Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2025.353263.3800 Volume 31, Issue 4, April . 2025
Manuscript ZUMJ-2501-3800
DOI: 10.21608/ZUMJ.2025.353263.3800
ORIGINAL ARTICLE

Nuclear Factor-κβ Expression in Invasive Breast Cancer

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Submit Date 15-01-2025
Revise Date 27-01-2025
Accept Date 28-01-2025

ABSTRACT

Background: The expression of nuclear factor kappa beta (NF-κβ/p65) has been investigated in breast cancer and its molecular subgroups to ascertain the association between NF-κβ/p65 expression and clinicopathological features of breast cancer and to find out how (NF-κβ)/p65 plays a role in breast cancer development. This study aimed to assess the immunohistochemistry expression of NF-κβ/p65 in invasive breast cancer.

Methods: The immunohistochemistry expression of NF-κβ/p65 receptor was examined in 82 paraffin-embedded slices from invasive breast cancer. Both clinicopathological characteristics and immunohistochemical-based subtypes of the patients under study were evaluated in relation to the expression of NF-κβ/p65.

Results: NF-κβ/p65 expression was found to be substantially linked with tumor size, grade, pathological stage, Her2-enriched and triple-negative subtypes, and Ki67 levels. Furthermore, there was a significant inverse association between the expression of NF-κβ/p65 and the expression of both progesterone and estrogen receptors. There was no significant correlation between age, axillary lymph node status, distant metastases, or tumor histological subtypes and NF-κβ/p65 expression.

Conclusion: The aggressive biological properties of breast cancer have been connected to the expression of NF-κβ/p65.

Keywords: Breast Cancer; HER2/neu; Invasive Breast Cancer.

INTRODUCTION

For women under 40, breast cancer (BC) ranks as the second most frequent cause of cancer-related mortality, representing over 12% of all cancer cases globally [1, 2]. With a mortality rate of about 11% in 2020, breast cancer ranks second in Egypt after hepatocellular carcinoma as the primary cause of mortality from cancer. Instead of being in the early stages, the majority of cases in Egypt appear as locally progressed or metastatic [3]. Gene expression categorizes breast cancer into three groups: triple-negative breast cancer (TNBC), Her2-enriched, and luminal (types A and B) [4].

The proinflammatory transcription factor, nuclear factor kappa beta (NF-κβ), is implicated in many biological processes, both healthy and unhealthy, including the emergence of cancer. The cytoplasm of quiescent cells contains the inactive form of NF-κβ, which is associated with the NF-κβ inhibitor (I-κβ). Upon activation, I-κβ is broken down in the proteosome, causing NF-κβ to be released and go to the nucleus [5].

It is rarely observed to be constitutively active in normal cells, with the exception of proliferative T cells, B cells, thymocytes, monocytes, and astrocytes. It is unknown what mechanism causes NF-κβ to be constitutively activated in tumor cells. Protease activity was increased when I-κβ was mutated [6]. All resting cells contain NF-κβ in an inactive form as a heterodimer in the cytoplasm, which is bound by a particular inhibitor for NF-κβ (I-κβ) [7]. When external stress breaks down the I-κβ

protein, the active form of NF-κβ is produced. After entering the nucleus, this form regulates the expression of genes involved in angiogenesis, adhesion, cell proliferation, and survival. Additionally, it helps chemoresistance to develop. Different chemotherapeutic agents seem to activate the anti-apoptotic pathway, which mediates this effect [8].

In breast cancer, this transcription factor regulates the growth of Her2 tumors, and in models, its blockage was adequate to lower the tumor microvessel density. I-κB mutants that constitutively inhibit the NF-κβ pathway were expressed by Her2 murine cells [9].

In this study, we attempted to assess how NF-κβ/p65 contributes to the development of breast cancer. In order to determine the relationship between NF-κβ/p65 expression and clinicopathological characteristics, the expression of NF-κβ/p65 was examined in instances of breast cancer and its subtypes.

METHODS

This cross-sectional study was carried out on 82 consecutive cases of female breast tissue with primary invasive breast carcinomas that were collected at the Pathology Center of Faculty of Medicine, Zagazig University in the period from October 2022 to December 2024. Modified radical mastectomy, lumpectomy, or true-cut biopsy (n = 22) (n = 60) were the methods used to collect the specimens. Primary invasive breast cancer cases with comprehensive clinico-pathological information were included. While instances with insufficient tissue for staining or those that had previously had adjuvant treatment, chemotherapy, or radiation therapy were excluded. The study protocol was approved by the Institutional Review Board at Faculty of Medicine, Zagazig University (IRB#10533/2023). Participants' written informed consent was acquired. The World Medical Association's Code of Ethics for Human Subjects Research was followed in the conduct of this study, which was developed in 1975 by the Helsinki Declaration and updated in 2000.

The pathology reports that came with the tissue specimens were used to obtain the clinicopathological information, which included the patient's age, tumor grade, size, stage, involvement of lymph nodes, distant metastases, and the immunohistochemistry status of estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and ki67.

The WHO classification of breast tumors was used to histologically classify all cases of invasive breast cancer [10]. The Bloom-Richardson system's Elstron/Nottingh classification was used to grade the tumors [11]. Both the tumor size and dissected lymph nodes were visually evaluated under a microscope using the AJCC TNM Staging of Breast Cancer [12]. ER, PR, and Her2 findings were categorized in accordance with the authorized CAP guidelines [13, 14]. A Ki67 index of more than 14% was regarded as high [15].

Immunohistochemistry protocol

ER/PR was immunohistochemically stained using the EnVision system approach using the Dako Autostainer (ER: Mouse, monoclonal, clone 1D5, Code IR657, Dako, Glostrup, Denmark, Ready-to-use) and the ER/PR pharmDx Kit (PR: Mouse, monoclonal, clone PgR 636, Code IR068, Dako, Glostrup, Denmark, Ready-to-use). According to the approved CAP standards, ≥10% of tumor cells with moderate to strong nuclear staining were considered positive [13].

HER2/neu immunostaining and evaluation

Every slide was subjected to the HercepTestTM kit K5207 (DAKO Cytomation, Glostrup, Denmark). According to the authorized CAP guidelines, HER2/neu was deemed positive if there was full and strong circumferential membrane staining within 10% of tumor cells [14].

Ki67 immunostaining and evaluation

Ki67 (mouse, monoclonal, and clone MIB-1, Glostrup, Denmark, Dako, Ready-to-use, Code: IS626) was immunohistochemically stained. A Ki67 value of more than 14% was regarded as high [15].

Based on the immunohistochemistry analysis of HER2/neu and hormone receptor expression, the molecular subtypes were identified. There are four immunohistochemical subtypes:

Luminal A [ER+, PR+, HER2/Neu−, low Ki67], Luminal B (ER+, PR+, HER2/Neu− or +, high Ki67), Her2-enriched (ER−, PR−, Her2/Neu+, high Ki67), and Triple negative (ER−, PR−, HER2/Neu−, high Ki67) [4].

NF-κβ/p65 immunostaining

The immunohistochemistry staining was performed using the EnVision system technique (DAKO, North America Inc., CA, USA). This two-step IHC detection method is enhanced by polymers and employs an antibody to NF-κβ/p65 (NP_068810.3). The optimal dilution for NF-κβ/p65 (L8F6) was 1:1000 using a rabbit monoclonal antibody (Abclonal technology, Leader in Biomolecular Solutions for Life Science), clone No. ARC51088, Gene ID 5970. Human lymphocytes were used as a positive internal control for NF-κβ/p65 using a synthetic peptide that matched a sequence in amino acids 450–551. By leaving the primary antibody out, a negative control was produced.

Interpretation of immunohistochemistry

Two researchers separately and blindly assessed and scored the NF-κβ/p65 immunoreactivity. Both the staining intensity (no staining = 0, straw yellow = 1, brown staining = 2, and tan staining = 3) and the percentage of stained cells from 0 to 5 (0 = 0-5%, 1 = 6-25%, 2 = 26-50%, 3 = 51-75%, and 4 = >75%) were assessed using a semi-quantitative scoring system. The sum of intensity-based and percentage-based scores was used to create the final scoring index, and three was chosen as the cut-off value, meaning that ≥ 3 was regarded as positive and < 3 as negative [16]. Cytoplasmic NF-κβ/p65 staining positivity was assessed as follows: three to four for weak expression, five to six for moderate expression, and seven for high expression [17].

Statistical analysis

We collected, tabulated, and statistically analyzed all data using Version 23 of the Statistical Package for the Social Sciences (SPSS), Chicago, Illinois, USA. To determine whether the data was normal, the Shapiro-Kolmogrov test was employed. Using the Student's t-test, two independent samples with a normal distribution were compared. Also, the Chi-square test and Spearman's correlation coefficient were used for data analysis. When P values were less than 0.05, they were considered statistically significant.

RESULTS

The mean age of patients was 50.4±12.4, with a range of 28 to 77 years. Most of the patients (70.73%) were older than 40 years. The majority of cases had IBC-NST (73.2%), grade II (47.6%), T2 (45%), N1 (43.3%), stage IV (36.7%), and molecular subtype basal-like (triple-negative) (43.9%). Metastases were present in 22 cases (36.7%), while the remaining cases were negative. The clinicopathological features are displayed in Table 1.

In 82 cases of breast cancer, 54 (65.9%) had NF-κβ/p65 expression, and 28 (34.1%) had negative NF-κβ/p65 expression. The correlation between NF-κβ/p65 expression and clinicopathological parameters is shown in Table 2. The expression of ER and PR had a statistically significant inverse correlation with NF-κβ/p65 expression (P=0.001, 0.002, respectively). ER-negative tumors had higher NF-κβ/p65 expression (68.5%) than ER-positive BC (31.5%), and PR-negative tumors had higher NF-κβ/p65 (75.9%) than PR-positive BC (24.1%). Additionally, there was a statistically significant correlation between NF-κβ/p65 expression and HER2 status (P = 0.008), with HER2/neu positive tumors having higher levels of NF-κβ/p65 (33.33%). Also, there was a statistically significant correlation between NF-κβ/p65 expression and Ki67 index (P=0.013). According to (Figures 1 and 2), NF-κβ/p65 was more positive in high Ki67 (79.6%) and histological grades II and III (P = 0.008), with 55.6% of the cases having second-grade NF-κβ/p65 expressions, while 40.7% had third-grade expressions.

Additionally, (Figure 2) shows a significant correlation between NF-κβ/p65 expression and molecular types, with the triple negative, HER2/neu, and luminal B groups having higher expression than the luminal A tumor (51.9%, 16.7%, 22.2%, and 9.3%, respectively) (P < 0.001).

The age (P=0.151) and IBC histological categories (P=0.561) did not significantly correlate with NF-κβ/p65 expression. The majority of ductal and lobular carcinoma malignant epithelial cells had NF-κβ/p65 immunoreactivity in their cytoplasm. Six specimens (11.1%) of invasive lobular carcinoma had NF-κβ/p65 expression, while three specimens (10.7%) had none. Four instances of mucinous carcinoma (14.3%) were negative for NF-κβ/p65 immunostaining, while two cases were positive (3.7%). Two cases of micropapillary carcinoma and one case of IDC with neuroendocrinal differentiation were positive for NF-κβ/p65 expression.

The NF-κβ/p65 expression was found to be statistically significantly correlated with large tumor size (P = 0.007), with NF-κβ/p65 expression in 36.84% and 15.79% of cases with T3 and T4, respectively. Also, there was a statistically significant correlation between the NF-κβ/p65 expression and high tumor stage (P <0.001), with NF-κβ/p65 detected in 28.95% and 52.63% of cases with stages III and IV, respectively. On the contrary, the NF-κβ/p65 expression didn't statistically significantly correlate with lymph node status (P=0.63) and distant metastasis (P = 0.55) (Table 3).

Table (1): Clinicopathological parameters of the studied cases

Variables	Number (82)		Frequency (%)
Age	**≤40**	24	29.27%
	>40	58	70.73%
	Mean ± SD	50.4±12.4	
	Range	28-77	
Histopathological Types	**IDC**	60	73.2%
	Others	22	26.8%
Histological grades	**I**	9	10.9%
	II	39	47.6%
	III	34	41.5%
Tumor size (60 cases)	**T1**	9	15%
	T2	27	45%
	T3	18	30%
	T4	6	10%
Axillary lymph node status (60 cases)	**N0**	16	26.7%
	N1	26	43.3%
	N2	12	20%
	N3	6	10%
Distant metastasis (60 cases)	**Negative**	38	63.3%
	Positive	22	36.7%
Tumor stage (60 cases)	**I**	4	6.7%
	II	20	33.3%
	III	14	23.3%
	IV	22	36.7%
Molecular subtypes	**Luminal A**	19	23.2%
	Luminal B	17	20.7%
	Her2 overexpression	10	12.2%
	Triple negative	36	43.9%

Table (2): Correlation between NF-κβ/p65 and clinicopathological parameters

Variables		NF-κβ/p65 expression		P value
		Negative (n=28)	Positive (n=54)	
ER	**Positive (n=36)**	19(67.9%)	17 (31.5%)	**0.001***
	Negative (n=46)	9 (32.1%)	37(68.5%)	
PR	**Positive (n=29)**	16(57.1%)	13 (24.1%)	**0.002***
	Negative (n=53)	12(42.9%)	41(75.9%)	
HER2/neu	**Positive (n=20)**	2 (7.14%)	18(33.33%)	**0.008***
	Negative (n=62)	26(92.86%)	36(66.67%)	
Ki67	**High (n=58)**	15 (53.6%)	43 (79.6%)	**0.013***
	Low (n=24)	13 (46.4%)	11 (20.4%)	
Tumor grade	**I (n=9)**	7 (25%)	2 (3.7%)	**0.008***
	II (n=39)	9 (32.1%)	30 (55.6%)	
	III (n=34)	12 (42.9%)	22 (40.7%)	
Molecular subtypes	**Luminal A (n=19)**	14 (50%)	5 (9.3%)	**<0.001***
	Luminal B (n=17)	5(17.9%)	12 (22.2%)	
	Her2 enriched (n=10)	1 (3.6%)	9 (16.7%)	
	Triple negative (n=36)	8(28.6%)	28 (51.9%)	
Age	**≤40 (n=24)**	11 (39.3%)	13(24.07%)	0.151
	>40 (n=58)	17 (60.7%)	41(75.93%)	
Histological types	**IDC (n=60)**	18 (64.3%)	42 (77.8%)	0.561
	ILC (n=9)	3 (10.7%)	6 (11.1%)	
	Tubular (n=2)	1 (3.8%)	1(1.9%)	
	Mucinous (n=6)	4 (14.3%)	2 (3.7%)	
	Micropapillary (n=3)	1 (3.8%)	2 (3.7%)	
	IDC with neuroendocrine differentiation (n=1)	0 (0.0%)	1(1.9%)	
	IDC with apocrine differentiation (n=1)	1 (3.8%)	0 (0.0%)	

** P value < 0.05 was considered statistically significant.*

Table (3): Correlation between NF-κβ/p65 expression and clinicopathological parameters

Variables		NF-κβ/p65 expression		P value
		Negative (n=22)	Positive (n=38)	
Tumor size	**T1 (n=9)**	7 (31.81%)	2 (5.26%)	**0.007***
	T2 (n=27)	11 (50%)	16 (42.11%)	
	T3 (n=18)	4 (18.19%)	14 (36.84%)	
	T4 (n=6)	0 (%)	6 (15.79%)	
Tumor stage	**I (n=4)**	4(18.18%)	0 (0.0%)	**< 0.001***
	II (n=20)	13(59.09%)	7(18.42%)	
	III (n=14)	3(13.64%)	11 (28.95%)	
	IV (n=22)	2(9.09%)	20 (52.63%)	
Lymph node status	**N0 (n=16)**	5 (23%)	11 (28.9%)	0.63
	N1 (n=26)	11 (50%)	15 (39.5%)	
	N2 (n=12)	5 (23%)	7 (18.4%)	
	N3 (n=6)	1 (4%)	5 (13.2%)	
Distant metastasis	**Negative (n=38)**	15 (68.2%)	23 (60.5%)	0.55
	Positive (n=22)	7 (31.8%)	15 (39.5%)	

** P value < 0.05 was considered statistically significant.*

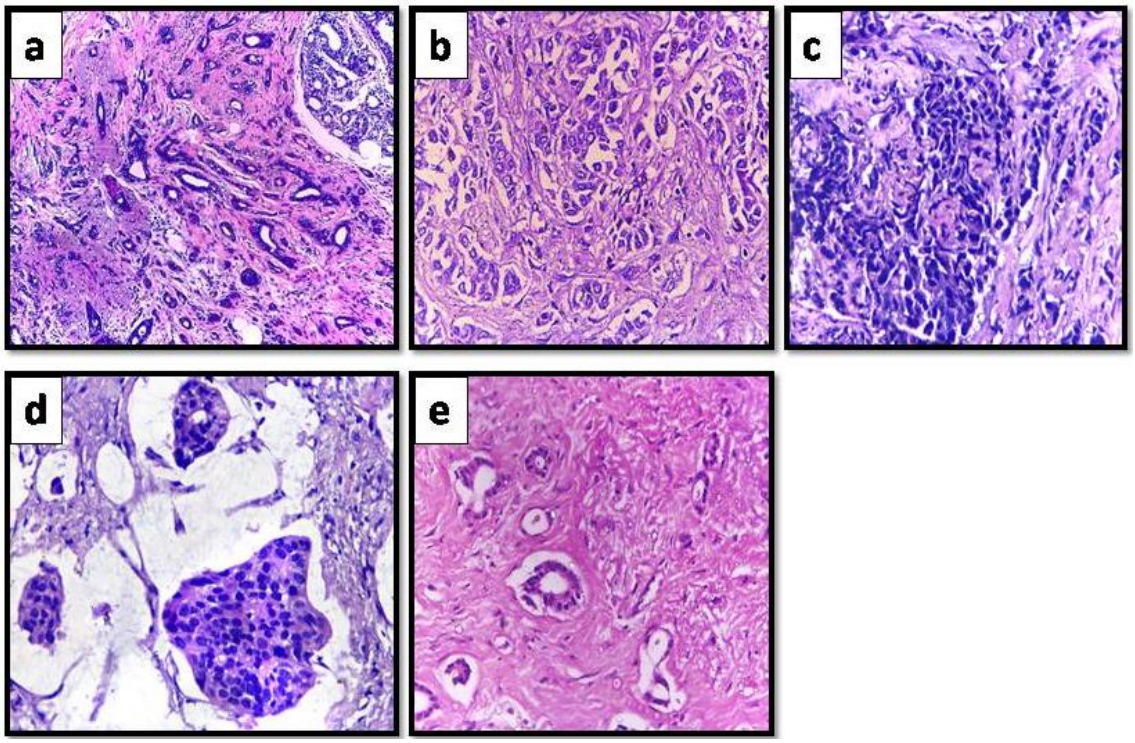

Figure (1): Histopathological findings of different types of invasive breast cancer with different grades in H&E-stained sections. (a) Luminal A subtype of IDC (NOS) grade II with solid nests, trabeculae, and open tubules separated by fibroblastic cellular stroma that infiltrated by chronic inflammatory cells (*H&E x200 original magnification*). **(b)** Luminal B subtype of IDC grade II showing nests and trabeculae of malignant ductal epithelial cells infiltrating collagenous stroma (*H&E x200 of original magnification*). **(c)** IDC (NOS) grade

III showing solid nests, trabeculae, and cords of pleomorphic malignant cells with intervening fibrocellular stroma (*H&E x400 of original magnification*). **(d)** Luminal A subtype of IDC grade II showing mucinous carcinoma with a large mass of pleomorphic malignant epithelial cells in mucinous pools (*H&E x400 of original magnification*). **(e)** TNBC subtype of IBC grade I showing tubular carcinoma with open tubules that have angulated outlines, irregulars arranged in dense fibroblastic stroma, and tumor cells showing some pleomorphism (*H&E x200 of original magnification*).

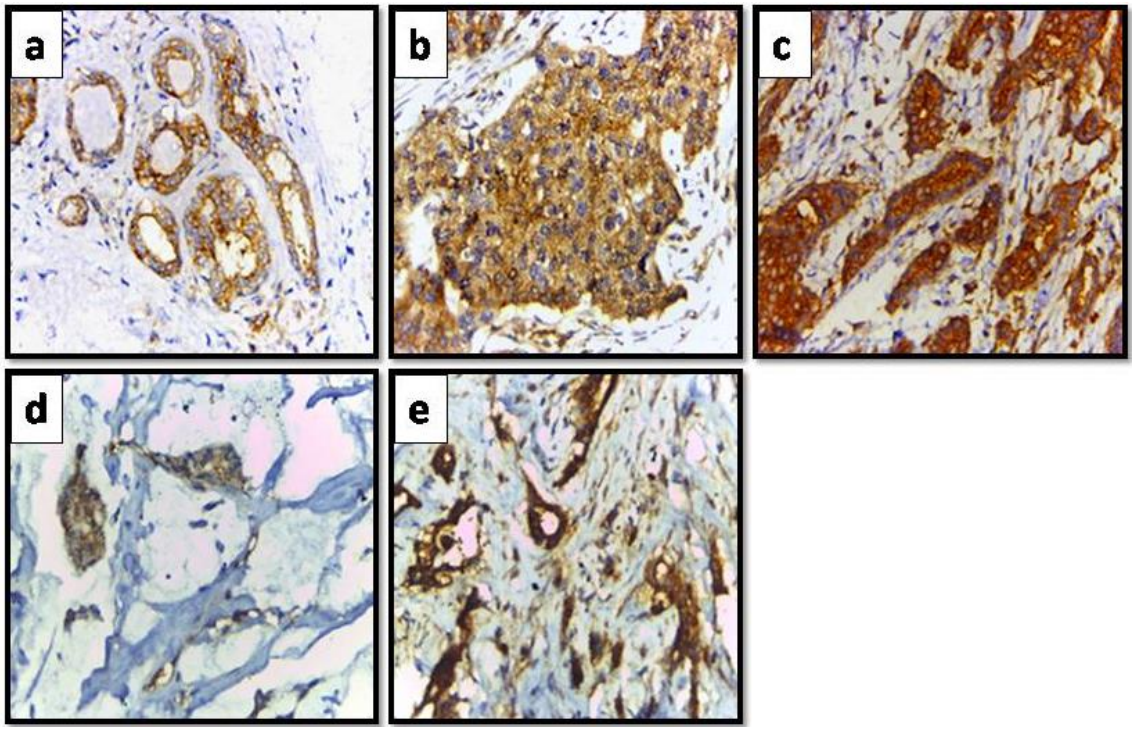

Figure (2): Histopathological findings of different types of invasive breast cancer in immunohistochemically stained sections. (a) A case of IDC showing low expression of NF-κβ/p65 immunostaining, with a straw yellow cytoplasmic staining score (3) (*ABC x200 of original magnification*). **(b)** IDC showing moderate expression of NF-κβ/p65 immunostaining with brown staining of cytoplasm, score (5) (*ABC x 400 of original magnification*)**. (c)** A case of HER2/neu overexpression showing high expression of NF-κβ/p65, score (6) with tan, brown cytoplasmic staining (*ABC x 400 of original magnification*)**. (d)** A case of mucinous carcinoma showing NF-κβ/p65 immunostaining with moderate expression, score (4), brown cytoplasmic staining (*ABC x200 of original magnification*)**. (e)** A case of IDC (NOS) subtype showing high expression of NF-κβ/p65 immunostaining with tan, brown nuclear staining score (7) (*ABC x of original magnification*).

DISCUSSION

Numerous chemotherapeutic drugs can cause NF-κβ/p65 translocation and target gene activation. This indicates that a poor pathological response to neoadjuvant chemotherapy is likely to occur [18]. NF-κβ/p65 expression was used to determine the prognostic relevance of the disease in 82 breast cancer patients at Zagazig University Hospitals and how it related to other molecular and clinical markers.

According to this study, NF-κβ/p65 was expressed in 65.9% of breast cancer tissue specimens, but it was not detected in the control

group, which consisted of patients with benign breast tumors (fibroadenoma). When Prajoko and Aryandono [18] examined 113 breast cancer samples, they discovered that 70.2% of the samples were positive and 29.8% had negative NF-κβ/p65.

It was further shown in this work that NF-κβ/p65 had a statistically significant inverse relationship with ER expression (P value = 0.001) and NF-κβ/p65 expression was more common in the ER-negative tumors (68.5%) than in ER-positive breast cancer (31.5%). Similarly, PR and NF-κβ/p65 had a statistically significant inverse relationship (P=0.002), with NF-κβ/p65 expression being 24.1% in PR-positive tumors and 75.9% in PR-negative tumors. Previous investigations by Sarkar et al. [19] had similar findings. They reported that the frequency of NF-κβ/p65 activation varied between ER/PR positive and ER/PR negative tumors in a statistically meaningful way. They claimed that the lack of estrogen function in breast cancer caused NF-κβ/p65 to be activated through epidermal growth factor receptor-2. Via the route of mitogen-activated protein kinase (MAPK), HER2/neu attaching to its receptor eventually activates NF-κβ/p65, increasing its activity in ER-negative breast cancer cells and promoting tumor cell survival. Progesterone receptor activation can reduce the transcriptional activity and DNA binding of NF-κβ/p65-driven genes by inhibiting their expression.

In contrast to the results of this investigation, studies by Barnes et al. [20], Al-Mutairi et al. [17], and Agrawal et al. [21] discovered no meaningful correlation between PR and NF-κβ/p65 expression in the ER.

The interactions between NF-κβ and ER might be advantageous. According to Frasor et al. [22], messenger RNA (mRNA) transcription is synergistically boosted after the ER/p65 complex's binding to its response elements is stable.

Inhibitory crosstalk may be mediated by tumor necrosis factor alpha (TNF-α). TNF-α-induced NF-κβ production may be inhibited by PR, and PR suppression may follow TNF-α-induced activation of NF-κβ [19].

NF-κβ/p65 and HER-2/neu expression were shown to be statistically significantly correlated in the current study (P=0.008). HER-2/neu positive tumors had a higher frequency of NF-κβ/p65 activation (33.33%). This result is consistent with the research by Cavalcante et al. [23] and Sarkar et al. [19], who discovered a statistically significant correlation between tumors overexpressing the HER-2/neu oncoprotein and p65 positivity.

Researchers studying breast cancer need to further investigate this discrepancy because Barnes et al. [20], Al-Mutairi et al. [17], and Agrawal et al. [21] discovered that HER2 negative tissues have a substantial expression of NF-κβ/p65.

Sarkar et al. [19] demonstrated that IKKα, which involves serine 536 phosphorylation of the p65 subunit, has a greater impact than IKKβ in HER-2 breast cancer cell activation of NF-κβ/p65. Therefore, the primary function of metastasis through IKKα and HER-2/neu activation is NF-κβ/p65 activation.

HER2 controls cell proliferation, differentiation, and survival and belongs to the family of receptor tyrosine kinases (RTKs). Because of elevated HER2 levels, 15–20% of individuals with breast cancer are categorized as HER2 positive. These tumors usually have a poor prognosis and develop quickly.

According to the current study, NF-κβ/p65 expression is prevalent in BCs with a high Ki67 proliferative index (79.6% and 20.4% instances, respectively). The expression of NF-κβ/p65 and Ki67 showed a statistically significant correlation (P=0.013). Similarly, NF-κβ/p65 and Ki67 expression were reported to be statistically significantly correlated by Barnes et al. [20], suggesting that tumor tissues with higher levels of NF-κβ/p65 expression have higher rates of proliferation. Studies by Al-Mutairi et al. [17] and Agrawal et al. [21] reported no significant correlation between NF-κβ/p65 and Ki67 level, which is in contrast to the current investigation.

NF-κβ/p65 expression and tumor grade were significantly correlated in this study (P=0.008). According to Barnes et al. [20], grade II tumors with strong NF-κβ/p65 expression were around ten times that of grade I tumors (54.2% vs. 5.1%) (P=0.029). Grade II revealed 55.6% positive cases, followed by grade III with 40.7%, compared to grade I (3.7%).

In research of 99 BC patients, Al-Mutairi et al. [17] discovered a considerable correlation between samples with high-grade malignancies and NF-κβ/p65 expression. Within the first five years, patients with grades II and III had a worse prognosis than those with grade I, demonstrating the effectiveness of tumor grading as a predictive and prognostic indication. Additionally, there was a significant association between histological grading, pathological response, and disease-free survival in patients with locally advanced stage BC who underwent chemotherapy, indicating the usefulness of tumor grading as a predictor of chemotherapy response.

High-grade tumors exhibit higher levels of NF-κβ/p65, indicating the usefulness of histopathological grading as a predictor of therapy response. It could also reflect the genetic background of the patients under study or indicate the part NF-κβ/p65 plays in tumor growth through dedifferentiation [17, 19].

There was a strong correlation between the expression of NF-κβ/p65 and the molecular subtype of breast cancer. NF-κβ/p65 was found to be substantially expressed in luminal B, HER2/neu, and TNBC subtypes (22.2%, 16.6%, and 51.9%, respectively) in this study compared to the luminal A subtype (9.3%) (P=0.001). This outcome is in line with research conducted by Barnes et al. [20]. The overexpression rates for TNBC, luminal A, luminal B, and HER2 cases were 17%, 25.4%, 8.5%, and 49.1%, respectively. They showed that those with TNBC have greater levels of NF-κβ/p65 expression. They suggested NF-κβ/p65 as a possible biomarker for cancer progression, prognosis, and staging.

Because NF-κβ/p65 is believed to stay in the cytoplasm, its deactivation is considered to improve outcomes for individuals with breast cancer, especially those with the triple-negative subtype. The chemical changes that activate NF-κβ/p65 are thought to be associated with increased expression of this protein [20].

One possible explanation for its aggression could be the control of NF-κβ/p65. It has been demonstrated that cancer cells with an active NF-κβ/p65 pathway are more resistant to ionizing radiation and chemotherapy, but cancer cells that have this route inhibited are far more susceptible to these treatments [21].

With a mean age of 50.4±12.4, patients with BC beyond 40 years old accounted for 70.7% of the study's cases. Age and NF-κβ/p65 expression did not significantly correlate (P=0.151). Similar results have been reported by Al-Mutairi et al. [17], who examined 99 BC cases and discovered that patients in their forties comprised a sizable age group, making up 75.8% of the total. This is in line with earlier research by Agrawal et al. [21]. In a different study, Sarkar et al. [19] discovered a statistically insignificant (p=0.973) association between postmenopausal status and high levels of NF-κβ/p65 expression in Indian patients (71.8%). Other factors, including sample size and the genetic background of the cases, may have contributed to this discrepancy.

Histological subtypes revealed that NF-κβ/p65 expression was present in 22.2% of other BC types and 77.8% of invasive ductal carcinoma (NOS). NF-κβ/p65 expression varied from low to moderate to high levels in the different BC histological subtypes, and the association between histological subtypes and expression was not statistically significant (P=0.561).

These results are consistent with previous studies by Barnes et al. [20], Al-Mutairi et al. [17], and Agrawal et al. [21], which did not discover a significant correlation between NF-κβ/p65 expression and histological types. In terms of tumor size, T2 (45%) and T3 (30%) tumors accounted for the majority of the cases in this study. Large tumors (≥5 cm) in T3 and T4 had a higher NF-κβ/p65 activation (36.84% and 15.79%, respectively) than small tumors (<2 cm) in T1 and T2 (5.26% and 42.11%,

respectively), with a statistically significant difference (p=0.007). This outcome was in line with earlier research by Al-Mutairi et al. [17], which discovered that the cytoplasm contained solely NF-κβ/p65 and that it was highly linked with tumor size (p = 0.018), with cancers larger than 2 cm showing positive staining in 81.4% of patients. According to previous studies, Indian patients had a statistically significant difference (p = 0.012) between the percentage of large (≥5 cm) cancers (89.3%) and little (<2 cm) tumors (42.9%) [19, 21].

This suggests that aggressive tumor traits, including big size, high grade, poor differentiation, and involvement in tumor development, are linked to NF-κβ/p65.

In contrast to the current study's findings, Barnes et al. [20] proposed that NF-κβ/p65 expression did not correlate with tumor development.

There was a statistically significant correlation between the tumor stage and NF-κβ/p65 expression (P=<0.001). This is consistent with earlier research conducted by Wang et al. [24].

In contrast to earlier research, Barnes et al. [20] found no statistically significant variations in NF-κβ/p65 activity according to the stage of the malignant tumor.

According to the current study, N1 exhibited greater levels of NF-κβ/p65 expression (39.5%) in relation to lymph node metastases than N0 (28.9%) and N2 (18.4%). There was no statistically significant correlation between NF-κβ/p65 expression and nodal status (P=0.63).

Similarly, there was no statistically significant association between NF-κβ/p65 expression and malignant lymph node metastasis, according to Agrawal et al. [21], Barnes et al. [20], Al-Mutairi et al. [17], and Sarkar et al. [19].

According to Barnes et al. [20], the majority of earlier research revealed no correlation between NF-κβ and p65 expression.

In addition to encouraging angiogenesis, inhibiting apoptosis, and promoting tumor cell proliferation, NF-κβ/p65 activation also triggers the epithelial mesenchymal transition (EMT), which makes distant metastasis easier [25]. In the current investigation, 15 individuals (39.5%) with distant metastases had NF-κβ/p65 expression. Distant metastasis and NF-κβ/p65 expression do not statistically significantly correlate (P=0.55). In contrast to our findings, Mirzaei et al. [26] discovered a strong correlation between distant metastasis and nuclear NF-κβ/p65 expression. They propose that NF-κβ/p65 is translocated in the nucleus as a result of high activation of the protein.

CONCLUSION

The prognostic value of NF-κβ/p65 in Egyptian breast cancer patients was investigated in this study. The present study indicates the importance of NF-κβ/p65 in breast cancer oncogenesis and tumor development, since it is associated with bigger, higher grade, and tumor stage. NF-κβ/p65 is linked to aggressive tumor biology, and its high expression in ER-negative, PR-negative, and HER2/neu overexpressing breast cancer tumors indicates a bad prognosis in breast cancer. Target genes that can prevent apoptosis, interfere with cell cycle regulation, and aid in the development of cancer were induced as a result of the NF-κβ/p65 signaling pathway's activation and exhibit chemoresistance and radio-resistance. When assessing the pathogenic response to neo-adjuvant chemotherapy, NF-κβ/p65 expression yields good findings. In clinical practice, it can be used to predict chemotherapy resistance. Inhibition of NF-κβ/p65 overexpression may prevent breast carcinogenesis and slow the growth of tumors in patients, lowering the risk of breast cancer in high-risk individuals. The development of NF-κβ/p65 inhibitors is being pursued vigorously in order to determine whether it is feasible to include its status as a new therapeutic target.

Conflict of interest: None.

Financial disclosures: None.

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Citation

Abdel Hay, H., Nasr, A., Atwa, H., Gobran, M. Nuclear Factor- κβ Expression in Invasive Breast Cancer. *Zagazig University Medical Journal*, 2025; (1407-1417): -. doi: 10.21608/zumj.2025.353263.3800