Prognostic Significance of p53 Expression in Breast Carcinoma

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ABSTRACT

Background & Aim: Breast cancer is the most frequently diagnosed cancer among females worldwide. p53 tumor suppressor gene regulates the cell cycle and DNA repair and its over-expression in breast carcinoma is associated with a worse prognosis.

The aims of the our study are; first, to determine the frequency of p53 overexpression in different types of infiltrating breast carcinoma. Second, to study the correlation between the p53 over-expression with different parameters, including the age, size, grade, histologic type. the status of lymph nodes, CD10 expression .Third, to compare our results with others

Methods: The study included 100 cases of breast carcinoma. Data were obtained from archives of the pathology department, at AL-Assad University Hospital in 2017 .p53 over-expression was assessed immunohistochemically. .

Results: The patients ages ranged from 33 to 79 years ,most of them were in the fifth decade (32%). There was a significant positive relation between p53 over-expression and KI67 expresssion (P=0.000), and HER2/neu (P=0.038). There was a significant direct correlation between p53 over-expression and molecular classification (p=0.000), grade (p=0.002), axillary lymph node metastasis (p=0.000) and CD10 expression(p=0.000). p53 over-expression was found in (100%) of micropapillary carcinoma , (93.3%) of invasive breast carcinoma (NST), (50%) of invasive cribriform, and (53.3%) of invasive lobular carcinoma.

Conclusions: p53 over-expression was significantly correlated with pathologic parameters refer to worse prognosis , so p53 overexpression was found to be poor prognostic factor and its detection may help in risk catergorization and designing future target approaches.

Keywords: Breast carcinoma, p53 over-expression

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الأهمية الإنذارية لتعبيرية p53 في سرطانة الثدي

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الملخص

خلفية البحث وهدفه:سرطانة الثدي هي أكثر السرطانات المشخصة تواتراً عند النساء حول العالم، وجينة p53 هي جينة كابحة للورم تنظم دورة الخلية وإصلاح الدنا، وإن فرط تعبيريته في سرطانة الثدي تترافق مع إنذار أسوء.

يهدف هذا البحث لتحديد تواتر فرط تعبيرية الواسم p53 في الأنكاط المختلفة من سرطانة الثدي الغازية ودراسة علاقة فرط تعبيرية هذا الواسم بعوامل سريرية و باثولوجية مختلفة كالعمر وحجم الورم والنمط النسيجي وحالة العقد اللمفاوية وتعبيرية الواسم CD10 ومقارنتها بدراسات أخرى.

المواد والطرائق: شملت الدراسة 100 سيدة لديها سرطانة ثدي من أرشيف مستشفى الأسد الجامعي بدمشق، وقيمت تعبيرية الواسم p53 باستخدام الكيمياء النسيجية المناعية .

النتائج: تراوحت أعمار المريضات بين عمر 33 و79 ومعظم المريضات في العقد الخامس تبين وجود ارتباط إيجابي مهم بين تعبيرية الواسم والمشعر التكاثري (P=0.000) و تعبيرية المشعر HER2/neu (P=0.038)، مع كل من نقائل العقد اللمفاوية (P=0.000) و الدرجة النسيجية (P=0.002) و التصنيف الجزيئي للأورام (P=0.000) و تعبيرية الواسم اللحمي CD10 (P=0.000) ، وشوهدت فرط تعبيرية الواسم في 100% من حالات السرطانة الحليمية المجهرية و93.3% من السرطانة الغازية بلا نمط خاص، و 50% من حالات السرطانة الغازية الغربالية، و 53.3% من حالات السرطانة الفصيصية الغازية.

الإستنتاج: نتائجنا تركز على أن تعبيرية الواسمp53 ترتبط بشكل مهم إحصائياً بعوامل باثولوجية دالة على إنذار أسوأ لذا يعد الواسم p53 كمشعر إنذاري ضعيف ، وإن كشفه يساعد في تصنيف الاختطار ووضع مقاربات علاجية مستقبلية.

الكلمات المفتاحية:سرطانة الثدي الغازية_ تعبيرية الواسم p53

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Background:

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide. [1] The large majority of breast cancers are detected during the postmenopausal years [2] .The term "invasive breast carcinoma (lBC)" refers to a large and heterogeneous group of malignant epithelial neoplasms of the glandular elements of the breast.[4,3]

Infitrating breast carcinoma is a group of malignant epithelial neoplasms characterized by invasion of adjacent tissues(stromal invasion) and a marked tendency to metastasize to distant sites. The vast majorities of these tumors are adenocarcinomas and exhibit a wide range of morphological phenotypes.[5]In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among the most important of these advances is the realization that apoptosis and the genes that control it have a profound effect on malignant phenotype. Changes in this cell loss factor could have a major impact on tumor growth or regression.[6] The number of cancer-related parameters available to predict the prognosis in breast cancer patients has grown considerably in recent years. Prognostic factors of breast cancer include histological features (histological type, histological grade, lymphovascular invasion), tumor size, lymph node status, steroid hormone receptors status and age.[7,8] Prognostic and predictive biomarkers including p53 was also identified in breast cancer.[1]

The TP53 gene is the most commonly mutated gene in human cancers and functions in many cellular pathways including cell cycle regulation, metabolism, angiogenesis, and DNA repair mechanisms. [9] Approximately 30% of breast tumors are believed to harbor mutations in TP53[9] and recent data suggested that the frequency, spectrum, and timing of these mutations varied according to the molecular subtype of the disease. [10] In general, TP53 mutations were less common in luminal (A and B) than basal-like tumors; occurring in 26% of luminal tumors (17% for luminal A and 41% for luminal B) as compared to 50% of HER2- enriched and 88% of basal-like breast tumors Based on gene-expression profiling, breast cancer can be classified into luminal, human epidermal growth factor receptor 2 (HER2)- enriched, normal-like, and basal-like subtypes,[1] with distinct clinical and epidemiological attributes.[11] In general, compared to non-luminal (HER2-enriched and basal-like) breast cancers, luminal tumors have better survival outcomes. [9]

Aim and Objectives:

To estimate the frequency of expression of p53 in infiltrating breast carcinomas. To assess prognostic significance of p53 expression and its correlation with other standered clinicopathological factors such as age, tumor size, histological grade, lymph node status, ER ,PR HER2/neu KI67, molecular classification, histological subtype.

To estimate its correlation with **novel tumor microenviroment factors** such as **CD10** expression

Materials and Methods:

This study was performed on specimen composed of 100 women who had undergone modified radical mastectomy for infiltrating breast carcinoma in Al-Assad University Hospital (Damascus ,Syria) during 2017. None of the patients had received neoadjuvant therapy.Tissue from primary tumor was processed, paraffin blocks were prepared and seven slides were cut from each:

Slide 1: Stained with haematoxylin and eosin.

Slide 2: Immunohistochemistry for estrogen receptor

Slide 3: Immunohistochemistry for progesterone receptor.

Slide 4: Immunohistochemistry for HER2/neu

Slide 5: Immunohistochemistry for CD10

Slide 6: Immunohistochemistry for ki-67 .

Slide 6: Immunohistochemistry for p53

Hematoxylin and-eosin stained microscopic slides of the primary mass were reviewed to

complete the study, define tumor subtype, and perform grading of infiltrating carcinomas according to the Nottingham modification of the Bloom and Richardson system. [12] **Tumor size** in this study means the greatest tumor diameter in milimeter.**Lymph node status** was defined according to tumor node metastasis (TNM) staging system for breast carcinoma in which 0 correspond negative nodes, 1-3 positivenodes (N1), 4-9 positive nodes(N2), and 10 or more positive nodes (N3), respectively.**Estrogen receptor (ER) and progesterone receptor (PR) markers** were considered as positive when at least 1% of tumor cell nuclei were immunoreactive for the marker. [12] **Expression of human epidermal growth factor receptor 2 (HER2/neu)** was scored as 0 to 3 as Follow: Score of 0 (negative): no staining or membrane staining in less than 10% of the tumor cells, 1+ (negative): a faint/barely perceptible partial staining in the membrane of more than 10% of the tumor cells,2+ (weakly positive): a weak to moderate complete membrane staining in more than 10% of the tumor cells, and 3+ (strongly positive) : a strong complete membrane staining in more than 30% of the tumor cells. [12]

 CD10 expression in myoepithelial cells of normal breast tissue was used as the positive control. CD10 expression in the tumor stroma (in both stromal cells and extracellular matrix) Tabel(1):CD10 Scoring. [13]

We used well-known prognostic biomarkers, such as ER, PR, and HER2/neu, as well as clinicopathological prognostic factors including age, tumor size , grade, lymph node status , KI67 ,histological subtype , stromal CD10 expression and molecular classification to investigate the prognostic potential of p53 expression in breast carcinoma.

The collected data was analyzed using SPSS. The relationships between p53 expression and all variables were evaluated by Chi-square. P-values of less than 0.05 were considered as significant.

Results:
Descriptive Statistics:
100 cases of infiltrating breast carcinoma were included in this study. Age of the patients ranged from 33-79 years ,the majority were in 5th decade.The size of the tumors ranged from 11 to more than 50 mm and the tumor size was more than 50 mm in 48% of cases. infiltrating ductal carcinoma, no special type(NST) comprised the majority of our study population (60 cases, 60%), followed by 30 cases (30%) of invasive lobular carcinoma (ILC), 4 cases (4%) of micropapillary carcinoma,4 cases (4%) of invasive cribriform carcinoma and 2 cases (2%) of tubular carcinoma. All cases of invasive breast carcinoma were graded based on the Bloom and Richardson grading system. Most cases (56 cases, 56%) were grade II, followed by 30 (30%) grade III, and 14 (14%) grade I cases .Regarding the lymph node status, 44 cases (44%) lacked lymph node involvement.

CD10 immunostaining was performed on all 100 cases. No stromal expression was detected in the normal tissue of breast. The myoepithelial cells lining the normal TDLUs components in normal breast parenchyma adjacent to the tumor were considered as the positive control for CD10 expression.

The staining of CD10 marker was scored as negative , weak, and strong as described previously in the methods section. CD10 was found to be positive in 60% (60 cases), out of which 28% (28 cases) showed weak immunoreactivity and 32% (32 cases) showed strong immunoreactivity.Most patients in this study were ER positive (74 cases, 74%) and PR positive (72 cases, 72%).

Resutls of p53 expression:
Compared with women with p53− tumors, those with p53+ tumors had higher frequencies of aggressive tumor features including : more

proliferative index of KI67+(P-value = 0.000)(figure 1). The same finding was observed about HER2 (P = 0.038) and the highest frequency of p53 positivity was observed in cases with HER2 positivity(100%) despite the fact that all categories showed p53 positivity(figure 2).

P53 associated with higher grade (P-value = 0.002), a higher percent in P53 positivity was seen in cases with gradeIII(100%) followed by grade II (75%) followed by grade I category(57.1%) (figure 3).
and aggressive molecular subtypes(triple negative , luminal B-like/HER2+, HER2_ positive)(P-value = 0.000). In general, the prevalence of p53 IHC positive staining differed by subtype, with luminal A-like tumors comprising of fewer (16.7%) p53+ tumors than women with the luminal B-like/HER2− (85.7%), luminal B/HER2+ (100%), TN (87.5%), and HER2-positive (100%) subtypes (P value=0.000).

Chi-square test showed a statistically significant correlation between p53 expression and PR (P = 0.045). the highest frequency of p53 positivity was observed in cases with PR negativity(92.9%).
Chi-square test showed a statistically no significant correlation between p53 expression and ER (P = 0.068).despite the highest frequency of p53 positivity was observed in cases with ER negativity(92.3%).
No statistically significant association was identified between p53 expression and lymphovascular invasion(LVI) (P=0.585) (figure 4), but the highest frequency of p53 positivity was observed in cases with lymphovascular invasion than those without invasion (81%).
No statistically significant association was identified between p53 expression and tumor size (P=0.102), but the highest frequency of p53 positivity was observed in cases with higher size , a higher percent in P53 positivity was seen in cases with T3(87.5%) followed by T2 (76.2%) followed by T1 category(20%).

A statistically significant association was identified between p53 expression and lymph node status (P=000), a higher percent in P53 positivity was seen in cases with N3(90.9%) followed by N2 (87.5%) followed by N1 category(77.8%) despite the fact N0 category showed positivity for p53(72.7%).
A statistically significant association was identified between p53 expression and age(p=0.015) ,despite the fact that all age groups showed positivity for p53.
A statistically significant association was identified between p53 expression and Histologic type (p=0.000) and the highest frequency of p53 positivity was observed in micropapillary carcinoma (100%) and invasive breast carcinoma (NST)(93.3%).
Chi-square test showed a statistically significant positive correlation between p53 expression and stromal CD10 positivity (P = 0.000), the highest frequency of p53 positivity was observed in cases with CD10 positivity(70%).Overexpression and intermediate expression of p53 were associated with stromal CD10 positivity(P=0.000) (figure 5).

Discussion:

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide [1] Breast carcinoma is highly heterogeneous disease with a wide range of biological, pathological and clinical characteristics. Role of hormone receptors such as ER/PR & HER-2/neu is well known in treatment of breast cancer whereas new studies has shown the role of p53 in prognosis and overall survival of patients of breast cancer. [1]
In our study :100 cases of infiltrating breast carcinoma were included in this study. Age of the patients ranged from 33-79 years ,the majority were in 5th decade.The size of the tumors ranged from 11 to more than 50 mm and the tumor size was more than 50 mm in 48% of cases. infiltrating ductal carcinoma, no special type(NST) comprised the majority of our study population (60 cases, 60%), followed by 30 cases

(30%) of invasive lobular carcinoma (ILC), 4 cases (4%) of micropapillary carcinoma,4 cases (4%) of invasive cribriform carcinoma and 2 cases (2%) of tubular carcinoma. All cases of invasive breast carcinoma were graded based on the Bloom and Richardson grading system. Most cases (56 cases, 56%) were grade II, followed by 30 (30%) grade III, and 14 (14%) grade I cases , the same results observed in Mohammadizadeh, et al [14] study about breast carcinoma.

In M. Abubakar et al. [9] study of 7226 Chinese women(2019) with invasive breast cancer, defined breast cancer subtypes using immunohistochemical (IHC) measures of hormone receptors and HER2 in conjunction with histologic grade. p53 expression status was then used to further stratify subtypes into p53-positive and p53-negative. The frequency of p53 protein expression varied by breast cancer subtype, being lowest in the luminal A-like and highest in the triple-negative and HER2- enriched subtypes (P-value < 0.01). In luminal A-like and B-like/HER2-negative subtypes, p53 positivity was associated with, high grade, high proliferative index , all these results are similar to our study (2021)

Our results aslo were similar to results In Eman M.S. Muhammad, et al. [5] study (2012) showed that P53 expression increased significantly with increased tumor grade of IBC (p<0.006), lymphovascular invasion (p<0.003).

our study revealed strong correlation between expression of P53 and **Stromal** CD10 positivity in **tumor microenviroment** (p=0.000) , the significance appeared that most researches regarding the role of p53 in cancer have focused on its ability to elicit apoptosis or growth arrest of cells that are prone to become malignant owing to DNA damage or oncogene activation, i.e. cell-autonomous activities of p53 and it is now well recognized that cancer initiation, growth and progression is dependent on tumor microenvironment of which tumor–stroma is an integral part [15], also more recently, attention has focused on the potential prognostic value that tumor–stromal ratio (TSR) described by some as proportion of tumour may have an increasing number of different cancer types and may be p53 activation within a cell can also exert a variety of effects upon neighboring cells, through secreted factors and paracrine and endocrine mechanisms according to many studies and stromal p53 plays many roles in angiogenesis and metastases by molecular mechanisms.[15]

Attila Patocs et al [16] study showed that ,Somatic TP53 mutations in stroma, but not epithelium, of sporadic breast cancers were associated with regional nodal metastases (P = 0.003)and p53 marker might help predict nodal status.

Conclusion:

This is the first study to show that p53 expression in tumor cell poor progonsis of patients. So the immunohistochemical evaluation of p53 expression in malignant tissue is an important parameter in the assessment of the prognosis , So better understanding of the underlying molecular mechanisms and its role and expression by immunohistochemistry study in stroma in tumor microenviroment are thus required. Hopefully, this will allow their manipulation towards better inhibition of cancer initiation, progression and metastasis.

p53 expression could be a very important target for tumor gene therapy for breast carcinoma ,suppressing tumor cell–stromal cell interactions arising from p53 gene abnormalities or p53-related tumor microenvironment reactions.
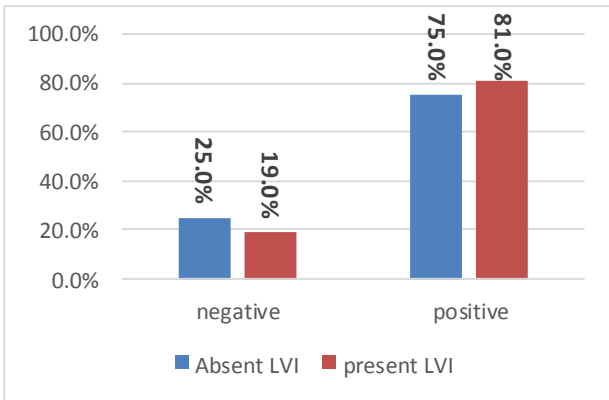
Table (1): CD10 scoring

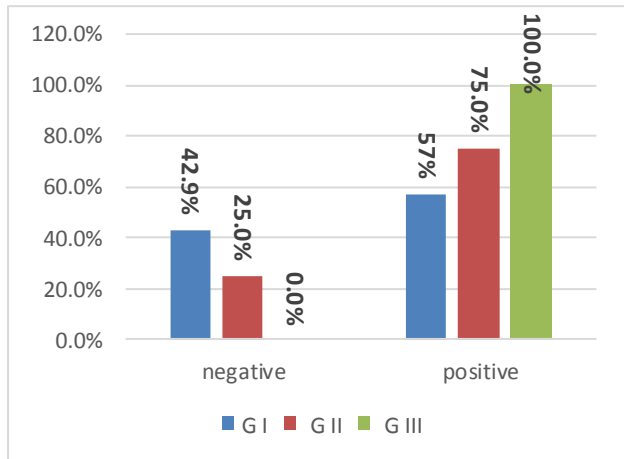| Score	Result	CD10 staining
0	Negative	<10% stromal positive cells (cytoplasmic and membrane positivity)
1	Weak	10%-30% stromal positive cells
2	Strong	>30% stromal positive cells
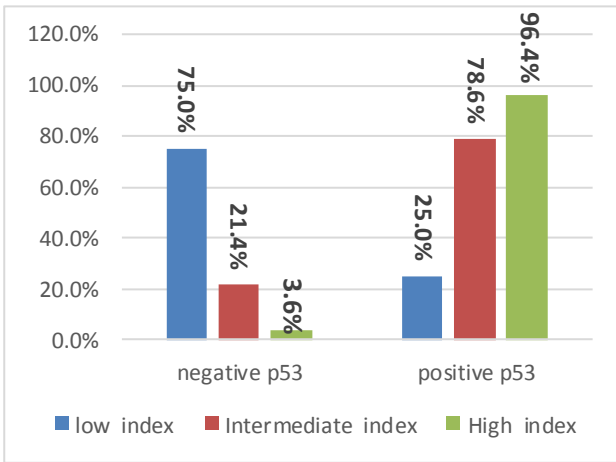
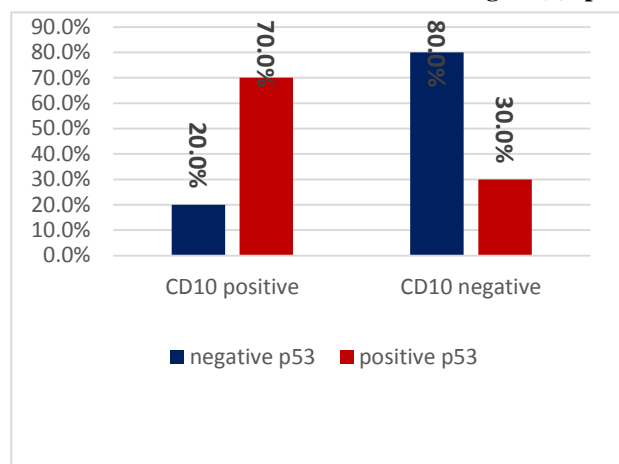**Figure(1): p53 and KI67 status**
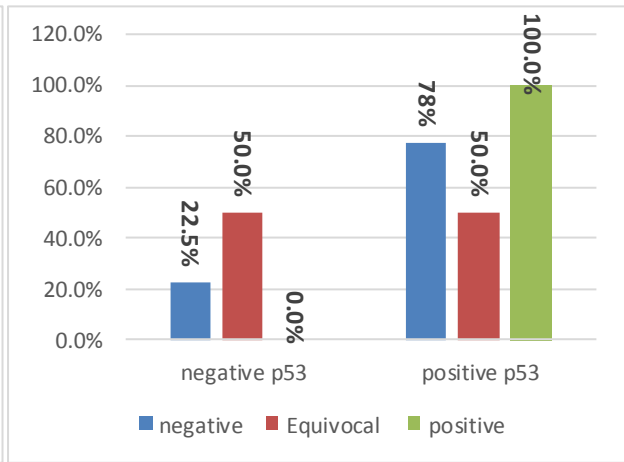
Figure(2): p53 and HER2/neu

Figure(3): p53 and tumor Grade

Figure(4): p53 and LVI status

Figure(5) :p53 and CD10 expression

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