PROLIFERATIVE INDEX AND P53 EXPRESSION IN BREAST CARCINOMA AND THEIR CORRELATION TO CLINICO-PATHOLOGICAL PARAMETERS

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ABSTRACT

Background: Breast cancer is the most frequently diagnosed cancer and the most common cause of cancer deaths in women worldwide. Ki67 is a biomarker that reflects cell proliferation. Despite a clear understanding of the structure and properties of this protein, its functional role remains elusive. Gene expression alteration confers the potential for invasive growth in the preinvasive stages of breast cancer. Altered expression of the tumor suppressor gene p53 is frequently seen in carcinomas of the breast and correlates with poor prognosis. This study aims to investigate Ki67 and p53 expressions in benign, preinvasive and invasive breast lesions and to correlate their expressions with the clinico-pathological parameters. **Materials and Methods:** This study included 74 specimens of breast lesions. Ki67 and p53 immunostaining expression was detected using avidin-biotin peroxidase method. **Results:** Ki67 and p53 increased progressively along the continuum of neoplastic changes from normal breast epithelium to invasive ductal carcinomas; IDC ($P<0.000$ & $P<0.01$ respectively). There was significant positive correlation between Ki67-labeling index (LI) and either tumor grade or lymph node metastasis in IDC ($P<0.03$ & $P<0.02$ respectively). P53 expression increased with increasing grade of both ductal carcinoma *in situ* (DCIS) and IDC ($P<0.01$ & $P<0.002$ respectively). There was significant correlation between p53 and tumor size, lymphovascular invasion, and lymphocytic infiltration ($P<0.05$, $P<0.02$, $P<0.03$ respectively). There was positive correlation between Ki67 and p53 in both DCIS ($r= 0.845$, $P<0.001$) and in IDC ($r=0.697$, $P<0.02$) of the breast.
Conclusion: Ki67 and p53 increased progressively along the continuum of neoplastic changes from normal breast epithelium to DCIS and IDC. Ki67 and p53 were increased with poor prognostic parameters; tumor size, tumor grade, lymphovascular invasion, lymphocytic infiltration, and lymph node metastasis.
Keywords: Breast cancer; DCIS, IDC, Ki67, proliferation index, and p53.
Abbreviation:
Invasive ductal carcinomas (IDC), labeling index (LI), ductal carcinoma *in situ* (DCIS), National Cancer Institute (NCI), invasive breast carcinoma (IBC), World Health Organization (WHO), Hematoxylin and Eosin (H&E), quantity score (QS), intensity score (IS), immunoreactive score (IRS).

INTRODUCTION

Breast cancer is the most common type of cancer and the most common cause of cancer-related mortality among women worldwide (Hortobagyi et al., 2005). According to the Egyptian National Cancer Institute (NCI), breast cancer represents 18.9% of total cancer cases; 35.1% in women and 2.2% in men (Elatar, 2002). The age-adjusted rate was 49.6 per 100 000 population (Seedhom and Kamal, 2011).

Mammary carcinogenesis is a multistep process with transformation of normal ductal epithelial cells→ benign proliferative breast disease→ DCIS→ IDC (Hussein and Hassan, 2006). Women with benign breast disease could be prevented from developing invasive breast carcinoma (IBC) if we can exactly identify patients with which subtype of benign lesions will subsequently develop IBC and treat them accordingly.

DCIS are immediate precursors of most breast cancer, but they are heterogeneous regarding morphology and invasiveness risk. The prevalence of DCIS has been rising in the last decades, probably due to better screening programs and now accounts for approximately 20–25% of all breast cancer diagnoses. The

understanding of the transition between the preinvasive and invasive stages in breast carcinomas is the key to more efficient strategies for early diagnosis and treatment, as well as it expands the knowledge about the complex mechanisms of carcinogenesis (Aguiar et al; 2013).

Ki67 protein is a large (395 kD) nuclear protein that is present during all active phases of the cell cycle except for the G0 phase (Varga et al., 2012). Ki67 is strictly associated with and may be necessary for cellular proliferation (Bonanni et al., 2012). Because proliferation status is closely correlated with tumor aggressiveness, the Ki67-LI is considered an established prognostic marker for various tumor types, including breast cancer (Yerushalmi et al., 2012).

Many studies investigated the clinical value of Ki67 in breast cancer and suggested that it had some prognostic or predictive role in clinical practice (de Azambuja et al., 2007; Viale et al., 2008a; 2008b; Stuart-Harris et al., 2009; Karanikas et al., 2010; Santisteban et al., 2010). However, the guidelines of the American Society of Clinical Oncology did not recommend Ki67 as a required routine biological marker of breast cancer (Harris et al., 2007), probably because some uncertainty remains on the value of Ki67 as a routine marker and further studies are needed.

P53 is a tumor suppressor gene that maintains genomic stability either by inducing cell cycle arrest or apoptosis. In malignant cells, its function can be compromised by various mechanisms; mutations, alteration of p53 regulators, alteration of p53 target genes (Lacroix et al, 2006). In ductal carcinomas, p53 gene is mutated with subsequent overexpression of p53 protein (Lai et al, 2004).

This study was conducted: a. to evaluate Ki67 and p53 expressions in the successive steps of breast carcinogenesis, b. to explore the prognostic value of Ki67-LI and p53, and their correlation to the clinico-pathological parameters in breast cancer.

PATIENTS AND METHODS

A total of 74 breast specimens, 4 with normal breast tissue, 5 with typical ductal hyperplasia, 11with DCIS, and 54 with IDC, were retrieved from the files of the Department of Pathology, Sohag University Hospital, Egypt in the period from 2010-2011. Availability of adequate tissue material and clinical data was the only criterion for selection of patients with DCIS and/or IDC.

According to World Health Organization (WHO) criteria (Tavasassoli and Devilee, 2003), DCIS were graded into, 2/11(18%) low grade, 3/11 (27%) intermediate grade, and 6/11 (55%) high grade. IDC were classified according to Elston and Ellis grading system (1998) into 6/54 (11%) low grade, 29/54 (54%) intermediate grade, and 19/54 (35%) high grade. All patients with carcinomas were treated by modified radical mastectomy.

Immunohistochemistry

After reviewing hematoxylin and eosin (H&E) stained slides, a representative blocks was chosen for the study. Serial sections from each block were used for immunohistochemistry. Five micron tissue sections mounted on sialinized glass slides were deparaffinized and rehydrated through descending graded alcohols to water. Tissue sections were incubated in hydrogen peroxide for 10 min to block endogenous peroxidase activity. Then slides were treated with antigen retrieval solution (citrate buffer; 10 mmol sodium citrate buffer solution, pH 6.0). The buffer was allowed to boil in microwave at 750 Watt for 15 min divided into 3 cycles. Non specific protein binding was blocked with 10 min exposure to 10% normal goat serum.

Sections were then incubated with mouse monoclonal antibodies ready to use for p53 (Cat # MS-738-R7, LABVISION corporation, Fremont, USA) for one hour at room temperature and 1/150 rabbit polyclonal antibody against human Ki-67 gene product (Catalogue; Cat # RB-9043-P0, 0.1ml, LABVISION Corporation) overnight at 4 C° in a humid chamber.

Then biotinylated goat polyvalent was applied on each section for 10 min with Streptavidin peroxidase. DAB (14-diaminobenzidine and 0.06 % H2O2) chromogen was applied to each tissue section for 10 min then washed in distilled water. Universal staining kit (Cat # TP-015-HD, LABVISION Corporation, Fremont, USA) composed of: Hydrogen peroxide block, Biotinylated goat anti-polyvalent, Streptavidine peroxidase, DAB chromogen, DAB

subsrate was used. Tissue sections were counterstained with Mayer's hematoxylin, dehydrated alcohols, cleared in xylene, and cover slipped.

Positive control: Previously diagnosed positive cases from breast and colon cancer for Ki67 and p53 respectively were used, and brownish nuclear staining was considered positive for each.

Negative control: The negative control slides were stained in parallel, but with omission of the primary antibody.

Assessment of Ki67:

For Ki67-LI; only cells had an undoubtedly positive nuclear staining was considered positive, while cells had unclear or equivocal staining was considered negative. The number of Ki67 positive nuclei in relation to the total number of tumor cells was counted, multiplying the result by 100. There were at least 1000 nuclei for each case (40X objective), and only brown to black nuclei being interpreted as positive. A Ki67-LI of 0-15% was considered low, between 16-30% was medium, and 31-100% index was high (Plesan et al., 2010).

Assessment of p53:

Nuclear staining is only considered (Oldenburg et al., 2006), and its expression in 5% of tumor cells was considered as the threshold of positive staining (Wikonkal et al., 1997). Quantity score (QS) was estimated as follows: negative = <5% stained cells, 1 = 5-25% stained cells, 2 = 26-50% stained cells, 3 = 51-75% stained cells and 4 = 76-100% stained cells. Staining intensity (SI) was scored on a scale of 0-3: 0 = no staining, 1 = weak staining, 2 = moderate staining and 3 = strong staining. Immunoreactivity score (IRS) was measured by multiplying QS by SI (McLendon et al., 2000). An IRS of 0-4 was considered weak, 5-8 was moderate, and 9-12 was considered strong (Hussein et al., 2002).

Statistical analysis: ANOVA test (Analysis of variance) and Pearson's Correlation Coefficient tests were used with p value <0.05 was considered statistically significant.

RESULTS

Ki67 expression and its relationship to clinico-pathologic features:

Ki67 was positive in all studied cases with different grades. Ki67-LI expression was weak in all cases of normal breast tissue with a mean value of 1±0.16 and an insignificant increase in Ki67-LI in hyperplastic lesions with a mean value of 1.29±0.46 (p<0.27). Ki67-LI was weak in all cases of DCIS except for one which had moderate Ki67-LI with a mean value of 8.59±4.01. There was significant increase in Ki67-LI on transition from hyperplasia to DCIS (P<0.001). There was an insignificant increase in Ki67-LI with increasing grade of DCIS (P< 0.17; Table 1). In invasive carcinoma Ki67-LI was weak in 14/54 (26%) cases, moderate in 14/54 (26%), and strong in 26/54 (48%). There was significant increase in Ki67-LI with increasing grade of IDC (P< 0.03; Table 2). On transition from DCIS to IDC, Ki67-LI showed highly significant increase (P<0.000). Ki67-LI increased gradually on the progression from apparently normal breast through hyperplasia to DCIS ending at IDC (P<0.000; Table 3).

There was statistically significant correlation between Ki67-LI and lymph node metastasis (P< 0.02). However, no significant correlation was found between Ki67-LI and age of the patient, tumor size, lymphovascular invasion, lymphocytic infiltration, or desmoplasia (Table 4). Figure (1) shows Ki67 expression in DCIS and IDC.

(Table 1): Ki67-LI in DCIS of the breast according to grade

Tumor grade	No. of positive cases	Ki67expression (IHCS)			IHCS mean (X±SD)
		Weak	Moderate	Strong	
Grade I	2/2	2	-	-	4.55±3.48
Grade II	3/3	3	-	-	7.49±2.08
Grade III	6/6	5	1	-	10.49±4.05
P value	0.17 (NS)				

ANOVA test is used, NS, not significant

(Table 2): Ki67-LI in IDC the breast according to grade

Tumor grade	No. of positive cases	Ki67 expression (IHCS)			IHCS mean (X±SD)
		Weak	Moderate	Strong	
Grade I	6	3	2	1	18.37±10.68
Grade III	29	8	10	11	23.39±11.35
Grade II	19	3	2	14	29.87±9.98
P value	0.03*				

ANOVA test is used, *= significant

Table (3): Ki67-LI in different breast lesions

Histological type	No. of positive cases	Ki-67-LI			IHCS mean (X±SD)
		Low (0-15%)	Medium (16-30%)	High (31-100%)	
Normal breast	4	4	0	0	1±0.16
Hyperplastic lesions	5	5	0	0	1.29±0.46
DCIS	11	10	1	0	8.59±4.01
IDC	54	14	14	26	25.11±11.3
P value	0.000				

ANOVA test is used

(Table 4): Ki67-LI in IDC of the breast in relation to clinico-pathological factors (No = 54).

Clinicopathological Parameter	NO of cases	Ki67-LI			P <
		Low (14)	Moderate (14)	High (26)	
Age					
<50	19	6	5	8	0.46 (NS)
>50	35	8	9	18	
Tumor size					
2-5	23	7	8	8	0.18 (NS)
>5	31	7	6	18	
Tumor grade					
Grade I	6	3	2	1	0.03*
Grade II	29	8	10	11	
Grade III	19	3	2	14	
Lymphovascular invasion					
Absent	33	7	12	14	0.91 (NS)
Present	21	7	2	12	
Lymphocytic infiltration					
Minimal	40	12	10	18	0.29 (NS)
Prominent	14	2	4	8	
Desmoplasia					
Absent	19	6	4	9	0.68 (NS)
Present	35	8	10	17	
Lymph node status					
Negative	18	8	5	5	0.02*
Positive	36	6	9	21	

ANOVA test is used, NS= Not significant, *= Significant

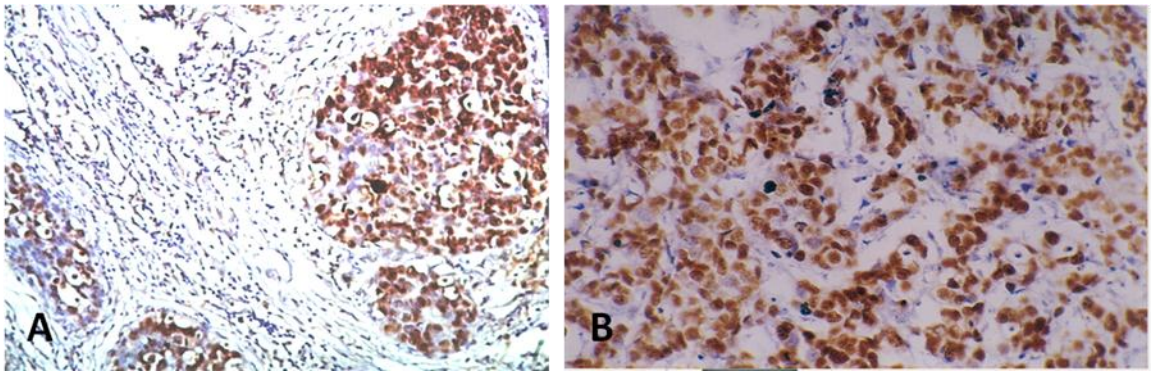

Figure (1): Moderate nuclear Ki67 expression in DCIS (A) & strong nuclear Ki67 expression in IDC grade II (B). Magnifications X 200 (A, B).

P53 expression and its relationship to clinico-pathological features:

P53 expression was negative in 3/4 (75%), and it was weak in 1/4 (25%) of normal breast tissue. P53 expression was negative in 4/5 (80%) and it was weak in 1/5 (20%) of typical ductal hyperplasia. P53 was negative in all 2/2 (100%) grade I, positive in 2/3 (66.7%) grade II, and in all 6/6 (100%) grade III DCIS (P< 0.01; Table 5). P53 was positive in 3/6 (50%) grade I, 20/29 (69%) grade II, and in 17/19 (89.5%) grade III IDC (P< 0.002; Table 6). P53 expression appeared to increased progressively along the continuum of neoplastic changes from normal breast epithelium to IDC (P< 0.01; Table 7).

There was statistically significant correlation between p53 expression and tumor size (P<0.05), tumor grade (P<0.002), lymphovascular invasion (P<0.02), and lymphocytic infiltration (P<0.03). However, there was no significant correlation between p53 expression and age, lymph node metastasis or desmoplasia (Table 9). Figure (2) shows p53 expression in DCIS and IDC.

(Table 5): P53 expression in DCIS of the breast according to grade

Tumor grade	No. of positive cases	P53 expression (IHCS)								IHCS mean (X)
		Weak				Moderate		Strong		
		1	2	3	4	6	8	9	12	
Grade I	0/2	0	0	0	0	0	0	0	0	0±0
Grade II	2/3	0	1	0	0	1	0	0	0	2.7±3.1
Grade III	6/6	0	0	0	1	1	1	2	1	8±2.8
P value	< 0.01*									

ANOVA test is used, *, significant

(Table 6): P53 expression in IDC of the breast

Tumor grade	No. of positive cases	P53 expression (IHCS)								IHCS mean (X±SD)
		Weak				Moderate		Strong		
		1	2	3	4	6	8	9	12	
Grade I	3/6	0	2	0	0	0	1	0	0	2±3.1
Grade II	20/29	0	2	0	4	4	6	1	3	4.7±4.1
Grade III	17/19	0	0	0	1	0	9	1	6	8.3±3.6
P value	< 0.002**									

ANOVA test is used, **; highly significant

(Table 7): P53 expression in different breast lesions:

Histological type	No. of positive cases	P53 expression (IHCS)								IHCS mean (X±SD)
		Weak				Moderate		Strong		
		1	2	3	4	6	8	9	12	
Normal breast	1/4	1	0	0	0	0	0	0	0	0.3±0.5
Hyperplasia	1/5	0	1	0	0	0	0	0	0	0.4±0.9
DCIS	8/11	0	1	0	1	2	1	2	1	5.1±4.2
IDC	40/54	0	4	0	5	4	16	2	9	5.7±4.3
P value	< 0.01*									

ANOVA test is used, *, significant

(Table 8): P53 expression in IDC of the breast in relation to clinico-pathological factors (No = 54).

Clinico-pathological Parameter	NO of cases	P53 expression					P <
		Positive cases (40cases)	Negative cases (14cases)	Weak IHCS≤4 (9cases)	Moderate 6≤IHCS≥ 8 (20cases)	High IHCS>8 (11cases)	
Age							0.8 (NS)
<50	19	15	4	3	9	3	
>50	35	25	10	6	11	8	
Tumor size							0.05*
2-5	23	15	8	6	6	3	
>5	31	25	6	3	14	8	
Tumor grade							0.002*
Grade I	6	3	3	2	1	0	
Grade II	29	20	9	6	11	3	
Grade III	19	17	2	1	8	8	
Lymphovascular invasion							0.02*
Absent	33	21	12	6	10	5	
Present	21	19	2	3	10	6	
Lymphocytic infiltration							0.03*
Minimal	40	27	13	7	14	6	
Prominent	14	13	1	2	6	5	
Desmoplasia							0.1(NS)
Absent	19	12	7	5	4	3	
Present	35	28	7	4	16	8	
Lymph node status							0.3 (NS)
Negative	18	12	6	3	7	2	
Positive	36	28	8	6	13	9	

ANOVA test is used, NS= Not significant, *= Significant

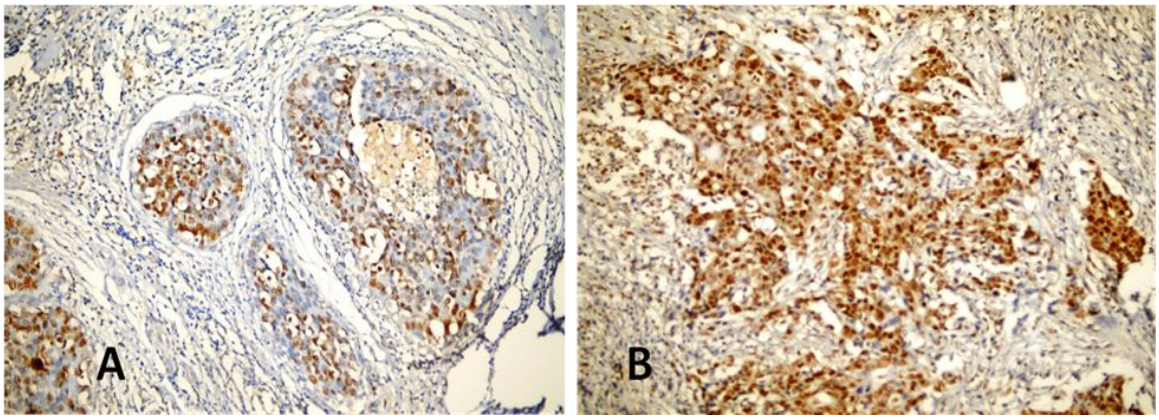

Figure (2): Moderate nuclear p53 expression in DCIS (A) & strong nuclear p53 expression in IDC grade II (B). Magnifications X 200 (A, B).

Correlation between the studied markers:
There was positive correlation between Ki67 and p53 in DCIS (r= 0.845, P <0.001) and in IDC (r=0.697, P <0.02) of the breast.

DISCUSSION

Breast cancer is a malignant tumor threatening women's health with an increasing incidence worldwide. There are still many deaths due to relapse or metastasis (Wang et al., 2009).

Proliferative activity has historically been assessed by counting mitotic figures at high magnification as well as by immunohistochemical detection of Ki67, a nuclear protein that is expressed in proliferating cells (Gerdes et al., 1983). Ki67 is a reliable marker of the mitotic activity. It is not expressed inside cells in cases whereas DNA repairs take place as another cell proliferation marker; PCNA, does (Plesan et al; 2010). It was assume that the correlation of Ki67 with breast cancer outcome involves a mixture of prognostic and predictive effects (Fasching et al. 2011). Proliferation has a major impact on calculating the risk of recurrence (Esteva et al., 2005) and predicting distant metastasis in breast cancer (Varga et al., 2012).

However, previous studies revealed Ki67-LI to be a good prognostic indicator for breast cancer patients (Cheang et al., 2009).

Immunohistochemical detection of Ki67 has gained increasing importance in routine breast cancer diagnosis and has recently been recommended by the St. Gallen Consensus Conference (Goldhirsch et al., 2009 & Dowsett et al., 2011).

Current study revealed that Ki67-LI was weak in all cases of normal breast tissue and hyperplastic lesions with a mean value of 1±0.16% and 1.29±0.46% respectively. This finding is near to that reported by Pavelic et al. (1992) who found that the mean value of Ki67-positive cells in 11 normal breast tissues was 0.91±0.31%.

DCIS are genuine precursors of breast cancer, but the mechanisms involved in this transition are mostly unknown (Aguiar et al; 2013). In DCIS Ki67-LI was weak in all cases except one which had moderate Ki67-LI with a mean value of 8.59±4.01%. This finding was different from what was found by Pevalic et al; (1992) and mean value of Ki67-positive cells in DCIS was 4.57±1.36%. This may be due to variable proportions of different tumor grades in their study.

In IDC Ki67-LI was weak in 14/54 (26%) cases, moderate in 14/54 (26%), and strong in 26/54 (48%) with a mean value of 25.11±11.3%. This finding consisted with Inwald et al; (2013) who found that the mean value of Ki67-LI in IDC was 20.3± 18.1%. Pevalic et al; (1992) found the mean value of Ki67-positive cells in IDC was 12.76±2.18%. This may be due to variable

proportions of different tumor grades in those studies.

Ki67-LI increased steadily and progressively on transition from normal breast to hyperplasia to DCIS and lastly to IDC ($p<0.000$). The increase in proliferation was significant on transition from hyperplasia to *in situ* component and then to invasive carcinoma. To the best of our knowledge no previous studies discussed this transition, so it needs further studies to be explained.

Several prognostic factors directly or indirectly are involved in determination of tumor aggressiveness, and very important among them are tumor proliferation markers which have been extensively investigated in determination of tumor metastatic potential in breast cancer patients (Dedić Plavetic´ et al; 2013). Consistent with Gonzalez-vela et al; (2001), Karanikas et al; (2010), Yang et al; (2011), and Inwald et al; (2013), we found positive correlation between Ki67-LI and tumor grade in IDC ($P< 0.03$).

Axillary lymph node metastasis is an important prognostic factor, and metastasis occurs through the lymphatic route (Lee et al., 2011). Current study showed positive correlation between Ki67-LI and lymph node positivity ($P< 0.02$), consistent with Pevalic et al; (1992), Karanikas et al; (2010), Yang et al; (2011), Bordea et al; (2012) and Inwald et al; (2013).

Although tumor size is one of the strongest predictive factors for local recurrence, and tumors greater than 2 cm leads to decreased disease free survival (Lee et al., 2011), our study revealed no significant correlations between Ki67-LI and tumor size in IDC. In the contrary Gonzalez-Vela et al; (2001), Karanikas et al; (2010), and Inwald et al; (2013) found significant correlation between Ki67 expression and tumor size.

Contrary to our findings of insignificant correlation between Ki67-LI and lymphovascular invasion, Inwald et al; (2013) found significant positive correlation between Ki67 expression and lymphovascular invasion.

Still the optimal value of the cutoff that makes the distinction between high proliferation and low proliferation activity in a clinically relevant manner when it is immunohistochemically determined in mammary cancers was not universally established (Plesan et al; 2010).

Our results showed that no significant correlation between Ki67-LI in one side and age of the patient, desmoplasia and lymphocytic infiltration in the other side. To the best of our knowledge, no previous studies discussing these relations and much more studies must be done to explore these correlations.

Nearly one-third of breast cancers have mutations in p53 gene (Fitzgibbons et al., 2000). 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 (Kerns et al., 1992, and Hurlimann et al., 1994).

In the current study p53 was expressed in 25% of normal breast tissue adjacent to IDC. P53 was expressed in one case of typical ductal hyperplasia adjacent to IDC consistent with Rohan et al. (2006) who found p53 accumulation and mutations in benign breast tissue and correlates this with a 2-fold increased risk of subsequent breast cancer.

Our study showed that, p53 was expressed in 8/11 (72.7%) of DCIS, and its expression correlated positively with higher tumor grade ($P <0.01$) in agreement with Done et al. (2001) Our finding indicated that p53 mutations usually occur before invasion during the progression of DCIS to IDC in agreement with Rajan et al. (1997).

In this study p53 was positive in 74% (40/54) of IDC. Different ratios for p53 expression were mentioned in the literature; Putti et al. (2005), Cho et al. (2006), and Lee et al. (2011) who found positive p53 in 60%, 25.3%, and 37.1% respectively. This difference is most likely due to variable proportions of different tumor grades in those studies.

The majority of studies support an association between worse survival and the presence of p53 mutations (Pharaoh et al., 1999). The possibility that p53 status influences the biological behavior was raised in an early study of Alsner et al. (2000) in which the presence of p53 mutation in aggressive breast cancer was demonstrated.

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., 2003), we found insignificant correlation between p53 expression and age of the patients. This observation concurs with the observations of; Michalides et al. (1996), and Zolota et al. (1999).

Current study showed significant correlation between p53 expression and larger tumor size (P<0.05) consisting with Ferrero et al. (2002), and Yamashita et al. (2004) In contrast, Noguchi et al. (1994) found no 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 IDC (P<0.002) in agreement with Redondo et al. (2003), Kourea et al. (2003), Yamashita et al. (2004), and Skarlos et al. (2005) and indicates that p53 is an indicator of poor prognosis in breast cancer.

We found significant positive correlation between p53 expression and vascular invasion in breast cancer (P<0.02). In agreement with Arisio et al. (2000) and Song et al. (2006) we found insignificant correlation between p53 expression and the presence of lymph node metastasis. In contrast, Noguchi et al. (1994), Gattuso et al. (1998), and Amila et al. (2002) found significant positive association between p53 expression and lymph node metastasis. 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.

Axillary lymph node status has repeatedly been shown to be the single most important predictor of disease-free survival and overall survival (Macchetti et al., 2006). Nodal involvement may be an indicator of metastatic disease, the cause of death in patients with breast cancer. Hence, the generally admitted conclusion is that tumor size loses its prognostic role in cases of nodal involvement (Verschraegen et al., 2005).

This study revealed insignificant correlation between p53 expression and the presence of desmoplastic stroma in IDC. However, Lipponen et al. (1993) found p53 expression more frequent in schirrous carcinoma than in other carcinomas with less extensive desmoplastic reaction. Ferrini and Rossi (2001) suggested that intense stromal reaction in IDC may modulate the p53 expression.

This study revealed positive correlation between p53 expression and lymphocytic infiltration in IDC (P <0.03) compatible to the findings of Lipponen et al. (1993) which implies that p53 may have a role in modulation of tumor immunity.

There was positive correlation between Ki67 and p53 in both DCIS (r= 0.845, P<0.001) and IDC (r=0.697, P<0.02) of the breast. Most cases that were p53 positive had an increased proliferation activity, as determined by increased Ki67-LI consistent with (Plesan et al; 2010) who found that most cases that were p53 positive had an increased proliferation activity, as determined by Ki67 expression in breast cancer patients.

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 IBC (Deng et al., 1996).

Conclusion: There was a steady progressive increase in the mean value of Ki67-LI and p53 expression on transition from normal breast to hyperplasia, to DCIS and lastly to IDC. Ki67 and p53 were increased with poor prognostic parameters; tumor size, tumor grade, lymphovascular invasion, lymphocytic infiltration and lymph node metastasis.

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المؤشر التكاثري وتعبير بي53 في سرطان الثدي وارتباطهما بمؤشرات الممارسة الطبية السريرية المرضية

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الخلفية: سرطان الثدي هو السرطان الأكثر شيوعا في التشخيص والسبب الأكثر شيوعا لوفيات السرطان في النساء في جميع أنحاء العالم. ويعد مؤشر كي ٦٧ من العلامات البيولوجية التي تعكس تكاثر الخلايا. وعلى الرغم من الفهم الواضح لهيكل وخصائص هذا البروتين، لا يزال دوره الوظيفي غير واضح. ويمنح تغييرتعبير الجينات إمكانات النمو الغازية في المراحل السابقة للغزو من سرطان الثدي . وقد وجد تغير في تعبير جين الورم القامع بي٥٣ في كثير من الأحيان وارتبط بسوء العاقبة في سرطان الثدي.
وتهدف هذه الدراسة إلى: تقييم تعبيرات المؤشر كي٦٧ وبي٥٣ في آفات الثدي الحميدة، والسابقة للغزو والغازية وكذلك تقييم علاقتهما بالقيم النذيرية الاكلينكية الباثولوجية المحتملة في سرطان الثدي.
المواد والطرق المستخدمة: شملت هذه الدراسة 74 عينة من آفات الثدي. وقد تم الكشف عن تعبير كي٦٧ وبي٥٣ باستخدام طريقة البيروكسيداز أفيدين بيوتين المناعية. **النتائج:** زادت تعبيرات كي٦٧ وبي٥٣ تدريجيا على طول سلسلة متصلة من التغيرات الورمية من ظهارة الثدي الطبيعي إلى سرطان الأقنية الغازية زيادة احصائية ذات دلالة. وكانت هناك علاقة إيجابية ذات دلالة إحصائية بين مؤشر كي٦٧ ودرجة الورم وكذلك الانبثاث في العقد الليمفاوية. وكانت هناك زيادة في تعبير بي٥٣ مع تزايد درجة كلا من سرطان الأقنية في الموقع وسرطانات الثدي الغازية. وكان هناك ارتباطا كبيرا بين تعبير بي٥٣ وحجم الورم، وغزو الاوعية الدموية الليمفاوية، والارتشاح الليمفاوي. وكان هناك ارتباطا إيجابيا بين تعبيرات كي٦٧ وبي٥٣ في كلا من سرطان الأقنية في الموقع وسرطانات الثدي الغازية.
الخلاصة: وقد ازدادت تعبيرات كي٦٧ وبي٥٣ تدريجيا على طول سلسلة متصلة من التغيرات الورمية من ظهارة الثدي الطبيعي مرورا بسرطان الأقنية في الموقع إلى سرطانات الثدي الغازية. وقد ازدادت تعبيرات كي٦٧ وبي٥٣ مع المؤشرات السلبية التي تنذر بسوء العاقبة مثل حجم الورم، ودرجة الورم، وغزو الاوعية الدموية الليمفاوية، والارتشاح الليمفاوي، والانبثاث إلى العقد الليمفاوية .