- Let-7b	0.995	<0.001*	0.988 – 1.000	≤2.373	98.67	97.33	97.4	98.6
mRNA SIRT 1	0.997	<0.001*	0.992 – 1.000	>6.158	97.33	96.0	96.1	97.3
CA 15-3(IU/ ml)	0.962	<0.001*	0.934 – 0.990	>23.5	90.67	88.0	88.3	90.4
Patients with benign tumor from control								
Mir- Let-7b	0.965	<0.001*	0.925 – 1.000	≤6.995	98.67	96.0	96.1	98.6
mRNA SIRT 1	0.920	<0.001*	0.868 – 0.972	>3.026	98.67	88.0	89.2	98.5
CA 15-3(IU/ ml)	0.566	0.160	0.474 – 0.659					

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value *: Statistically significant at $p \leq 0.05$

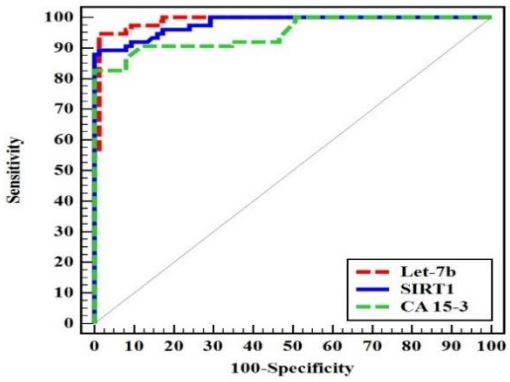

Figure (1): Receiver Operating Characteristic Curve (ROC) Analysis of Mir- let-7b, mRNA SIRT 1 and CA-15-3 for discriminating breast cancer patients from benign tumor patients

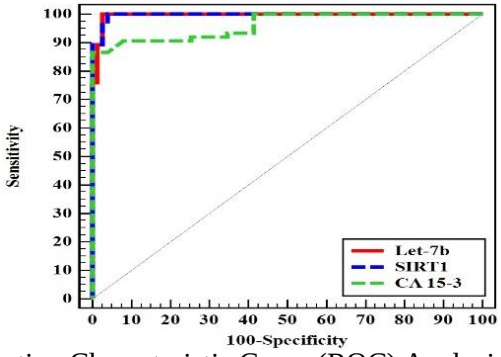

Figure (2): Receiver Operating Characteristic Curve (ROC) Analysis of Mir- let-7b, mRNA SIRT 1 and CA-15-3 for discriminating breast cancer patients from control

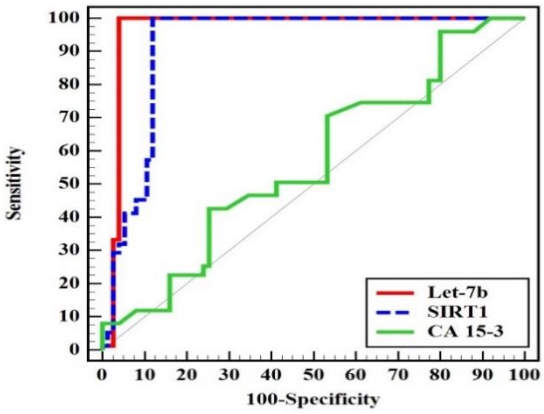

Figure (3): Receiver Operating Characteristic Curve (ROC) Analysis of Mir-let-7b, mRNA SIRT 1 and CA-15-3 for discriminating benign tumor patients from control

DISCUSSION:

Breast cancer is one of the most common malignancies in women globally. In 2020, it was responsible for near 24.5% of all malignancies and 15.5% of cancer deaths in women. The leading cause of death in breast cancer is metastasis[18]. Therefore, it is important to identify biomarkers for early diagnosis and prediction of recurrence.

MicroRNAs are non-coding RNAs, 17–25 base pairs long. They regulate genes post-transcriptionally through binding to the 3' or 5' untranslated regions of certain messenger RNA (mRNA), prevent-ing degradation or inhibiting translation of

the mRNA. Many researches are focusing on microRNAs as biomarkers in cancer diagnosis. Many studies proved that microRNAs play role in the formation and metastasis of breast cancer[18]. So, they can be used as diagnostic tools, predictors, and therapeutic targets.

BMI was significantly higher among patients with breast cancer in comparison to benign tumor patients and controls. There is conflicting evidence that BMI and breast cancer are related. In agreement with our study, results of Liu et al. [19] meta-analysis showed that a 5 kg/m^2 rise in BMI was associated with a 2% increase in breast cancer risk, according to a weakly positive correlation between the two variables. Conversely, Schoemaker et al.[20] study suggested that increased adiposity is associated with a reduced risk of premenopausal breast cancer.

CA 15-3 serum level when compared to patients with benign tumors was considerably higher in patients with breast cancer. In agreement with what we discovered, Atoum et al.[21] found compared to females with benign tumors, breast cancer patients had higher serum levels of CA 15-3. CA 15-3 serum level was significantly higher among patients with breast cancer when compared with controls. This was in consistency with what reported by Fejzić et al.[22] studies.

In this study, let-7b microRNA expression level was significantly lower in patients with breast cancer when compared with controls. Following our study, Li et al. [23] reported the same results. Moreover, Lu et al.[24] and Bozgeyik. [25] reported that let-7b microRNA was low in tumor tissue compared to adjacent normal tissue. Hu et al. [26] reported that let-7b microRNA reduced breast cancer cell migration by inhibiting several genes involved in the cytoskeleton pathway. Shao et al.,[27] reported that let-7b microRNA modulates the proliferation and tumorigenicity of cancer cells as well as the self-renewal of embryonic stem cells. Furthermore, Encarnación et al. [28] found that as a tumor suppressor, let-7b microRNA works through a number of methods to stop cell adhesion, proliferation, and invasion. In addition, Al-Harbi, et al. [29] presented an evidence that the persistent stimulation of breast stromal fibroblasts and their functional interaction with cancer cells are important outcomes of let-7b microRNA, a tumor suppressor microRNA. They showed that let-7b microRNA was downregulated in cancer-associated fibroblasts as compared to their corresponding normal adjacent fibroblasts, and by stimulation of the IL-6-related positive feedback loop, temporary selective let-7b microRNA inhibition permanently stimulated breast fibroblasts[29].

In our study, microRNA expression levels of let-7b were downregulated in patients with breast cancer when compared to patients with benign tumor. This was in agreement with results of Joosse et al.[30] and Ma et al.[31].

SIRT1 mRNA expression level was upregulated in patients with breast cancer than benign tumor patients and than control group. In addition, SIRT1 mRNA expression level was upregulated in patients with benign tumor when compared to control group. In agreement with our study, Zhang et al.[32] results showed that SIRT1 mRNA is more expressed in breast cancer than in their normal adjacent tissues. They demonstrated that cortactin's deacetylation by SIRT1 promotes cell motility and the development of breast tumors. Moreover, Xu et al.[33] reported SIRT1 mRNA overexpression in (ER+) luminal breast cancer cell line Michigan Cancer Foundation-7 (MCF-7).

Xu et al.[33] revealed that MCF-7 cells' proliferation, migration, and invasion are encouraged by SIRT1 mRNA overexpression, whereas same actions are inhibited by SIRT1 knockdown. They demonstrated that, in contrast to SIRT1 silencing, SIRT1 mRNA overexpression positively correlates with decreased p53

expression and higher expression of DNA polymerase delta1 (POLD1), an oncogene played a role in genomic instability and cell proliferation. They concluded that SIRT1 mRNA is involved in breast cancer development by suppressing p53 and stimulating *POLD1*.

Furthermore**,** Jin et al.[34] revealed that in contrast to SIRT1 mRNA deficiency, SIRT1 overexpression strongly encourages breast cancer growth both in vitro and in vivo. They established that SIRT1 mRNA promotes the activity of the oncogenic PI3K/Akt signalling pathway in vitro, which has impacts on breast cancer development, and that SIRT1 is positively linked with P-Akt expression in vivo.

The most important conventional prognostic factors for breast cancer are tumor size, grade, and metastasis[35]. We found that let-7b microRNA expression level in breast cancer patients was significantly related to tumor grade, tumor stage and lymph node stage. Similarly, let-7b microRNA expression was negatively correlated with tumor grade and stage in Quesne et al. [36] study. Additionally, Lu et al.[24] reported that clinical stage III/IV patients had lower level of let-7b microRNA when compared with clinical stage I/II.

Furthermore, Bozgeyik [25] reported that the expression level let-7b-5p decreased as the grade of breast cancer increases. Let-7b microRNA downregulates the expression of oncogenes in cell growth and proliferation[37]. Let-7b microRNA expression level in the current study was negatively correlated with tumor size. This agrees with Quesne et al.[36] results. In addition, Jonsdottir et al. [38] reported that the most differentially expressed miRNA, let-7b microRNA showed an inverse relationship with the traditional prognostic factors (proliferation and tumor size), and its low expression was substantially correlated with high proliferation.

Let-7b microRNA expression level in this study was negatively correlated with affected LN. This was in agreement with results of Lu et al.[24], Ma et al.[31] and Quesne et al.[36] studies.

According to the current study, SIRT1 mRNA expression level was positively correlated with tumor size and affected LN. SIRT1 mRNA expression level was also significantly related to tumor grade. In line with our findings, Cao et al. [39] meta-analysis revealed that SIRT1 mRNA expression was substantially connected with lymph node metastases and high TNM stage, indicating that SIRT1 mRNA expression is closely related to biological aggressiveness. Additionally, Song et al.[40] reported similar results.

Interestingly, we observed that Let-7b microRNA expression inversely correlated to SIRT1 mRNA expression in the breast cancer patients group. Wang et al. [14] reported that let-7b microRNA inhibitor obviously upregulated SIRT1 mRNA expression levels in chronic obstructive pulmonary disease patients. Additionally, it was discovered that let-7b-5p targets SIRT1 in BEAS-2B cells. Moreover, they discovered let-7b-5p mimic reduced SIRT1 mRNA expression. Univariate logistic regression analysis was performed, let-7b microRNA, Sirtuin-1 mRNA expression levels and CA 15-3 levels were independent determinants affecting breast cancer patients from control group. For comparing the diagnostic efficacy of let-7b microRNA, SIRT1 mRNA and the traditional breast cancer tumor marker; CA15-3. Diagnostically, both let-7b microRNA and SIRT1 mRNA perform better than CA 15-3 in discriminating patients with breast cancer from both benign breast disease patients and control group.

Conclusion:

Finally, our findings revealed that let-7b microRNA and SIRT1 mRNA are potentially useful biomarkers for the early detection of

breast cancer patients. In this regard, they are more accurate than CA 15-3. Let-7b microRNA and SIRT1 mRNA may also be used as biomarkers to predict the aggressiveness and metastasis of tumor cells in breast cancer. Let-7b microRNA may be a potential target for breast cancer treatment through its effect on SIRT1 mRNA expression.

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none.

Authors' contributions:

Amany M. Wahb (the corresponding author), designed the research and was a major contributor in writing the manuscript and performed the laboratory investigations. Moatez M. Kamel performed the laboratory investigations beside to selecting the study design and contributed in writing the manuscript. Doaa M. Gharib performed laboratory investigations. Galal Ghaly performed the case selection and the clinical and radiological evaluations. Mohamed G. Elhelbawy performed the laboratory investigations and performed the data analysis. Hadeel M. Shalaby performed the laboratory investigations and performed the data analysis. All authors participated in writing and revision of the paper and approved the final manuscript.

Abbreviations list:

Area under a Curve (AUC)
Carbohydrate antigen (CA-15-3)
Confidence Intervals (CI)
Enzyme-linked immunosorbent assays (ELISA)
Estrogen receptor (ER)
Interquartile ranges (IQRs)
Intralobular carcinoma (ILC)
Invasive duct carcinoma (IDC)
Michigan Cancer Foundation-7 (MCF-7).
Negative predictive value (NPV)
Nicotinamide adenine dinucleotide (NAD+)
Positive predictive value (PPV)
Progesterone receptor (PR)
Sirtuin-1 (SIRT1)
Tumor Node Metastasis (TNM)

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التعبير الجيني MicroRNA let-7b و Sirtuin-1 mRNA كمؤشرات حيوية محتملة لسرطان الثدي للمرضى المصريون

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الخلفية والأهداف: الورم الخبيث الأكثر شيوعًا الذي يهدد الحياة عند النساء هو سرطان الثدي. كنا مهتمين بتقييم التعبير الجيني sirtuin-1 mRNA و microRNA let-7b في مرضى سرطان الثدي المصريين.

المرضى وطرق البحث: تم تشكيل ثلاث مجموعات من المشاركين: المجموعة الأولى: ٧٥ مريضًا من الإناث ثبتت إصابتهم بسرطان الثدي ، والمجموعة الثانية: ٧٥ مريضًا تم إثبات إصابتهم بأورام الثدي الحميدة. والمجموعة الثالثة: ٧٥ مطابقة في السن للإناث الأصحاء ، تم اختيارهم من أقسام الجراحة العامة وعلم الأمراض السريري في مستشفى جامعة المنوفية في مصر. تم استخدام RT-qPCR لتقييم مستويات التعبير عن Sirtuin-1 mRNA و let-7b microRNA ، بينما تم استخدام ELISA لتقييم CA 15-3.

النتائج: كانت مستويات تعبير Let-7b microRNA أقل بشكل ملحوظ في مرضى سرطان الثدي مقارنة بالمرضى الذين يعانون من أورام الثدي الحميدة والمجموعة الضابطة ($P < 0.001$) ، وكانت أقل بشكل ملحوظ في المرضى الذين يعانون من أورام الثدي الحميدة مقارنة بالمجموعة الضابطة ($P < 0.001$)). كانت مستويات تعبير Sirtuin-1 mRNA أعلى بشكل ملحوظ في مجموعة مرضى سرطان الثدي مقارنة بمرضى أورام الثدي الحميدة ($P < 0.001$) ومجموعة التحكم ($P < 0.001$). ارتبط مستوى تعبير Let-7b microRNA، let-7b عكسيًا مع حجم الورم ($P = 0.001$ ؛ $r = 0.377$) والعقد الليمفاوية المصابة ($P < 0.001$ ؛ $r = 00.569$). علاوة على ذلك ، ارتبط مستوى تعبير Sirtuin-1 mRNA طرديا مع حجم الورم ($P < 0.001$ ؛ $r = 0.438$).

الاستنتاج: يمكن استهداف Let-7b microRNA و Sirtuin-1 mRNA للعلاج واستخدامهما كمؤشرات حيوية محتملة للكشف المبكر عن مرضى سرطان الثدي.