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" Accuracy of combined serum micro RNA 21 and micro RNA let 7g expression level in breast cancer diagnosis "

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Abstract:

Micro RNAs are single-stranded non-coding RNA molecules that regulate biological processes by inhibiting post-transcriptional activity. Many diseases including breast cancer have uncontrolled levels of microRNA. The current work **aims** to highlight the importance of miRNA let7g and miRNA 21 expression in blood for early detection of breast cancer. **Methodology**: Using quantitative real-time polymerase chain reaction the serum expression levels of micro RNA 21 and micro RNA let7g were evaluated in 52 female patients with primary breast cancer and 28 matched healthy females as a control group. **Results:** Serum expression level of micro RNA 21was highly increased in breast cancer patients when compared to the control group, while the serum expression levels of micro RNA let7g recorded a lower level in breast cancer patients when compared to control group and these differences were statistically significant. On studying the relation between the expression levels of the measured parameters and the different clinicopathological characteristics; a positive significant correlation was found between CA15.3 and microRNA 21 levels. A negative significant correlation was present between miRNA let7g levels and HER2, as well as with molecular subtype prognostic ranking. Receiver operating characteristic curve of both markers were exploited at a cut off points 0.82 and 1.785 respectively and showed a 100% sensitivity, 67.8% specificity. **Conclusion:** The effectiveness of combined serum mi RNA21 and mi RNA let7g expression level as diagnostic biomarker for detecting breast cancer has been convincingly proven. This innovative idea could be used to create a complementary tool for disease diagnosis and screening.

1. Introduction: An estimated 1.5 million new cases of breast cancer are reported worldwide each year, making it one of the most prevalent malignant diseases. (1) It is one of the most prevalent major causes of cancer-related death in women, according to research conducted worldwide. (2) In 2013, there were 110.3/100,000 females and 115.7/100,000 males diagnosed with cancer in Egypt, according to estimates. (3,4) At the moment, the classification of breast cancer is based on the expression of hormone receptors and the human epidermal growth factor receptor HER2, which might influence the method of diagnosis and the selected course of treatment. Meanwhile, other classification techniques based on global gene expression are now gaining traction [6,7, 8]

Micro RNAs (miRNAs), which are significant regulators of gene expression, are class of evolutionarily conserved tiny (18–24 nucleotides) non-coding RNA molecules that by specifically targeting the 3' untranslated region (UTR) of mRNA transcripts, they affect the stability of RNA and the effectiveness of translation, respectively, by degrading RNA or by inhibiting protein translation (9,10, 11). As a result, changes in miRNA expression may have an impact on key biological processes involved in the genesis and progression of cancer, including apoptosis, differentiation, and proliferation (12, 13).

According to Andorfer et al. (14), aberrant miRNA expression associated with the cancer process makes for a good starting point for examining the functional role of miRNA in the development of cancer. Sequencing and molecular biology tools have developed quickly, making it simple to detect amplified or decreased miRNA and quickly identify functional miRNAs. It is well known that down-regulated "tumor suppressive microRNAs" like micro RNAlet7g promote the augmentation of downstream signal pathways involved for tumor growth, whilst up-regulated "oncogenic microRNAs" like micro RNA21 restrict the production of putative tumor suppressive genes. Both have the potential to accelerate the onset and spread of cancer. By focusing on the same or various molecules in a complex regulatory network, these two populations may take part in pathogenic pathways. (7, 15, 16)

Numerous miRNAs have been found to be tumor suppressors or oncogenes in breast cancer, and they have been described as important regulators of tumor initiation, metastasis, and chemoresistance. They have also been found to be specifically dysregulated in the blood plasma of breast cancer patients when compared to healthy subjects. (17, 18)

From C. elegans to humans, the evolutionary conserved let 7 miRNA family has been thoroughly documented (19). Let-7g, one of their 13 members, is involved in gene regulation, cell adhesion, and muscle development under physiologically normal circumstances. (20) Meanwhile, it was discovered that they prevented the progression of cancer by concentrating on important mitogenic pathway regulators such RAS(166) and high mobility group A2 (HMGA2). (21,22) It has been found that the expression of members of the let-7 family is significantly reduced in various cancers, including stomach, ovarian, colon, and lung cancer. Additionally, it improves the capacity of lung cancer cells to form colonies in vitro, and reduced expression is associated with a significantly shorter survival time for those who have the disease. (21, 23) It's interesting to note that the gene for another micro RNA, miRNA-21, is found on the plus strand of chromosome 17q23. 2. It is a particular microRNA that is up-regulated in almost all solid tumors generated from epithelial cells, such as head and neck, breast, pancreatic, lung, gastric, prostate, colon, and esophageal cancers. In hematological malignancies such leukemia, lymphoma, and multiple myeloma, it is also said to be up-regulated. (16, 24, 25) As a result, miRNA21 appears to be the only microRNA or gene that is discovered to be overexpressed in all major classes of human cancers derived from epithelial cells, connective tissues, hematopoietic cells, germ cells, or nervous cells. This finding supports the idea that miRNA21 is a widespread oncogene. MicroRNA21 primarily exerts its oncogenic effect by targeting a number of genes that suppress cellular death. (26, 27) Our work aims to emphasize the value of microRNA21 and microRNAlet7g serum expression level in Egyptian breast cancer patients as an aid in diagnosis and clinical decision-making and treatment.

2. Subjects and methodology:

After the approval of the Ethics Committee of the Medical Research Institute an informed consent was taken from all subjects participating in the study. Sample size was calculated and eighty subjects were included in this study and divided as **control group** including twenty eight healthy female volunteers matched for age and socioeconomic status to the **patients group** which included fifty two female patients with breast cancer before receiving adjuvant chemotherapy. They were selected from the Oncology Department of the Medical Research Institute, University of Alexandria. The study was planned to be over 6 months. Medical records were selected weekly for extraction of patient's data till completing sample size.All the studied subjects were subjected to full history taking where no cardiovascular disease or other malignancy was present. Clinical examination and radiological examination including chest x-ray, abdominal ultrasound and bone scan were done. All patients included in the study had a histopathological confirmed diagnosis of breast cancer including staging, according to the TNM classificationsystem[(28)].Immunohistochemical examinations were done to all patients to determine:[(29,30)]

- Estrogen receptor status (ER).
- Progesterone receptor status (PR).
- Human epidermal growth factor2 (HER2).

Estimation of serum CA15.3 level using ELISA techniques. [(31)]

The kit was purchased from biovision (Catalog # K4804-100) Milpitas, USA.

This assay employed an antibody (Ab) specific for human CA15-3 coated on a 96-well plate. Standards and samples were pipetted into the wells and CA15-3 present in a sample was bound to the wells by the immobilized antibody. The wells were washed, and biotinylated anti-human CA15-3 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of CA15-3 bound. The Stop Solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm.

Sample collection

Five milliliters of whole venous blood sample were obtained from every participant under aseptic conditions before chemotherapy. Blood was collected in gel clot activator vacutainers and each sample was centrifuged at 3,000 g for 10 min to separate serum and then stored at −80°C until RNA extraction.

Relative quantification of serum microRNA21 and microRNAlet7g expression levels. [(32)]

Total RNA extraction, and reverse transcription

Total RNA containing small RNAs was isolated from 100 µl of serum using miRNeasy kit (cat. no. 217004; Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then, 40 µl of RNase-free water was used for RNA elution NanoDrop 2000/2000c (Thermo Scientific, Wilmington, DA, USA) was used to check RNA quality and quantity.

Complementary DNA (cDNA) was synthesized from RNA samples using TaqMan® MicroRNA Reverse Transcription Kit with miRNA primers specific for miR-21 (ID: 000397) and miR-let7g (ID: 002282) and miR-16 as an endogenous internal control (Assay ID 000391) (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Thermal profile was as follows: 16°C (30 min), 42°C (30 min), followed by 85°C for 5 min and a final hold at 4°C.

Real-time PCR was performed using Applied Biosystems Step One™ Real-time PCR System, thermal cycler (Block, foster City, CA, USA) using TaqMan MicroRNA Assay reagents purchased from Applied Biosystems, CA. miR21 Assay (ID 000397) and miRlet7g Assay (ID: 002282) and miRNA16 Assay (ID 000391) which was used as an endogenous internal control. Amplification was carried out in a final volume of 20 µl including 2× TaqMan Universal Master Mix no AmpErase UNG**,** 20× TaqMan Assay for miR21 and RT product in a concentration of up to 10 ng per reaction and similarly for miRlet7g. Thermal cycling conditions were as follow: an initial hold at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s (denaturation step) and 60°C for 1 min (annealing/extension). Comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method was used to calculate miR21 and miRlet7g expression in serum samples normalized to miR16 expression as endogenous control and relative to healthy controls. [33]

2.3 Statistical analysis: Statistical analysis was performed using SPSS program version 22.

- Qualitative data were described using number and percent.
- Quantitative data (micro RNA let7g, micro RNA21 expression levels) were described using median (minimum-maximum). As data are abnormal distributed using Kolmogorov Smirnov test.
- Chi- square test and Fisher exact test were done to detect association between Micro RNA21 and let7g expression categorized at specific cut off and breast cancer.
- Statistical significance was accepted at 5% two tailed level of significance.
- Accuracy measures were calculated at specific cut off points against the gold standard (histopathological examination): accuracy, sensitivity, specificity, positive and negative predictive values.
- ROC curve was constructed and overall diagnostic accuracy was related to the area under the curve.

3. Results:

3.1 Description of the study sample: The present study was conducted on a total of 80 individuals; including 52 female patients presented with primary breast cancer and 28 healthy age and sex matched individuals as a control group. Table (1) represents the clinicopathological characteristics of the patients. The age of the patients ranged from 31-70 years with a median of 52 years. As regard the histopathological features 51 of the patients had infiltrating duct carcinoma (IDC) and only one patient with lobular carcinoma(98.1% and 1.9% respectively) 23 of the cases were of the intrinsic type luminal A (44.2%), luminal B were 20 patients(38.5%),overexpression were 7 (13.5%) and triple negative were 2 (3.8%).According to the tumor grade 43 (82.7%) of the patients were grade II, 8(82.7) were grade III and 1 (1.9%) grade I. For the hormonal receptors and HER2 expression; 43 (82.7%) had positive estrogen and progesterone receptors and 9(17.3%) were negative while, 27 (51.9%) had positive HER2 and 25 (48.1%) had negative HER2 receptor. Concerning the stage of the disease 35 (67.3%) of the patients were stage II, 14(26.9%) stage I and 3(5.8%) stage III.the measured serum CA15.3 (IU/ml) in the patients showed a median of 16.50IU/ml (range 6-29 IU/ml).

Table (1): Description of the study sample according to age and Clinico-pathological characteristics of the breast cancer patients' group.

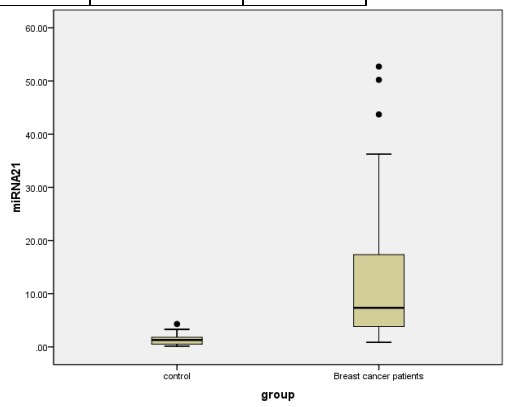
	Number (n)	Percent %
Positive estrogen receptor	43	82.7
Positive progesterone receptor	43	82.7
Positive HER2 receptor	27	51.9
Molecular subtypes		
LA	23	44.2
LB	20	38.5
OE	7	13.5
TN	2	3.8
Grade		
Grade 1	1	1.9
Grade 2	43	82.7
Grade 3	8	15.4
Stage		
Stage 1	14	26.9
Stage 2	35	67.3
Stage3	3	5.8
Pathological type		
IDC	51	98.1
IDC+ILC	1	1.9
Age (years)		
Median (Range)	52.0 (31-70)	
CA15.3 (IU/ml)		
Median (Range)	16.50 (6-29)	
Affected Lymph node %		
Median (Range)	41.8 (0-92.8)	

LA=luminal A, LB=luminal B, OE=over expression, TN Triple negative, IDC=intra ductal carcinoma, ILC=intraluminal carcinoma *Significant (P<0.05)*

3.2 The molecular expression levels of MicroRNAlet7g and MicroRNA21 in the serum in groups included in the study were presented in table (2) and figure (1 a & b). A highly significant difference was noticed between both groups where the serum expression level of micro RNA21 was higher in breast cancer patients when compared to the control group (median= 7.3 and 1.28 respectively) ($p \leq 0.05$). The serum expression levels of micro RNA let7g recorded a lower level in breast cancer patients when compared to the control group (Median= 0.165 and 1.225 respectively), showing a statistically significant difference ($p \leq 0.05$).

Table (2): Comparison between breast cancer patients and control regarding serum microRNA 21 and micro RNA let7g expression levels.

	BC patients (n=52)	Controls (n=28)	P value (Mann Whitney test)
Micro RNA21 Median (Mini-Max)	7.3 (0.85-52.7)	1.28 (0.14-4.3)	0.001*
Mi RNA let7g Median (Mini-Max)	0.165 (0.03-1.75)	1.225 (0.25-6.41)	0.0013*



Significant(P<0.05)

Figure (1a): Box plot showing the comparison between breast cancer patients and controls as regard microRNA 21 serum expression level.

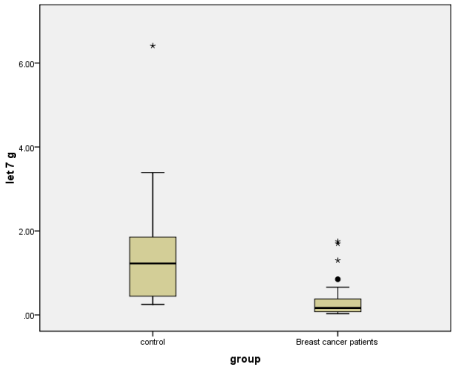


Figure (1b): Box plot showing the comparison between breast cancer patients and controls as regard miRNA let7g serum expression levels

3.3 Comparisons between different basic clinical and sociodemographic characteristics of the breast cancer patients as regards the expression of microRNA and the MicroRNAlet7g: There was no statistically significant difference between any of the parameters except between HER2 receptor expression and Micro RNAlet7g as presented in table**(3).**There was a highly statistically significant difference between them; a lower expression level of microRNAlet7g was noticed in Her2 positive patients than Her2 negative patients (median=0.09 & 0.28 respectively).

Table (3): Comparisons between basic clinical and characteristics of the breast cancer patients as regards the Micro RNA21 and miRNA let7g serum expression levels

Variable	No. (%)	micro RNA21 *Median (min-max)*	Mann Whitney test (P value)	miRNA let 7g serum *Median (min-max)*	Mann Whitney test (P value)	
Age(years) ≤50 50-70	22 (42.3) 30 (57.7)	7 (1.3-52.7) 7.7 (0.85 - 50.2)	0.431	0.165 (0.04-1.3) 0.160 (0.03-1.75)	0.87	
ER positive negative	43 (82.7) 9 (17.3)	7.4(0.85-52.7) 6 (1.72-50.21)	0.87	0.17 (0.03-1.75) 0.09(0.03-0.85)	0.885	
PR positive negative	43 (82.7) 9 (17.3)	7.4(0.85-52.7) 6 (1.72-50.21)	0.87	0.17 (0.03-1.75) 0.09(0.03-0.85)	0.88	
Her 2 Positive Negative	27 (51.9) 25 (48.1)	6.7 (0.9-50.21) 8 (0.85-52.7)	0.91	0.09(0.03-1.7) 0.28(0.03-1.75)	0.003*	

** Significant (P<0.05)*

3.4.The spearman's correlation between the basic clinicopathological parameters and the expression level of both micro RNAs in the serum of the breast cancer patients are presented in table **(4)** ; a positive significant correlation between CA15.3 and microRNA 21 serum expression levels (r=0.468,p=0.000) .Meanwhile, a negative significant correlation was present between miRNA let7g serum expression levels and HER2 (r=-0.416,p=0.002*)(fig.2a), as well as with molecular subtypes the luminal classification according to the Ki 67 proliferation index was taken from the patients pathological reports since it is done as routine work in the pathology department, prognostic ranking (r=-0.34,p=0.014*)(fig.2b). While no significant correlation was found between miRNA let7g serum expression levels and the rest of the studied parameters.

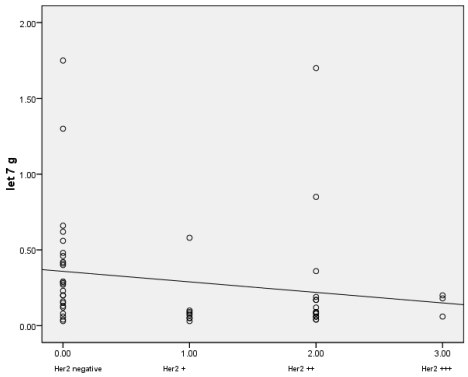


Figure (2a): Scatter plot showing the correlation between miRNA let7g serum expression levels and HER2 receptor.

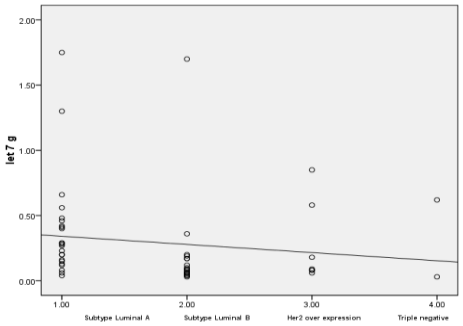


Figure (2b): Scatter plot showing the correlation between miRNA let7g serum expression levels and molecular subtypes.

Table (4): Correlation between both Micro RNA21 and miRNA let7g and different parameters

	Micro RNA21		**Micro RNA let 7g**	
Variable	**Correlation coefficient (r)**	**significance**	Correlation coefficient (r)	**Significance**
CA15.3	0.468	0.0001*	0.076	0.594
Progesterone receptor	0.022	0.87	0.02	0.88
Estrogen receptor	0.022	0.87	0.02	0.88
Her 2 receptor	0.015	0.914	-0.416	0.002*
Age	0.19	0.89	0.169	0.231
Stage	0.258	0.064	0.166	0.239
Grade	-0.0.037	0.793	-0.067	0.636
Affected Lymph node percentage	0.119	0.402	0.051	0.722
Molecular subtypes (Prognostic ranking)	0.021	0.885	-0.34	0.014*

*significant **(p≤0.05)**

3.5.1 Accuracy of each of micro RNA 21, micro RNA let7g serum expression levels and both of them in diagnosis of breast cancer at specific cut off points: is demonstrated in table (5). It reveals that 100% of breast cancer patients have serum miRNA let7g expression levels less than or equal to 1.78 compared to 67.9 % of controls. This association was statistically significant (Fisher exact test, P=0.000). Also there was a significant association between micro RNA21and breast cancer as 100% of breast cancer patients had serum microRNA21 expression level more than or equal to 0.82 compared to 64.3% of controls. (Fisher exact test, P=0.000). Concerning the combined tests, 100% of the cancer patients had micro RNA21 **≥ 0.82 and micro RNA let7g ≤1.785 (both tests were positive) compared to 32.1 % of the control. This was statistically significant** (Fisher exact test, P=0.000).

Table (5) Accuracy of each of Micro RNA21, Let 7 g and combined both tests in diagnosis of Breast cancer

Test at specific cut off point	**Cases**	**Control**	**Test of significance** (P value)	**O.R** (95% C.I)
Micro RNA 21 ≥ 0.82 <0.82	 52 0	 18 10	 Fisher exact test (0.000)*	 3.8 (2.6-5.8)
Micro RNA Let 7G ≤1.785 >1.785	 52 0	 19 9	 Fisher exact test (0.000)*	 3.7 (2.5-5.5)

Combined both tests: **-Both tests are positive** **-One of them is positive**	 52 0	 9 19	Fisher exact test (0.000)*	6.7 (3.7-12.4)

*** P value is significant (< 0.05)**

3.5.2. Regarding accuracy and diagnostic measures of Micro RNA21 at cut off point 0.82, Let 7 G at cut off point 1.785 and both combined tests in diagnosis of cancer breast (table 6& figures 3a, 3b). It was shown that the combined accuracy of both tests at 0.82 and 1.785 cut off points of microRNA21 and microRNAlet7g respectively is higher (88.8%) than the accuracy of each one separately (77.7% and 76.2% respectively).

Table (6) Accuracy measures of Micro RNA21 at cut off point 0.82, Let 7 G at cut off point 1.785 and both combined tests in prediction of cancer breast .

Accuracy measures	MicroRNA21 at cut off point0.82	Micro RNA Let7G at cut off point 1.785	Combined tests
Sensitivity: Specificity: P.PV.: N.P.V.: Accuracy at the specific cutoff point Overall accuracy: 95% C.I: P value:	100% 35.7% 74.3% 100% 77.5% 94.3% (99.5% – 99%) 0.000	100% 32.1% 73.2% 100% 76.2% 89.9% (83.4% – 96.4%) 0.000	100% 67.8% 85.2% 100% 88.8%

PPV=positive predictive value,NPV=negative predictive value

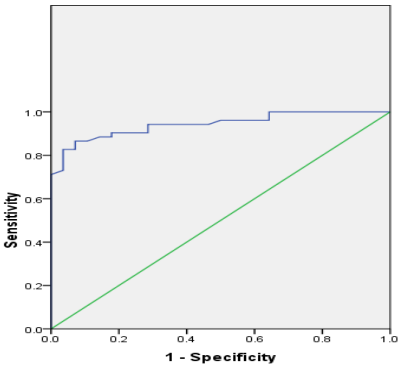


Figure (3a) ROC curve of the microRNA21 in prediction of Breast cancer microRNA21

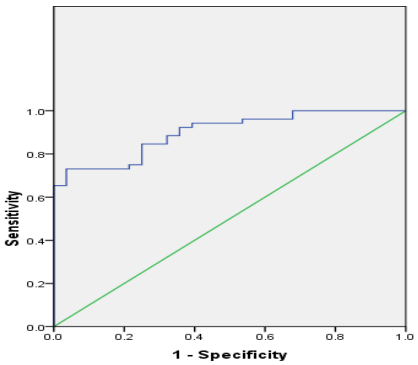


Figure (3b) ROC curve of the microRNA Let 7 G in prediction of Breast cancer

Discussion:

The most prevalent kind of cancer in women and the second greatest cause of cancer-related death is breast cancer. (3, 4, 34) The traditional clinicopathological variables, which are now insufficient for an effective diagnosis of breast cancer, are primarily responsible for the diagnosis of breast cancer outcomes and treatment approaches. Since DNA and micro RNA can be readily separated, they are stable under a variety of settings, and they have been discovered to be dysregulated in many different diseases, including breast cancer, gene expression profiles have recently emerged as a novel tool for the management of breast cancer. (34, 35). By targeting mRNAs implicated in downstream signaling cascades, microRNAs, a type of tiny, non-coding single-stranded RNA molecules, govern a variety of cellular activities, including early embryonic development, proliferation, apoptosis, and differentiation. (36) In the present study the serum expression level of miRNA21 and miRNAlet7g have been quantified using q RT-PCR in Egyptian breast cancer patients and compared to an age-sex matched control group. The expression level of miRNA21in breast cancer patients was higher than in the control group and this difference was highly significant. On the other hand, serum expression level of miRNAlet7g was lower in breast cancer patients than its expression in the control group and showing a statistically significant difference. The upregulation of miRNA21 and downregulation of miRNAlet7g demonstrates their role in breast cancer as novel biomarkers for early detection of breast cancer. Several studies [34, 37, 38.39, 40] have been in accordance with our results, where Iorio et al[37] and Wang et al[34] demonstrated that the serum expression level of microRNA21 was significantly higher in BC than in healthy control. Moreover, Usmani et al [39], conducted a study including breast cancer patients, daughters of the patients as a control group and a healthy matched control group. They showed high serum expression of microRNA21 in breast cancer patients. MicroRNA21 serum expression level studied in daughters of the cases was also significantly higher as compared to the healthy individuals but lesser than the full-blown disease of breast cancer. As regard the downregulation of miRNAlet7g have been supported by several studies [41, 42, 43] where the serum expression level of miRNAlet7g was lower in breast cancer patients than in control group.

Numerous studies have discovered the expression of miRNA21 and miRNAlet7g in breast cancer patients' serum as well as in breast cancer tissue, indicating that tumor-specific microRNAs in serum are derived from both cancer cells and circulating blood cells. (34, 44, 45, 46) They have shown that these microRNAs have aberrant expression in tissue or serum and are implicated in tumorigenesis acting as either oncogenes or tumor suppressors. More than 50% of human microRNA genes reside in fragile locations, which are typically shown to be amplified in several tumor tissues, including the lung, breast, and lung, or in genomic areas associated with cancer. (45,47). One of the most widely expressed miRNAs in breast cancer, miRNA21 is an oncogenic miRNA that suppresses many tumor suppressor genes, promoting cell proliferation, invasion, and tumor metastasis. Upregulation of miRNA21 is linked to tumor development and a poor prognosis. Tropomyosin 1 and PTEN68 are two of the many targets of miRNA21, which encourage the proliferation of MCF-7 breast cancer cells. (45,47,48). Let-7 is known to target mRNA and is itself a target of negative feedback regulation in humans. It is known to be overexpressed in differentiated epithelial tissues and is frequently downregulated during tumorgenesis. Targeting HRAS and HMGA2, Let-7 was discovered to control breast cancer tumor initiating cells (T-IC). (45,49)
We looked at the relationship between these measured parameters and the various clinicopathological factors in breast cancer patients, including tumor grade and molecular subtypes, as well as HER2, and hormonal expressions (ER and PR), and stage of the disease in order to highlight the role of miRNA21 and miRNAlet7g in breast cancer prognosis. Even though there was a higher level of miRNA21 expression in the HER2, ER, PR, positive lymph

node groups, and stage groups, neither a significant association nor correlation between miRNA and these parameters was discovered. However, a positive correlation was discovered between miRNA21 serum expression and CA15.3 serum level in breast cancer patients, indicating the potential role of serum microRNA21 expression level as a prognostic marker.CA15.3 is considered one of the conventional tumor markers in breast cancer but it is not sensitive or specific enough for screening asymptomatic women thus, miRNA21 may have a role in breast cancer diagnosis and screening.[40] our results were in consistent with other studies [40,50] meanwhile, the serum expression level of miRNAlet7g showed a negative correlation with tumor hormonal and tumor HER2 receptors expression ,these findings suggest that miRNAlet7g can be used as prognostic marker. This result matched that concluded by Mattie et al [51] who studied the relation between different miRNAs expressions and hormonal receptors expressions in breast cancer patients and found that unique sets of miRNAs are associated with Her 2 status (let7g, let7f, miRNA107, miRNA10b, miRNA126, miRNA154 and miRNA195).

In the present work, ROC curve analysis for the diagnostic power of serum microRNA21 yielded an AUC of 0.94 with 100% sensitivity and 35.7% specificity in differentiating female patients with breast cancer from healthy control group at a cutoff point of 0.82. The confidence interval was 89.5-99, positive predictive value was74.2% and negative predictive value was 100%. Gao J et al [40] evaluated serum expression level of micro RNA21 in breast cancer patients and controls. They got an area under the curve=0.92 with sensitivity and specifity of 87.6%, 87.3% respectively. Similarly, Aguilar F et al [38] measured the serum expression levels of the microRNA 21 in BC patients and healthy controls and they performed ROC curve analysis to evaluate the diagnostic value for serum microRNAs. They found at the cutoff value of 6.48 for microRNA21, the sensitivity and specificity were 94.40% and 80.00% respectively. In conclusion our study provides evidence that microRNAs serum expression level was higher in breast cancer patients than in normal controls. This result can mark microRNA21 as a potential diagnostic biomarker for breast cancer.
Studying the diagnostic performance of miRNA let7g through constructing a ROC curve, the area under the curve was (0.899). At the cut off level 1.78 the diagnostic sensitivity for miRNA let7g was 100% and its diagnostic specificity was 32.1%. This means that miRNA let7g might be considered as one of the biomarkers that could play an important role in breast cancer. Meanwhile on investigating the accuracy measures of combined tests of microRNA21 and microRNAlet7g at a cut off points 0.82 and 1.785 respectively data revealed that they can both detect the presence of breast cancer with 100% sensitivity, 67.8% specificity, positive predictive value of 85.2% against 100% for negative predictive value and accuracy at cut off points was 88.8%..($p \leq 0.05$). Thus, we can conclude the usefulness of circulating micro RNAs as predictive biomarkers in breast cancer. our results of the current study are supported by several reports [104,105,106 2017] where Heneghan and colleagues[104] assessed the diagnosis potential of a panel of seven cancer-associated miRNAs in the circulation of patients with various cancer types. The authors found that let-7a and miR-10b and -155 levels were upregulated in the majority of cancer patients, whereas circulating miR-195 level distinguished those with breast cancer from other cancer types and from normal control with a sensitivity of 88% and a specificity of 91%. The sensitivity was further increased to 94% when using a combination of circulating levels of miRNAs.
A good place to start when investigating the functional role of microRNA in the development of cancer is with its abnormal expression during the cancer process. Down-regulated "tumor suppressive microRNAs" increase downstream signal pathways that are involved in tumor growth, whereas up-regulated "oncogenic microRNAs" suppress the expression of putative tumor suppressor genes. Both have the potential to accelerate the onset and spread of cancer. These two categories of miRNAs might take involvement in pathogenic processes. The

combined measurement of miRNA let7g and miRNA21 serum expression levels may be considered promising biomarker that is crucial for breast cancer screening, prognosis, and treatment plans since they are non-invasive, simple to quantify, inexpensive, and acknowledged for sensitivity. However, more research with a larger number of patients is strongly advised.

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