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THE ROLE AND PROGNOSTIC SIGNIFICANCE OF p53 EXPRESSION IN BREAST CANCER

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Background: ***Alterations in the p53 tumor suppressor gene are most frequent genetic changes found in breast cancer, with an incidence reported in a range of 15 to 50%. This high rate of alteration suggests that the gene plays a central role in the development of breast cancer. Since p53 alteration can reflect a more advanced state of progression and a higher rate of proliferation, breast tumors that have a p53 alteration could have a greater probability of having micrometastasis. p53 alteration could therefore be a prognostic factor for recurrence after primary local therapy.***
Consistent with this hypothesis, this study was performed. Aminig at assessing p53 protein expression in different breast lesions to evaluate its significance and its correlation with other established prognostic factors.
Methods: ***The study involved 40 patients with invasive mammary carcinoma and 10 patients with benign breast lesions.***
Results: ***p53 positive immunostaining has been correlated significantly with the tumor grade, but, did not correlate with tumor size, lymph node status, the age of patients and estrogen and progesterone receptors.***
Conclusion: ***The use of immunohistochemical detection of p53 protein overexpression is a simple, useful and reproducible technique. Unfortunately, its absence does not by itself define a group of patients whose risk of relapse is low enough that most physicians would consider not giving adjuvant therapy. The use of other factors in combination with p53 will be needed to achieve this goal.***

Key words: p53, breast cancer, tumor suppressor genes, prognosis, p53 expression.

INTRODUCTION

Broca established in the 19th century that breast cancer may have a familial pattern in high incidence of cases (5 to 10%). Such families have been thought to inherit a defective or lost allele encoding a tumor-suppressor gene. The first tumor-suppressor gene to be shown to have an association with inherited breast cancer was termed p53 (on chromosome 17). Mutations in this locus was inherited in families with the rare breast cancer and sarcoma syndrome termed Li-fraumeni syndrome [(1)].

Alterations in the p53 tumor suppressor gene are the most frequent genetic changes found in a wide variety of malignancies, including breast cancer [(2)]. This high rate of alteration suggests that the gene plays a central role in cancer development in general and in breast cancer in particular. 15-50% of breast cancers contain a p53 alteration, depending on the stage and method of detection[(3-5)]. As with other cancer types, non-invasive or less advanced breast tumors have a lower incidence of alteration[(4,6)] for in situ disease, the incidence of mutation is approximately 15%, while for invasive node- positive carcinoma it is 2-3 times higher[(4)].

Although p53 is a tumor suppressor gene, when mutated in one of several regions, the conformation of its encoded protein changes. Its stability increases and it functions as inhibitor of unmutated p53. The p53 gene is generally mutated in a distinct position in African-American women with high mortality from the disease compared with a corresponding control population with breast cancer[(7)].

Mutation can result in a prolonged protein half –life and accumulation of the altered protein in the nucleus.

Immunohistochemical (IHC) staining can detect this accumulation and is therefore thought to be an indirect indication of a mutation. 30-50% of breast tumors have accumulation of p53 protein as measured by IHC[3,4,6,8]. By DNA-based methods like single-strand conformation polymorphism (SSCP), fewer abnormalities are detected, between 15-45%[5,9,10]. Possible reasons for the lower detection rate by this method include presence of mutation outside the area screened, or a large portion of stromal or non-mutated tumor cells resulting in a dilution of the signal from mutated tumor DNA to below the level of detectability.

Breast tumors frequently show loss of heterozygosity (LOH) of the short arm of chromosome 17[11,12], the region that contains the p53 locus. If p53 behaved as a classical tumor suppressor gene, one would expect that both alleles would have to be inactivated. Of tumors that have LOH of 17p, only approximately 50% or less show the remaining allele to be mutated[10,13]. The fact that the remaining allele is apparently wild type in more than 50% of cases could have a number of explanation first, it could simply be the result of an inability to detect a mutation experimentally, even though it is present, because of tumor heterogencity. Second, loss of even one allele of p53 may give a cell a slight proliferative advantage over cells with two intact alleles. Third, LOH at 17p without a p53 mutation could reflect the presence of another tumor suppressor gene on this portion of the chromosome. Fourth, it might simply be the result of the random genomic instability of cancer cells.

Since the discovery that germline p53 mutation can cause familial cancer syndrome[1], it has been of interest to determine if p53 germline mutations contribute to familial breast cancer, especially the premenopausal or bilateral type, or if those mutations are often found in women with breast cancer diagnosed at a relatively young age, in their 20's or 30's. Several studies have addressed this issue. The incidence of p53 germline mutations as a cause of familial[14], early onset[15], or bilateral breast cancer[16] is low ≤1%. Because of the very low incidence of germline mutations, the screening of these groups would not be justified.

Testing for p53 alterations in breast tumors, however, may have a prognostic clinical application. Alterations in the p53 gene lead to loss of its negative growth regulatory function and hence to more rapid cell proliferation[17]. Also p53 alterations are more often found in invasive or advanced malignancies [4,6,18]. This suggests the possibility that p53 alterations occur more often as a late event in the transformation process, or are associated with an increase in metastatic potential. For these reasons, and because p53 mutations are common in breast cancer, it was our hypothesis that p53 mutations could be an indicator of the likelihood of occult distant micrometastasis in node-negative breast cancer. The association observed between IHC staining and clinical outcome are exciting and one might be tempted to add p53 to the list of the biomarkers that should be routinely obtained for treatment decisions. In this study, a clinicapathological study of p53 gene mutations with the different pathological entities of breast carcinoma was performed and was compared with the expression of the gene in some cases of benign breast diseases.

PATIENTS AND METHODS

Patients

The study comprised forty patients with primary infiltrating mammary carcinoma and ten patients with benign breast disease diagnosed between 1999 and 2002 at the department of surgery of El Menia University hospital. Patients were all females and their age ranged from 30 to 72 years. All patients had a full preoperative laboratory work up and the patients with malignant pathology were submitted for metastatic work up in the form of chest x-ray, abdominal ultrasound and bone scan. The patients with breast carcinoma were either treated by modified radical mastectomy (n=25) or by conservation treatment with axillary clearance and radiotherapy (n=15). Benign breast lesions were treated by simple lumpectomy. Histologic assessment was performed by one pathologist (N.M.) at the International Cancer Institute of Cairo University. Tumor types were determined according to the World Health Organization classifrcation [!9] and infiltrating ductal carcinoma grades were determined according to the criteria deseribed by Nottingham modification of bloom and Richardson system[20]. There were 30 cases of infiltrating ductal carcinoma, 10 cases of infiltrating lobular carcinoma, 5 cases of fibroadenoma and 5 cases of fibrocystic disease. Steroid hormone receptor status was determined by the Ligand-binding assay according to the method of king et al.[21].

Immunotistochemical staining

Four-micrometer sections were cut from a representative paraffin block from each case, air dried overnight, then stained using a monoclonal antibody to p53 protein (clone DO-7) conjugated avidin-biotin immunoperoxidase technique[22]. In this system three reagents were utilized: the primary antibody specific for the antigen to be localized, the biotinylated secondary anti-immunogloblulin or the link which is capable of binding to both the primary antibody and avidine-biotin enzyme complex or the label, since both the primary antibody and the antibody of label are produced in the same animal species (this link provides additional steps for amplification of the antigen binding event), finally, the reaction can be visualized by an appropriate substrate/chromogen reagent.

Scoring system

Positive staining was identified when the nucleus shown brown staining. Cases of the study were divided into two groups: negatively stained, where tumor cells failed to show any nuclear reactivity to the antibody and the positively stained. Positively stained tumors were scored semiquantitavely for both the intensity and proportion of cells staining, where at least five representative high power microscope fields were examined in all cases. Intensity was given scores from 1 to 3 expressed as weak, moderate and strong intensity; and the proportion of positive cells scores from 1 to 4 as follows (1% to 25% = 1; 26% to 50% = 2; 51% to 75% = 3 and > 75 % = 4). Score 1 was described as mild (+), while 2 was moderate (++) and 3&4 designated as marked (+++). In cases where significant field to field variation in the percentage of positive cells existed, the major score was considered.

Statistical Analysis

A commercial statistical package (stat view SE, Abacus concepts Inc, USA. 1988) for the Macintosh was used for data management and analysis. Microsoft Excel version 4 (Microsoft Inc., USA 1992) was used for ambulation. Harvard graphics (version 2.0 software publishing cooperation, USA, 1993) was used for figures generation. Data were summarized as means and percentages. Comparison between groups of means was done using student's test. For comparing more than two groups, a non parametric test equivalent to analysis of variants (ANOVA test) was used. Comparison between percentages was done by chi-square test or fishers Exact test for small number (Armitage and peri, 1987).

RESULTS

I Clinical and Pathological Characteristics

Forty female patients were operated upon for breast carcinoma in the period between 1999 and 2002 either by modified radical mastectomy (n=25) or by conservation surgery with formal axillary clearance (n=15). Ten patients with benign breast lesions were treated by lumpectomy. The distribution of clinical and pathologic characteristics in the malignant group were as follows:

1) Age distribution

The mean age of patients with invasive carcinoma was 45.9 years ranged from 30 to 72 years. For invasive duct carcinoma it was higher 47.2 years ranged from 31 to 72 years with the highest age incidence in the 5th dectade (36%). As for invasive lobular carcinoma, the mean age was 42.8 years ranged from 30 to 55 years with the highest age incidence in the 4th and 5th decades (36.4% for either). The number of patients with older age (> 35 years) was higher in the two groups. (Table & Fig. 1), show the frequency distribution of age groups.

Table (1): ***Age distribution of malignant cases***

Age group	*IDC*		*ILC*	
	No.	*%*	*No.*	*%*
≤ 35	6	20	3	30
> 35	24	80	7	70
Total	30	100	10	100

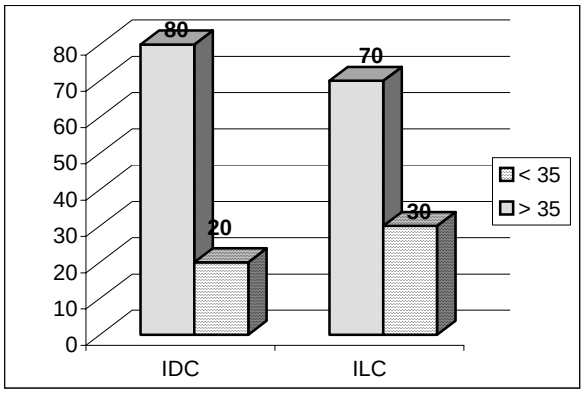

Fig. (1): Frequency distribution of age groups in malignant cases.

2) Tumor size.

The tumor size was evaluated as its largest diameter in centimeters and the cases were divided into three groups according to the tumor size (T) included in the TNM staging system. T2 tumors (> 2 cm - ≤ 5 cm) consitituted the majority of cases (80%) in invasive lobular carcinoma compared to (63.3%) for invasive duct carcinoma. Generally, the percentage of patients with a large tumor size was higher in I.L.C cases than in I.D.C, yet, the difference between the two means of tumor size was found to be statistically insignificant (P > 0.05). (Table & Fig. 2), demonstrate the distribution according to tumor size.

Table (2): ***Distribution of malignant cases according to tumor size.***

Tumor size (cm)	*IDC*		*ILC*	
	No.	*%*	*No.*	*%*
≤ 2	7	23.3	1	10
> 2 - ≤ 5	19	63.3	8	80
> 5	4	13.3	1	10

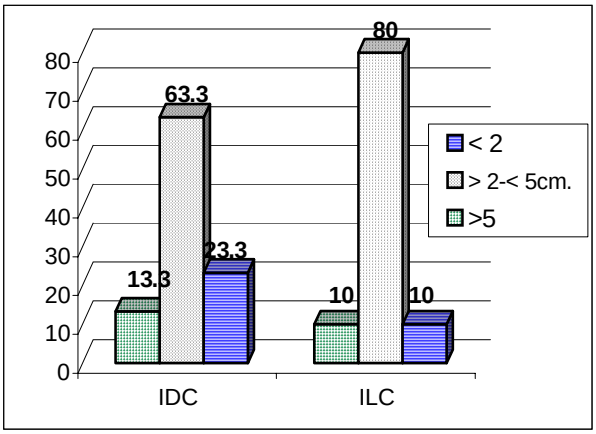

Fig.(2): Distribution of malignant cases according to tumor size.

3- Tumor grade

The histopathologic grade of the invasive duct carcinoma subgroup (n=30) was done according to the Nottingham modifications of the Bloom-Richardson grading system. The majority of the studied cases were of grade II (60%). (Table & Fig. 3), show the distribution of the histopathologic grades of I.D.C. subgroup.

Table (3): ***Histopathologic grade of invasive duct carcinoma.***

Histopathologic	*No.*	*%*
Grade I	5	16.6
Grade II	18	60
Grade III	7	23.3

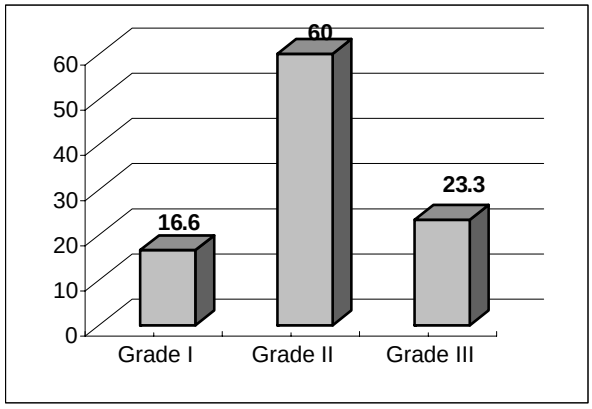

Fig. (3): Histopathologic grade of invasive duct carcinoma

4) Axillary lymph node involvement

Axillary lymph nodes obtained by formal axillary dissection in all forty cases were available for pathological evaluation. Evaluation was done according to the presence or absence of metastasis and not for capsular invasion or the number of lymph nodes affected. For IDC the pathological lymph nodes were detected in 60% (n=18) and for I.LC they were detected in 70% (n=7). The difference between the two subgroups was not found to be statistically significant (P > 0.05). (Table & Fig. 4), show the axillary lymph node status distribution in the forty cases.

Table (4): ***Axillary lymph node status***

Lymph node status	*IDC*		*ILC*	
	No.	*%*	*No.*	*%*
Positive	18	60	7	70
Negative	12	40	3	30

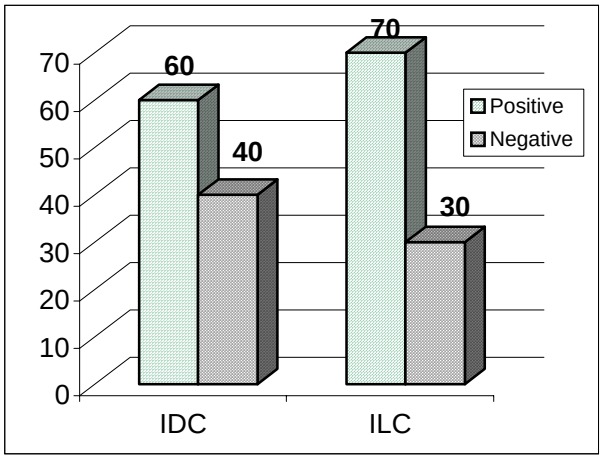

Fig. (4): Axillary lymph node status

5) Estrogen and progesterone receptors status

ER and PR status was evaluated for all specimens. ER was positive in 53.3% of IDC (n=16) and in 60% of ILC (n=6). PR was positive in 40% of IDC (n=12) and in 40% of ILC (n=4). The difference between the results in the two pathological categories was not found to be statistically significant. (Table & Fig. 5), show the results of the ER and PR staus in all cases.

Table (5): *Evaluation of steroid hormone receptors in malignant cases*

Histopathology	Malignant cases		ER+Ve		Er -Ve		PR+ve		PR-ve	
	No	%	No	%	No	%	No	%	No	%
IDC	30	100	16	53.3	14	46.6	12	40	18	60
ILC	10	100	6	60	4	40	4	40	6	60
All types	40	100	22	55	18	45	40	40	24	60

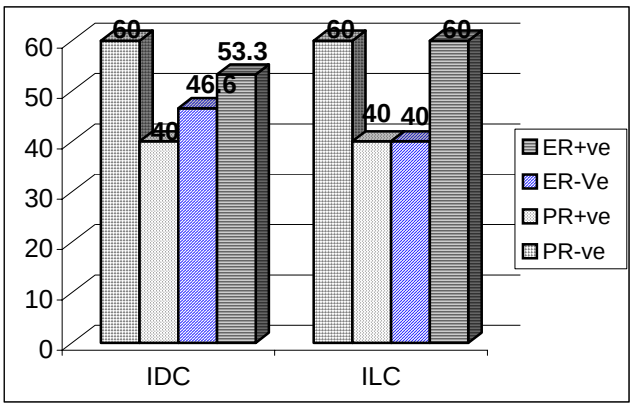

Fig. (5): ER and PR status in malignant cases

Summary of the clinical and pathologic characteristics of the malignant group is shown in Table (6).

Table (6): *Distribution of Clinical and Pathologic Characteristics of the malignant group.*

	Total No. tumors.	Percentage
Patients age		
≤ 35 years.	9	22.5
> 35 years	31	77.5
Tumor size		
≤ 2 cm	8	20
> 2- ≤ 5 cm	27	67.5
> 5 cm	5	12.5
Histologic type		
Infiltrating ductal	30	75
GI	5	16.6
GII	18	60
GIII	7	23.4
Infiltrating lobular	10	25
Node Status		
Negative	15	37.5
Positive	25	62.5
Receptor status		
ER positive	22	55
ER negative	18	45
PR positive	16	40
PR negative	24	60

II p53 Immunostaining

p53 immunostaining positivity was found in 37.5% of the total forty malignant cases (n=15) compared to 10% of the benign breast lesions (n=1) (only one case of fibroadenoma showed weak positivity), the difference between the two groups was found to be statistically highly significant ($p > 0.01$). p53 overexpression was detected in 45.5% of IDC cases (n=14) and in only 10% of ILC cases (n=1) (only one case of lobular carcinoma showed diffuse moderate "++" intensity of nuclear staining), again the difference between the two invasive subgroups was statistically significant ($P < 0.05$). In IDC cases, marked nuclear positivity (+++) was found in 57.1% of the total positive cases (n=8), moderate positivity (++) in 28.5% (n=4), and weak positivity (+) in 14.4% (n=2). (Tables 7,8 & Fig. 6), show the p53 imunostaining in the malignant cases and the degree of positivity distribution among them.

Table (7): *p 53 immunostaining in malignant cases*

Histopatho -logic type	Total cases		- ve for p53		+ ve for P 53	
	No.	%	No.	%	No.	%
IDC	30	100	16	53.3	14	46.6
ILC	10	100	9	90	1	10
All types	40	100	25	62	15	37.5

Table (8): *Distribution of p53 degree of positivity in malignant cases*

Histologic type	Total No. of p53+ve cases		Mild (+)		Moderate (++)		Marked (+++)	
	No.	%	No.	%	No.	%	No.	%
IDC	14	100	2	14.4	4	28.5	8	57.1
ILC	1	100	0	0	1	100	0	0
All types	15	100	2	13.3	5	33.3	8	53.3

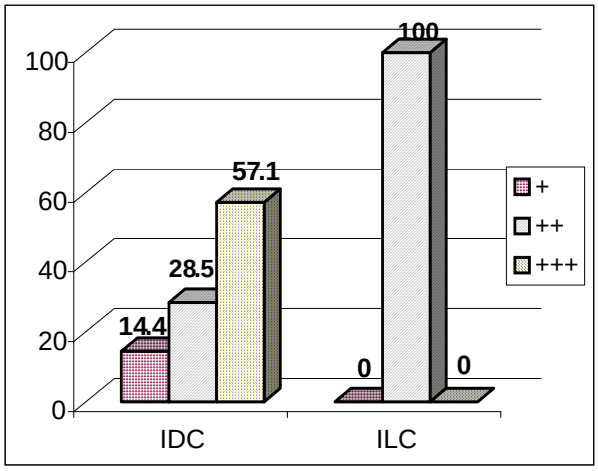

Fig. (6): Distribution of p53 degree of positivity in malignant cases.

III-Correlation With Established Prognostic Factors:

Correlations were applied to the thirty cases of IDC. ILC cases were not included as only one case was p53 immunoreactive.

1) Correlation of p53 overexpression with patient age:

p53 positivity did not correlate with patient age although p53 immunostaining was higher (50%) in younger age group (≤ 35 years) compared to (45.8%) for the alder age (> 35 years). The difference was not found to be statistically significant (p>0.05). (Table 9) shows the distribution of p53 immunostasning in different age groups.

Table (9): ***Distribution of p53 immunostaining in different age groups.***

Age years	*IDC*		
	No. of cases	*+ ve cases for p53*	*%*
≤ 35	6	3	50
> 35	24	11	45.8

2) Correlation of p53 overexpression with tumor size:

Tumor size was not statistically correlated with percentage of p53 immunopositivity in IDC cases. Chi-square test was applied and yielded a p-value 0.67 which was considered to be insignificant. (Table 10), shows the distribution of p53 immunostaining according to tumor size.

Table(10):*Distribution of p53 immunostatining according to tumor size*

Tumor size	*IDC*					
	Total		*Negative*		*Positive*	
	No.	*%*	*No.*	*%*	*No.*	*%*
≤ 2 cm	7	100	4	57.1	3	42.9
> 2- ≤ 5 cm	19	100	10	52.6	9	47.4
> 5 cm	4	100	2	50	2	50

3) Correlation of p53 overexpression with tumor grade:

All grade III IDC cases (n=17) were immunoreactive (100%), 33.3% (n=6 of 18 cases) of grade II, while only one case of 5 patients (20%) of grade I were immunoreactive. The percentage of p53 immunoreactivity correlated significantly with tumor grade (P < 0.01). 85.7% of grade III tumors (n=6) showed marked p53 immunoreactivity compared to only 33.3% (n=2) of grade II tumors, yet the difference did not reach the statistical significance (p=0.39). (Table 11 & Fig.7), show the p53 immunostaining in different grades of IDC.

Table (11): ***p53 immunostaining in different grades of IDC*.***

	Grade I		*Grade II*		*Grade III*	
Histologic grade	*No.*	*%*	*No.*	*%*	*No.*	*%*
Negative	4	80	12	66.6	0	0
Positive	1	20	6	33.3	7	100
Mild (+)	1	100	0	0	1	14.3
Moderate (+)	0	0	4	66.6	0	0
Marked (+++)	0	0	2	33.3	6	85.7

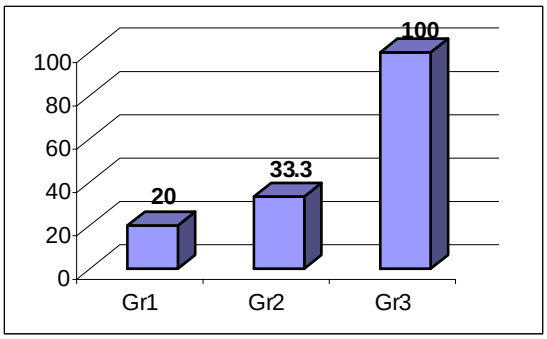

Fig. (7): p53 immunostaining in different grades of IDC

4- Correlation of p53 overexpression with lymph node status:

Eight cases of 18 node-positive patients (44%), and six

cases of 12 node-negative patients (50%) showed p53 immuno reactivity. There was no significant correlation between percentage of p53 immuno reactivity and lymph node status (p > 0.05). (Table 12), shows this correlation.

Table (12): ***Correlation of P53 overexpression with lymph node status***

Lymph node status	*No. of cases*	*+ ve cases for p53*	*%*
- ve nodes	12	6	50
+ ve nodes	18	8	44

5- Correlation of p53 overexpression with ER and PR status.

p53 positivity did not correlate with ER and PR status, although positive ER & PR cases showed higher p53 immunostaining (50%) than negative ER and PR cases (42%), yet the difference was found statistically insignificant (P > 0.05). (Table 13), shows this correlation.

Table (13): ***Correlation of P 53 overexpression and ER and PR status.***

ER and PR status	*No. of cases*	*+ve cases for P53*	*% of p53 positivity*
+ ve ER & PR	16	8	50
-ve ER & PR	14	6	42

(P > 0.05)

DISCUSSION

Breast carcinoma is the most common malignant tumor in females[20]. An approach that combines several molecular genetic markers with established pathologic criteria may help physicians to make more accurate predictions of prognosis, especially in patients with early stage breast cancer[23]. Meanwhile reliable prognostic markers are needed to help clinicians identify a subset of axillary node-negative breast cancer patients who can benefit from adjuvant therapy. The product of p53 tumor suppressor gene is one of these prognostic markers [24].

In this study, the mean age for patients with breast cancer was 45.9 years. For IDC cases, it was 47.2 years and for ILC it was 42.8 years. These figures are comparable to those reported by other studies done on breast cancer in Egyptian females [25,26]. The mean age is however about 10 years younger than those reported in western countries[27,28]. The mean tumor size for IDC cases was 4.00 cm and the majority of tumors categorized in T2 (56%), which coincide with other studies [29]. Histopathologic grading of invasive duct carcinoma showed a high incidence of 60% for grade II tumor followed by 23.3% for grade III tumors and the least incidence 16.6% for grade I tumors. These results are in concordance with those reported by Mokhtar[29] who recorded 66%; 28.5%, 5.4% for grades II, III, I respectively. Bahnasy [26] in 1996 also reported similar figures (58.3% for grade II, 26.7% for grade III, 15% for grade I). However, these figures were different from those reported by Ismail[25] in 1995 where grade II constituted 84.2%, grade III were 15.8% and none had grade I.

The p53 gene is a tumor suppressor gene negatively regulating the cell cycle via the p53 gene protein. The p53 gene is located on human chromosome 17 p13[30]. To date, p53 gene mutation appears to be the most common mutation identified in carcinoma[2], its role in initiation and progression of carcinogenesis remains to be defined[31]. Tumor cells that show p53 mutation or possess p53 protein inactivated by binding to host or viral proteins cannot arrest in G1 phase of the cell cycle to allow DNA repair. This would imply genetic instability of the tumor cells lacking functioning p53 protein and rapid selection of neoplastic clone[32]. There is an association between p53 anomalies and tumor aggressiveness, and p53 mutation generally precedes invasion and metastasis [33]. p53 protein accumulation has been considered to be an indicator of a poor prognosis in most series[8,34]. Moreover, Barnes and colleagues[27] have found that patients with carcinomas who express p53 protein in the majority of their tumor cells have a considerable worse prognosis. This effect is most apparent in patients with ILC that have a rather heterogenous clinical behavior and are difficult to subdivide on the basis of currently available markers. However, it is not yet entirely clear at what stage of transition from benign to malignant breast disease p53 gene mutations occur and how the levels of p53 protein overexpression change during tumor progression[35].

In this work, the overall incidence of p53 protein overexpression was 37.5%. for the total malignant breast cancers (duct and lobuler carcinoma). This figure was found to be highly significant and denoted that wild-type p53 inactivation, which is reflected as p53 protein overexpression, is involved in the molecular pathogenesis of mammary carcinoma. This statistically significant percentage was comparable to other studies who detect p53 protein overexpression in 35.6%, 24%, 28%, 33%, by Ostrowski et al.[36], Scimmelpening et al.[37], Domagala et al.[38], and Pelosi et al.[39] respectively. However, it was lower than those of Bartek et al.[3], Walker et al.[40], and Ismail[25] who were able to detect p53 protein accumulation in 54%, 53% and 66.7% respectively. In general the frequency of p53 protein expression varied in different series ranging from 23% to 58% for primary invasive carcinomas. This discrepancy in the literature could be attributed to several reasons; first, various fixation methods and length of storage time and this explains the difference in frequency of p53 positive tumors between the fixed and the frozen sections [41]. Secondly, the use of different methods

and antibodies which are known to recognize distinct epitopes in the linear sequence of p53 [42]. Third the use of different scoring methods for interpretation of p53 reactivity [43].

p53 protein overexpression has been correlated significantly with histopathologic tumor type; ILC showed a very low frequency of P53 immunoreactivity (10% compared to 46.6% in IDC). These results are in agreement with those reported by Domagala et al.[38], Marks et al.[23], and Rosen et al.[44], who detected p53 immunopositivity in 2/47 (4.2%), 8/174 (4.5%), and 9% respectively. Davidoff et al.(45), and Bartek et al. [3], have reported that none of the lobular carcinomas in their series was positive for p53. Domagala et al.[38], reported that if cases of ILC were collected in the various studies, the overall incidence of p53 protein overexpression would have been 9%, this figure is in accordance with our result.

In this study, all the non-malignant breast lesions showed 10% positive nuclear staining for p53 monoclonal antibody, this result is coinciding with those reported by Walts et al.[46], in benign fibrocystic disease and by Barbareschi et al.[47], in hyperplasia without atypia, and by Schmitt et al.[31], in atypical hyperplasia. However, it was concluded that the p53 positivity of breast lesions should not be used as an exclusive evidence of malignancy and that p53 immunoreactivity in benign breast lesions may not identify a subset of patients at increased risk for breast carcinoma, as was concluded by Younes et al.[48], from their study that showed p53 expression in 16% of non malignant cases and 30% of breast cancer, follow-up information showed that 12% of those p53 positive and 7% of those p53 negative benign lesions developed breast carcinoma with insignificant difference between the two groups.

The frequency of p53 overexpression detected by immunohistochemical techniques, in this study, as well as other studies [3,40] was higher than those recorded by molecular biologic techniques which delect changes in the p53 gene. These higher figures of p53 overexpression obtained by IHC suggest that p53 inactivation on the protein level is an important mechanism in the pathogenesis of mammary carcinoma. Such a finding may suggest the utilization of p53 immunastaining as a functional assay for p53 in human tumors; as it shows a higher sensitivity rate compared to the molecular biologic techniques, which detect only p53 inactivation on the genetic level, but not other conditions of p53 inactivation [49].

In this work, p53 overexpression was 46.6% in IDC cases with marked nuclear positivity encountered in 57.1% of them. A highly significant correlation was found between p53 protein overexpression and the tumor grade in IDC cases. These results are in agreement with studies reported by Charpin et al.[50], Haersley and Jacobson[28], Rosen et al.[44], in 1995. Eriksson et al.[35], in 1994 stated that the p53 gene might play an important role as a mechanism for the differentiation and aggressive biologic behavior of mammary neoplasms, being associated with a more aggressive phenotype. Conversely, others failed to establish such correlation [39].

In this study, p53 immunopositivity did not correlate with either the age of patients or tumor size in IDC cases, although p53 positivity was higher (50%) in the younger age group (≤ 35 years) versus 45.8% in the older age (> 35 years), yet the difference was not significant. These results agree with those of Barnes et al.[27], Charpin et al.[50], Ismail[25], and Rosen et al.[44]. On the other hand it contrasted with those of Marks et al.[23], regarding the relation between p53 accumulation and tumor size. Allred et al.[8], have found a significant association between p53 overexpression and younger age.

No significant correlation could be established between p53 positive staining and axillary lymph nodes status; whether negative or positive. These results are comparable to those of Walker et al.[40], Barnes et al.[27], Eriksson et al[35]., and Marks et al.[23], However it contrasted those of Davidoff et al.[45], and Bahnasy [26], who were able to detect that the percentage of p53 positive tumors was significantly higher in patients with positive lymph nodes. Davidoff et al.[45], concluded that alteration of p53 expression appear to have occurred prior to metastatic spread and were maintained during this process, from their finding that a comparable p53 protein level was detected in both primary tumor and its metastasis in lymph nodes; whether elevated, low or none.

In this study, there was no significant correlation could be established between p53 positive staining and steroid hormone receptors status. Barnes et al.[27], found a weak association between the proportion of stained cells and steroid hormone receptors, with more of the receptor-negative tumors being majority stainers than the receptor-positive tumors (Estrogen receptor, P=.07; progesterone receptor, P=.03).

CONCLUSION

There are two main clinical reasons for measuring biologic tumor marker: to provide information on the likely course of the disease and to help with treatment selection. In addition, there is much interest in the effect of the presence of oncogenes and tumor suppressor genes on the etiology and the progression of many carcinomas. From this study we concluded that the use of immunohistochemical detection of p53 protein overexpression is a simple, useful and reproducible technique. Unfortunately, its absence does not by itself define a group of patients whose risk of relapse is low enough that most physicians would considere not

giving adjuvant therapy. The use of other factors in combination with p53 will be needed to achieve this goal. New prognostic markers are being developed every day and it is impossible to measure them all. For a new marker to attract real interest it should provide more information than is currently available. Although this is only a pilot study using a small number of patients, the results are encouraging and hopefully, will stimulate the additional research necessary before the measurement of p53 can fulfill the evaluation guidelines for prognostic factors.

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