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Immunohistochemical P53 Expression in Breast Carcinoma with Correlation to Clinico-Pathological Parameters

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Abstract

Breast cancer has emerged as a grave danger in the last 50 years. It represented 35.1% of total cancer in women in Egypt National Cancer Institute (NCI) series. Changes in cell loss factor could have a major impact on tumor growth or regression. A large percentage of cell loss from tumors was due to apoptosis. P53 is a tumor suppressor gene that plays a critical role in preventing human cancer formation. In response to a variety of stress signals p53 becomes activated and induces cell cycle arrest and/or apoptosis.

We Aimed to: 1. Study the immunohistochemical profile of p53 in breast carcinoma. 2. Assess its prognostic value in relation to clinico-pathological prognostic factors of breast carcinoma.

Subjects and Methods: This study included 45 specimens of breast carcinoma. Patient's age, tumor size and local aggressive changes, history of recurrence and/or presence of distant metastasis were obtained. H&E stained sections were evaluated for the presence of benign breast disease, histopathological tumor type, and tumor grade, presence of in situ component, lymphocytic infiltration, lymphovascular invasion, and axillary lymph node status. P53 immunostaining was done to detect its expression using the avidin-biotin peroxidase method.

Results: P53 was weakly expressed in 11% of areas of benign breast disease. P53 was negative in all cases of low grade ductal carcinoma in situ (DCIS), positive in 2/3 of intermediate grade DCIS, and positive in all cases of high grade DCIS. All grade I invasive breast carcinoma (IBC) were negative for p53, 50% of grade II and 91% of grade III IBC were positive for p53. P53 expression increased significantly with increased tumor grade of IBC (p<0.006), lymphovascular invasion (p<0.003) and lymphocytic infiltration (p<0.004). No significant correlation between p53 expression and lymph nodal status.

Conclusions: P53 is an indicator for poor prognosis in breast cancer being positively correlated to tumor grade, presence of lymphovascular invasion. P53 may modulate the immune response in breast cancer being positively correlated with prominent lymphocytic infiltration.

Key Words: *P53 – Breast cancer – Apoptosis – DCIC – IBC.*

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Background

BREAST cancer is the most common cause of cancer death among women worldwide. Invasive breast cancer is the most common carcinoma in women. It accounts for 22% of all female cancers. Incidence in developed countries is 6%, which is more than twice the incidence at other sites. The risk of disease had been increasing until the early 1980s in both developed and developing countries [1].

Invasive breast carcinoma is a group of malignant epithelial tumors characterized by invasion of adjacent tissues 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 [2].

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 [3].

P53 is the most frequently mutated gene in human malignancies. Its protein plays a central role in maintaining genomic integrity. It does so

Abbreviations:

NCI:	National Cancer Institute.
DCIS:	Ductal carcinoma in situ.
IBC:	Invasive breast carcinoma.
IDC NOS:	Infiltrating duct carcinoma, not otherwise specified.
PBS:	Phosphate buffered solution.
SI:	Staining intensity.
PP:	Percentage of positive tumor cells.
IHCS:	Immunohistochemical scores.

by occupying a nodal point in DNA damage control pathway [4,5]. The wide occurrence of defective p53 derives from 3 properties. First, wild type p53 is highly vulnerable to dysfunction caused by even a single base change in the coding sequence. Second, in contrast with classical tumor suppressor gene theory a single abnormal p53 allele or allele loss can alter phenotype. Depending on the gene lesion, this manifests by, a gene-dose dependent reduction in certain p53 function (s), a dominant negative inhibition of the remaining wild type allele's function, or gain of a novel function (s) not associated with wild type. Third, the participation in multiple pathways of fundamental importance to carcinogenesis makes it an 'Achilles heel' of cancer suppression, a defect in which can radically diminish cellular defenses against carcinogenesis [6].

The tumor suppressor p53 plays a critical role in preventing human cancer formation. In response to a variety of stress signals, p53 becomes activated and induces cell cycle arrest and/or apoptosis. By eliminating damaged and potentially dangerous cells that might otherwise become cancerous, p53 suppresses tumor formation [7,8]. This specific action is exerted mainly through triggering of apoptosis [9]. According to this important function, p53 activity is controlled in a very complex manner, through the intervention of several modulator proteins [10]. So, p53 has been called the "gatekeeper of genome" [7,8].

P53 mutations are observed in some cases of breast tumors. In the remaining cases, alterations of p53 regulating components or target genes contribute to reduce the ability of p53 to efficiently manage stress events. The qualitative and quantitative activity of p53 depends on its integrity (mutation status), its amount, and its specific post-translational modifications produced by different stress-induced signaling pathways [10].

Although immunohistochemical detection of p53 ptotein accumulation does not always coincide with the presence of the p53 gene mutation, it has been used as a marker of p53 abnormalities because the p53 antigen level in normal cells is very low owing to its short half-life [11,12].

Patients and Methods

This study included 45 specimens of breast carcinoma; 13 prospective cases were retrieved from Department of General Surgery, Sohag University Hospital, and 32 retrospective specimens were retrieved from Department of Pathology Laboratory, Sohag University Hospital, in the period from 2001-2007. Clinical data were obtained from hospital data sheets including: Patient age, tumor size, evidence of axillary lymphadenopathy, presence of distant metastasis, local changes of aggressiveness, and history of recurrence. The tumor was located on the right side in 22/45 (49%) cases, on the left side in 15/45 (33%) cases, bilateral in 2/45 (5%) cases. Tumor side was unknown in 6/45 (13%) cases. Specimens included 14 modified radical mastectomies, 6 lumpectomies with axillary clearance, 12 excisional biopsies, 10 incisional biopsies and 3 simple mastectomies. Lymph node status was assessed in: 14 cases of Patey operation, 6 cases of lumpectomy and axillary clearance, and additional 5 cases in which there were strong clinical evidence of lymph node metastasis (enlarged hard fixed axillary lymph nodes). Five micron formalin fixed, paraffin embedded tissue sections mounted on poly L lysine coated slides and dried overnight at 37°C were prepared. Sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol to distilled water and stained with H&E.

Histopathological evaluation:

Tumors were classified according to their size into three groups; 2cm, >2-5cm, >5cm after Guerra et al. [13]. Tumors were histopathologically typified according to the World Health Organization (WHO), "Classification of Breast Tumors" Ellis et al. [2] as shown in Table (1). DCIS was classified according to the criteria of Holland et al. [14], into: Well differentiated (Grade I), intermediately differentiated (Grade II) and poorly differentiated (Grade III) DCIS. In specimens showing more than one histological grade, DCIS was graded according to the highest grade. IBC were classified according to the Elston and Ellis grading system [15] into; well differentiated (Grade I), moderately differentiated (Grade II), and poorly differentiated (Grade III). Lymph nodal status was classified according to the number of the affected lymph nodes into, no lymph node affection (grade 1), one to three affected nodes (grade 2) and four or more affected nodes (grade 3), after Guerra et al. [15]. Lymphocytic infiltration was evaluated as follows; <50% (+), equal to 50% (++), or >50% (+++) per field at low magnification (X10) after Mañes et al. [16]. Lymphovascular invasion (LVI) was considered evident when at least one tumor cell cluster was clearly visible inside a vascular channel lined by a single layer of endothelial cells without red blood cells [17]. Desmoplastic stroma was considered evident when dense collagen stroma is present with apparently few stromal cells [18].

Immunohistochemical procedures:

Immunohistochemical staining with p53 antibody was performed using immuno-peroxidase technique. Sections were pre-treated by boiling for 9-15min in citrate buffer; pH 6.0. Sections were incubated for 1-2 hours in humid chambers at room temperature with 1/25 rabbit monoclonal antibody for p53 (clone Y5, catalogue RM- 2103-R7, Lab Vision) in 1% blocking anti-goat serum. Then sections were incubated for 15 min with biotinylated secondary antibody (Ultravision Detection System, anti-polyvalent, HRP, Catalogue TP-060-HLX, LabVision). Dilution was made in phosphate buffered solution (PBS), pH 7.2. Finally, the sections were lightly counterstained in Mayer's Hematoxylin and mounted on glass slides using DPX (BDH Ltd, Poole, United Kingdom). A known positive specimen of breast cancer was used as positive control for p53, [19]. Negative controls were performed by omitting the primary antibody.

Immunohistochemical analysis of p53 in tissue sections:

Immunostaining p53 was analyzed and evaluated in 10 different tumor fields. For p53, nuclear staining only is considered [20]. Expression of p53 in 5% of tumor cells was considered positive, as the presence of more than 5% immunoreactivity may be associated with p53 mutations [21]. Percentage of positive tumor cells (PP) was evaluated and scored as: 0 for <5%, (1) for 5-25%, (2) for 25-50, (3) for 50-75 and 4 for >75 following Hussein et al. [22] and Baltaziak et al. [23]. Staining intensity (SI) was considered as (1) for weak, (2) for medium and (3) for intense staining in evaluation of p53 according to Hussein et al. [22]. Immunohistochemical scores (IHCS) were calculated by multiplying PP with the SI. Hence, the following formula was used; IHCS=PP X SI. Validation of this method has been described by Damron et al. [24].

Statistical analysis:

Chi-Square test was used to evaluate statistical significance of various parameters as predictors for prognosis, individually and in relation to each other, with a statistical significance of $p<0.05$ [25].

Results

Clinical findings:

The age range of the 45 studied patients was 31-87 years. Mean age was 52 years; 16 (36%) cases below or equal to the age of 50 years and 29 (64%) cases after the age of 50 years. The tumor size was 2-5cm in 21/45 (47%) cases, and >5cm in 24/45 (53%). Local aggressive manifestations e.g. Fixation, pau d' orange, nipple retraction, skin ulceration and fungation were present in 10/45 (22%) cases. Axillary lymph node metastasis was assessed in 25/45 (56%) of patients. They were positive in 17/25 (68%) cases, and negative in 8/25 (32%). One case had distant bone metastasis.

Histopathological findings:

WHO classification of breast tumors Ellis et al. [2] revealed IDC NOS in 33/45 (73.3%) cases, lobular carcinoma in 2/45 (4.4%) cases, medullary carcinomas in 3/45 (6.7%) cases, neuroendocrine differentiation in 1/45 (2.2%) cases, papillary carcinomas in 1/45 (2.2%) case, micropapillary carcinomas in 3/45 (6.7%) cases, glycogen rich carcinoma in 1/45 (2.2%) case, cribifom carcinoma in 1/45 (2.2%) case.

Benign breast disease was found in 18/45 (40%) cases. In situ component was present in 16/45 (36%). DCIS was found in 13/33 (39%) cases of infiltrating duct carcinoma, not otherwise specified (IDC NOS); of solid, commedo, papillary, micropapillary, clinging, and cribriform patterns. In situ commedo, and micropapillary carcinoma was seen in 1/2 cases of micropapillary carcinoma. In situ cribriform carcinoma was seen in the case of invasive cribriform carcinoma. In situ lobular carcinoma was found in 1/2 case of lobular carcinomas. Lymphovascular invasion was present in 23/45 (51%) cases. Prominent lymphocytic infiltration was present in 5/45 (11%) cases. Desmoplasia was present in 29/45 (64%) as shown in Table (2).

Histopathological grading of breast carcinomas studied:

DCIS was grouped according to the published criteria of Holland et al., (1994), into; 5/13 (42%) of low grade, 3/13 (16%) of intermediate grade, and 5/13 (42%) of high grade cases. IBC was grouped according to histopathological grading system of Ellis et al., (2003) into; 6/45 (13%) grade I, 28/45 (62%) grade II and 11/45 (25%) grade III cases (Table 1).

Immunohistopathological findings:

Fig. (1A-F) and Table (5) show the results of histological assessment of p53 expression in different breast lesions. P53 was weakly expressed in 2/18 (11%) areas of benign breast disease (IHS=3) (Fig. 1A). P53 was negative in all low grade DCIS (Fig. 1B), positive in 2/3 (66.7%) intermediate grade DCIS, and positive in all cases (100%) of high grade DCIS (Fig. 1C) (Table 2, Graph 1). All (6) grade I IBC were negative for

p53, 14/28 (50%) cases of grade II IBC were positive for p53 (Fig. 1D), 10/11 (91%) of grade III IBC were positive for p53 (Fig. 1E) (Table 3, Graph 1).

P53 expression increased significantly with increased tumor grade of IDC (p<0.006), lymphovascular invasion (p<0.003) and lymphocytic infiltration (p<0.004) as shown in Table (4). No significant difference in p53 expression in lymph nodal positive or negative cases (Table 5). P53 expression in special types of breast cancer was shown in Table (6) and Figs. (Fig. 1F).

Table (1): Histopathological data of studied patients (n=45).

Parameter	No. of cases
Histological types:	
IDC NOS	33 (73.3%)
Lobular carcinoma	2 (4.4%)
Medullary carcinoma	3 (6.7%)
Neuroendocrine carcinoma	1 (2.2%)
Micropapillary carcinoma	3 (6.7%)
Papillary carcinoma	1 (2.2%)
Glycogen rich	1 (2.2%)
Cribriform carcinoma	1 (2.2%)
Tumor grade of DCIS (13):	
Low grade	5 (42%)
Intermediate grade	3 (16%)
High grade	5 (42%)
Tumor grade of IBC (45):	
Grade I	6 (13%)
Grade II	28 (62%)
Grade III	11 (25%)
Lymphovascular invasion:	
Absent	22 (49%)
Present	23 (51%)
Lymphocytic infiltrate:	
Minimal	40 (89%)
Prominent	5 (11%)
Desmoplasia:	
Minimal	16 (36%)
Prominent	29 (64%)

Table (2): P53 expression in DCIS according to the tumor grade.

Tumor grade	P53 expression (IHCS)							IHCS (X±SD)
	0	2	4	6	8	9	12	
Grade I (5)	5	0	0	0	0	0	0	0±0
Grade II (3)	1	0	0	1	1	0	0	4.7±4.2
Grade III (5)	0	0	0	0	0	2	2	10.5±1.7
IHCS	p<0.004							

Table (3): P53 expression in IBC according to tumor grade.

Tumor grade	P53 expression (IHCS)							IHCS (X±SD)
	0	2	4	6	8	9	12	
Grade I (6)	6	0	0	0	0	0	0	0±0
Grade II (28)	14	0	3	3	4	3	1	3.6±4.0
Grade III (11)	1	0	0	0	2	4	4	9.1±3.4
p-value	p<0.006							

Table (4): P53 expression in IBC in relation to clinicpathological factors.

Clinicopathological parameter		P53 expression: Low (IHCS 6) (27 cases)	P53 expression: High IHCS>6 (18 cases)	p-value
Age:				
<50	16	9	7	0.9
>50	29	18	11	
Tumor size:				
2-5	21	12	9	0.2
>5	24	15	9	
Tumor grade:				
Grade I	6	6	0	0.006
Grade II	28	20	8	
Grade III	11	1	10	
Lymphovascular invasion:				
Absent	22	18	4	0.003
Present	23	9	14	
Lymphocytic infiltration:				
Minimal	40	27	13	0.004
Prominent	5	0	5	
Desmoplasia:				
Absent	16	8	8	0.3
Present	29	19	10	

Table (5): P53 expression in IBC in relation to lymph node status.

	P53 expression: Low (15)	P53 expression: High (10)
Negative (8)	5	3
Positive (17)	10	7
p-value	<0.9	

Table (6): Expression of p53 in special types of breast carcinoma.

Tumor type	Mean values of IHS: No. of cases	Mean values of IHS: P53
Lobular carcinoma	2	0.0
Medullary carcinoma	3	10.0
Papillary carcinoma	1	10
Neuroendocrine carcinoma	1	0
Micropapillary carcinoma	3	8
Glycogen rich carcinoma	1	6
Cribriform carcinoma	1	0.0

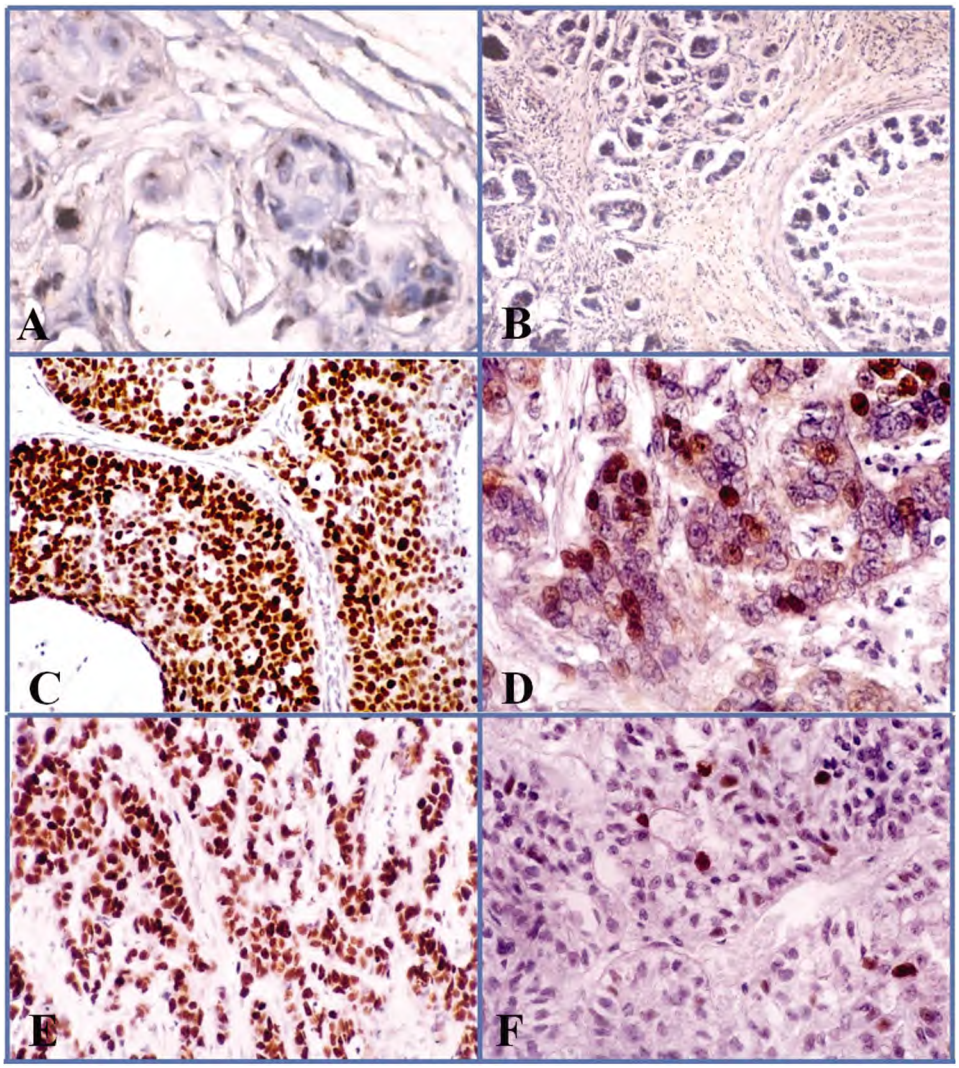
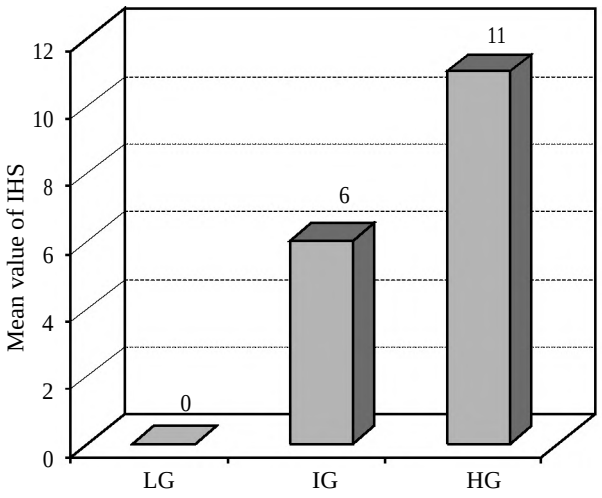
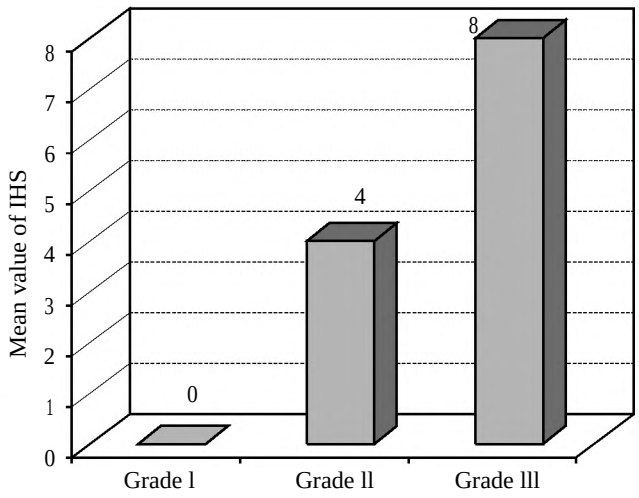


Fig. (1A-F): A- Weak p53 expression in area of benign breast disease (X400). B- Weak p53 in in situ and infiltrating micropapillary carcinoma (X200). C- High p53 expression in high grade commedo DCIS (X200). D- High p53 expression in grade II IDC NOS of the breast (X400). E- Moderate p53 expression in grade III IDC NOS (X200). F- Weak p53 expression in cribriform carcinoma of the breast X400).



(Graph 1): P53 expression in DCIS according to tumor grade.



(Graph 2): P53 expression in IBC according to tumor grade.

LG: Low grade, IG: Intermediate grade, HG: High grade.

Discussion

It has been suggested that gene expression studies offer the greatest promise for refining prognostication in breast cancer [26]. The current hypothesis of tumorigenesis in humans suggests that cancer cells acquire their hallmarks of malignancy through the accumulation of gene activation and inactivation events over long periods of time. For breast cancer, this multistep process may manifest itself as a sequence of pathologically defined stages. It is widely held that breast cancer initiates as the pre-malignant stage of atypical ductal hyperplasia (ADH), progresses into the pre-invasive stage of DCIS, and culminates in IDC [27].

In our study, 18/45 (40%) specimens of breast carcinoma were accompanied with benign breast disease which is a known risk factor for breast cancer. Elmore et al. [28], stated that; for women with proliferative changes, about 10 out of 100 will develop breast cancer.

In situ component was evident only in 16/45 (36%) of IBC and in 13/45 (33.3%) of IDC NOS. This percentage is much lower than the findings of Tavassoli [29], who found foci of DCIS in up to 80% of cases of IDC. This marked difference may be explained at least in part by late discovery of cases in our locality. However, Omar et al. [30], found even smaller percentage of carcinoma in situ component (1.5%), in breast cancer patients in Upper Egypt.

Invasive ductal carcinoma and all other invasive breast carcinomas are routinely graded based on an assessment of tubules/gland formation, nuclear pleomorphism, and mitotic Figs. [2]. Tumor grade has been a highly valuable prognostic factor for breast cancer, as poorly differentiated lesions are associated with significantly poorer clinical outcome [31]. In our study tumor grades were; 6/33 (13%) grade I, 28/33 (62%) grade II, and 11/33 (25%) grade III IBC. These finding was consistent with Omar et al. [30], who reported a low incidence of grade I tumors (5.4%) in Egyptian patients, while grades II and III tumors were 66.0% and 28.6% respectively. This could be explained by presence of certain genetic or environmental carcinogens which lead to development of aggressive tumor phenotypes in our locality.

IDC NOS of the breast is the most commonly encountered form of IBC Saxena et al. [32]. In our series most of the specimens were IDC NOS (73.3%). This ratio looks intermediate between (72.8%); found by Li et al. [33], and (88.2%); found by Saxena et al. [34].

Axillary lymph node examination is an important staging procedure for invasive breast carcinoma (Sebastian et al, 2002). In our study axillary lymph nodes were involved in 17/25 (68%) of cases. In agreement with the findings of El-Bolkainy, [35]; the frequency of axillary lymph node metastases was 75%, of Egyptian patients, and in large series studied by Jatoi et al. [36], nodal metastasis was present in 63.3% (1,068/1,696). In contrast Silverstein [37]; found nodal metastasis in 36% (680/1891) of cases. These findings may indicate that the incidence of lymph node metastasis is relatively lower in more recent studies, which may reflect earlier detection of the tumor.

Invasion of the lymphovascular channels is a necessary gateway to the metastatic process and is an independent prognostic indicator in breast cancer [38]. In our study, lymphovascular invasion was present in 23/45 (51 %) of breast cancer patients. Similar incidence (31.6%) 56/177 was found by Mohammed et al. [39]. However, other investigators found higher incidence of lymphovascular invasion in breast cancer, e.g. 78% (54/69) by Ito et al. [40], which is most likely due to the use of lymphatic endothelial markers; D2-40 and podoplanin in the latter study. These are markers useful in accurate detection of lymphovascular invasion by tumor cells.

The prognostic significance of inflammatory cell infiltrates is controversial, with some studies noting an adverse effect on clinical outcome Lee et al. [41], and others observing either no significant effect Hussein and Hassan, [42], or a beneficial effect Schumacher et al. [43].

There is no definitive conclusion regarding the efficacy of T cell-dependent immune mechanisms or regarding the correlation between the extent and type of lymphocyte infiltration and tumor progression in most subtypes of breast carcinoma [44]. However, the presence of certain T-cell subsets have also shown to negatively impact prognosis in other studies, suggesting that the exact composition of the immune infiltrate and/or its specific interactions with tumor cell biology may vary significantly in different tumors [41,46]. Our study showed that lymphocytic infiltration was prominent in 5/45 (11%) of breast cancer patient. A finding shared with Demaria et al. [47], who found that lymphocytic infiltrate in breast carcinoma was minimal in the majority of patients.

Desmoplastic reaction is characteristic of IBC especially IDC, and the intensity of this reaction can be different from case to case. The interactions between the tumor stroma and the neoplastic cells

are very important, and the tumor stroma can act as a regulator of neoplastic behavior [48]. In our study, desmoplastic stroma was evident in 29/45 (64%) of cases, in agreement with Ferrini and Rossi [41] who found a ratio of 74% of tumors with prominent desmoplasia.

Immunohistochemical findings:

Nearly one-third of breast cancers have mutations in p53 gene [49]. Immunohistochemical assays generally detect nuclear accumulation of p53 protein, which is often related to conformational alterations and a prolonged half-life of the encoded protein [50,51]. We studied the relation between p53 expression and several clinic o-patological prognostic factors in invasive breast carcinoma, namely; age of the patient, tumor size, tumor grade, nodal status, lymphovascular invasion, lymphocytic infiltration, and desmoplastic stroma.

In our study p53 was expressed in 2/18 (11%) of areas of benign breast disease adjacent to invasive tumor tissue. This finding is supported by the findings of Rohan et al. [52], who investigated the association between p53 protein accumulation and p53 mutations detected in benign breast tissue and increased the risk of subsequent breast cancer. P53 protein accumulation was detected in 82/104 cases using in situ hybridization technique, and they came to the conclusion that p53 protein accumulation was associated with a 2-fold increase in risk of progression to breast carcinoma.

Previous findings support the hypothesis that breast cancer evolves by clonal selection of cells that acquire multiple molecular changes through a defined progression of morphologically distinguishable stages, beginning with benign hyperplasia, which progresses to atypical hyperplasia, then to in situ carcinoma, and finally to invasive breast cancer [53].

Our study showed that, p53 is expressed in areas of ductal in situ carcinoma 7/13 (48%), and its expression correlated positively with higher tumor grade (p<0.004). This is in agreement with Done et al. [54], who found that the frequency of p53 missense mutations increased significantly with increasing grade categories of DCIS; 0/49 (0%) of low-grade DCIS, 1/23 (4.35%) of intermediate-grade DCIS, and 9/22 (40.9%) of high-grade DCIS (p<.000). This finding indicated that p53 mutations usually occur before invasion during the progression of breast cancer, and that p53 protein expression in DCIS is an important parameter to evaluate the cellular biology and prognosis of DCIS [55].

The association between p53 alterations and clinical outcome in breast cancer has been the subject of numerous investigations [56]. The possibility that p53 status influences biological behavior was raised in an early study in which the presence of p53 mutations in aggressive breast cancer was demonstrated [57]. The majority of studies support an association between worse survival and the presence of p53 mutations. This association was confirmed in a comprehensive meta-analysis of the effect of somatic p53 mutations on prognosis in breast cancer [58].

Despite the hypothesis that a reduction in apoptotic response to DNA damage with increasing age may play a significant role in the age-related increase in cancer Camplejohn et al. [59], we found no significant correlation between p53 expression and age of the patients. This observation concurs with the observations of; Michalides et al. [60], and Zolota et al. [61]. However, positive association to patient's age more than 65 years was found by Hahm and Davidson, [62]. In our study, there are 3 patients only exceeding 65 years, which may explain the difference between our findings and the previous study. Another explanation is the small number of the studied patients in this study.

Consisting with the findings of Noguchi et al. [63], this study showed no significant correlation between p53 expression and larger tumor size. Contradictory findings was observed by Yamashita et al. [64] and Ferrero et al. [65], who reported positive correlation between p53 expression and tumor size. This difference may be due to the presence of other molecules that affect tumor cell apoptosis and proliferation.

This study showed significant positive correlation between p53 expression and higher tumor grade of IBC (p<0.006). This finding is in agreement with several publications Redondo et al. [66], Kourea, et al. [67], Yamashita et al. [64], and Skarlos et al. [68], and indicates that p53 is an indicator of poor prognosis in breast cancer.

The serpin family member "maspin" is an inhibitor of angiogenesis, invasion and metastasis. A step-wise decrease in the expression of maspin in the sequence DCIS > invasive cancer > lymph node metastasis has been described, strongly supporting an important role in breast cancer progression. Maspin is directly transcriptionally induced by wild-type p53, thus providing an interesting connection between p53 and progression in ductal breast carcinoma [69]. Concurring with this notion, we found significant positive correlation between

p53 expression and presence of vascular invasion in breast cancer (p<0.003).

Axillary lymph node status has repeatedly been shown to be the single most important predictor of disease-free survival and overall survival [70]. Nodal involvement may be an indicator of metastatic disease; the cause of death of patients with breast cancer. Hence, the generally admitted conclusion is that tumor size loses its prognostic role in cases of nodal involvement [71].

In agreement with Arisio et al. [72], and Song et al. [73], we found insignificant correlation between p53 expression and presence of lymph node metastasis. However, Noguchi et al. [63], Gattuso et al. [74], and Amila et al. [75], found significant positive association between lymph node metastasis and p53 protein expression. This controversy may be attributed to the interplay of other genes which can alter the metastatic potential of breast cancer cells e.g bcl-xl.

This study revealed insignificant correlation between p53 expression and the presence of desmoplastic stroma in invasive carcinoma. This finding is contradictory to previous study of Lipponen et al. [76] who found that schirrous carcinoma of the breast expresses p53 oncoprotein more frequently than other carcinomas with less extensive desmoplastic reaction. Our findings suggest that; intense stromal reaction in IBC may modulate the expression of p53 [48].

This study revealed positive correlation between p53 expression and lymphocytic infiltration in IBC (p<0.004). This is compatible with the findings of Lipponen et al. [76], who stated that lymphocytic infiltration is positively correlated to p53 protein expression, which implies that p53 may have a role in modulation of tumor immunity.

Immunohistochemistry of p53 according to the type of breast carcinoma:

We studied the expression of p53 in the different histopathological variants of breast carcinoma; however the small number of the studied cases did not enable adequate statistical evaluation of the differences between p53expression in IDC NOS and other variants.

Infiltrating lobular carcinoma:

We found 3/45 cases of ILC (6%), which lies within the range found in literature; 4.7% in the study of Bane et al. [77], and 7.6% in the study of Li et al. [33]. ILC showed an immunoprofile different from IDC. Specimens of studied lobular carcinoma lacked p53 immunoreactivity, a finding similar to Coradini et al. [78], Bane et al. [77], and Arpino et al. [79]. This feature could be used in differentiation between the ILC and IDC in difficult cases, and may indicate a better outcome for lobular carcinoma in comparison with duct carcinoma.

Medullary carcinoma:

Medullary carcinoma is a poorly differentiated breast cancer with a high histological grade and paradoxically good prognosis [80]. In the current study, three medullary carcinomas were found (6%), which is slightly higher than the ratio found by de Cremoux et al. [80], who stated that typical medullary carcinoma of the breast is a rare histological form of breast carcinoma representing less than 5% of cases.

The three studied specimens of medullary carcinoma showed, high p53 expression (mean IHS=10). The pattern of p53 expression concurs with findings of Kajiwara, [81], and de Cremoux et al. [80], who found that typical medullary breast carcinomas exhibited significantly positivity and high frequency of p53 mutation (100%) than other types of breast carcinoma.

This high expression of p53 indicate that the immune-profile of medullary carcinoma reflect its aggressive histo-pathological features rather than its favorable outcome [80,81].

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