THE EMERGING ROLE OF MICRORNA-138 AS NOVEL DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC TOOLS FOR BREAST CANCER

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Breast cancer is the second most common cancer among women overall, after lung cancer (Miller *et al.*, 2021 and Giaquinto *et al*., 2022). A two million plus increase in new cases was predicted for 2020. With more than 680,000 fatalities, it also ranks first among cancers that kill women (Sung *et al*., 2021). According to Ibrahim *et al*. (2014), it accounts for 33% of female cancer cases in Egypt and more than 22,000 new cases are reported every year. A variety of methods are used in an effort to find breast cancer early. Mammography, which is regarded as the gold standard approach among them, is the most used tool for finding breast cancer (Zubor *et al*., 2019). According to Francis *et al*. (2014), mammography does have certain drawbacks, including the fact that it is a painful procedure that requires exposure to ionizing radiation as well as difficulties in detecting tiny tumors. Therefore, use of additional non-invasive sensing methods beneficial for those working in the field of breast cancer detection and diagnosis, which also have to cope with mammography's limitations. This is crucial because, in the end, it increases the chances of survival (Mahmoudzadeh *et al*., 2015).

Messenger RNAs (mRNAs) are known to be extensively influenced at the post-transcriptional level by small noncoding RNAs called microRNAs (miRNAs), which are short noncoding RNAs with an average length of 22 nucleotides (nt) (Bahari Khasraghi *et al*., 2023 and Shirvani *et al*., 2023). According to investigation of the miRNA expression in human breast tumors, some miRNAs may behave as potential tumor suppressors or oncogenes and control the immune response that distinguishes these tumors (Dvinge *et al*., 2013). In fact, the state of the hormone receptors in breast cancer is associated with certain miRNA expression profiles (Lowery *et al*., 2009; Klinge, 2012 and Kunc *et al*., 2020) have characterized three classes of miRNA signatures matching with oestrogen receptor (ER), progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2/neu) status. MiR-138 has recently come to light as a significant tumor suppressor miRNA among the abundant miRNAs (Ding *et al.*, 2018 and Yeh *et al*., 2019). MiR-138 is an appropriate biomarker to identify triple-negative breast cancer (TNBC) from other breast cancer subtypes and develop miR-138 as a predictive biomarker for TNBC since it is highly and specifically expressed in this form of breast cancer (Nama *et al*., 2019). Increased miR-138 expression is positively correlated with a poor prognosis among patients with triple-negative breast tumors, suggesting that miR-138 expression level can be useful in determining the best course of cancer treatment. Some of the variations in miR-138 expression between TNBC patients may be the consequence of sampling from various subtypes of TNBC (Lehmann *et al*., 2011). The impact of the TNBC subtype on patient survival is another factor that should be closely correlated with the expression of the miR-138 that is specific to the subtype (Nama *et al*., 2019).

The importance of miR-138 as a non-invasive diagnostic for breast cancer will be the main topic of this article. It offers a tool for early identification, which improves patient outcomes, and permits a thorough knowledge of the molecular processes producing breast cancer. Additionally, to assess the relationship between miR-138 expression and treatment responsiveness and influence on survival.

SUBJECTS AND METHODS

Study design and population

This case-control study was conducted by the Medical Biochemistry and Molecular Biology Department in association with the Clinical Oncology and Nuclear Medicine Department at the Menoufia University Faculty of Medicine. Seventy-five with breast cancer were included in the study, based on histology and the possibility of collecting blood samples from the participants, and 75 healthy women who visited the clinic for a checkup. Age and sex were equal between the two groups. Every participant in the study supplied their free and informed permission. All patients provided written consent after being fully informed. The medical school's ethics committee at Menuofia

University gave the study protocol their blessing.

The study excluded patients who had received preoperative chemotherapy or radiotherapy, as well as those who had current or prior histories of primary malignancies other than breast cancer. All participants' personal histories were collected, including information on the patient's age, menopausal state, and breast cancer in the patient's family. Tumor sidedness, histological type, tumor grade, TNM staging, tumor immunohistochemistry including ER and PR status, HER2/neu and Ki67 expression, and various molecular subtypes (Triple negative, Her2neu overexpression, luminal A and luminal B) are all examples of clinicopathological data. Measurements of serum tumor markers, CA15.3 (cancer antigen 15.3), and CEA (carcinoembryonic antigen) are performed in laboratories. Following that, qRT-PCR was used to find miR-138 expression.

Data on treatments include Metastatic status, the type of surgery, the type of treatment chemotherapy or biological and hormone therapy received, the occurrence of treatment toxicity, and the severity of the toxicity. For the purposes of calculating progression free survival (PFS) and overall survival (OS), progression status and living status are recorded. PFS is the period from the time of diagnosis till the time of relapse, and OS is the period from the date of diagnosis to the date of death, according to National Cancer Institute (NCI) standards for survival criteria.

Sampling and laboratory investigations

Each participant had two sterile vacationer tubes used to draw five milliliters of venous blood from them. Two milliliters were collected for RNA extraction in the first tube, which contained ethylene diamine-tetraacetic acid (EDTA), and the second tube, which lacked an anticoagulant. The samples were allowed to clot in the first tube before being centrifuged and the serum was separated using the chemiluminescence method (ECLIA) to evaluate the levels of CA15.3 and CEA.

Extraction and reverse transcription (RT) of RNA

Total RNA, including miRNAs, was extracted using the RNeasy Mini Kit and Qiazol Reagent (Qiagen, USA), as directed by the manufacturer. The NanoDrop 1000 (Thermo Scientific, USA) was used to assess RNA quality. Using the miScript II RT Kit (Qiagen, USA), create single-stranded cDNA from the extracted materials as directed by the manufacturer. The cDNA product is then kept at 20ºC until the real-time PCR stage.

Quantitative real-time PCR

Using the StepOne Real-Time PCR machine (Applied Biosystems) and the miScript Primer Assay (forward primer) for miR16 (reference miR) and miR-138, as well as the miScript SYBR Green PCR Kit, which contains the QuantiTect SYBR Green PCR Master Mix, according to the manufacturer's instructions, real-time PCR was carried out on 100 nanograms of total

RNA. The following real-time PCR protocol was used: 40 cycles of 94ºC for 15 s, 55ºC for 30 s, and 70ºC for 30 s were performed after 15 minutes at 95ºC. Inter-assay controls, verified endogenous controls, and samples were all utilized. By using the $2^{-\Delta\Delta Ct}$ method ($\Delta\Delta Ct$ = {[Ct (miRNA of interest) – Ct (reference miR-16 of interest)] − [Ct (miRNA of control) – Ct (reference miR-16 of control)]}, the relative quantification (RQ) of miRNA gene expression was evaluated.

Statistical analysis

The computer-fed data were examined using the IBM SPSS software program, version 20 (IBM Corp., New York's Armonk). In order to describe quantitative data, percentage and number were used. The Kolmogorov-Smirnov test was used to determine if the distribution was normally distributed. Quantitative data were described using interquartile range (IQR), mean, standard deviation, and range (minimum and maximum). The significance of the results was calculated at the p value less than 0.05. The Kaplan-Meier curve, Fisher's Exact or Monte Carlo correction, Student t-test, Mann Whitney test, Receiver operating characteristic curve (ROC), and Chi-square test were the tests used for the survival study.

RESULTS AND DISCUSSION

Breast cancer has surpassed lung cancer as the most frequent disease in the world for the first time, according to the most recent global cancer data for 2020 provided by the World Health Organization's International Agency for Research on Cancer (IARC). Continuous improvements in early detection, individualized treatment, and chemotherapy strategies have considerably boosted the survival rate of breast cancer patients. But it continues to be the top reason for women's cancer-related deaths globally (Volovat *et al*., 2020). To estimate the pace of metastatic spread, the effectiveness of treatment, and even to develop innovative therapeutic strategies, it is required to discover the disease's prognostic biomarkers. Among the promising molecular targets for breast cancer treatment are microRNAs (miRNAs) (Wu and Chu, 2022). In light of this scenario, our goal was to assess miR-138 expression level's potential as a biomarker that may be used in conjunction with other tools for diagnosis, prognosis, and therapy.

According to earlier research, miR-138-5p may have a role in controlling the development, progression, and invasiveness of certain tumor types. MiR-138-5p expression is barely detectable in tissues and cells from breast cancer. Overexpression of miR-138-5p significantly decreases the capacity of breast cancer cells proliferation, invasion and migration (Bockhorn *et al*., 2014 and Zhao *et al*., 2019). The miR-138-activated signal thus might serve as a novel independent prognostic marker (Liang *et al*., 2017).

With 150 participants overall, the median age was 51 years, and the mean age ± SD was 49.16± 10.17. Participants' ages ranged from 27 to 73 years. Two

groups of cases ((≤51 and >51) were created based on the median age of the cases. At the time of diagnosis, 31 (41.3%) and 44 (58.7%) patients, respectively, were postmenopausal and premenopausal, respectively. Only five (6.7%) of the total cases under investigation had a positive family history of breast cancer. Age, menstrual status, and family history between the tested groups did not show any discernible differences.

Table (1) displays the clinical features of the breast cancer group. Patients with positive oestrogen receptor (ER) levels (58.7%) and positive progesterone receptor (PR) levels (56%) had positive hormone receptor status. Human epidermal growth factor receptor 2 (HER2/neu) levels were high in (60%) of the patients, low in (21.3%) of the patients, and missing in (18.7%) of the patients. The molecular subtypes revealed that Luminal B biological type was identified in higher instances (41.3%) compared to Luminal a (17.3% of patients). Ten cases (13.3%) died before the end of the trial, and 25 cases (33.3%) reported illness progression. Regarding the tumor markers, the levels of CEA and CA15.3 showed a statistically significant difference between the cases group compared and the control group (P=<0.001, 0.002 respectively, (Fig. 1).

In the current study, patients with breast cancer had substantially higher levels of miR-138 expression than did controls, with a p value of <0.001. In comparison to the control group, triple negative patients and other types of cases had considerably higher levels of miR-138 low expression (<13.48) (Table 2). This result is consistent with (Wang *et al*., 2022), who observed that miR-138 was downregulated in TNBC. Additionally, (Zhao *et al*., 2019) found that miR-138-5p was considerably less abundant in breast cancer and that its overexpression could prevent BC cells from spreading by inhibiting Rhomboid Domain-Containing Protein 1 (RHBDD1).

To the best of our knowledge, this is the first study demonstrated a relationship between the level of miR-138 expression and clinicopathological traits and survival. It also found that there was no statistically significant relationship between miR-138 expression and age, menopausal status, family history of breast cancer, or other histopathological parameters. Increased TNM stage was significantly correlated with a considerable decline in miR-138 expression (P=0.001), while high miR-138 expression was positively correlated with non-metastatic, low-grade (GI & GII) disorders (P=0.006 & P=0.019, respectively),. A significant inverse relationship between miR-138 and the levels of the tumor markers CEA and CA15.3. When compared to individuals that were dead, still-alive subjects had significantly higher miR-138 expression (P=0.002) (Table 3). Our current investigation verified the independent predictive usefulness of miR-138 expression level for breast cancer in terms of its connection with survival result. Furthermore, the Kaplan-Meier survival curve showed that

miR-138 may be the poorest predictive indicator when taking its expression level into account. When compared to patients with low expression (mean= 31.703, overall survival time=94.6%), patients with high expression of miR-138 had a substantially higher overall survival (mean= 26.987 months, overall survival time=78.9%) (P=0.049) (Fig. 2).

The serum CEA, CA153, and CA125 levels have been shown in earlier research to be very helpful in clinical diagnosis and to offer insights into how to manage breast cancer metastasis and recurrence. High blood levels of CEA and CA153 have been linked to poor prognoses (Zhao *et al*., 2016 and Uygur and Gümüş, 2021). Three tumor markers linked to breast cancer (CEA and CA153) were chosen for this investigation. The tumor marker levels between the breast cancer patients were compared using ROC curves. The breast cancer patients and healthy controls were more easily distinguished by CEA with AUC (0.796 vs. 0.647 and 0.686) than by the other tumor marker CA.153 and the miR-138. These markers' specificities (81.33%, 69.33%, and 61.33%, respectively,) were broadly acceptable (CEA, CA 153, and miR-138) Table 4. Additionally, the sensitivities were (72%, 56.0%, and 60.0%, respectively). According to the association between these biomarkers, the expression level of miR-138 significantly correlated negatively with CEA and CA15.3 (P=0.008, 0.038, respectively), but CEA and CA153 significantly correlated positively (r=0.369, P=0.001) (Fig. 3).

Following adjustment for additional clinical variables, univariate Cox analysis showed that metastasis status, CA15.3.value, chemotoxicity, and miR-138 were all significant predictors for mortality in the univariate COX regression analysis. The other factors, however, were negligible mortality predictors. Metastasis Status, CA15.3, value, chemotoxicity, and miR-138 were not significant predictors for mortality in the multivariate COX regression analysis (Table 5). The relationship between clinicopathological characteristics and relapse: Metastasis status, PT status (≥3), PN status (≥3), CEA value, CA15.3.value, and miR-138 were significant predictors of relapse in the univariate COX regression analysis. The other factors, however, were negligible relapse predictors. PT status (≥3), PN status (≥3), miR-138, metastatic status, and CA15.3 value were not significant predictors of recurrence in the multivariate COX regression analysis (Table 6).

CONCLUSION

The current study supported the use of miR-138 as a significant and unique marker with high specificity and sensitivity for the diagnosis of breast cancer as well as its potential significance in prognosis and therapy. But in order to confirm these results, further in-depth functional evaluations and prospective population-based studies with larger sample sizes and a variety of ethnic groups are needed.

SUMMARY

Background and objectives: A multistep process called breast carcinogenesis is characterized by genetic and epigenetic changes. It is believed that miRNAs have a role in the onset and spread of breast cancer and also present attractive targets for such novel treatments. This study's goal was to investigate at the role of miR-138 in breast tumor invasion, metastasis, diagnosis, prognosis and treatment in Egyptian women, as well as their relevance to the molecular types of those processes.

Methodology: A total of 150 individuals were included in the present study, including 75 breast cancer and 75 supposedly healthy women who were age and gender matched. All historical data mammogram, and laboratory tests were performed on each patient. These tests included the determination of miR-138 expression levels by real-time PCR, serum CEA and CA15-3 levels, and general clinical examination.

Results: The expression level of miR-138 was considerably higher (P<0.001) in breast cancer patients than control group. In triple negative cases and instances of other types, low expression (13.48) miR-138 was reported with a significant difference with control group (p^2=0.002*, $p^3$0.001*). A substantial inverse relationship between miR-138 and the levels of the tumor markers CEA and CA15.3. In patients with high expression miR-138 had substantially higher overall survival (P=0.049). Metastasis status, CA15.3.value, chemotoxicity, and miR-138 were all significant predictors for mortality and metastasis status, PT status (≥3), PN status (≥3), CEA value, CA15.3 and miR-138 were significant predictors of relapse.

Conclusion: The miR-138 suppression may promote metastasis. Consequently, the restoration of miR-138 in breast cancer may have therapeutic potential, so the miR-138 may play a role in breast cancer development.

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Table (1): Distribution of the breast cancer patient based on clinicopathological Features and hormonal receptors.

Clinicopathological Features	No.	%
Tumor side		
Right	33	44.0
Left	42	56.0
Pathological subtype		
IDC	65	86.7
ILC	6	8.0
Mixed IDC and ILC	2	2.7
Other	2	2.7
Pathological stage		
Stage 1	6	8.0
Stage 2	25	33.3
Stage 3	30	40.0
Stage 4	14	18.7
Metastasis Status		
No	54	72.0
Yes	21	28.0
Grade		
Grade I	1	1.3
Grade II	68	90.7
Grade III	6	8.0
PT status		
T1	9	12.0
T2	36	48.0
T3	23	30.7
T4	7	9.3
PN status		
N0	15	20.0
N1	29	38.7
N2	17	22.7
N3	14	18.7
Toxicity grade		
No Toxicity	56	74.7
Grade 1	7	9.3
Grade 2	4	5.3
Grade 3	8	10.7

Table (1): Cont’		
ER	44	58.7
PR	42	56.0
HER2 /neu	16	21.3
Ki 67		
Not done	14	18.7
Low (<14)	16	21.3
High (equal or > 14)	45	60.0
Molecular subtype		
Basal (Triple negative)	25	33.3
HER2 overexpressed	6	8.0
Luminal A	13	17.3
Luminal B	31	41.3

Table (2): miR-138 expression level in the studied groups.

Micro RNA 138	Triple negative (n= 25)	Other (n= 50)	Control (n = 75)	Test of sig.	p
Low expression (≤13.48)	14(56.0%)	31(62.0%)	30(40.0%)	χ^2= 6.240*	0.044*
High expression (>13.48)	11(44.0%)	19(38.0%)	45(60.0%)		
Min. – Max.	0.48 – 23.96	1.49 – 18.90	10.30 – 19.87	H= 15.684*	<0.001*
Mean ± SD.	10.11 ± 7.36	11.71 ± 4.76	14.85 ± 2.72		
Median (IQR)	8.26 (1.82 – 16.50)	12.67(7.28 – 14.90)	14.81(12.48 – 16.78)		
Sig. Bet. Groups	p^1=0.626, p^2=0.002*, p^3=0.001*				

IQR: Inter quartile range, SD: Standard deviation χ^2: Chi square test, H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test) p: p value for comparing between the studied groups, P1: p value for comparing between Triple negative and other p2: p value for comparing between Triple negative and control p3: p value for comparing between other and control *: Statistically significant at $p \leq 0.05$.

Table (3): The association between miR-138 and patient characteristics (clinicopathological and treatment) in the breast cancer patients.

Variables	N	Micro RNA 138			U&H	p
		Min. – Max.	Mean ± SD.	Median		
Menstrual status						
Premenopausal	44	0.48 – 23.96	11.94 ± 5.63	12.94	U=573.0	0.241
Postmenopausal	31	1.25 – 18.90	10.10 ± 5.86	9.87		
Family history						
Negative	70	0.48 – 23.96	11.34 ± 5.70	12.10	U=144.0	0.528
Positive	5	1.33 – 14.90	9.02 ± 6.80	12.90		
Tumor side						
Right	33	0.48 – 23.96	11.46 ± 6.30	12.67	U=667.50	0.785
Left	42	1.25 – 18.90	10.96 ± 5.36	12.10		
Pathological subtype						
IDC	65	1.25 – 23.96	10.96 ± 5.59	12.01	U=267.50	0.370
Other	10	0.48 – 22.56	12.61 ± 6.90	14.44		
Pathological stage						
Stage 1+2	31	5.26 – 18.90	13.14 ± 4.01	13.34	U=467.50*	0.021*
Stage 3+4	44	0.48 – 23.96	9.80 ± 6.41	9.24		
Metastasis Status						
No	54	1.25 – 23.96	12.39 ± 4.89	13.27	U= 335.0*	0.006*
Yes	21	0.48 – 22.56	8.07 ± 6.72	6.35		
Grade						
Grade I+ II	69	0.48 – 23.96	11.71 ± 5.46	12.67	U= 87.0	0.019*
Grade III	6	1.49 – 16.76	5.10 ± 6.01	2.20		
PT status						
T1 + T2	45	1.33 – 18.90	11.45 ± 5.50	12.89	U= 604.0	0.443
T3+ T4	30	0.48 – 23.96	10.78 ± 6.19	9.79		
PN status						
N0 + N1	44	1.25 – 18.90	11.75 ± 4.56	12.78	U= 608.50	0.429
N2+N3	31	0.48 – 23.96	10.37 ± 7.12	8.20		
ER						
No	31	0.48 – 23.96	11.02 ± 6.89	13.50	U= 677.50	0.961
Yes	44	1.49 – 18.90	11.30 ± 4.88	12.10		
PR						
No	33	0.48 – 23.96	10.67 ± 6.82	11.76	U= 649.50	0.642
Yes	42	1.49 – 18.90	11.59 ± 4.80	12.43		
HER2 neu						
Negative	59	0.48 – 23.96	11.31 ± 5.89	12.18	U= 444.50	0.722
Positive	16	1.49 – 17.78	10.70 ± 5.36	12.33		

Table (3): Cont'						
Ki 67						
Not done	14	1.49 – 17.78	11.48 ± 5.26	13.20	H= 2.972	0.226
Low (<14)	16	8.12 – 18.90	13.38 ± 3.12	13.79		
High (equal or > 14)	45	0.48 – 23.96	10.31 ± 6.45	11.78		
Molecular subtype						
Basal (Tripple negative)	25	0.48 – 23.96	10.11 ± 7.36	8.26	H= 5.252	0.154
HER2 overexpressed	6	11.76 – 16.78	14.79 ± 1.92	15.08		
Luminal A	13	8.12 – 18.90	13.27 ± 3.36	12.89		
Luminal B	31	1.49 – 18.90	10.47 ± 5.22	11.89		
CEA						
Normal (<5)	32	1.89 – 23.96	13.40 ± 4.35	14.05	U= 433.50*	0.006*
Elevated (Equal or >5)	43	0.48 – 22.56	9.53 ± 6.15	8.12		
CA15.3						
Normal (<30)	49	1.33 – 23.96	12.71 ± 4.98	13.50	U= 360.0*	0.002*
Elevated (Equal or > 30)	26	0.48 – 22.56	8.30 ± 6.11	7.28		
Toxicity grade						
No Toxicity	56	1.25 – 23.96	13.12 ± 4.70	13.42	H= 5.252	0.154
Grade 1	7	1.56 – 14.03	5.90 ± 5.12	4.11		
Grade 2	4	1.57 – 6.80	5.01 ± 2.34	5.84		
Grade 3	8	0.48 – 16.78	5.34 ± 5.77	2.29		
Relapse or progression status						
Not progressed	50	1.33 – 23.96	12.06 ± 5.03	12.78	U= 479.0	0.101
Progressed	25	0.48 – 22.56	9.43 ± 6.76	8.20		
Living Status						
Dead	10	0.48 – 17.78	5.36 ± 5.93	1.73	U= 126.50*	0.002*
Alive	65	1.33 – 23.96	12.08 ± 5.22	12.89		

SD: Standard deviation, U: Mann Whitney test, H: H for Kruskal Wallis test, p: p value for comparison between the studied categories, *: Statistically significant at $p \leq 0.05$

Table (4): Prognostic performance for different parameters to discriminate patients from control.

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Micro RNA 138	0.686	<0.001*	0.600 – 0.772	≤13.36	60.0	61.33	60.8	60.5
CEA value	0.796	<0.001*	0.718 – 0.875	>3	72.0	81.33	79.4	74.4
CA15.3.value	0.647	0.002*	0.552 – 0.743	>17.5	56.0	69.33	64.6	61.2

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

Table (5): Analysis of the characteristics determining breast cancer patients' mortality using uni- and multivariate COX regression.

	Univariate		#Multivariate	
	p	HR (LL – UL 95%C.I)	p	HR (LL – UL 95%C.I)
Age (years)	0.216	0.962(0.905 – 1.023)		
Menstrual status (Postmenopausal)	0.879	0.906(0.256 – 3.211)		
Family history	0.572	0.045(0.0 – 2170.328)		
Tumor side (left)	0.817	1.162(0.328 – 4.117)		
Pathological subtype (IDC)	0.770	1.362(0.172 – 10.749)		
Pathological stage (≥3)	0.067	6.915(0.876 – 54.596)		
Metastasis Status	0.006*	6.711(1.732 – 25.998)	0.527	1.894(0.262 – 13.699)
Grade (III)	0.175	2.923(0.620 – 13.780)		
PT status (≥3)	0.201	2.282(0.644 – 8.088)		
PN status (≥3)	0.342	1.927(0.498 – 7.453)		
ER	0.207	0.443(0.125 – 1.570)		
PR	0.280	0.498(0.140 – 1.765)		
HER2 neu	0.935	0.938(0.199 – 4.417)		
Ki 67	0.884	0.891(0.189 – 4.195)		
Molecular subtype (Triple negative)	0.066	0.305(0.086 – 1.083)		

Table (5): Cont'				
CEA value	0.698	1.003(0.986 – 1.021)		
CA15.3.value	0.002*	1.010(1.003 – 1.016)	0.190	1.005(0.998 – 1.012)
Chemotherapy status	0.309	29.137(0.044 – 19423.36)		
Chemo toxicity	0.042*	4.060(1.049 – 15.707)	0.782	0.751(0.098 – 5.734)
Toxicity grade	0.133	2.638(0.744 – 9.352)		
Table (5): Cont'				
Hormonal treatment	0.078	0.296(0.077 – 1.146)		
Biological treatment	0.483	1.742(0.370 – 8.204)		
Micro RNA 138	0.002*	0.799(0.694 – 0.921)	0.062	0.862(0.738 – 1.008)

HR: Hazard ratio C. I: Confidence interval LL: Lower limit UL: Upper Limit.
#: All variables with p<0.05 was included in the multivariate *: Statistically significant at $p \leq 0.05$.

Table (6): The characteristics influencing relapse in breast cancer patients: a multivariate and univariate COX regression analysis.

	Univariate		#Multivariate	
	p	HR (LL – UL 95%C.I)	p	HR (LL – UL 95%C.I)
Age (years)	0.653	0.991(0.953 – 1.030)		
Menstrual status (Postmenopausal)	0.853	1.077(0.489 – 2.374)		
Family history	0.350	0.044(0.0 – 30.685)		
Tumor side (left)	0.156	0.564(0.256 – 1.243)		
Pathological subtype (IDC)	0.605	0.754(0.259 – 2.198)		
Pathological stage (≥3)	0.095	2.105(0.878 – 5.048)		
Metastasis Status	<0.001*	5.633(2.509 – 12.650)	0.909	0.948(0.383 – 2.351)

Table (6): Cont’				
Grade (III)	0.373	1.731(0.518 – 5.787)		
PT status (≥3)	0.020*	2.590(1.163 – 5.772)	0.769	1.160(0.432 – 3.116)
PN status (≥3)	0.005*	3.244(1.426 – 7.379)	0.798	0.881(0.334 – 2.323)
ER	0.664	0.839(0.381 – 1.850)		
PR	0.916	0.958(0.435 – 2.112)		
HER2 neu	0.818	0.891(0.334 – 2.375)		
Ki 67	0.867	0.920(0.345 – 2.451)		
Molecular subtype (Tripple negative)	0.138	1.818(0.825 – 4.010)		
CEA value	<0.001*	1.020(1.011 – 1.030)	0.014*	1.015(1.0 – 1.028)
CA15.3.value	<0.001*	1.013(1.009 – 1.018)	0.004*	1.010(1.0 – 1.016)
Chemotherapy status	0.796	1.138(0.427 – 3.034)		
Chemo toxicity	0.105	1.916(0.874 – 4.202)		
Toxicity grade	0.215	1.643(0.749 – 3.603)		
Hormonal treatment	0.446	0.737(0.336 – 1.616)		
Biological treatment	0.885	0.915(0.274 – 3.057)		
Micro RNA 138	0.033*	0.923(0.858 – 0.993)	0.059	0.934(0.870 – 1.0)

HR: Hazard ratio C. I: Confidence interval, LL: Lower limit, UL: Upper Limit, #: All variables with $p<0.05$ was included in the multivariate *: Statistically significant at $p \leq 0.05$.

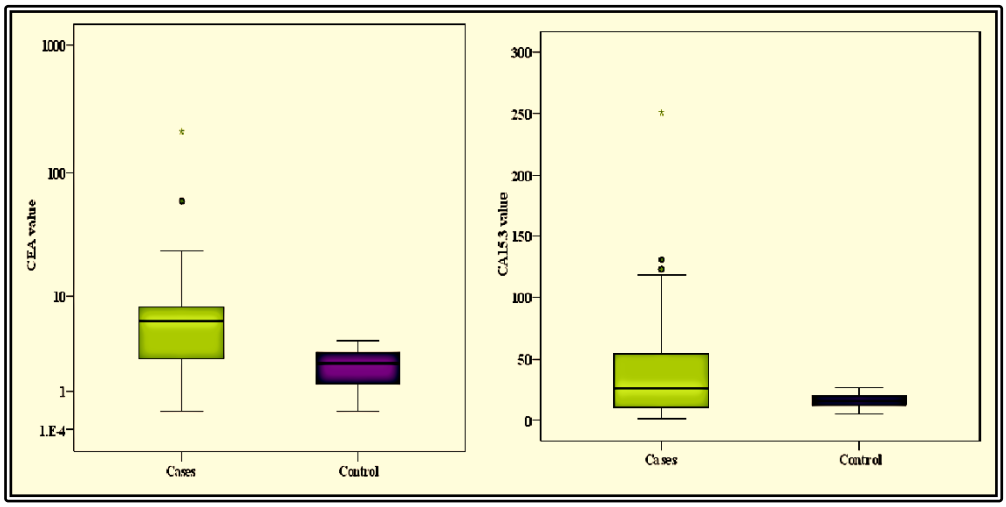

Fig. (1): Comparison between the tumor markers CEA and CA15.3 in the studied groups.

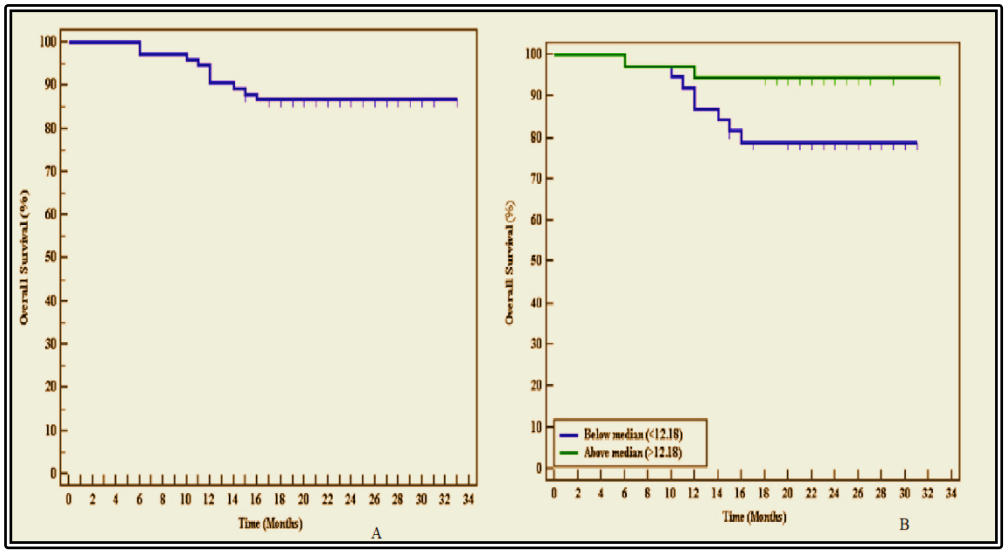

Fig. (2): Kaplan-Meier survival curve for A: Overall Survival B: Overall Survival with miR-138.

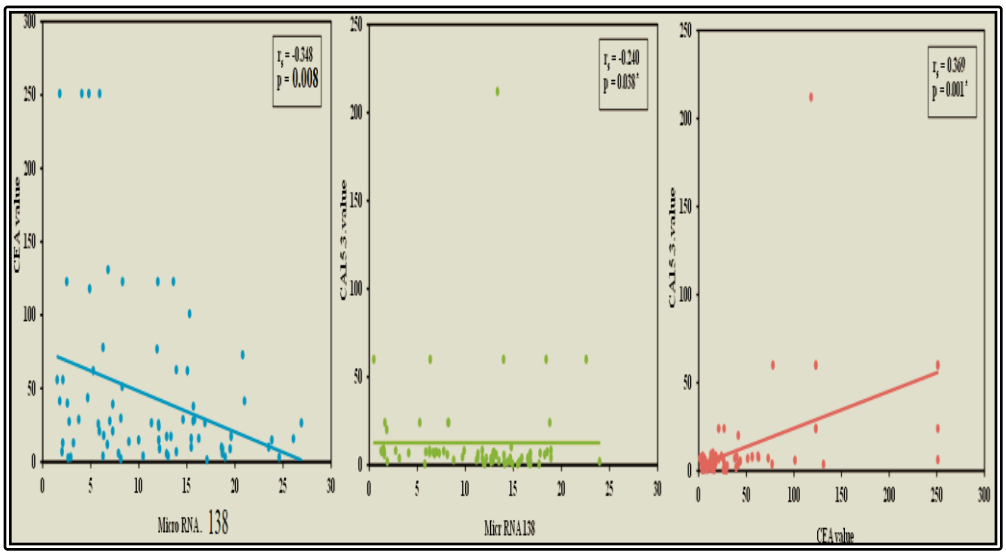

Fig. (3): The correlation between the studied biomarkers in breast cancer patients.