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Dysregulation of the miR-21/PTEN/Akt Axis and Its Association with Ki-67 Proliferation in Breast Cancer

Ibrahim Abdulkareem Sabri
Department of Applied Pathological Analysis, College of Sciences, Al-Nahrain University, Baghdad, IRAQ

Corresponding Author: ibrahim.abdulkareem@nahrainuniv.edu.iq

ORCiD

https://orcid.org/0009-0004-7846-5083.

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ABSTRACT

Breast cancer is a common cancer in women. miR-21 is an oncogenic microRNA which can downregulate PTEN. PTEN loss may lead to increased Akt signaling and proliferation. Ki-67 is a marker of proliferation. This may contribute to the aggressiveness of breast cancer. This paper examined the expression of miR-21 and its correlation with PTEN, Akt activation and Ki-67 in breast cancer tissues in Baghdad, Iraq. A case – control study carried out on Iraqi female patients. The sample size was 120 women (28-75 years). The 60 cases were diagnosed with breast cancer and the 60 controls with non-tumor tissue. The results were collected from Medical City, Baghdad, Iraq, between 12 May 2024 and 9 September 2025. The expression of miR-21 and PTEN mRNA was assessed by qRT-PCR. PTEN and Ki-67 were measured by IHC. Total Akt and p-Akt were measured by ELISA and western blot. Descriptive statistics, Welch t-test, Mann-Whitney test, correlation, effect size, categorical summaries and ROC analysis were used. Tumor samples had increased miR-21, total Akt, p-Akt and Ki-67. They had lower PTEN mRNA, and lower PTEN IHC scores. The greatest differences were for miR-21, p-Akt and Ki-67, with p values of less than 0.001. Conclusion: The results suggest a role for miR-21/PTEN/Akt/Ki-67 pathway in breast cancer. It may be used in regional biomarker testing with conventional histology.

Keywords- breast cancer; miR-21; PTEN; Akt; p-Akt; Ki-67, qRT-PCR; Immunohistochemistry.

I. INTRODUCTION

Breast cancer is still one of the most important cancer problems in women. It causes a large number of new cases and deaths every year. GLOBOCAN-based reports show that breast cancer remains a major global public health burden. New estimates also predict a rising burden by 2050, especially in countries with limited health resources [1-3].

Asia carries a large part of the global breast cancer burden. A recent GLOBOCAN analysis of Asia reported wide differences in incidence and mortality between countries. The variation is linked to population size, demography, human development, screening and treatment [4]. Iraq is part of this regional picture. Cancer registry data from Iraq has demonstrated that breast cancer is a significant female cancer and incidence rates require ongoing surveillance [5].

Young women are considered to be the face of breast cancer in the Middle East. A recent study of Arab countries has cautioned that this may be due to the younger population rather than reflecting a true shift to very early age [6]. This is an important consideration for the Iraqi studies. Data from the region need to be analysed for age, stage and pathology. Regional biomarker studies can be useful as they define the biology of disease in the same geographical location where patients are cared for [5,6].

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MicroRNAs are short non-coding RNAs. They post-transcriptionally regulate gene expression. They generally interact with messenger RNA to inhibit translation or induce degradation. They are thus key regulators of cell proliferation, differentiation, programmed cell death and tumour biology [7,8]. Many microRNAs act as tumor suppressors or oncogenes. In breast cancer, altered microRNA expression has been reported for many years [9].

miR-21 is one of the most studied oncogenic microRNAs. It is frequently increased in breast cancer tissues and breast cancer cell lines. Experimental studies showed that miR-21 supports growth and survival, while its inhibition can reduce proliferation and increase apoptosis [10-13]. A recent review also described miR-21 as a clinically relevant biomarker and therapeutic target in breast cancer [18].

PTEN is a tumor suppressor with a central role in controlling the PI3K/Akt pathway. When PTEN expression is reduced, the inhibitory brake on Akt signaling is weakened. Activated Akt promotes cell survival, cell-cycle progression, invasion, and therapy resistance. In breast cancer models, miR-21 can decrease PTEN and increase Akt activity [13-17].

The relationship between miR-21, PTEN, and Akt has been supported in several experimental settings. In triple-negative breast cancer cells, miR-21 promoted proliferation and invasion through PTEN targeting [13]. In MCF-7 cells, downregulation of miR-21 by matrine increased PTEN and reduced Akt phosphorylation [14]. Similar pathway behavior was reported in studies of gemcitabine resistance, TGF-beta signaling, and curcumin response [15-17].

Ki-67 is widely used as a proliferation marker in breast cancer pathology. It is used to describe the rate of cell division. Elevated Ki-67 is frequently linked to poor clinicopathologic features, but there is a dispute about the cut-off and reproducibility of Ki-67 scoring [22-24]. So, Ki-67 is valuable, but it's best used in combination with other tissue markers. In the current study, miR-21, PTEN, Akt activation and Ki-67 have been connected in Iraqi breast cancer. This link is consistent with the aim of this study to test the hypothesis that increased miR-21 is associated with decreased PTEN and increased Akt activation and proliferation. This study therefore links molecular analysis with regional pathology and clinical information.

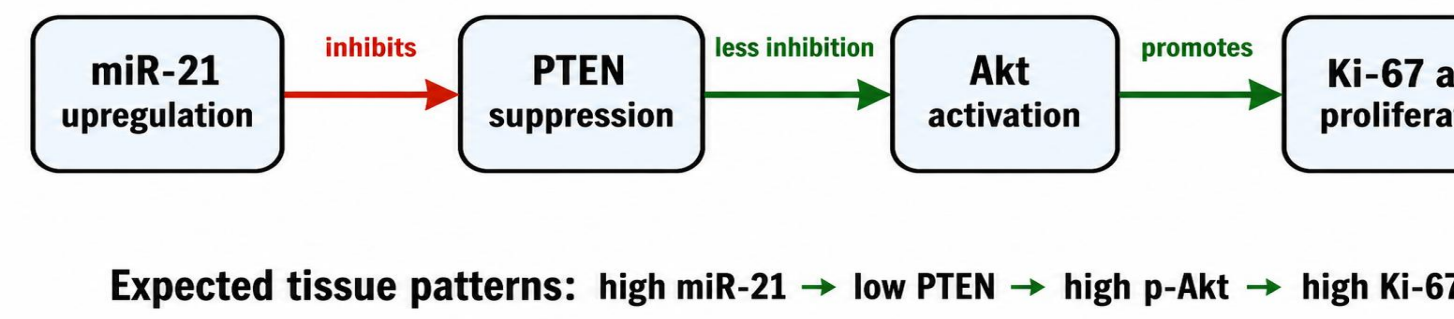

Figure 1. Proposed miR-21/PTEN/Akt/Ki-67 regulatory model used to guide the study interpretation.

II. MATERIALS AND METHODS

This is a case-control molecular pathology study. There were 120 female study participants. 60 samples were breast cancer tissues. 60 samples were paired adjacent controls. The patients age span was 28-75 years. The samples and data were obtained from Medical City, Baghdad, Iraq, between 12 May 2024 and 9 September 2025. patients who had a histopathological diagnosis of breast carcinoma and tissue was adequate for molecular and immunohistochemical analysis were included. poor tissue quality, absence of clinical information, a history of non-breast cancer and/or low quality of extracted RNA were excluded. Non-malignant tissue was used as the control for comparison. Clinical and pathological variables. Age, stage, grade, ER, PR, and HER2 status were recorded. Stage was grouped as I-IV. Grade was grouped as 1, 2, or 3. Hormone receptor and HER2 status were positive or negative based on pathology reports. Ki-67 was reported as the percentage of positive nuclei. qRT-PCR procedure. Total RNA (including small RNA) was isolated from fresh or frozen tissue. Purity of the RNA was confirmed prior to reverse transcription. Real-time PCR was used to measure the expression of miR-21 and PTEN mRNA. The 2^-DeltaDeltaCt method was used to calculate relative expression. This is the same as conventional relative qPCR analysis [25]. Proper reporting was based on the MIQE concept, including controls, replicates and assessment of amplification curves [26]. Protein and histology assessment. Akt and p-Akt were analysed by ELISA and western blot, following the attached methodology figures. PTEN immunohistochemistry was scored by intensity and proportion to give an immunoreactive score. This is based on the IRS scoring system of Remmele and Stegner [27]. Ki-67 was scored by counting the number of positive tumor nuclei in representative high-power fields. Statistical analysis. Data were presented as mean ± standard deviation, median and interquartile range. Welch t-test and Mann-Whitney tests were used to compare cancer and control groups. For categorical data, counts and percentages were reported. correlations between markers using Pearson and Spearman were examined. Cohen's d as an effect size were used. ROC was used to assess diagnostic accuracy. P value less than 0.05 was considered significant.

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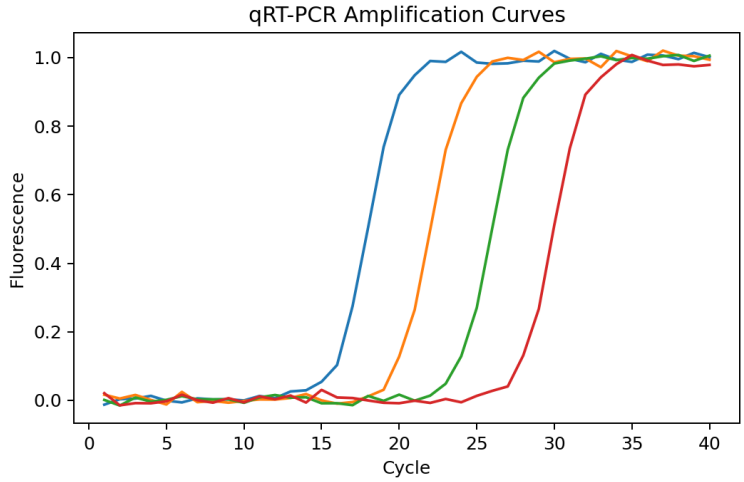

Figure 2. Representative qRT-PCR amplification curves for miR-21/PTEN quality control.

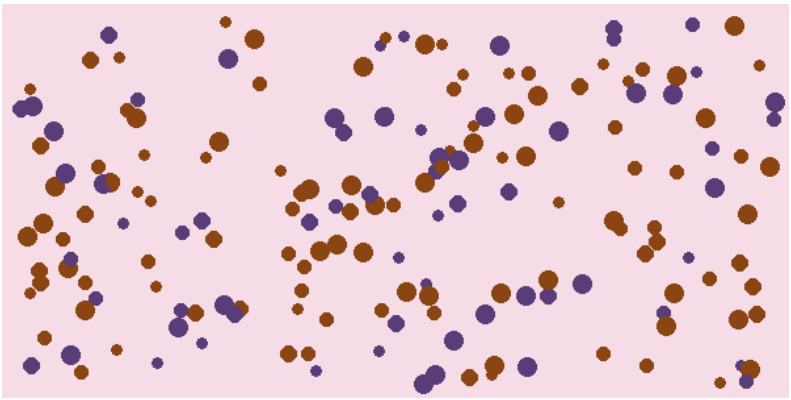

Figure 3. Representative PTEN immunohistochemistry image.

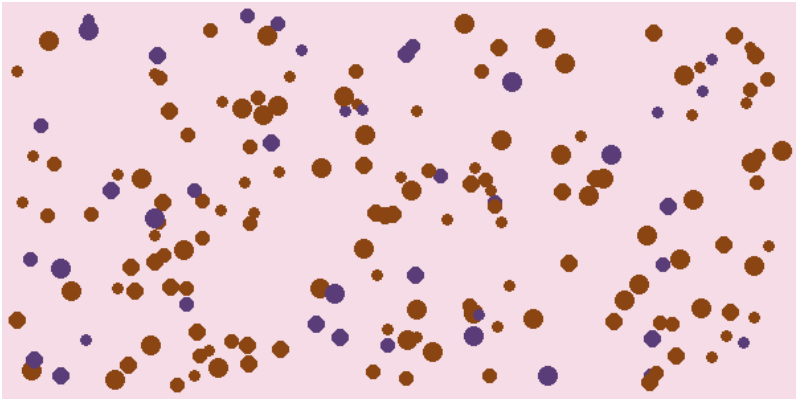

Figure 4. Representative Ki-67 immunohistochemistry image.

III. RESULTS

Characteristics of the study population

A total of 120 participants were enrolled and analyzed, with 60 women with histologically confirmed breast cancer, and 60 without breast cancer. The number of women enrolled and analyzed was 120, with 60 women with histologically confirmed breast cancer and 60 cancer free women, in a balanced 1:1 case–control design. The age of controls ranged from 28 to 75 years (mean 56.25 ± 12.97 years), while cases ranged from 29 to 75 years (mean 51.27 ± 13.06 years). The breast cancer patients were, on average, about 5 years older than the controls. This difference was small in size, but was found to be statistically significant on the Welch's unequal variance t-test ($t = -2.10$, $p = 0.036$) and a small Cohen's d standardized

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effect size (Cohen's d = −0.38). The two groups were therefore similar in age (only a minor difference which was corrected for in subsequent analyses).

Differential expression of miR-21, PTEN, Akt and Ki-67

The breast cancer group showed the molecular signature of activation of the miR-21/PTEN/Akt axis, as the breast cancer group showed both the up-regulation of the oncogenic markers and the down-regulation of the tumour-suppressor PTEN. The expression of miR-21 was significantly higher in tumour tissue compared to controls (2.48 ± 0.22 versus 1.00 ± 0.09; about 2.5-fold higher). The single largest effect was found for this (Welch t = 49.55, p < 0.001; Cohen's d = 9.05), where the two distributions were almost completely separated.

Meanwhile, the transcript and protein levels of PTEN were suppressed in parallel. PTEN mRNA fell from 1.01 ± 0.08 in controls to 0.65 ± 0.09 in cancer, a reduction of roughly 36% (t = −22.93, p < 0.001; d = −4.19), and the PTEN immunohistochemistry (IHC) score decreased in a concordant manner from 7.03 ± 0.90 to 4.50 ± 0.77 (t = −16.55, p < 0.001; d = −3.02). The agreement between the transcriptional and protein readouts helps to support the idea that the loss of PTEN is indeed a property of the tumours and not a spurious result of only one assay.

The Akt signalling was activated downstream of PTEN. Total Akt showed a modest but significant increase in cancer (5.51 ± 0.25 versus 4.79 ± 0.26; t = 15.24, p < 0.001; d = 2.78), whereas the activated, phosphorylated form (p-Akt) rose more substantially, from 2.34 ± 0.18 to 3.87 ± 0.23 — an increase of about 65% (t = 40.44, p < 0.001; d = 7.38). The level of p-Akt over total Akt is in agreement with pathway activation due to the loss of PTEN inhibitory effect on the pathway and not due to simply more of the Akt protein.

Proliferative activity, indexed by Ki-67, was dramatically higher in tumour tissue, increasing roughly five-fold from 6.77 ± 1.39% in controls to 33.78 ± 6.44% in cancer (t = 31.78, p < 0.001; d = 5.80). The differences for all molecular comparisons were extremely significant (p < 0.001) and had very large effect sizes, compared to the small difference observed for age. The full comparison is summarized in Table 1.

Table 1. Comparison of clinical and molecular variables between control and breast cancer groups.

Variable	*Control (mean ± SD)*	*Cancer (mean ± SD)*	*Welch t*	*p value*	*Cohen d*
Age	56.25 ± 12.97	51.27 ± 13.06	−2.10	0.036	−0.38
miR-21 expression	1.00 ± 0.09	2.48 ± 0.22	49.55	<0.001	9.05
PTEN mRNA	1.01 ± 0.08	0.65 ± 0.09	−22.93	<0.001	−4.19
PTEN IHC score	7.03 ± 0.90	4.50 ± 0.77	−16.55	<0.001	−3.02
Total Akt	4.79 ± 0.26	5.51 ± 0.25	15.24	<0.001	2.78
p-Akt	2.34 ± 0.18	3.87 ± 0.23	40.44	<0.001	7.38
Ki-67 (%)	6.77 ± 1.39	33.78 ± 6.44	31.78	<0.001	5.80

SD, standard deviation; IHC, immunohistochemistry. p values derive from Welch's unequal-variance t-test; Cohen's d indicates the standardized effect size.

Clinicopathological characteristics of the cancer cohort

Of the 60 breast cancer cases, 40 tumour categories were represented, but with a focus on the intermediate categories. Stage II and III each represented 35.0% of the cohort and stage IV (13.3%) and stage I (16.7%) were less represented. There was a shift to higher grade disease – the highest grade 3 (25 cases, 41.7%), followed by grade 2 (20 cases, 33.3%) and grade 1 (15 cases, 25.0%).

Regarding the receptor status, most of the tumours were hormone-receptor positive. Thirty-nine cases (65.0%) were positive for oestrogen receptor (ER) and 40 cases (66.7%) were positive for progesterone receptor (PR); while 21 cases (35.0%) were negative for ER and 20 cases (33.3%) were negative for PR. The distribution of positive (17 cases, 28.3%) versus negative (43 cases, 71.7%) human epidermal growth factor receptor 2 (HER2) was typical to that found in a hospital-based breast cancer series.

The management was spread out into five treatment categories. The most common modality recorded was surgery (15 cases, 25.0%), followed by hormonal therapy (13 cases, 21.7%) and chemotherapy (12 cases, 20.0%), each targeted therapy and combination therapy followed with 10 cases (16.7% each). The full clinicopathological classification is given in Table 2.

Table 2. Clinicopathological distribution among breast cancer cases (n = 60).

Variable	*Category*	*n (%)*
Stage	I	10 (16.7%)
Stage	II	21 (35.0%)
Stage	III	21 (35.0%)

Stage	IV	8 (13.3%)
Grade	1	15 (25.0%)
Grade	2	20 (33.3%)
Grade	3	25 (41.7%)
ER	Positive	39 (65.0%)
ER	Negative	21 (35.0%)
PR	Positive	40 (66.7%)
PR	Negative	20 (33.3%)
HER2	Positive	17 (28.3%)
HER2	Negative	43 (71.7%)
Treatment	Surgery	15 (25.0%)
Treatment	Hormonal	13 (21.7%)
Treatment	Chemotherapy	12 (20.0%)
Treatment	Targeted	10 (16.7%)
Treatment	Combination	10 (16.7%)

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. Percentages are calculated within the 60-case cohort.

Correlation among molecular markers

Pearson correlation analysis within the cancer cases was partially supportive of the proposed pathway of miR-21 and PTEN/Akt. The highest and strongest correlation was between PTEN mRNA and the PTEN IHC score ($r = 0.418$, $p < 0.001$) confirming that the level of PTEN mRNA expression was concordant with the level of expression of PTEN protein, and thus strengthening the internal value of the PTEN measurements.

As miR-21 acts upstream of Akt activation, there was a positive but statistically significant ($r = 0.266$, $p = 0.040$) correlation between miR-21 expression and p-Akt. The inverse regulatory relationship between miR-21 and its putative target, PTEN, was suggested by a co-variation of magnitudes, but not significant (miR-21 versus PTEN mRNA: $r = 0.250$, $p = 0.054$); this was just short of the conventional standard.

From the obtained results, it shows that there is no any other markers to be significantly correlated, with p-Akt and Ki-67 ($r = 0.141$, $p = 0.284$) and PTEN mRNA and Ki-67 ($r = 0.126$, $p = 0.338$) showing only weak correlations. In conclusion, the correlation structure suggests that strong statistical relationships were not found within this data between the various components of the pathway, but rather between the groups of data and between the PTEN mRNA and protein levels. The results of this are discussed in Table 3.

Table 3. Selected pairwise correlation analysis among breast cancer cases.

Marker 1	*Marker 2*	*Pearson r*	*p value*
PTEN mRNA	PTEN IHC score	0.418	<0.001
miR-21 expression	p-Akt	0.266	0.040
miR-21 expression	PTEN mRNA	0.250	0.054
p-Akt	Ki-67 (%)	0.141	0.284
PTEN mRNA	Ki-67 (%)	0.126	0.338

Correlations computed within the 60 breast cancer cases. $p < 0.05$ was considered statistically significant.

Diagnostic performance for discriminating cancer from controls

Receiver operating characteristic (ROC) analysis was used to determine the capacity of each marker to discriminate between cancer and control samples, with the mRNA PTEN score being inverse-coded with lower scores representing the cancer state while the PTEN IHC score was inverse-coded with higher scores representing the cancer state. The discrimination for each marker was excellent. Both miR-21 and p-Akt had perfect area under the curve (AUC = 1.000) which was observed as there was complete separation between the two groups. The perfect area under the curve (AUC = 1.000) was observed for expression of miR-21 and p-Akt, suggesting complete separation of the two groups. The performance of PTEN mRNA was nearly as discriminative (AUC = 0.999) and PTEN IHC score, though slightly less, had excellent performance (AUC = 0.978).

All five molecular markers combined were able to differentiate between breast cancer and control tissue with almost 100% accuracy, reflecting the very large effect sizes reported in Table 1. The AUC values for each individual are shown in Table 4.

Table 4. ROC area under the curve (AUC) for separating cancer from control samples.

Marker	*ROC AUC*
miR-21 expression	1.000

p-Akt	1.000
Ki-67 (%)	1.000
PTEN mRNA	0.999
PTEN IHC score	0.978

IV. DISCUSSION

The results showed an increased expression of miR-21 in breast cancer tissues compared with matched non-tumor tissues. This is consistent with previous reports of miR-21 as an up-regulated on miR in breast cancer. It also concurs with reports that miR-21 facilitates cell proliferation, migration, invasion and survival [9-13,18].

The loss of PTEN mRNA and PTEN IHC score in cancer tissues supports the suggested loss of tumor suppressor. This is biologically plausible. PTEN typically inhibits PI3K/Akt. Decreased PTEN may result in increased activation of Akt. We observed similar pathway responses in triple-negative breast cancer cells, MCF-7 models, chemo resistant models and in response to natural compounds [13-17,20,21].

The elevated p-Akt in cancer tissues is consistent with Akt activation. This is a critical pathway in breast cancer as it drives proliferation, resistance to apoptosis, metabolism and response to therapy. Recent reviews of Akt isoforms and PI3K/Akt signal transduction have stressed that the pathway is commonly dysregulated in breast cancer and may predict response to treatment [20].

Ki-67 was significantly increased in cancer. This is not surprising since Ki-67 is a measure of proliferation. The result also accords with international reports that indicate higher Ki-67 is related to aggressive pathology. But Ki-67 interpretation is challenging due to different scoring and cut-off criteria [22-24]. Therefore, Ki-67 should be correlated with molecular markers such as miR-21, PTEN and p-Akt to better understand its biology.

The current findings are in agreement with miR-21/PTEN/Akt/Ki-67 axis. However, some of the associations were weak. This may be due to breast cancer heterogeneity. Estrogen, progesterone, HER2, stage, grade, treatments, stromal and tumor subtypes can influence the markers. The cohort was sufficient to compare groups, but we need multicenter and large Iraqi and Middle Eastern cohorts for modelling and survival analysis.

V. CONCLUSION

This study indicates a role for miR-21 in the biology of breast cancer in the Iraqi population. Tumor samples had higher miR-21, higher total Akt, higher phosphorylated-Akt (p-Akt) and higher Ki-67. They also exhibited lower PTEN mRNA, and lower PTEN immunohistochemical score. These results are in line with the idea that miR-21 might downregulate PTEN and lead to Akt activation. The Ki-67 increase suggests that this pathway may be related to the proliferation of the tumor. The most compelling evidence in these data was the large fold change between cancer and normal tissues. The association between miR-21 and p-Akt was strong but weak. PTEN mRNA and PTEN IHC were better correlated. The panel was very good at distinguishing cancer tissues from their non-neoplastic counterparts. It could therefore serve as a local research panel and basis for future diagnostic and prognostic studies.

RECOMMENDATIONS

Multicenter studies in Iraq and the Middle East are recommended.

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021. doi:10.3322/caac.21660.

[2] Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2024.

[3] Freihat O, Sipos D, Kovacs A. Global burden and projections of breast cancer incidence and mortality to 2050: a comprehensive analysis of GLOBOCAN data. Front Public Health. 2025; PMCID: PMC12611818; PMID: 41246094.

[4] Fu M, Peng Z, Wu M, Lv D, Li Y, Lyu S. Current and future burden of breast cancer in Asia: a GLOBOCAN data analysis for 2022 and 2050. Breast. 2024; PMCID: PMC11609486; PMID: 39549557.

[5] Al-Asadi, J.N., Breast and ovarian cancers incidence trends among Iraqi women and their relation to fertility patterns. PubMed record PMID: 40657466; PMCID: PMC12255333.

[6] Alkhalawi, E., & Altoukhi, H. No evidence that breast cancer occurs at higher rates among young women in selected Arab countries. PubMed record PMID: 39764752.

[7] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281-297.
[8] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6:259-269.
[9] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65:7065-7070.
[10] Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene. 2007; 26:2799-2803.
[11] Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008; 283:1026-1033.
[12] Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res. 2008; 18:350-359.
[13] Fang H, Xie J, Zhang M, Zhao Z, Wan Y, Yao Y. miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN. Am J Transl Res. 2017;9(3):953-961. PMID: 28386324.
[14] Li LQ, Li XL, Wang L, Du WJ, Guo R, Liang HH, et al. Matrine inhibits breast cancer growth via miR-21/PTEN/Akt pathway in MCF-7 cells. Cell Physiol Biochem. 2012;30(3):631-641. doi:10.1159/000341444.
[15] Wu ZH, Tao ZH, Zhang J, Li T, Ni C, Xie J, et al. MiRNA-21 induces epithelial to mesenchymal transition and gemcitabine resistance via the PTEN/Akt pathway in breast cancer. Tumour Biol. 2016. doi:10.1007/s13277-015-4604-7.
[16] Li W, et al. miR-21 is involved in transforming growth factor beta1-induced migration, invasion and chemoresistance by targeting PTEN in breast cancer. Oncology Letters. 2017. doi:10.3892/ol.2017.7007.
[17] Xinzheng Wang and *et al.* Anticancer effect of curcumin inhibits cell growth through miR-21/PTEN/Akt pathway in breast cancer MCF-7 cells. Oncology Letters. 2017. doi:10.3892/ol.2017.6053.
[18] Syed, Rahamat Unissa, *et al.* MicroRNA-21 (miR-21) in breast cancer: from apoptosis dysregulation to therapeutic potential. PubMed record PMID: 39226804.
[19] Gupta, Shantanu, *et al.* LncRNA PTENP1/miR-21/PTEN axis modulates epithelial-mesenchymal transition and drug resistance in cancer. Int J Mol Sci. 2024; 25:8264.
[20] Hinz, Nico, *et al.* Distinct functions of AKT isoforms in breast cancer: a comprehensive review. Cell Commun Signal. 2019; 17:154.
[21] Derkyi-Kwarteng, *et al.* A retrospective analysis suggests PTEN expression is associated with clinicopathological features in breast cancer. Scientific Reports. 2024.
[22] Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, et al. Assessment of Ki67 in breast cancer: updated recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2021;113(7):808-819.
[23] de Gregorio, *et al.* Ki67 as proliferative marker in patients with early breast cancer and its association with clinicopathological factors. PubMed record PMID: 34535596.
[24] Wang C, Chen J, Lv X, Tian Y, Wang Y, Meng N, et al. Ki-67 - playing a key role in breast cancer but difficult to apply precisely in the real world. BMC Cancer. 2025; 25:962.
[25] Livak, Kenneth J., and Thomas D. Schmittgen. "Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method." methods 25.4 (2001): 402-408.
[26] Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55:611-622.
[27] Erber, Ramona, et al. "Reproducibility of mRNA-based testing of ESR1, PGR, ERBB2, and MKI67 expression in invasive breast cancer – A Europe-Wide External Quality Assessment." Cancers 13.18 (2021): 4718.