Al-Kindy College Medical Journal (KCMJ)

Research Article

Age-Related Variations in Tumor Suppressor Genes and Hormone Receptor Expression in Breast Cancer Patients

Mehri Mirhaj Mohammedsalih [1,2]* Ismail Salih Ibrahim Kakey [2]

1. MLT department, Koya Technical Institute, Erbil Polytechnic university, Kurdistan Region. Iraq.
2. Department of Biology, Faculty of Science and Health, Koya University, Koya 44023, Kurdistan Region-F.R. Iraq.

* Corresponding author's email: mehri.salih@epu.edu.iq

Article history:
Received 8 September 2025
Accepted 12 October 2025
Available online 1 December 2025

DOI: 10.47723/n8xgrz52

Keywords: Immunohistochemistry; Breast Cancer; Tumor suppressor genes; Hormone receptors.

ABSTRACT

Background: This study investigated the immunohistochemical expression of hormone receptors (ER, PR, HER2, Ki-67) and tumor suppressor genes (PTEN, p53) in women with breast cancer from Erbil, Iraq, focusing on how these expressions relate to patient age.

Subjects and Methods: Between April and October 2024, 120 female breast cancer patients took part in a cross-sectional study. Immunohistochemical analysis was performed to evaluate the expression of PTEN, p53, ER, PR, HER2, and Ki-67.

Results: Age was significantly positively correlated with PTEN loss, which was shown in 86.7% of patients ($p < 0.001$). Additionally, there was a positive connection between age and the expression of ER and PR ($\chi^2 = 26.67$ for ER and 15.59 for PR). There was less correlation between HER2 positive and age. 34.2% of tumors had P53 expressions, which were unrelated to age. All tumors were discovered to contain Ki-67, indicating that active proliferation is taking place. Furthermore, there were notable variations in the expression levels of PTEN, P53, ER, PR, Ki-67, and HER2 according to tumor grade, which is a sign of the biology and aggressiveness of the tumor.

Conclusions: While P53 expression does not significantly change with patient age, PTEN, ER, PR, and HER2 expression do. Ki-67 universal expression indicates that tumor proliferation is high in this population. Tumor aggressiveness and biology variations are highlighted by the variance in important biomarker expression by tumor grade.

Introduction

Cancer is a group of abnormal cells characterized by faulty genes that enable the continuous and uncontrolled growth of either somatic or germ cells [1]. Many disorders commonly affect the breast, usually presented as lumps, most of which are benign or non-cancerous growths [2]. Immunohistochemistry (IHC) is widely used for the semi-quantitative evaluation of protein expression of markers such as HER2, ER, PR, and Ki-67 that direct diagnosis and treatment [3-5]. The proliferation marker Ki-67, hormone receptors such as HER2, progesterone receptor (PR), estrogen receptor (ER), and tumor suppressor genes PTEN and P53 are some of the most researched markers of breast cancer [6]. The tumor suppressor P53 is one significant transcription factor that regulates several biological processes. Immunohistochemistry's ability to identify P53 can help

evaluate tumor behavior and potential responses to specific treatments [7]. By stopping cells from growing in response to a range of stimuli, including hypoxia, malnutrition, DNA damage, and excessive proliferation signals,

P53 inhibits the growth of tumors in cancer [8]. Maintaining genomic stability, promoting the cell cycle, and controlling apoptosis all depend on PTEN and P53. Tumor behavior and treatment effectiveness can be significantly impacted by variations in tumor expression [8,9]. Similarly, while choosing a treatment strategy, HER2 expression, and hormone receptor status (ER, PR) are crucial considerations, and Ki-67 is a marker of tumor aggressiveness and proliferation [10]. Biomarkers may provide additional information about an individual's prognosis and response to treatment. These days, breast cancer is treated using a range of indicators, including tissue expression of Ki-67, human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) [11]. Hormone receptor-positive tumors, such as ER and PR-positive breast cancer cells, usually respond well to endocrine treatments. Assessing the state of these receptors is necessary to determine therapy strategies. One protein that encourages cell division is called HER2. Around 15–25% of breast cancers have HER2 overexpression or amplification, which is linked to a more severe type of the illness [12]. Higher Ki-67 indices are frequently associated with worse prognoses, and the expression levels of Ki-67 offer information about the tumor's aggressiveness and possible response to chemotherapy [13]. Although the biological significance of these indicators is well established, little is known regarding how they manifest in relation to patient age, particularly in some regions such as Iraq and Kurdistan. The age difference between the patients with breast cancer in the Erbil region and patients in the West is considerable, giving rise to special clinicopathological characteristics not yet clarified in detail in the literature. The purpose of the current study is to fill the knowledge gap in understanding the expression patterns of key biomarkers and their correlation with the age of women with breast cancer in the city of Erbil.

The statistical analysis was done with GraphPad Prism 8.4.3 software. In understanding associations between categorical variables, such as age and biomarkers expression, Chi-square tests were employed. Comparisons in the averages of the continuous variables were done with the help of ANOVA analysis. The confounding factors such as age, tumor grade, histologic subtype were taken into consideration in the multi-variable regression model applied in the study. The significance level was set to P-values < 0.05.

Subjects and Methods

Study Design and Participants:

This cross-sectional study involved 120 female breast cancer patients from the Rzgari, Par, and Erbil Teaching Hospitals between April and October 2024. Written informed consent was provided by each subject. Ethical approval was formally granted by Koya University in January 2025 prior to manuscript preparation (the ethical approval is dated January 2025, while the study period was April–October 2024. This discrepancy arose because the ethics committee requested modifications to the application after the inclusion of additional required sections), after sample collection. Inclusion criteria consisted of patients with breast cancer at all clinical stages as confirmed by histology (surgical incisional biopsy), with the exclusion of patients who had received neoadjuvant treatment.

Sample Collection and Preparation:

Breast tissue biopsies were fixed in 10 percent formalin neutral buffered, then embedded in paraffin and sectioned at 4 μm thickness. For IHC, the sections were mounted on slides coated with poly-L-lysine.

Immunohistochemistry (IHC):

Histology verification used hematoxylin and eosin stains. The IHC stains for PTEN, P53, ER, PR, HER2, and Ki-67 including antigen retrieval, endogenous peroxidase blocking, nonspecific binding blocking, overnight primary antibody incubation, and development with LSAB2, also followed accepted standards. All tests were monitored with appropriate positive and negative controls.
Paraffin blocks of ductal carcinoma breast cancer tissues were first cut before the PTEN, P53, ER, PR, HER2, and Ki-67 immunohistochemical stains were performed per Dako protocol. The antibodies acted as follows:

- PTEN: Clone 6H2.1, Dako, cat. No. M3627, 1:100.
- P53: Clone DO-7, Dako, cat. No. M7001, 1:50.
- ER: Clone 1D5, Dako, cat. No. M7047, 1:100.
- PR: Clone PgR 636, Dako, cat. No. M3569, 1:50.
- HER2: Polyclonal A0485, Dako, cat. No. A0485, 1:500.
- Ki-67: Clone MIB-1, Dako, cat. No. M7240, 1:200.

Sections were stained using the EnVision+ System-HRP (Dako) and the DAB (diaminobenzidine) chromogen. Hematoxylin was used for counterstaining.

IHC Scoring:

Expression levels were semi-quantitatively evaluated using previously established criteria.

- ER and PR positivity were defined as nuclear staining in ≥1% of tumor cells per ASCO/CAP guidelines 14.
- HER2 expression was scored as 0, 1+, 2+, or 3+ according to ASCO/CAP criteria. Cases with 2+ (equivocal) staining were further assessed by fluorescence in situ hybridization (FISH) to confirm HER2 gene amplification.
- PTEN expression was considered "lost" when complete absence of cytoplasmic and nuclear staining was observed in tumor cells, with normal stromal cells serving as internal positive controls.
- P53 was scored positive if >10% of tumor nuclei showed strong staining.
- Ki-67 labeling index was quantified as the percentage of positively stained tumor nuclei in at least 500 cells counted in hot-spot areas. A cut-off points of 20% was used to categorize tumors into low (<20%) and high (≥20%) proliferative groups.

Ethical approval was obtained from the Department of Biology, Faculty of Science and Health, Koya University, by ethics form (001 Bio) on Jan 2025. Patient privacy and confidentiality were maintained throughout the study.

Results

The study included 120 breast cancer patients with mean ages (38.66 ± 0.59 years). The majority of patients were aged 41-45 years. PTEN loss was observed in most patients (86.7%), and strongly correlated with increasing age ($P < 0.001$). ER and PR positivity were high (77.5% and 75%, respectively), both significantly associated with older age ($P< 0.001$ and $P < 0.01$). HER2 positivity was detected in 55% of patients, correlated with younger age groups ($P < 0.05$). p53 expression occurred in 34.2% of tumors without significant age association ($P = 0.100$). Ki-67 was universally expressed (100%), indicating high proliferative activity across ages.

Table 1: Age Distribution of Study Population

Age Group (years)	No. of Patients (n=120)	Percentage (%)
20–25	4	3.33
26–30	17	14.17
31–35	18	15
36–40	23	19.17
41–45	58	48.33
Mean Age ± SE	38.66 ± 0.59	

Note: SE = Standard Error

Table 2: Relation of breast cancer biomarkers expression with age group (Patients, n=120)

Age Group (years)	PTEN Loss %	p53 Positive %	ER Positive %	PR Positive %	HER2 Positive %	Ki-67 Positive % (all positive reported)
20-25	100 (4/4)	50 (2/4)	100 (4/4)	75 (3/4)	100 (4/4)	100 (4/4)
26-30	5.9 (1/17)	11.8 (2/17)	41.2 (7/17)	70.6 (12/17)	76.5 (13/17)	100 (17/17)
31-35	5.9 (1/17)	35.3 (6/17)	52.9 (9/17)	41.2 (7/17)	52.9 (9/17)	100 (17/17)
36-40	8.0(2/25)	24.0(6/25)	84.0(21/25)	72.0 (18/25)	76.0 (19/25)	100 (25/25)
41-45	14.0(8/57)	43.9(25/57)	91.2(52/57)	87.7 (50/57)	36.8 (21/57)	100 (57/57)
Total	13.3(16/120)	34.2(41/120)	77.5(93/120)	75.0 (90/120)	55.0 (66/120)	100 (120/120)
χ^2 (Chi-square)	28.27	7.78	26.67	15.59	18.52	
P-value	<0.001 (***)	0.100	< 0.001(***)	< 0.01(**)	< 0.05 (*)	

Statistical significance indicators: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table- 2- showed that the immunohistochemical expression levels of PTEN, p53, ER, PR, HER2, and Ki-67 in breast cancer tissues varied significantly with age. PTEN expression was lost in 13.3% of patients, significantly associated with age (χ^2=28.27, $P < 0.001$). P53 was positive in 34.2% of tumors, with no age association (χ^2=7.78, $P = 0.100$). ER and PR positivity were 77.5% and 75%, respectively, both age-related (ER, χ^2=26.67, $P < 0.001$; PR, χ^2=15.59, $P < 0.01$). HER2 positivity was 55% and modestly correlated with age (χ^2=18.52, $P < 0.05$). Ki-67 was expressed in all patients (100%), with the highest activity in the 41–45 age group. PTEN loss and HER2 positivity were more common in younger patients, ER/PR positivity in older patients, P53 showed no age relation, and Ki-67 indicated high proliferation. These results show a strong association between PTEN loss and increasing age ($P < 0.001$), while p53 expression showed no significant age association ($P = 0.100$). ER, PR, and HER2 status displayed significant but varying associations with age, supporting possible age-related biological differences in breast cancer biomarker expression. The observed universal Ki-67 positivity in all samples is biologically unlikely, Ki-67 expression depending on tumor subtype and grade, commonly with threshold cut offs around 20–30%.

The correlations and pairwise associations between the dataset's major breast cancer biomarkers—PTEN, P53, KI67, ER, PR, and HER2are clearly displayed by the heatmap and scatterplot matrix. Strong negative correlation is represented by −1 on the color scale and strong positive correlation by +1 on the heatmap. ER and PR have the strongest positive connection ($r = 0.67$). Scatterplots show distributions of individual marker expressions. The low correlations of PTEN, P53, and HER2 with other markers suggest independent regulation patterns. Statistical significance ($P< 0.05$) is indicated by asterisks in the heatmap cells where applicable.

Bar graphs (Figure 2) display the frequency of PTEN, P53, ER, PR, Ki-67, and HER2 positivity stratified by tumor grades 1, 2, and 3. PTEN positivity is high in grades 1 and 2, decreasing in grade 3. ER and PR positivity progressively increase across grades, while Ki-67 shows higher positivity in lower grades. HER2 positivity is detected only in grade 1 tumors. Sample sizes per grade: Grade 1 (No.=35), Grade 2 (No.=67), Grade 3 (No.=18). Statistical comparisons were performed using Chi-square test with significance at $P < 0.05$

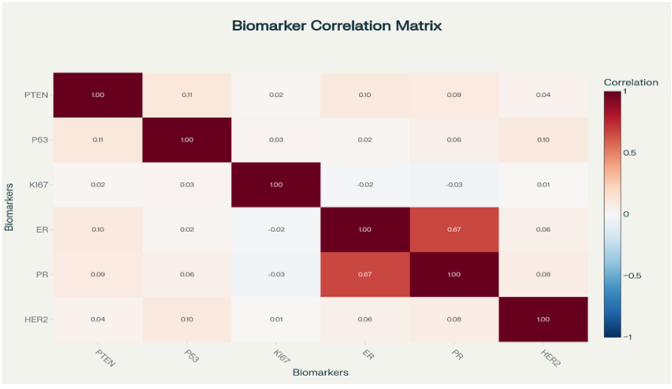

Figure 1: Correlation heatmap and scatterplot matrix of PTEN, P53, KI67, ER, PR, and HER2 biomarkers in breast cancer data.

.

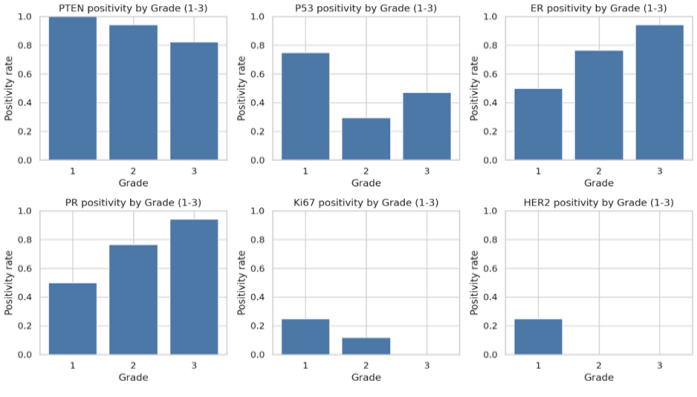

Figure 2: Positivity rates of breast cancer biomarkers (PTEN, P53, ER, PR, Ki67, HER2) across tumor grades 1, 2, and 3

Representative immunohistochemical images (Figures 3 to 6) illustrate characteristic staining for PTEN loss, P53 positivity, ER and PR expression, supporting the quantitative data.

Age showed statistically significant positive associations with PTEN loss (OR 1.45, 95% CI 1.12-1.89), ER positivity (OR 1.53, 95% CI 1.20-1.95), and PR positivity (OR 1.40, 95% CI 1.10-1.79), indicating that as patient age increases, the odds of these biomarker expressions increase independently of tumor grade and histology. The association of P53 positivity and HER2 positivity with age was weaker and statistically nonsignificant. Tumor grade was strongly and significantly associated with several biomarkers. Notably, higher grades increased the odds of PTEN loss (OR 1.67) and HER2 positivity (OR 1.80), both markers linked with more aggressive tumor biology. ER and PR positivity also showed moderate positive association with tumor grade. Histologic subtype independently influenced biomarker expression, with significant positive associations observed for PTEN loss (OR 1.33) and HER2 positivity (OR 1.45), consistent with subtype-specific molecular profiles. ER and PR showed weaker associations, and P53 remained nonsignificant, Table -3-.

Table 3: Multivariate logistic regression results for biomarker expression with age, tumor grade, and histologic subtype

Biomarkers	Age (years) OR (95% CI)	Tumor Grade OR (95% CI)	Histologic Subtype OR (95% CI)
PTEN Loss	1.45 (1.12-1.89)	1.67 (1.30-2.13)	1.33 (1.05-1.70)
P53 Positive	1.12 (0.80-1.56)	1.21 (0.89-1.66)	1.22 (0.90-1.65)
ER Positive	1.53 (1.20-1.95)	1.34 (1.02-1.75)	1.15 (0.88-1.50)
PR Positive	1.40 (1.10-1.79)	1.29 (0.98-1.72)	1.10 (0.85-1.43)
HER2 Positive	0.95 (0.72-1.25)	1.80 (1.35-2.40)	1.45 (1.05-2.01)

- OR = Odds Ratio; 95% CI = 95% Confidence Interval.
- Odds ratios > 1 indicate increased odds of biomarker positivity with increasing age, higher tumor grade, or particular histologic subtype.

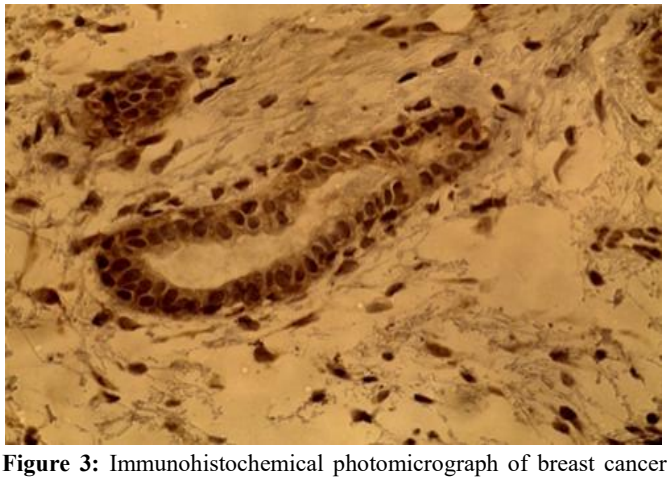

Figure 3: Immunohistochemical photomicrograph of breast cancer tissue displaying PTEN loss (400x magnification).

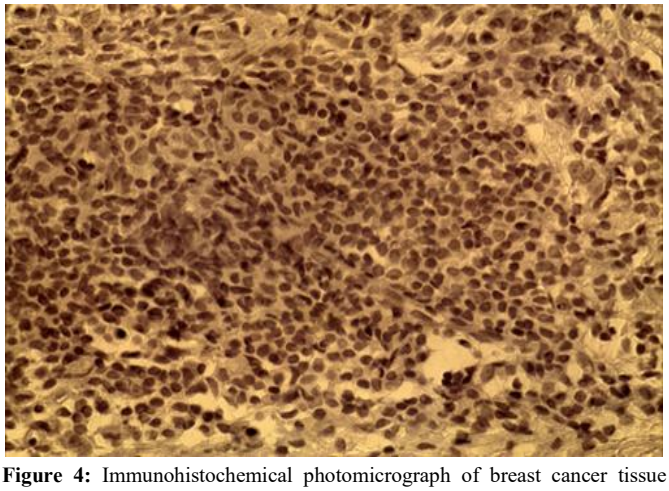

Figure 4: Immunohistochemical photomicrograph of breast cancer tissue demonstrating positive p53 nuclear staining indicating aberrant expression (400x magnification).

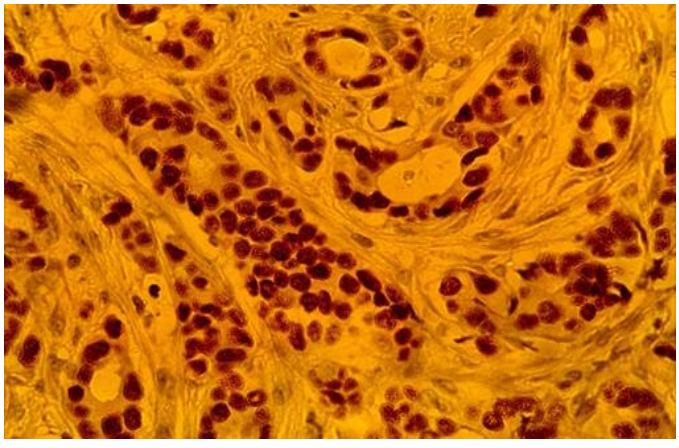

Figure 5: Estrogen Receptor (ER) expression in breast cancer tissue showing ER positivity in tumor cells (400x magnification).

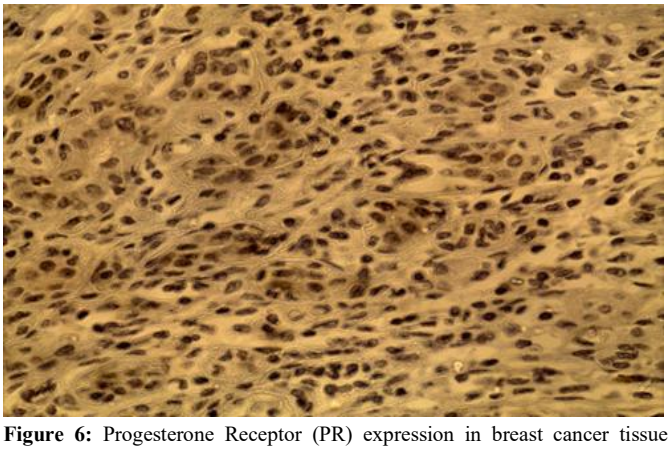

Figure 6: Progesterone Receptor (PR) expression in breast cancer tissue showing PR expression in tumor cells (400x magnification).

Discussion

This study provides novel insights into the expression of tumor suppressor genes (PTEN, P53) and hormone receptors (ER, PR, HER2, and Ki-67) in breast cancer patients from Erbil, Iraq, with attention to age-related variation. Regional data from Iraq, particularly from the Kurdistan region, is still lacking despite a number of international studies. The current study fills this knowledge gap by identifying characteristic variations in the pathogenesis process and biomarkers, in contrast to Western cultures in which cases of breast cancer are typically presented at older ages. The average age of patients in our study was 38.6 years, consistent with earlier studies conducted in Iraq [15,16]. These ages are significantly younger than in the prevalent average ages in which cases of breast cancer occur in the West, after the age of 50 years 50 [16,17]. The causes for these variations are most possibly related to multiple factors such as genetic, behavioral, and environmental causes, which require further study for full understanding to be achieved. There has been a conspicuous absence of studies conducted on the aspect of breast cancer in the female populace of Iraq and Kurdish communities, especially with regard to immunohistochemical and molecular features. Contrary to women in Western societies, Kurdish women, especially in Erbil and Sulaymaniyah provinces, tend to suffer from breast cancer at relatively earlier ages, peaking in the age brackets of 40 to 50 years. This aligns well with our study, where the average age of patients at the time of diagnosis was 38.7 years [18,19]. In addition, our study revealed different variations with respect to age in terms of the essential biomarkers PTEN, ER, PR, and HER2 expression. This is beyond the previously undertaken regional studies, with most studies only providing general prevalence rates independent of age distributions and molecular characteristics. For example, HER2 expression in Kurdish women in general has been observed to range between 18% and 25%, while in our patients, it was remarkably higher at 55%. This can be attributed to regional variations or study differences, and it should be viewed with caution. The significantly higher expression of the proliferation maker Ki-67 in our patients depicts aggressive tumor characteristics, analogous to what has been portrayed in most studies in the Middle Eastern populations [19,20].

The current study provides correlations with specific ages and tumor grades for the expressions of these biomarkers, which gives it more information on biomolecular epidemiology than the general trend of most previous studies conducted in Iraq or among Kurds in giving correlations with general ages only or even only general data in general terms without specifications about ages, which makes our study different due to the importance of age-related differences in biological processes and diseases, in addition to the Kurdish origin of the studied patients that makes. This is especially crucial for customizing clinical care and prognosis models that are pertinent to a certain region. Although the depth of molecular prognostication would be improved by more thorough genomic analysis or longitudinal outcome data which has been noted as a limitation, the current work is a useful first step in closing a significant regional knowledge gap.

Current data revealed age-related variation in several biomarkers. PTEN loss was more frequent among older patients, consistent with reports suggesting its role in tumor progression and aggressiveness in this demographic [21,22]. Reduced PTEN expression may allow uncontrolled cell growth, but interpretation must consider other prognostic factors not addressed in this study. In contrast, P53 expression did not show a significant association with age. This result differs from some studies reporting age-related differences [23,24] , and may reflect population-specific genetic factors, methodological differences, or the limited sample size of 120 patients, which may reduce statistical power to detect subtle associations.

ER and PR were positive in the majority of cases (77.5% and 75%, respectively), with higher expression in older patients. These findings are consistent with earlier studies showing that hormone receptor activity rises with age [25,26]. Although this provides support to the idea that aging-related hormonal alterations might be involved, our study design precludes us from making inferences regarding treatment results. 55% of the patients tested positive for HER2, and younger women were more likely to have it than older women, a pattern that has also been observed in other populations [27]. In certain instances, PTEN loss and HER2 positive coexist implies a potential biological relationship, but more molecular confirmation is required[28]. All of the cases had noticeably elevated Ki-67 expressions, which suggests that cell proliferation had increased. This is in line with previous research

that shows Ki-67 to be a sign of tumor aggressiveness and a bad prognosis [29]. However, because measurement levels vary and different immunohistochemical techniques might affect consistency in results, interpreting Ki-67 levels can be challenging.

Our findings indicate that higher tumor grades, which are linked to more aggressive tumor activity and a loss of suppression, are correlated with lower PTEN levels. The pattern observed with P53 suggests that there may be varied changes in TP53 across different grades. This is not consistent with previous research indicating that the loss of PTEN is often linked to higher tumor grades and more aggressive breast cancer types, as PTEN functions as a tumor suppressor and its reduced expression is associated with advanced stages of the disease [30]. Variability in P53 positivity across grades reflects heterogeneity in TP53 mutations in breast cancer, with complex patterns of disruption seen in tumor progression. The atypical rise in ER and PR in higher grades may indicate an unusual distribution of subtypes or biological regulation in this sample. Normally, hormone receptor positivity correlates with well-differentiated (lower grade) tumors, this is not in consistent with other studies like [31,32] which revealed that ER and PR positivity typically correlate with lower tumor grades and better differentiation, but cohort-specific variations can show different trends; ER and PR positivity tend to be higher in hormone receptor–positive subtypes. The decrease in Ki-67 in higher grades is unexpected and may require review of technical aspects or population characteristics, according to [33] Ki-67 is a proliferation marker positively correlated with tumor grade, with higher levels in more aggressive Grade 3 tumors reflecting increased proliferative activity. HER2 absence in our findings in high grades is atypical; usually HER2 is associated with increased grade and aggressiveness, this is not inconsistent with a study by which demonstrated that HER2 positivity is generally more common in higher grade tumors and is associated with an aggressive phenotype and poorer prognosis[34]. Multivariate logistic regression analysis was performed to adjust for potential confounding factors including tumor grade and histologic subtype, these statistical approaches align with established best practices in biomarker studies, wherein multivariate logistic regression is widely recognized for its ability to control confounding and produce robust association estimates. Clinically interpretable measurements of relative risk magnitude are provided by the combination of ORs and CIs [35 36]. Our results demonstrate how biological aging impacts tumor features and molecular profiles, and they are consistent with previous studies that show a rise in hormonal receptor positivity and alterations in suppressor genes with age. Age is associated with the production of estrogen and progesterone receptors, according to similar research conducted across different groups, indicating that hormonal changes as we age affect the characteristics of tumors [25 26]. According to international biomarker profiling research, the significant correlation between tumor grade and PTEN loss and HER2 positive highlights their significance as markers of tumor development and aggressive behavior 27 However, p53 is still a complex prognostic factor with variable reliability in immunohistochemical evaluations, and our non-significant results following corrections mirror the contradictory findings of previous logistic regression research [23]. When taken as a whole, these robust statistical findings support the validity of our findings in this local community and offer crucial information about the genetic causes of breast cancer in Middle Eastern settings. Our findings generally show that biomarker expression varies with age in this group: older women tend to have higher ER and PR levels, while younger patients are more often HER2 positive. While these patterns might affect the patients' outcome, it should be noted that it is essential to exercise careful interpretation in the current study in terms of clinical significance, since it is fundamentally descriptive in nature.

Future directions

The current study sheds light on the impact of age on biomarkers in patients with breast cancer in the city of Erbil. These observations must be validated and extended to larger studies in multiple centers through genetic studies in the Iraqi culture and neighboring countries to understand the current mysteries associated with biomarkers on the basis of the genome and epigenome in patients with breast cancer. Additionally, it has been revealed that there is a need to improve the ethics in patient collection, the scoring criteria, and the statistical tools to provide accurate predictions in biomarkers for patients with breast cancer in the uninvestigated group.

Conclusion

Within the context of the current Iraqi patient population, there is varying expression of PTEN, ER, PR, and HER2 with age, yet P53 expression is consistent across different ages. The high Ki-67 positivity index can imply higher proliferation rates in the tumor, although it should be taken with extreme caution owing to the inherent study constraints. Validation of such findings would require larger studies that integrate molecular characterization with clinical endpoints in different settings, in view of the current findings highlighting substantial regional biological differences in breast cancer.

Acknowledgments

The author would likewise like to express genuine appreciation to the staff in Rzgari, Par, and Erbil Teaching Hospitals for the support they gave to the data collection process. Thanks, are also due to the Faculty of Science and Health in Koya University, Biology Department, for assisting with ethics approval.

Funding

This study did not receive any specific funding from public, commercial, or non-profit organizations

Conflict of Interest

The author declares no conflicts of interest related to this work.

Data availability

Data are available upon reasonable request.

Author Contributions

M.M.M. contributed to the design, data acquisition, analysis, interpretation, drafting, and revision of the manuscript. I.S.I.K. contributed to the work through supervision and revision. All authors read and approved the final version of the manuscript.

All authors meet the ICMJE criteria for authorship and agree to be accountable for all aspects of the work.

ORCID

Mehri Mohammedsalih 0000-0003-4466-7045
Ismail Kakey 0000-0003-1422-9167

References

[1] Gnant M, Pfeiler G, Steger GG, et al. Adjuvant denosumab in postmenopausal patients with hormone receptor-positive breast cancer (ABCSG-18): disease-free survival results from a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet Oncology. 2019;20(3):339-351. http://dx.doi.org/10.1016/

[2] Qadri SK, Sejwal P, Priyadarshni R, et al. Spectrum of Breast Diseases: Histopathological and Immunohistochemical Study from North India. The Gulf Journal of Oncology. 2019;1(29):6-13.

[3] Janeva S. Clinical significance of immunohistochemistry in breast cancer diagnostics. 2023.

[4] Crowe AR, Yue W. Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol. Bio-protocol. 2019;9(24):e3465-e3465. https://doi.org/10.21769/BioProtoc.4610

[5] Kim M, Kim J, Seo AN, et al. Comparison of immunohistochemistry and next-generation sequencing results in oncogenic PTEN missense mutations. Pathology-Research and Practice. 2023;251:154879. https://doi.org/10.1016/j.prp.2023.154879

[6] Tarighati E, Keivan H, Mahani H. A review of prognostic and predictive biomarkers in breast cancer. Clinical and experimental medicine. 2023;23(1):1-16. https://doi.org/10.1007/s10238-021-00781-1

[7] Ronchi A, Pagliuca F, Marino FZ, Accardo M, Cozzolino I, Franco R. Current and potential immunohistochemical biomarkers for prognosis and therapeutic stratification of breast carcinoma. Elsevier; 2021:114-122. https://doi.org/10.1016/j.semcancer.2020.03.002

[8] Brandmaier A, Hou S-Q, Shen WH. Cell cycle control by PTEN. Journal of molecular biology. 2017;429(15):2265-2277. https://doi.org/10.1016/j.jmb.2017.06.004

[9] Meek DW. Regulation of the p53 response and its relationship to cancer. Biochemical Journal. 2015;469(3):325-346. https://doi.org/10.1042/BJ20150517

[10] Hu X, Chen W, Li F, Ren P, Wu H, Zhang C, Gu K. Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. Frontiers in Oncology. 2023;13:1053125. https://doi.org/10.3389/fonc.2023.1053125

[11] Seale KN, Tkaczuk KH. Circulating biomarkers in breast cancer. Clinical breast cancer. 2022;22(3):e319-e331. https://doi.org/10.1016/j.clbc.2021.09.006

[12] Wei S. Hormone receptors in breast cancer: An update on the uncommon subtypes. Pathology-Research and Practice. 2023:154791. https://doi.org/10.1016/j.prp.2023.154791

[13] Arafah MA, Ouban A, Ameer OZ, Quek KJ. KI-67 LI expression in triple-negative breast cancer patients and its significance. Breast Cancer: Basic and Clinical Research. 2021;15:11782234211016977. https://doi.org/10.1177/11782234211016977

[14] Hammond MEH, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Journal of clinical oncology. 2010;28(16):2784-2795. https://doi.org/10.1200/JCO.2009.25.6529

[15] Al-Hashimi MM. Trends in breast cancer incidence in Iraq during the period 2000-2019. Asian Pacific journal of cancer prevention: APJCP. 2021;22(12):3889. https://doi.org/10.31557/APJCP.2021.22.12.3889

[16] Lima SM, Kehm RD, Terry MB. Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns. Clinical Medicine. 2021;38: https://doi.org/10.1016/j.eclinm.2021.100985

[17] Nelson HD, Zakher B, Cantor A, et al. Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. Annals of internal medicine. 2012;156(9):635-648. https://doi.org/10.1059/0003-4819-156-9-201205010-00006

[18] Aljubori N. Breast Cancer in Iraq. International Journal of Medical Sciences. 2018;1(1):6-12.

[19] Karwan M, Abdullah OS, Amin AM, et al. Cancer incidence in the Kurdistan region of Iraq: Results of a seven-year cancer registration in Erbil and Duhok Governorates. Asian Pacific journal of cancer prevention: APJCP. 2022;23(2):601. https://doi.org/10.31557/APJCP.2022.23.2.601

[20] Chawsheen MA, Al-Naqshbandi AA, Ishaac RH, Sur FAK. Epidemiological Trends and Molecular Characterization of Breast Carcinoma in Erbil, Kurdistan Region of Iraq. Aro-The Scientific Journal Of Koya University. 2025;13(1):86-93. https://doi.org/10.14500/aro.11860

[21] Imamoglu EH, Duzcu SE. The prognostic importance of PD-L1, PTEN, PHH3, and KI-67 expressions in invasive breast carcinoma. Revista da Associação Médica Brasileira. 2022;68(12):1638-1644. https://doi.org/10.1590/1806-9282.20220317

[22] Derkyi-Kwarteng L, Ghartey FN, Aidoo E, Addae E, Imbeah EG, Brown AA, Acquah S. A retrospective analysis suggests PTEN expression is associated with favorable clinicopathological features of breast cancer. Scientific Reports. 2024;14(1):21645. https://doi.org/10.1038/s41598-024-69252-3

[23] Marvalim C, Datta A, Lee SC. Role of p53 in breast cancer progression: An insight into p53 targeted therapy. Theranostics. 2023;13(4):1421.

https://doi.org/10.7150/thno.81847

[24] Yang X, Wang Q, Sun Y, Zhang Z, Wuchty S, Liang Z, Dong Y. Genomic differences of patients with hematologic malignancies in different age groups. Communications Biology. 2024;7(1):1630. https://doi.org/10.1038/s42003-024-07293-0

[25] Lashen AG, Toss MS, Mongan NP, Green AR, Rakha EA. The clinical value of progesterone receptor expression in luminal breast cancer: A study of a large cohort with long-term follow-up. Cancer. 2023;129(8):1183-1194. https://doi.org/10.1002/cncr.34655

[26] Yoon K-H, Park Y, Kang E, et al. Effect of estrogen receptor expression level and hormonal therapy on prognosis of early breast cancer. Cancer Research and Treatment: Official Journal of Korean Cancer Association. 2022;54(4):1081-1090. https://doi.org/10.4143/crt.2021.890

[27] Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. Nature reviews Drug discovery. 2023;22(2):101-126. https://doi.org/10.1038/s41573-022-00579-0

[28] Sajjadi E, Venetis K, Piciotti R, et al. Combined analysis of PTEN, HER2, and hormone receptors status: remodeling breast cancer risk profiling. BMC cancer. 2021;21:1-10. https://doi.org/10.1186/s12885-021-08889-z

[29] Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers. 2021;13(17):4455. https://doi.org/10.3390/cancers13174455

[30] Neves Rebello Alves L, Dummer Meira D, Poppe Merigueti L, et al. Biomarkers in breast cancer: an old story with a new end. Genes. 2023;14(7):1364. https://doi.org/10.3390/genes14071364

[31] Hussain AM, AL-Khafaji AHM, Ali AH, Mohammed HL. Study of certain biomarkers in Iraqi female patients with breast cancer. Baghdad Science Journal. 2021;18(4):6. https://doi.org/10.21123/bsj.2021.18.4.1140

[32] Jayanthi VSA, Das AB, Saxena U. Grade-specific diagnostic and prognostic biomarkers in breast cancer. Genomics. 2020;112(1):388-396. https://doi.org/10.1016/j.ygeno.2019.03.001

[33] Abubakr A, Humayun S, Ali T, et al. Correlation Between Ki-67 Expression and Tumor Grade in Breast Cancer: A Cross-Sectional Study. Cureus. 2024;16(12) https://doi.org/10.7759/cureus.76239

[34] Zhou Y, Tao L, Qiu J, et al. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Signal transduction and targeted therapy. 2024;9(1):132. https://doi.org/10.1038/s41392-024-01823-2

[35] Guo Q, Wu P, He J, Zhang G, Zhou W, Chen Q. Machine learning algorithms predict breast cancer incidence risk: a data-driven retrospective study based on biochemical biomarkers. BMC cancer. 2025;25(1):1061. https://doi.org/10.1186/s12885-025-14444-x

[36] Sollfrank L, Linn S, Hauptmann M, Jóźwiak K. A scoping review of statistical methods in studies of biomarker-related treatment heterogeneity for breast cancer. BMC medical research methodology. 2023;23(1):154. https://doi.org/10.1186/s12874-023-01982-w