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Research Article:

The Role of Immunohistochemical Expression of p63 in Atypical Proliferative Lesions and Carcinoma In Situ of the Breast

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Abstract

Background: The transcription factor p63 is a member of a family of transcription factors p53, which play a fundamental role in cell growth, multiplication, programming, differentiation and apoptosis, henceforth, aberrantly involved in the form of different variants in breast cancers. **Objectives:** The study aimed to determine if p63 could serve as a novel method for identifying nuclear myoepithelial cells in a normal growing cell setting for both breast cancer in situ and lesions. **Methods:** This study is a combination of retrospective and prospective case series analysis. It involved examining 86 samples of breast lesions obtained from private clinics and laboratories in Ninawa Province (Iraq). The study was done on the excisional biopsy, lumpectomy, and mastectomy specimens. We performed an immunohistochemical (IHC) analysis to examine the intensity of the p63 nuclear protein. The extent was scored based on the percentage of positive cells and assigned a score of negative (0%), score 1 (<25%), score 2 (26–90%), and score 3 (91-100%). **Results:** 61 of the 86 cases examined had benign diagnosis, while 25 were malignant, Nuclear positivity was found in all benign lesions when using IHC staining with p63 and in the malignant cases 19 were found to be positive while 6 were negative. **Conclusions**: Based on the aforementioned data, we can conclude that p63 serves as a valid IHC marker for distinguishing between challenging cases, carcinoma in situ cases, cases in the grey area, and cases with an unclear histomorphological diagnosis

1. Introduction

[1]Because of its high rates of illness and death, breast cancer is a disease that primarily affects women and requires substantial attention. Breast cancer accounts for the majority of female cancer deaths and is the most common type of cancer in women, with an annual incidence of 2.1 million cases (1). In proliferative lesions, invasive lesions loosens the myoepithelial cell layer surrounding the benign mammary gland, p63 antibody is a myoepithelial cell marker that selectively stains cell nuclei. Interstitial cells, myofibroblasts, and adipocytes exhibit a negative response as well (2). This attribute renders p63 more precise and superior compared to other myoepithelial markers (1).It is essential to distinguish benign lesions from in situ and invasive breast cancer to assess a patient's condition and decide the best course of action for each unique case. The risk of developing breast cancer is a concern(2). Clear morphologic criteria can be used to classify most breast lesions, but in certain cases, additional testing, like immunohistochemistry, is required because of the unclear morphology (3).

Using one or more marker proteins, researchers can use IHC techniques to characterize various tumour subtypes, confirm the origin tissue, and distinguish between primary and metastatic tumours. It also offers helpful details about the prognosis, treatment response, and assessment of any leftover tumours following treatment. Several antibodies can assess the prognosis of breast lesions, and their response to

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treatment, and aid in making a final diagnosis using IHC in challenging cases (4).

Studies have demonstrated that p63, a homolog of p53, reliably indicates tubular myoepithelial cells. Because only myoepithelial cells in normal breast tissue express p63, this marker is extremely useful for identifying benign lesions. p63 is very helpful because it shows nuclear staining and does not show staining in smooth muscle cells like myofibroblasts and blood vessels (5). Over the past decade, researchers have made great progress in discovering and applying antibody markers for immunohistochemical identification of myoepithelial cells. These markers have proven highly useful in distinguishing between benign and malignant lesions (2).

Even though all current markers remain incomplete due to cross-reactivity, its unique and almost absolute specificity for breast tissue makes p63 a better choice (6). This study sought to enhance our understanding of the differences in p63 expression between non-cancerous and cancerous breast abnormalities.

2. Materials and Methods

2.1 Study Design

This case-series study is both prospective and retrospective. We gathered 86 samples of breast lesions over 10 months, from November 2022 to August 2023, from various private clinics and private laboratories in Ninawa Province (Iraq). The study was done on the excisional biopsy, lumpectomy, and mastectomy specimens.

2.2 Inclusion criteria:

Any cases with proliferative diseases diagnosed through a period of study were included in the study these were fibrocystic disease and epithelial hyperplasia with or without atypia, papilloma, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC) and lobular carcinoma.

2.3 Exclusion criteria:

Any case with inadequate tissue samples, inflammatory cases, and non-proliferative diseases were excluded from the study.

2.4 Histological and immunohistochemical study

The formalin-fixed paraffin-embedded (FFPE) tissue were sliced into 4-mm-thick sections. Next, these sections were subjected to a process of clearing and rehydration using a sequence of alcohols and xylene. Finally, the tissues were stained using the P63 immunohistochemical stain. Prepare the Dako Target Retrieval Solution (50x) by diluting the concentrate at a ratio of 1:50 in either distilled or deionized water (7).

The Dako Target Retrieval Solution (50x) was used in combination with a water bath-based Dako PT (pre-treatment) Link tank for the processes of deparaffinization, epitope retrieval, and re-hydration. To enhance the staining intensity, we carried out these processes by identifying the antigens using single primary antibodies. The procedure was done as per the manufacturer's instructions. Positive control: Including the tonsil as a positive control in the testing is justified because it is cost-effective.Negative Control: non-reactive sections of the positive control tissue. The conversion of glass slides into electronic digital images involves a series of steps, which include drying the slides to prevent adherence to racks, transferring the slides to scanning racks and loading the racks into scanners. An initial process was carried out to capture tissue and ensure the scanning of all tissue on the slide. We captured the slide images at a high level of detail and then removed the rack from the scanner. Finally, we visually inspected the images to ensure satisfactory quality.

Evaluation of p63 expression: The degree of p63 expression were assessed by categorizing it into four distinct groups: continuous positive, less continuous positive, discontinuous positive, and negative. The extent was assessed by calculating the percentage of positive cells and assigning a score as follows: score 0 for 0% negative cells, score 1 for less than 25% positive cells, score 2 for 26–90% positive cells, and score 3 for 91–100% positive cells (1) (**Figure 1** and **Figure 2**).

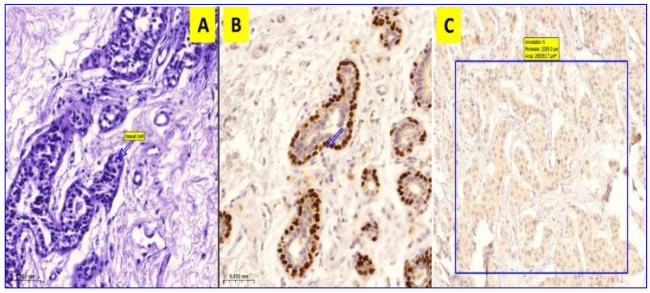

Figure 1. Normal breast acini and ducts stained by (A) hematoxylin and eosin (B) p63 immunostain showing continuous expression of p63 this picture used as positive control for our study (200x). (C) p63 immunostain (100x) showig no expression of p63 (negative control).

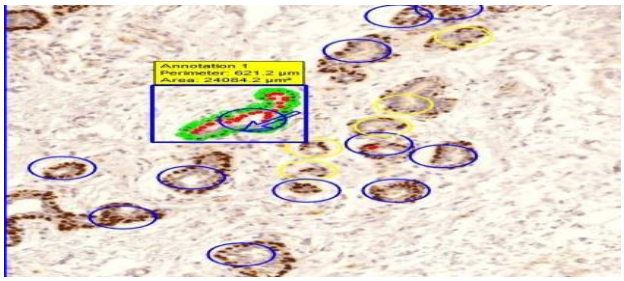

Figure 2. Showing the method of counting the myoepithelial cells in each duct that circuled by blue circles using 3DHISTECH slide viewer software to facilitate assessment of

assigned a score of negative (0%), 1 (<25%), 2 (26–90%), and 3 (91–100%) based on the percentage of positive cells.

2.5 Statistical analysis

The statistical analysis of the quantitative data conducted using the descriptive statistics utilizing the chi-square test, more specifically, Fischer's exact test. The statistical analysis were performed using the software Statistical Package for the Social Sciences (SPSS) version 20 and calculated the p-value, p-value less than 0.05 considered as significant.

3. Results

61 of the 86 cases examined had benign diagnosis. Out of the 61 (70.9%) patients, 20 (23.3%) cases were diagnosed with usual ductal hyperplasia (DH) , while 18 (20.9%) cases were found to have atypical DH, making them the most common types of benign tumours detected in the study compared to 16, 5, and 2 cases of sclerosing adenosis, intraductal papilloma, and atypical lobular hyperplasia. Of the total number of patients, 25 (29%) had a malignant tumour, with DCIS being the most common type, found in 17 (19.8%) patients. Out of the total of 86 cases, 38 (44.2%) were lumpectomy specimens, 19 (22%) were modified radical mastectomy (MRM) specimens, 10 (11.7%) were excisional biopsy specimens, 13 (15.2%) were simple mastectomy specimens, and 6 (6.9%) were of an unknown type of biopsy (**Table 1**).

Table 1. Distribution of study sample according to diagnosis.

	Diagnostic types	**N(%)**
Benign (n=61)	Usual DH	20(23.3)
	Atypical DH	18(20.9)
	Sclerosing Adenosis	16(18.7)
	Intraductal Papilloma	5(5.8)
	Atypical Lobular Hyperplasia	2(2.3)
Malignant (n=25)	DCI	18(20.9)
	cinomaInvasive Ductal Car	6(6.9)
	Lobular Carcinoma in Situ	1(1.2)
Total		86(100)

It revealed that the positivity rate for p63 was 100% for benign tumors and 76% for malignant tumors. Conversely, the negativity rate for p63 was 24% for malignant tumors (**Table 2**).

Table 2. Presents the levels of p63 expression in both benign and malignant cases, with a total sample size of 86.

	Positivity for p63 n (%)	Negativity for p63 n (%)
Benign (n=61)	61(100)	0(0)
Malignant (n=25)	19(76)	6 (24)
Total	80(93.1)	6(6.9)

These results showed that less continuous intensity was found in all types of benign tumours except intraductal papilloma which showed continuous intensity. The intensity for the malignant tumour was discontinuous and negative in IDC (**Table 3**).

Table 3. The presence of p63 was detected in 86 distinct histological subtypes of both benign and malignant lesions.

	Diagnosis	n	P63 intensity
Benign (n=61)	Usual DH	20	Less continuous
	Atypical DH	18	Less continuous
	Sclerosing Adenosis	16	Less continuous
	raductal PapillomaInt	5	Continuous
	Atypical Lobular Hyperplasia	2	Less continuous
Malignant (n=25)	Ductal Carcinoma in Situ	18	Discontinuous
	Invasive Ductal Carcinoma	6	Negative
	Lobular Carcinoma in Situ	1	Discontinuous

The study revealed that all types of benign tumours had score 2 except intraductal papilloma which had score 3. While the malignant tumour had a score 1. Apart from 6 patients with Invasive DC who had scores 0 (**Table 4**).

Table 4. p63 scoring in various histological types of benign and malignant lesions (n=86).

	Diagnosis	n	Score for p63%			
			%0	%25≥	%90-26	%100-91
			0-Score	1-Score	2-Score	3-Score
Benign (n=61)	Usual Ductal Hyperplasia	20	0	0	20	0
	Atypical Ductal Hyperplasia	18	0	0	18	0
	Sclerosing Adenosis	16	0	0	16	0
	Intraductal Papilloma	5	0	0	0	5
	Atypical Lobular Hyperplasia	2	0	0	2	0
Malignant (n=25)	Ductal Carcinoma in Situ	18	0	1	0	0
	Invasive DC	6	6	0	0	0
	Lobular Carcinoma in Situ	1	0	1	0	0

Among a total of 86 cases, 61 cases (70.9%) were benign lesions, 20 (23.3%) cases of usual DH,18 (20.9%) atypical DH,16 (18.6%) sclerosing adenosis, and 2 (2.3%) atypical lobular hyperplasia were less continuous p63 positivity with scoring 2, while 5 (5.8%) intraductal papilloma showed continuous p63 positivity with scoring 3. Out of 25 (29%) malignant cases, 19 (76%) showed discontinuous p63 positivity with scoring 1, while 6 (24%) were p63 negative with a score of 0 (**Table 5** and **Figure 3**).

Table 5. The expression of p63 and its score in different types of breast lesions with different histopathological characteristics

	sDiagnosi	n	Expression of p63	P63 scoring
Benign (n=61)	Usual Ductal Hyperplasia	20	Less continuous	2
	Atypical Ductal Hyperplasia	18	Less continuous	2
	Sclerosing Adenosis	16	Less continuous	2
	Intraductal Papilloma	5	Continuous	3
	lasiaAtypical Lobular Hyperp	2	Less continuous	2
Malignant (n=25)	Ductal Carcinoma in Situ	18	Discontinuous	1
	Invasive DC	6	Negative	0
	Lobular Carcinoma in Situ	1	Discontinuous	1

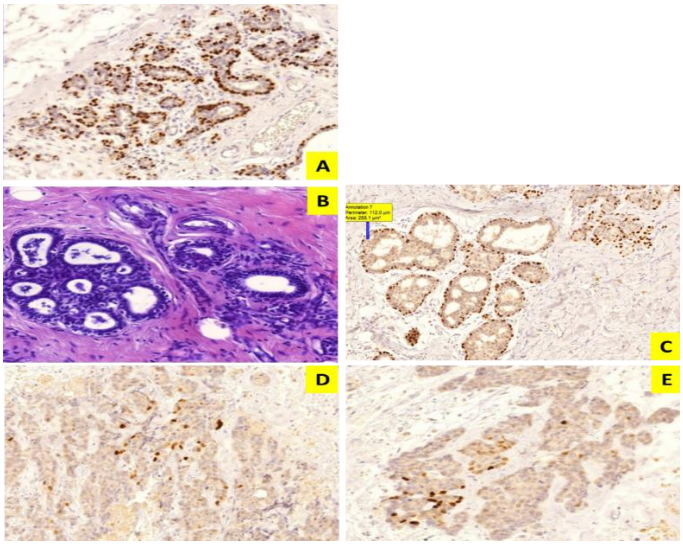

Figure 3. (A) Sclerosing Adenosis demonstrating less continuous immunostaining by p63 and score 2 mainly at the left bottom. (B) Atypical DH showing the growth of cells in an abnormal pattern (H&E) (C) Atypical DH of the breast revealing less continuous staining by p63 score 2. (D and E) DCIS showing discontinuous positive staining by p63 and score 1. (100x).

The age range of the study participants was 14–75 years old. The study revealed that the fifth decade of life had the greatest number of cases, closely followed by the fourth decade. 38.9 years was the average age of presentation. The distribution of the tumour types according to the size of the lump (**Table 6**) and revealed that a lump size of below 2 cm was found in 10 cases (16.4%) of the benign tumours but not in malignant tumours while the lump was more than 5 cm was found in 6 (9.8%) and 14 (56%) of benign and malignant tumours respectively. Among the types of tumor 4 (6.6%) of benign lesions and 6 (24%) of malignant lesions were not assessed in that order. The average size of benign tumours was 3.5, while the average size of malignant tumours was 7.5.

Table 6. Type of tumour according to the size of the lump.

	Size of lump (cm)	2>	2-5	5<	Not assessed
Type of tumour	Benign n(%)	10(16.4)	41(67.2)	6(9.8)	4(6.6)
	Malignant n(%)	0(0)	5(20)	14(56)	6(24)

The findings demonstrated a highly significant disparity (p=0.0001) in the levels of p63 expression between benign and malignant tumours about positivity and negativity. Benign and malignant breast lesions exhibited a notable discrepancy in p63 expression, emphasizing a significant difference between the two categories. **Table 7** demonstrates a persistent and favorable presence of p63 in non-cancerous growths. Hence, the immunohistochemical (IHC) detection of p63 can reliably differentiate between benign and malignant breast abnormalities.

Table 7. Presents a comparison of p63 about different tumour categories.

Category	Positivity for p63 (n=80) n(%)	Negativity for p63 (n=6) n(%)	p value
Benign	61 (76.3)	0 (0)	0.0001
Malignant	19 (23.7)	6 (100)	
*Fisher Exact test has been used			

4. Discussion

Typically, healthy breast myoepithelial cells exclusively exhibit p63 expression. As such, p63 can be a useful marker for benign lesions' differentiation. Researchers have found that p63 exhibits a sensitivity of 90% and a specificity of approximately 100% (8). Previous studies have shown that p63 immunostain was present in the myoepithelial cell layer surrounding the luminal epithelial cells in all benign breast lesions. In DCIS, a less continuous rim myoepithelial cells (MEC) was detected by p63 staining, while there was a complete loss of p63 immunostain in all invasive breast cancers (9). In de Souza Queiroz et al. (2006), myoepithelial cells of all benign sclerosing lesions were expressed p63-like sclerosing adenosis (10). Pavlakis et al. (2006) found that p63 is not a reliable method for identifying myoepithelial cells in large sclerosing breast lesions. However, in smaller lesions, it remains highly valuable (11). Therefore, the specificity of p63 is approximately 100%, and its sensitivity has been determined to be around 90%(5). In Werling et al. (2003) study, 11 (11.6%) cases of adenosclerosis, 33 (34.7%) cases of DCIS, of which 10 (10.5%) presented micro invasion, 6 (6.3%) cases of lobular carcinoma in situ (LCIS) and 35 (36.8%) cases of IDC (6), no case showed p63 expression by myofibroblasts or vascular smooth muscle cells because greater part of cases 21(39.6%) were in the range of 30-50 years old.

Based on histopathological characteristics, 70.9 cases in our study were determined to be benign and 29.1 cases were malignant. Our study's findings closely align with the research conducted by Modi et al. (2014), who reported 72 cases of benign tumours and 16.7% cases of malignant tumours (12). P63 immunostaining of the myoepithelial cells

was positive in most of the cases. Some problems with the myoepithelial cell layer can cause scattered positive immunostaining around noninvasive groups of epithelial cells. This can happen in DCIS, for example. Vascular smooth muscle cells, or myofibroblasts, did not express p63 (6). Thakkar et al. (2014) and Bukhari et al. (2011) discovered that 54.16% and 60% of the cases, respectively, were benign (13,14). Chaudhary et al. (2012) and Rahman and Islam (2013) reported that malignant cases occurred at frequencies of 14.7% and 19.23%, respectively (15,16).

In our study, the most prevalent cases consisted of usual DH, which is a benign lesion and accounted for 23.3% of the total. The finding was in line with the prevailing body of literature on benign breast masses, which indicates that usual DH accounts for 46.6%–55.6% of all cases (17). Typical DH, a benign lesion, was the most common case in our study, accounting for 23.3% of all cases. The majority of research on benign breast masses shows that normal DH accounts for 46.6%–55.6% of cases (17). Another study concluded that DCIS-associated myoepithelial cells have a different phenotype from their normal counterparts (18). The IDC was 6.9 % of all malignant tumours however, This result was different from the study of Verma et al. (2018)(87.5%), Bhagat et al. (2012) (94.82%), and Juneja et al. (2019) (92.72%) (8,19,20).

The Reis-Filho study differed from the present study because p63-positive myoepithelial cells were observed in all DCIS cases and 9 of 15 IDC cases ($p = 0.0375$) (21). We analyzed a total of twenty cases, which consisted of three cases of DCIS and five cases of IDC. In our investigation, we identified scattered malignant epithelial cells that expressed the p63 protein(20). Out of 174 invasive carcinomas in Koker and Kleer's (2004) study, only one (0.6%) tested positive for p63 (22). LCIS in our study was 1.2%. LCIS has characteristic morphological features: "Cells lose polarity, and change shape while maintaining a surprisingly uniform size.

LCIS is multifocal: It is always a multifocal disease. The features mentioned above characterize the "classic" form of LCIS. Although classic LCIS is both a risk factor and an optional precursor to invasive breast cancer and does not require complete excision and/or assessment of margin status (23).

In Abdallah and El-Deeb (2017) study, 40 cases of DCIS ± invasive ductal or lobular carcinoma were analyzed for myoepithelial markers, in all types of lesions; non-invasive breast, anti-P63 antibodies were positive on the majority of myoepithelial cells differences were noted in the immunostaining of low-grade DCIS compared with high-grade DCIS. However, in a minority of cases, myoepithelial cells showed incomplete positivity (score 2) for the antibody, resulting in apparent gaps in the myoepithelial cell layer, considered a “discontinuous” positive signal around DCIS nests are defined as a distance equivalent to two or more nuclei between two positive nuclei (24). All benign tumours in our study showed a 100% positive presence of p63. P63 was not detected in 6 cases (6.9%) of cancerous tumours, while it was detected in 19 cases (22%) of cancerous growths. There were a combined total of 19 cases, with 18 cases classified as DCIS and one case classified as LCIS.

Similar to our findings, Barbareschi et al. (2001) reported p63 positivity in all benign lesions but not in invasive breast cancer in 95% of cases (25). Wang et al. (2002) investigated the expression of p63 in benign and malignant breast tumours. Only normal breast myoepithelial cells express the p63 gene. Only partially, infrequently, and never present in carcinoma in situ (CIS), DH is absent in invasive carcinoma (26). Stefanou et al. (2004), researchers found p63 immunoreactivity in the layer of myoepithelial cells that envelop the luminal epithelial cells in all benign lesions(9). In every case of CIS, P63 staining consistently showed a discontinuous border of myoepithelial cells. Peripheral p63 staining was absent from all of the invasive cancers. Interestingly, only a small percentage (5–15%) of epithelial cells showed p63 nuclear immunoreactivity in all cases of papilloma, 62.5% of papillary carcinoma in situ, and 33.3% of invasive papillary carcinoma(9). Yanping and Qiurong (2006), detected p63 in the actively dividing cells in each of the 38 cases of noncancerous breast abnormalities. In their study (27), Yanping and Qiurong (2006), found that in 19 cases of DCIS and 7 cases of intraductal papillary carcinoma, there was a distinct layer surrounding the duct. Researchers identified these cells as p63-positive and observed regular separation. Verma et al. conducted a study on a total of 151 cases and showed that p63 expression was negative in 100% of malignant cases, positive in 88.6%, and negative in 11.4% of benign cases (8). Furthermore in the benign category, proliferative lesions were discontinuous

positivity for p63, ecxept 5 cases of intraductal papilloma revealed continuous positivity for p63 (8).

Among total 86 cases in our study,61 cases (70.9%) were benign lesions, 20(23.3%) cases of usual ductal hyperplasia,18(20.9%) atypical ductal hyperplasia, 16(18,6%) sclerosing Adenosis, and 2(2.3%) atypical lobular hyperplasia were less continuous p63 positivity with score 2, but 5 (5,8%) intraductal papilloma were continuous positive p63 with score 3. Out of 25 (29%) malignant cases, 19 (76%) showed discontinuous positive p63 with a score 1, while 6 (24%) showed a negative p63 with a score 0 (8).

Our study's findings were identical to those of the SAInI et al. (2021) investigation, which found that p63 was positive in 100% of benign tumors. Thirty four cases (89.5%) of malignant tumors did not show p63 staining, which is a significant percentage. Only four cases (10.5%) out of the total malignant lesions, or a lower percentage, had positive p63 staining. DCIS was the diagnosis in three of these four cases, and metaplastic carcinoma was the diagnosis in one (17).

Furthermore, Barbareschi et al. (2001) reported findings that align with our study. They discovered that all benign lesions exhibited p63 positivity, whereas invasive breast cancers showed a lack of nuclear p63 staining in 95% of cases (25). The findings of our investigation differ from those of Verma et al. (2018), who have subjected a total of 50 cases to p63 immunostaining in our study, 35 cases (70%) were determined to be benign, while 15 cases (30%) were classified as malignant. Between 35 benign cases, 31 (88.6%) demonstrated positive p63 staining, but 4 (11.4%) revealed negative p63 stain (8). In the study of Verma et al. (2018), all cases of fibroadenoma showed continuous positivity, whereas fibrocystic disease showed discontinuous positivity with a score 2, which means the percentage of p63 positive cells between (26-90%) but phyllodes tumor, gynaecomastia and fibroadenosis were negative for p63 immunostain in benign category score 0. Out of 15 malignant cases, all lacked a p63 expression score 0 (8).

The study conducted by Hill and Yeh (2005) identified a total of 25 cases of intraductal papillomas and 18 cases of papillary carcinomas, including 4 cases of invasive carcinoma, 5 cases of micropapillary carcinoma in situ, and 5 cases with p63 immunostaining. All intraductal papillomas and micropapillary DCIS cases showed the presence of p63-labeled MEC. All cases of invasive papillary carcinoma showed consistent negative results for all stains (28). Our present investigation yields comparable findings to those of Wang et al. (2002), who analyzed 40 cases encompassing normal breast tissue, ductal hyperplasia (DH), DCIS, and IDC, in which the esearchers found that p63 is exclusively present in the myoepithelial cells of healthy breast tissue (26).

Specifically, we found a continuous positive score of 3. P63 expression was partial with a score of 2 in cases of ductal hyperplasia. In carcinoma in situ, p63 expression was less continuous, with a score of 1. Finally, p63 was discontinuous and not expressed in invasive carcinoma, score 0 (26). Ranjan et al. (2021) observed the presence of p63 exclusively in the myoepithelial cells of healthy breast tissue. Specifically, we found a continuous positive score of 3. P63 expression was partial, with a score of 2 in cases of ductal hyperplasia. In carcinoma in situ, p63 expression was less continuous, with a score of 1(29). Finally, p63 was discontinuous and not expressed in invasive carcinoma. However, among the 17 malignant lesions, 11 cases showed no p63 staining in any clusters of epithelial cells (30).

Researchers found that p63 is exclusively present in the myoepithelial cells of healthy breast tissue (31,32). Patients included in this study were between 14 and 77 years old. The highest number of cases occurred in the 50th years of life, followed by the 40th years of life. The mean age of appearance was 38.9 years. The findings were marginally higher than those reported by SAInI et al.(2021), where the average age of presentation was 37.1 years (17). Results of our study vary from a study done by Werling et al. (2003), Verma et al. (2018), Stefanou et al. (2004), and in which the greater part of cases 21(39.6%) were in the range of 30-50 years old (6,8,9).

The limitations of the present study include a small sample size, which affects the generalizability of the results. Being a retrospective study, the results biased limit the ability to draw causal inferences. Other biomarkers were neglected and the study was based on P63 immunostain expression in carcinoma in situ of the breast which might be overlooked and other markers need to be included to characterize the type of cancer. The findings do not express other populations and lack external validity.

5. Conclusion

As a myoepithelial marker, P63 is very sensitive and specific. This means that it can be used in immunohistochemical panels that are meant to find myoepithelial cells in difficult breast lesions.. very useful in differential diagnosis involving benign and malignant lesions.

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دور التعبير المناعي الكيميائي لبروتين الورم 63 في الآفات التكاثرية غير النمطية والسرطان الموضعي للثدي

الخلاصة

المقدمة: عامل نسخ البروتين الورمي 63 هو عضو في عائلة من عوامل نسخ البروتين الورمي 63، والتي تلعب دورا أساسيا في نمو الخلايا ، والتكاثر ، والبرمجة ، والتمايز ، وموت الخلايا المبرمج ، من الآن فصاعدا ، تشارك بشكل شاذ في شكل متغيرات مختلفة في سرطانات الثدي. **الأهداف**: هدفت الدراسة إلى تحديد ما إذا كان البروتين الورمي 63 ، وهو عامل نسخ نووي مرتبط بالبروتين الورمي 53 ، يمكن أن يكون بمثابة طريقة جديدة لتحديد الخلايا الظهارية العضلية النووية في بيئة خلية طبيعية النمو لكل من سرطان الثدي الموضعي والآفات. **المواد وطرائق العمل**: هذه الدراسة عبارة عن مزيج من تحليل سلسلة الحالات بأثر رجعي ومستقبلي. وشملت فحص 86 عينة من آفات الثدي التي تم الحصول عليها من العيادات والمختبرات الخاصة في محافظة نينوى. امتد جمع البيانات لمدة تسعة أشهر ، من نوفمبر 2022 إلى أغسطس 2023. أجريت الدراسة على عينات الخزعة الاستئصالية واستئصال الكتلة الورمية واستئصال الثدي. أجرينا تحليل الكيمياء الهيستولوجية المناعية لفحص شدة البروتين النووي الورمي 63 .تم تسجيل الشدة بناء على النسبة المئوية للخلايا الإيجابية وتم تعيينها الى درجات، درجة سلبية (0٪) ، ودرجة 1 (<25٪) ، ودرجة 2 (26-90٪) ، ودرجة 3 (91-100٪. **النتيجة**: 61 من أصل 86 حالة تم فحصها كانت تشخيصات حميدة ، بينما كانت 25 حالة خبيثة ، تم العثور على إيجابية نووية في جميع الآفات الحميدة عند استخدام تلطيخ الكيمياء الهيستولوجية المناعية مع البروتين الورمي 63 وفي الحالات الخبيثة تم العثور على 19 إيجابية بينما كانت 6 سلبية. **الاستنتاجات**: بناء على البيانات المذكورة أعلاه ، يمكننا أن نستنتج أن البروتين الورمي 63 يعمل كعلامة كيميائية مناعية صالحة للتمييز بين الحالات الصعبة، وحالات السرطان الموضعي، والحالات في المنطقة الرمادية، والحالات ذات التشخيص النسيجي غير الواضح.

الكلمات المفتاحية: ورم حميد ، ورم خبيث ، خلايا عضلية ظهارية ، فحص البروتين الورمي 63 ، سرطان الثدي.