

The Role of the Let-7 microRNA Family as Biomarker Prognostic Indicator Against Breast Cancer

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Abstract

Breast Cancer is considering the most common cancer in Iraq and the major cause of cancer related deaths among women Iraqi Cancer Registry and Ministry of Health, Republic of Iraq, and also Among women in Iraq, breast cancer is the most frequent type of cancer. Material AND Methods: This study was designed to focus on microRNAs expression in breast cancer, by Measuring the levels of circulating microRNAs in all samples by determining the fold expression of the detected microRNAs by RT qPCR. The study, involved 49 specimens were collected from the point of view of patients, particularly women with breast cancer, and blood samples were fully taken, in addition to taking samples in the same way as combining those healthy women (control group) 40 samples, and the work of extracting the RNA in the method of extracting the organic Chloroform of samples of the full blood of all samples in EDTA tubes. The study includes take information's patient (age, city, history, and to adding information from histopathology examination). The results were referred to increase in gene expression to miRNA let-7 family (type b) in patient samples (BC females) compared with control group (healthy women) by q-RT-PCR also measured IL-8 levels by ELISA technique and the results were referred to raise in IL-8 levels of BC females for serum samples compared with control group (healthy women). The cut off value for miR-let-7 was > 11.18-fold change with low sensitivity (58.5 %), high specificity (97.5 %) and good accuracy (81.8 %). Therefore, can be used MiRNA Let-7 as biomarker predictors of diagnosis of breast cancer. Conclusion: The current study was concluded, significant over productions of all investigated parameters referred to increase in levels microRNA let-7 family (let-7 type-b) seems to have potential role in breast prognosis of cancer progression when compared with healthy people. This study has found that all the investigated parameters were associated with moderate to high grade carcinoma.

Keywords: Let-7 microRNA, Breast Cancer, Biomarker, Interleukin Family, prognostic.

INTRODUCTION

In Iraq, breast cancer is the most prevalent type of the disease and the main reason why people die from it. (Sunita Srivastava et al., 2022). And Iraqi Cancer Registry 2013 and Ministry of Health, Republic of Iraq, 2017. Breast cancer is the most frequent type of female cancer in Iraq, accounting for almost one-third of all recorded according to statistics on female malignancies, there are the most recent Iraqi Cancer Registry (Alwan, 2016). In general, cancer is the second biggest cause of mortality worldwide, accounting for an estimated 9.6 million deaths in 2018. Cancer One in six fatalities around the world is attributed to smoking. Nearly two-thirds of all cancer deaths occur in countries with a low or middle income. (Ravindra et al., 2011; WHO, 2018).

Local studies in Iraq have shown that the short survival of affected individuals is primarily due to advanced stages at the time of presentation as a result of late diagnosis and treatment (Alwan, 2016; Alwan et al., 2017). Despite the fact that a national program for early detection of breast cancer has been in place in Iraq since 2001, a lack of knowledge, attitudes, and practice towards breast cancer has been demonstrated even among the educated population (Dange et al., 2017). Urging prompt action to raise community awareness about the importance of early detection of breast cancer and to extend coverage (Alwan et al., 2018).

Breast cancer diagnosis in women poses considerable challenges for early detection, correct staging, and breast cancer surveillance (Ganesh et al., 2022). As a result, a cost-effective and accurate screening strategy for this malignancy is still needed, as is the discovery of novel biomarkers to improve diagnosis, prognosis, and prediction (Sandip et al., 2020). According to the American Cancer Society (2012), the purpose of early breast cancer detection screening is to locate the cancer before it causes

symptoms. Screening is a method of detecting an illness in people who have no symptoms, such as cancer. Early detection refers to a method that allows breast cancer to be detected earlier than it would have been otherwise (Hashmi et al., 2018).

Small RNA molecules without codons are known as microRNAs (miRNAs) that are thought to play a significant role in the regulation of gene expression and cell function (Abbas et al., 2015). A variety of disease processes, including the development of cancer, have been reported to exhibit abnormal patterns of miRNA expression. With translational research efforts increasing dramatically in recent years (Casey et al., 2015).

The immune system's ability to identify and eradicate embryonic tumors during cancer development, and hence to operate has been disputed as a primary cancer defense for a long time. (Kosaka et al, 2013. in breast cancer patients, both the innate and adaptive immune systems have a role in avoiding relapse (Pandya et al., 2016).

The growing number of research demonstrating the presence of miRNAs in circulating serum/plasma raises the possibility of employing miRNAs as a biomarker for cancer and other disorders (Sampoornam , 2014). For example, studies by (Edwin et al., 2019) found that miRNA gene expression levels are abnormally altered in breast cancer, with miR-21, miR-155, and miR-10 b being overexpressed and miR-221, miR-125b, and miR-145 being down regulated, as mentioned by and (Padmaja et al., 2020). The goal of this study is to detect the expression of some microRNAs in Iraqi breast cancer women to be used as an early diagnostic marker (Li et al., 2020).

MATERIALS AND METHODS:

Patient and Control Samples: The study, involved 49 samples were collected from patients, particularly women with breast cancer, during period from October 2021 to April 2022 and blood samples were fully taken, in addition to taking samples in the same way as combining those healthy women (control group) 40 samples, and the work of extracting the RNA in the method of extracting the organic Chloroform of samples of the full blood of all samples in EDTA tubes. The study includes take information's patient (age, city, family history, number of children and to adding information from histopathology examination). In certain cases, first-degree relatives provided the information.

The samples were extracted less than a week in a hurry to prevent damage and were save at (-20 C), the RNA would break faster and be more damaged than DNA, as well as the micro-RNA level was measured for the MicroRNA Let-7 family compared to healthy women

Primers:

The qPCR Primers for miRNA let-7b (MIMAT0000063) were developed in this work by using the miRNA primer design tool and (The Sanger Center miRNA database Registry) to choose miRNA sequences. Whereas, qPCR housekeeping gene (GAPDH) (NM_001256799.3) were design in this study by using NCBI-Database and Primer3 plus design online. These primers and probe were provided by (Macrogen company, Korea)

RESULTS

The various ages of breast cancer patients were 37 to 80 years, with a mean of 52.33 8.31 years. The age range for the control group was 32 to 61 years, with a mean age of 49.33 7.01 years. Between the sick group and control group, the mean age did not change ($p = 0.073$). also, the frequency distribution of patients by age, and it is obvious that patients older than 50 accounted for 64.4 percent of cases, while patients younger than 50 accounted for 35.6 percent of cases.

Table (1) Age differences between breast cancer patients and healthy controls are compared.

Characteristic	Breast cancer group	Control group	<i>p.v</i>
Age			
Men \pm SD	52.33 \pm 8.31	49.33 \pm 7.01	0.073 I NS
Range	37 -80	32 -61	
30-39, <i>n</i> (%)	5 (10.2 %)	3 (7.5 %)	
40-49, <i>n</i> (%)	12 (24.5 %)	14 (35.0 %)	
50-59, <i>n</i> (%)	20 (40.8 %)	20 (50.0 %)	
60-69, <i>n</i> (%)	11 (22.4 %)	3 (7.5 %)	
70-80, <i>n</i> (%)	1 (2.0 %)	0 (0.0 %)	

The mean BMI for the patient group and control group, 28.21 \pm 2.28 kg/m² versus 28.44 \pm 3.42 kg/m², did not differ significantly ($p = 0.714$). According to BMI classification, 4 (8.2 percent) of the patients were classified as normal weight, 36 (73.5 percent) as overweight, and 9 (18.4 percent). All control participants and the majority of cancer cases in our study were from the governorate of Al-Diwaniyah.

Table (2): According to domicile, the frequency distribution of patients and controls

Characteristic	Breast cancer group <i>n</i> = 49	Control group <i>n</i> = 40
Residence		
Al-Diwaniyah, <i>n</i> (%)	39 (79.6 %)	40 (100.0 %)
Wasit, <i>n</i> (%)	4 (8.2 %)	0 (0.0 %)
Al-Najaf, <i>n</i> (%)	1 (2.0 %)	0 (0.0 %)
Babel, <i>n</i> (%)	2 (4.1 %)	0 (0.0 %)
Al-Muthana, <i>n</i> (%)	3 (6.1 %)	0 (0.0 %)

The frequency distribution of patients and controls by marital status as well as a comparison of the number of children in each group. control group was 1 to 10, while the patients with breast cancer had children between the ages of 1 and 9.

The frequency distribution of patients and control according to additional potential risk variables. Additionally, there was no discernible difference in the smoking rates between the sick group and the control group, 5 (10.2%) versus 2 (5.0%), respectively ($p = 0.609$). Additionally, there was no discernible difference in the prevalence of chronic medical conditions.

Table (3): The frequency distribution of patients and controls by marital status, as well as a comparison of the number of children in each group.

Characteristic	Breast cancer group <i>n</i> = 49	Control group <i>n</i> = 40	<i>p</i>
BMI (kg/m²)			
Mean \pm SD	28.21 \pm 2.28	28.44 \pm 3.42	0.714 I NS
Range	22.77 -33.3	22.49 -37.04	
Normal weight, <i>n</i> (%)	4 (8.2 %)	4 (10.0 %)	
Overweight, <i>n</i> (%)	36 (73.5 %)	26 (65.0 %)	
Obese, <i>n</i> (%)	9 (18.4 %)	10 (25.0 %)	
Characteristic	Breast cancer group <i>n</i> = 49	Control group <i>n</i> = 40	<i>P</i>
Marital status			
Married, <i>n</i> (%)	49 (100.0 %)	40 (100.0 %)	-----

Number of children			
Mean \pm SD	5.12 \pm 1.49	3.33 \pm 1.79	<0.001 I**
Range	1 -9	1 -10	

Table (4): The frequency distribution of cases compared to controls and other potential risk variables

Risk factor	Breast cancer group <i>n</i> = 49	Control group <i>n</i> = 40	<i>P.V</i>
Family History			
Positive, <i>n</i> (%)	7 (14.3 %)	3 (7.5 %)	0.505 Y NS
Negative, <i>n</i> (%)	42 (85.7 %)	37 (92.5 %)	
Smoking			
Smoker, <i>n</i> (%)	5 (10.2 %)	2 (5.0 %)	0.609 Y NS
Not smoker, <i>n</i> (%)	44 (89.8 %)	38 (95.0 %)	
Chronic illness			
Positive, <i>n</i> (%)	6 (12.2 %)	3 (7.5 %)	0.700 Y NS
Negative, <i>n</i> (%)	43 (87.8 %)	37 (92.5 %)	

several of the cases enrolled in the present study received chemotherapy accounting for 42 (85.7 %) and some received only single dose at time of starting the study while others received multiple doses with a range of 0-9 doses and a median of 2 doses.

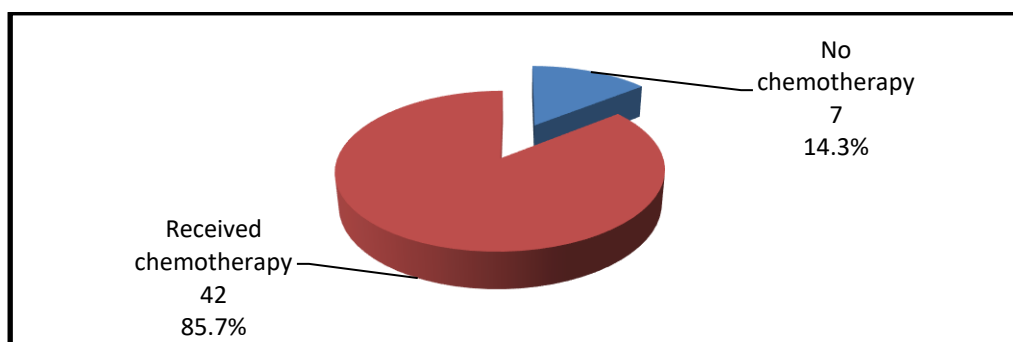


Figure (1): Pie graph illustrating the frequency distribution of breast cancer patients by chemotherapy

The gene expression of miR-let 7 and serum IL-8 in control group, the gene expression of miR-let-7 was considerably greater in the breast cancer group (13.93). (19.84) fold change compared to 4.31 (8.33) fold change, respectively, as indicated in the figure (p 0.001).

Table (5): miR-let 7 gene expression in cancer patients and the control group

Characteristic	cancer group	Corol group	<i>P</i>
miR-let-7			
Median (IQR)	13.93 (19.84)	4.31 (8.33)	< 0.001 M **
Range	0.37 -57.28	0.19 -19.97	

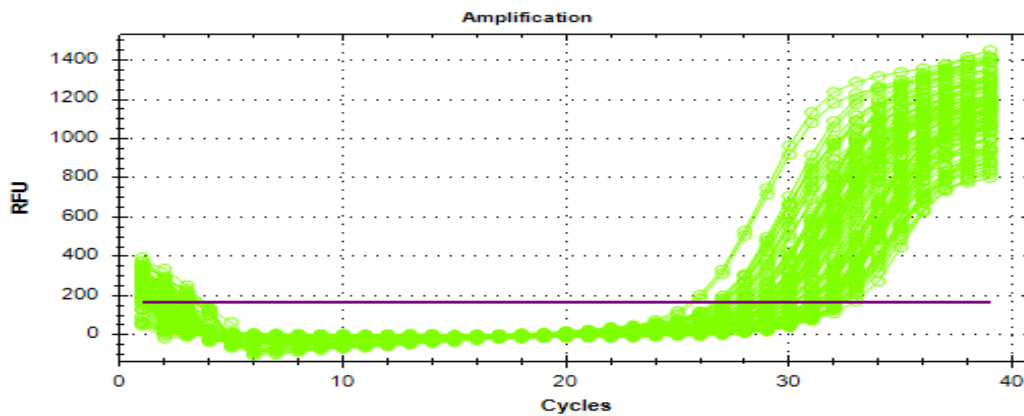


Figure (2) The Real time amplification plots of let 7 miRNA molecules in patients and healthy control samples

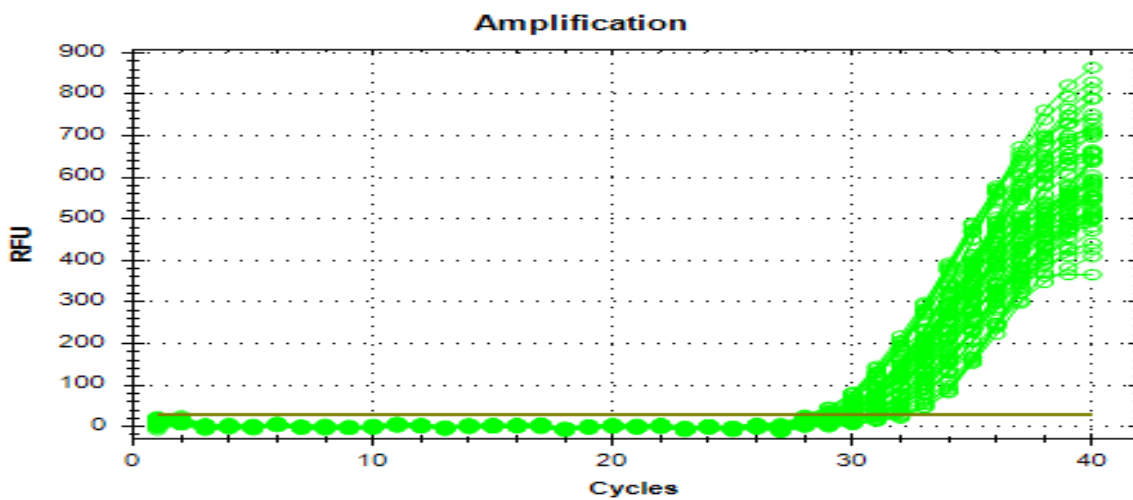


Figure (3) The Real time amplification plots of housekeeping GAPDH gene in patients and healthy control samples.

DISCUSSION

The lack of for this type of case-control study to be valid, there must be a statistically-significant age gap between the patients and the control group. significant variation in mean age. In our study, the mean age was 52.33 ± 8.31 years with a range of 37 to 80 years and this mean is nearly similar to another Iraqi study done in Basra in which the the average age of a 50 probability of a fatal outcome was (range 22-85 years) (Abood, 2018) and this study included 1,128 patients with breast cancer. According to (Dabakuyo-Yonli, and Arveux, 2020), the average age at diagnosis is 63 years old and this study was done France which revealed that the mean age of women with breast cancer in France is higher than that in our study.

It has been claimed that patients from the Arabian region present with breast cancer a full ten years before most of Countries in the West (Albeshan et al., 2018). In other Arab countries, age at diagnosis is the average was also 50 (Bahrain: Hamada et al. 2014; Oman: Mehdi et al. 2014; Lebanon: (Mehdi et al., 2014). It was discovered by (Chahine et al., 2015). However, the United States and Western Europe have substantially later average ages of diagnosis for breast cancer (61 and 63 years, respectively) (Sarav et al., 2011). According to these findings, breast cancer appears to strike younger women in Iraq and its neighbors than it does in the United States and Europe to the West. This underscores the necessity, for expanding screening programs for younger age groups of Iraqi women, while studies into age-specific incidence rates are required to determine a high risk at a younger age. In the current study we found no significant difference in mean BMI between control and patient's

groups and most of our patients and control subjects were either overweight or obese. This indicates that most Iraqi women above 50 of age are at high risk of obesity because of lack of exercise and sedentary lifestyle (Arti Tiwari et al., 2022).

In line with our study an Iraqi author has found that Seventy-five percent of patients had a body mass index (BMI) of 25 or more, making them either overweight or obese. BMI on average was 29.82. (SD, 6.2). (Mutar et al., 2019). Multiple studies have found that obesity increases the chance risk of having breast cancer after menopause (Neuhouser et al., 2015; Sebastiani et al., 2016). A meta-analysis of 34 studies involving over 2.5 million women and 23,909 cases of postmenopausal breast cancer found a positive association between an increase of 5 kg/m² in body mass index and an increased chance of developing this disease (Renehan et al., 2008). Menopausal women who haven't tried hormone replacement therapy (HT) are more likely to experience an increased risk of developing breast cancer after menopause (Picon-Ruiz et al., 2017).

In our study, we found that the prevalence rates of positive family history of breast cancer, smoking and chronic medical illness were more in patients with breast cancer than in control group, but statically speaking we found no significant difference. In one Iraqi study, it has been found that out of 1081 women with breast cancer, 204 have a history of breast cancer in their family making the rate of positive family history (18.9 %) (Alwan, 2017) and this rate is nearly similar to that found in our study (14.3 %). In another Iraqi study, positive family history was reported in 15.9 % in women with breast cancer and in 10 % of control group and there was a noticeable variation ($p = 0.023$) (Ali et al., 2019) while in our study they were 14.3 % versus 7.5 %, but the difference was not significant; and this difference is due to the larger sample size included in the study of (Ali et al., 2019) ($n = 338$) in comparison with our study ($n = 49$).

In one Iraqi study which enrolled 1093 women, it was found that the lifetime risk of breast cancer to be 13.4 % and that family history was a positive predictive of breast cancer meaning that family history is a risk factor (Hashim et al., 2019). The rate of positive family history in the study of (Hashim et al., 2019) is very close to our finding.

Regarding smoking, in one previous Iraqi study on 199 patients with breast cancer and 160 controls, it has been found that The prevalence of smoking among patients and the control group was not significantly different. (Naif et al., 2018); therefore, we agree with Naif et al in that rate of smoking is not significantly different between patients and control group. In another Iraqi study, rate of smoking was 30 % among women with breast cancer (Almoula et al., 2022) which is higher than that reported in our study (10.2 %) and this is probably because most of women in the study of Almoula et al., were before menopause and therefore, smoking plays more role as a risk of breast cancer in these women than age.

In line with our study, Ilic et al in 2014 reported no significant difference in rate of smoking between patients with breast cancer and control group, 31.9 % versus 35.1 % ($p > 0.05$). In Unites States, in a study enrolling 5791 patients with breast cancer and 17376 control subjects, it has been found that smoking in pre-menopausal women was not significant predictor of breast cancer; however, in post-menopausal women, smoking plays a significant role as a risk factor for breast cancer (Park et al., 2016). With respect to chronic medical illness, a recent study done in Canada by Arneja and Brooks in 2021 has found that the prevalence rate of chronic medical illness in patients with breast cancer was 57 %. This rate is higher than that reported in our study (12.2 %) and the difference is probably is due to variation in sample size since the sample size in the Canadian study ($n = 3372$) in comparison with our study ($n = 49$).

In another Iraqi study that included 570 patients with breast cancer, tumors that were positive for ER, PR, and HER-2 had correspondingly high rates of 66.8%, 64.2%, and 29.3%. (Alwan et al., 2017).

In our study, we found that miR-let-7 and serum IL-8 had higher levels in breast cancer in comparison with control and they should be used in combination to be markers of breast cancer. In line with our observation, a study done in Saudi Arabia found elevated level of miR-let-7 expression in patients with breast cancer in blood samples (Qattan et al., 2017). In addition, previous studies in Europe (Heneghan et al., 2010) and in India (Thakur et al., 2016) have shown increased expression of miR-let-7 in association with breast cancer.

Correlations of miR-let-7 gene expression with patients' characteristics are shown in table 3.8. The gene expression of miR-let-7 showed significant positive correlation to stage of disease ($r = 0.530$; $p < 0.001$), her2neu immunohistochemical expression ($r = 0.609$; $p < 0.001$), estrogen receptors (ER) ($r = 0.426$; $p = 0.002$) and progesterone receptors (PR) ($r = 0.426$; $p = 0.002$).

CONCLUSION

On the basis of the current study, significant over productions of all investigated parameters referred to increase in levels micro RNA let-7 family (let-7 type-b) seems to have potential role in breast cancer progression and prognosis when compared with healthy people. This study has found that all the investigated parameters were associated with moderate to high grade carcinoma. When this study correlates the investigated parameters microRNA let-7 family (type b) which found in a very high percentage in patient with breast cancer females. This study may clarify with microRNA let-7family could be a very important predictive marker for breast cancer and treatment responsiveness.

DATA AVAILABLE STATEMENT:" The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request."

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