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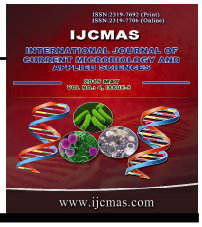

Original Research Article

Interaction between Altered P53 and PTEN Inactivation has a biological predictive implication in assessment of aggressive breast cancer

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A B S T R A C T

Breast cancer represents the most common cancer in women worldwide, constituting 23% of female cancers. In Iraq, It is considered as the first cause of death in women, accounting approximately one-third female cancers. The incidence increased dramatically, especially after Gulf War 1 and 2, probably due to exposure to environmental hazards as depleted uranium. Other factors such as life style may play a role breast cancer. The present study was designed to investigate the genetic alteration in two tumor suppressor genes(p53 and PTEN) and their possible role in breast cancer progression. The current study included 132 sample of Paraffin-embedded breast cancer tissues which were analyzed for PTEN and p53 expression by immunohistochemistry. We also studied the correlation between PTEN and p53 expression in relation to clinicopathological parameters. The loss expression of PTEN protein was found in 76 (63.8%) of 119 breast cancer tumors, while, The overexpression of P53 protein was found in 65.2% (86 out of 132).The loss expression of PTEN was correlated with high grade and stage, lymph node involvement, large tumor sizes and age of patient less than 50 years compare with low grade, lymph node negative, small tumor sizes and age patient more than 50 years. The p53 expression was significantly correlated grade, stage and lymph node status as well as age group less than 50 years.The loss function of p53 and PTEN geneswas found in more of half breast cancer cases and the expression of PTEN protein decreased in p53-deficient cells compared with that in p53 normally expressed cells. The loss function of p53 and inactivation of PTEN genes are well with high grade and stage as well as lymph node positive. The genetic alteration of p53 and PTEN genes play important role in progression of breast cancer.

Introduction

The breast cancer is originated from epithelial cells lining ducts or lobules of the breast. It is the most common cancer among women, constituting 23% of all cases worldwide (Armstrong *et al*, 2000 ; parkin., 2006; El-Ghannam *et al*.,2011). In Iraq, breast cancer is the first leading cause of cancer death in women and accounts approximately one-third of the registered female cancers (Iraqi cancer registry,2010).

Breast cancer is a complex molecular disease that occurs as a result of alterations in the genes that control cell growth and proliferation , particularly HRE2/neu, c-MYC, K-RAS, RB, P53,PTEN,BRCA1 and BRCA2(Sledge and Miller.,2003; Ingvarsson., 2004).Both P53 and PTEN tumor suppressor genes are the main regulatory genes involved (lost or inactivated) in the pathogenesis of in human cancers, including breast cancer (Stambolic *et al*., 2001). p53 gene is located on chromosome 17p13, consists of 11 exons and encodes a53-kDa nuclear phosphoprotein, that has a very important function in many cellular processes, such as cell-cycle control, DNA repair , apoptosis and gene transcription (Pim and Banks, 2004; Zhu *et al*., 2010). p53 is the most common mutated gene in human cancers , including breast cancer , accounting 30- 50 % of sporadic breast cancer (Ozcelik *et al* ,2007; Tsuda,2009).Patients with the Li–Fraumeni syndrome, who have an inherited germline mutation in one of the two p53 alleles, are at very high risk of developing breast cancer throughout their lifetimes (oliver *et al*., 2010).PTEN gene is located on chromosome 10q23, consisting of 9 exons, that encodes a 403 amino acids dual-specificity phosphatase with lipids and protein phosphatase activities. PTEN plays a major role in control multiple cellular functions such as cell metabolism, cell cycle progression and cell survival. The PTEN gene is inactivated in a high percentage of cancer such as breast cancer(**Steck***et al*., 1997; Cristofano and Pandolfi.,2000; Leslie and Downes.,2004; Parsons.,2004).PTEN protects wild p53 protein from degradation by Mdm2 through it restriction of Mdm2 in the cytoplasm and promotes degradation, but loss PTEN function maycause more rapid p53 degradation by Mdm2 protein. The p53 also induces PTEN gene expression through binding to the PTEN promoter region(Stambolic *et al.,* 2001; Mayo *et al,* 2002)**.**

Material and Methods

Patients and tissue samples

Paraffin-embedded tissues from 132 breast cancer patients werecollected from the private laboratories and the laboratories of AL-sadr Teaching hospital in Najaf over a period from 2012-2015. Their ages were ranging from 25 to 81 years, with a mean age of (44.5) years. confirmation of histopathological diagnosis, grade and stage of tumor was carried out after reviewing all slides before proceeding to immunhistochemical approach.

Immunohistochemistry analysis

Paraffin-embedded sections (5*m*) of tumor blocks were placed on positively charged slides**.** These sections were deparaffinized with xylene, rehydrated in serial alcohol solutions and were pre-treated with antigen retrieval solution (0.01 M, citrate buffer, pH9.0, DakoCytomation/Denmark) in water-bath at 95°C for 30 minutes. The sections were incubated in 0.3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. Then, the slides were incubated with Monoclonal Mouse Anti-

Human p53 Protein1 ml DAKO, Clone DO-7, Code N7001, DAKO Cytomation/ Denmark A/S. produktionsve lj42. DK-2600 Glosrup, Denmark with (dilution 1:25) or PTEN Protein(0.2ml, Clone 6H2.1, Code M3627, Dako North America) (dilution 1:50) 25 min in a humidified chamber at 37c. The slides were subsequently incubated with a biotinylated universal secondary antibody and with Streptavidin-Biotin horseradish peroxidase label. After, the sections were incubated with 3,3'-diaminobenzidine (DAB) substrate chromogen solution and counterstained with hematoxylin. sections of breast cancer tissue well known to be positive for PTEN and p53 were used as positive control for each run of immunostaining while negative control slides were incubated with phosphate buffered saline (PBS) instead of primary antibody. The normal epithelial duct and myoepithelial cells were used as internal control for PTEN and p53 expression.

Immunostaining scoring

The scoring of immunoreactive Staining was done by calculated the percentage of immunoreactive cells per total number of malignant cells. The staining intensity was evaluated by calculating the percentage of positive cells in 100 malignant cells at objective 40 total magnifications: The nuclear reactivity p53 protein was classified in following for categories(Esrig et al., 1993; 1994): **(-):** No nuclear reactivity, **(+/-):** Few focally positive cells (1 to <10% tumor cells), **(+):** Heterogeneous nuclear reactivity (10 to 50% tumor cells) and **(++):** Homogenous intense nuclear reactivity (50 to 100% tumor cells).

The PTEN immunostaining patterns was cytoplasmic and/ or nuclear expression. Evaluation of PTEN expression was semiquantitative based on staining intensity and distribution according to previous studies (Depowski *et al,2001*; Park *et al, 2004*):Distribution was scored as diffuse (>50% tumor staining), regional (15-50% tumor staining) and focal (<15% tumor staining). Intensity staining was scored in comparison to the internal positive control as follows : strong (staining equal to or stronger than the internal positive control), moderate (less than the internal positive control but still positive staining) and weak(race or no expression).

Tumors cells consider as positive for PTEN expression include intense reactivity(strong) with any distribution and moderate intensity to high proportion(>50%), whereas tumors showed moderate intensity to regional, moderate to focal, or weak staining with any distribution were considered as negative for PTEN expression, therefore; the scoring represent in the following: score 0: weak intensity with diffuse, regional and focal distribution. score1: moderate intensity with regional and focal distribution, score 2: moderate intensity with diffuse distribution, score 3: strong stain with diffuse, regional and focal distribution.

Statistical analysis

Statistical Package of Social Science software (SPSS, version 20) used to calculate Fisher's exact probability and Odds ratios (ORs).The Fisher's exact probability used to test the relationships between studied groups and considered statistically significant at P-value $\leq$ 0.05 while the strength of associations was measured by calculating Odds ratios (ORs). The categories for OR include greater than 1 and less than 1, in which a value greater than 1 indicates positive association and a value less than 1 indicates negative association.

Results and Discussion

In normal cells, Transcription factors p53 and phosphatase PTEN are two tumor suppressor genes that play essential roles in regulating cell proliferation and cell death as well as suppression of carcinogenesis. The relationship between p53 and PTEN is not well understood. However, recent studies suggest that there is a tight link between PTEN and p53. loss P53 and PTEN function play the main role in progression of breast cancer (Stambolic *et al.,* 2001; Yamada and Araki., 2001; Bargonetti and Manfredi., 2002; Mayo *et al.*,2002).

This study were included 132 cases of breast cancer patients, The patients' characteristics are summarized in (Table-1). The mean age of breast cancer patients was (44.5) with a range of 25 to 81years and 91(68.6%)cases were below the age of fifty years, while the 41(31.1%) cases were more than 51-years. Among 132 cases. This age distribution frequency is similar to other studies done in Egypt, Kuwait, Jordan and other countries in the region, Unlike in the United States of America where women aged 50 years and older are the most commonly affected(El saghir et al.,2007). This is because of the lifestyle changes including dietary habits, delay of ages of marriage and first pregnancy from the late teens and early twenties to the late twenties in many Arab countries (Parkin et al., 2002). Most cases in present study were larger tumor sizes, more positive lymph nodes involvement and advance stage and grade. This result agree with other authors who found the breast cancer patients in Iraq and Arab world at initial diagnosis were observed to have larger tumor sizes, more positive lymph nodes involvement and advance stage and grade (Chouchane et al., 2013; Lakkis et al., 2010). These observations obviously reflect the poor health education of the general population and their ignorance regarding the significance of clinical breast examination, breast self examination and early medical consultation(Etzioni et al.,2003).

Our results explained that expression of PTEN protein was cytoplasmic and/or nuclear expression of the tumor cells as well as normal ductal epithelial cells, and myoepithelial cells were useful as internal positive controls(Fig.1). we observed a decrease of PTEN expression in 76(63.8%) of 119 breast cancer tumors while PTEN protein expression was normal in 43 (36.1%) of 119 tumors, which is higher than that reported by Tsutsu et al.,(2005), Park et al., (2004) and Chang *et al*.,(2005), who recorded that loss of PTEN expression were found in 28% ,36.5% and 48% of breast cancer tumors respectively.

The relationships between PTEN protein expression and clinicopathological factors(gender of patient, stage, grade, tumor size, tumor site and histological types) in the 119 tumors are shown in Table (3).The loss PTEN expression was more frequent in lymph node positive breast cancer cases than in lymph node negative cases, with significant difference between these two groups(P=0.05). which is similar to that of other researchers (Chung *et al*.,(2004), Chang *et al.,(*2005) and Tsutsu *et al*.,(2005)), who reported that reduced expression of PTEN protein was significantly correlated with lymph node metastasis in the breast cancer patients. While Zhang *et al*.,(2013) mentioned that no significant correlation between loss expression of PTEN gene and the presence of lymph node metastasis. Our explanation for this result is probably related to the mutation of PTEN gene as well as other mechanisms such as promoter methylation, translational and post-translational regulation may also play important role on

the expression silencing of PTEN protein. Our results also reported PTEN expression was reduced in 25% in grade II compare with 67% in grade III and in 37.5%, 57.7% and 75.9% of stage I,II and III respectively. This result differs from previous studies on breast cancer done by Depowski *et al*.,(2001) and Park *et al*., (2004). They had found no significant difference between advance stage and grade and loss PTEN expression in breast cancer, but agrees with that reported by Chang *et al.,(*2005**)**who had found a signification correlation between stage and loss PTEN expression.

Furthermore, a highest percentage of loss PTEN expression was observed in the tumor size of >5cm (72.4%) and age above 50 years(73.1%) compared to tumor size <5 cm (48.8%) and age group below 50 years old(56.9%), This result agrees with Zhang *et al.*,(2013) who demonstrated positive correlation between PTEN expression and tumor sizes, but differs from that recorded by Park *et al*, (2004), Chang *et al*.,(2005), Chung *et al*.,(2004), Tsutsu *et al.*, (2005) and Yang *et al.,(*2010). They had showed no significant correlation between tumor size and age of patient with loss PTEN expression. Our explanation for this results is probably related to increasing accumulation mutation in PTEN with age.

On the other hand, our results revealed that expression of P53 protein was localized inside nuclei of malignant cells of breast cancer whereas lymphocytes, stromal cells and endothelial cells showed negative to p53 expression, therefore were used as internal negative control. (Fig.2). it has been showed that overexpression of P53 protein was 65.2% (86 out of 132) of breast cancer tumors(Table.4). this result is similar to that recorded by other researchers AL-Janabi (2004) and Hong *et al.*, (2006), they had reported that 44.3% and 51.6% respectively, of breast carcinoma were P53 positive, but differs from that study carried out by Ryujw *et al*., (2000) and Al-joudi *et al.,(*2008), they found that 25.9% and 29.6% respectively of breast carcinoma were P53 positive. Gursan, (2001) also reported (69%) of breast cancer were over expression to P53 protein. Such differences may reflect the variant immunohistochemical techniques applied in the various studies and to the different sample sizes.

The present study revealed that p53 positivity was more frequency in grade III than grade II(68.3% and 33.3% respectively) and in stage III than stage II and stage I (70.2% , 61.3% and 33.3% respectively) Table(3).Similar results were reported by Brano *et al*., (2002); Gurkan *et al*.,(2004), Hassan (2008) who mentioned that p53 over expression was correlated with high grade and stage of tumor. This reflects that the more abnormally accumulated P53 protein in nuclei represents an indicator of the accumulation of mutations which present in cases with high stage and grade.(Gluck *et al*,2003; Sidoni *et al*,2003). Furthermore, among the p53 positive cases; 71.7% were associated with lymph node involvement whereas 52.8% of the cases had no lymph node involvement, with significant difference between these two groups. This finding agrees with that reported by Kourea *et al*.,(2003) who mentioned that P53 expression is significantly associated with lymph node involvement, and this may be attributed to the aggressive behavior of node positive breast cancer, while it was against that reported by Mohamed., (2006). The highest percentage of p53 positive cases was observed in the tumor size of>5cm(60.9%) compared to tumor size less than <5 cm, without significant difference (p>0.05).

Table.1 Characteristic Clinic pathological of breast cancer patients

Parameters		**Number**	**Percentage**	**Total**
Gender	Male	2	1.5 %	132
	Female	130	98.5%	
Age	<50	91	68.9%	132
	≥50	41	31.1%	
Grad	II	9	6.8%	132
	III	123	93.2%	
Stage	I	8	6.1%	132
	II	31	23.4%	
	III	57	43.2%	
	Unknown	36	27.3%	
Lymph node status	Positive	60	45.4%	132
	Negative	36	27.3%	
	Unknown	36	27.3%	
Tumor sizes	<5 cm	45	34.1%	132
	>5 cm	87	65.9%	
Histological types	Ductal	112	84.8%	132
	Lobular	7	5.3%	
	Medullary	13	9.9%	
Tumor site	Left	70	53%	132
	Right	62	47%	

Table.2 Correlation between PTEN and p53 in breast cancer tissues

PTEN expression	**p53 expression**		**Total**	**p-value=0.2 OR=1.4 95CI=0.62-3.1**
	-ve	**+ve**		
-ve	21 (27.6%)	55 (72.4%)	76 (63.9%)	
+ve	15 (34.9%)	28 (65.1%)	43 (36.1%)	
Total	36 (30.3%)	83 (69.7%)	119	

Table.3 Correlation between histopathological parameters and PTEN expression in 119 breast cancer patients

Parameter		**Total**	**PTEN expression**						
			Negative			**Positive**			
Gender			0	1	Total	2	3	Total	P-value=0.05 OR=1.78 95% CI=0.1-29
	Male	2	0 0%	1 50%	1 50%	1 50%	0 0%	1 50%	
	Female	117	32 (27.4%)	43 (36.8%)	75 (64.1%)	18 (15.4%)	24 (20.5%)	42 (35.9%)	
		117							
Age patient	≤50	65	17 (26.1%)	20 (30.8%)	37 (56.9%)	12 (18.5%)	16 (24.6%)	28 (43.1%)	P-value=0.05 OR=2 95% CI=**0.93**-4.5
	≥51	52	15 (28.8%)	23 (44.3%)	38 (73.1%)	6 (11.5%)	8 (15.4%)	14 (26.9%)	
Histological Types	Ductal	102	29 (28.4%)	35 (34.3%)	64 (62.7%)	17 (16.7%)	21 (20.6%)	38 (37.3%)	P-value=0.5
	Lobular	5	1 (20%)	2 (40%)	3 (60%)	1 (20%)	1 (20%)	2 (40%)	
	Medullary	10	2 (20%)	6 (60%)	8 (80%)	0 (0%)	2 (20%)	2 (20%)	
		117							
Grade	I	0 (0%)	0 (0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	P-value=0.02 OR=**6.0** 95% CI=**1.16**-31
	II	8(%)	0(0%)	2(25%)	2(25%)	1(12.5%)	5(62.5%)	6(75%)	
	III	109	32 (29.4%)	41 (37.6%)	73 (67%)	17 (15.6%)	19 (17.3%)	36 (33%)	
		117							
TNM stage	I	8	2 (25%)	1 (12.5%)	3 (37.5%)	3 (37.5%)	2 (25%)	5 (62.5%)	P-value=0.001
	II	26	8 (30.8%)	7 (26.9%)	15 (57.7%)	4 (15.4%)	7 (26.9%)	11 (42.3%)	
	III	54	15 (27.8%)	26 (48.1%)	41 (75.9%)	7 (13%)	6 (11.1%)	13 (24.1%)	
	Unknown	29	7 (24.1%)	9 (31.1%)	16 (55.1%)	4 (13.8%)	9 (31%)	13 (44.8%)	
		117							
Tumor site	Left	64	20 (31.3%)	22 (33.3%)	42 (65.6%)	11 (17.2%)	11 (17.2%)	22 (34.4%)	P-value=**0.4** OR=**0.86** 95% CI=**0.40**-1.8
	Right	53	12 (22.7%)	21 (39.6%)	33 (62.3%)	7 (13.2%)	13 (24.5%)	20 (37.7%)	
Lymph node status	Positive	58	15 (25.9%)	28 (48.2%)	43 (74.1%)	8 (13.8%)	7 (12.1%)	15 (25.9%)	P-value=0.05 OR=**0.39** 95% CI=**0.15**-1
	Negative	30	10 (33.3%)	6 (20%)	16 (53.3%)	6 (20%)	8 (26.7%)	14 (46.7%)	
	Unknown	29	7 (24.1%)	9 (31.1%)	16 (55.1%)	4 (13.8%)	9 (31%)	13 (44.8%)	
		117							
Tumor size	≤5	41	9 (22%)	11 (26.8%)	20 (48.8%)	8 (19.5%)	13 (31.7%)	21 (51.2%)	P-value=0.01 OR=2.7 95% CI=1.2-6
	≥5	76	23 (30.3%)	32 (42.1%)	55 (72.4%)	10 (13.1%)	11 (14.5%)	21 (27.6%)	

Table.4 Correlation between histopathological parameters and p53 expression in 132 breast cancer patients

Parameter		Total	P53 expression						
			Negative			Positive			
Gender		132	-	-/+	Total	+	++	Total	P-value=0.57 OR=0.52 95% CI=0.032-.6
	Male	2	0 (0%)	1(50%)	1(50%)	1(50%)	0(0%)	150%)	
	Female	130	24 (18.5%)	21 (16.1%)	45 (34.6%)	35 (26.9%)	50 (38.5%)	85 (65.4%)	
		132							
Age patient	≤50	91	16 (17.6%)	10 (10.9%)	26 (28.57%)	25 (27.5%)	40 (43.95%)	65 (71.4%)	P-value=**0.03** OR=**2.15** 95% CI=1-4.6
	≥51	41	8 (19.5%)	11 (26.8%)	19 (46.3%)	12 (29.2%)	10 (24.5%)	22 (53.7%)	
		132							
Histological Types	Ductal	112	18 (16.1%)	19 (17%)	37 (33%)	33 (29.5%)	42 (37.5%)	75 (67%)	P-value=0.412
	Lobular	7	4 (57.1%)	0 (%)	4 (57.1%)	1 (14.3%)	2 (28.6%)	3 (42.9%)	
	medullary	13	2 (15.4%)	2 (15.4%)	4 (30.8%)	3 (23.1%	6 (46.2%)	9 (69.2%)	
		132							
Grade	I	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	P-value=**0.04** OR=**0.23** 95% CI=**0.05**-**0.97**
	II	9	4 (44.4%)	2 (22.2%)	6 (66.6%)	0 (0%)	3 (33.3%)	3 (33.3%)	
	III	123	20 (16.3%)	19 (15.4%)	39 (31.7%)	37 (30.1%)	47 (38.2%)	84 (68.3%)	
		132							
TNM stage	I	8	2 (25%)	3 (37.5%)	5 (62.5%)	2 (25%)	1 (12.5%)	3 (37.5%)	P-value=0.02
	II	31	9 (29%)	5 (16.2%)	14 (45.2%)	9 (29%)	8 (25.8%)	17 (54.8%)	
	III	57	7 (12.3%)	8 (14%)	15 (26.3%)	20 (35.1%)	22 (38.6%)	42 (73.7%)	
	Unknown	36	6 (16.7%)	5 (13.9%)	11 (30.6%)	6 (16.7%)	19 (52.8%)	25 (69.4%)	
		132							
Tumor site	Left	70	15 (21.4%)	14 (20%)	29 (41.4%)	16 (22.9%)	25 (35.7%)	41 (58.6%)	P-value**0.04** OR=**0.06** 95% CI=**0.23**-1
	Right	62	9 (14.5%)	7 (11.3%)	16 (25.8%)	21 (33.9%)	25 (40.3%)	46 (74.2%)	
		132							
Lymph node status	Positive	60	11 (18.3%)	6 (10%)	17 (28.3%)	19 (31.7%)	24 (40%)	43 (71.7%)	P-value=**0.04** OR=**2.2** 95% CI=**0.95**-5.3
	Negative	36	7 (19.4%)	10 (27.7%)	17 (47.2%)	12 (33.3%)	7 (19.4%)	19 (52.8%)	
	Unknown	36	6 (16.7%)	5 (13.9%)	11 (30.6%)	6 (16.7%)	19 (52.8%)	25 (69.4%)	
		132							
Tumor size	≤5	45	6 (13.3%)	5 (11.1%)	11 (24.4%)	16 (35.6%)	18 (40%)	34 (75.6%)	P-value=0.06 OR=1.9 95% CI=0.8-4.4
	≥5	87	18 (20.7%)	16 (18.4%)	34 (39.1%)	21 (24.1%)	32 (36.8%)	53 (60.9%)	

Figure.1 Immunostaining for PTEN in breast tissues, Invasive ductal carcinoma.(A) poorly differentiated (Grade III)Tumor cells show strong nuclear staining.(B) poorly differentiated (Grade III)Tumor cells show strong cytoplasmic staining.(C) poorly differentiated (Grade III) Tumor cells show strong both cytoplasmic and nuclear staining.(D)Moderately differentiated (Grade II))Tumor cells show moderate nuclear staining (40X)

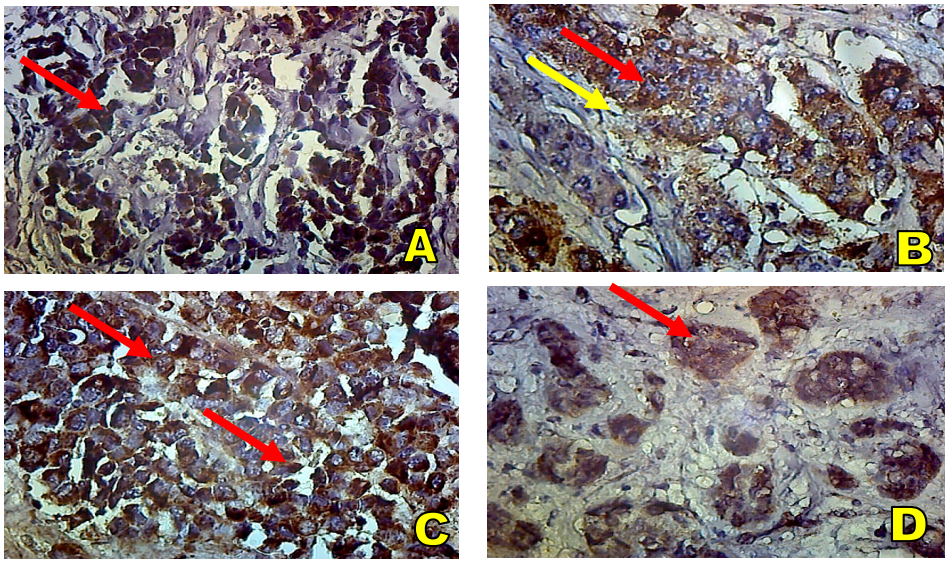
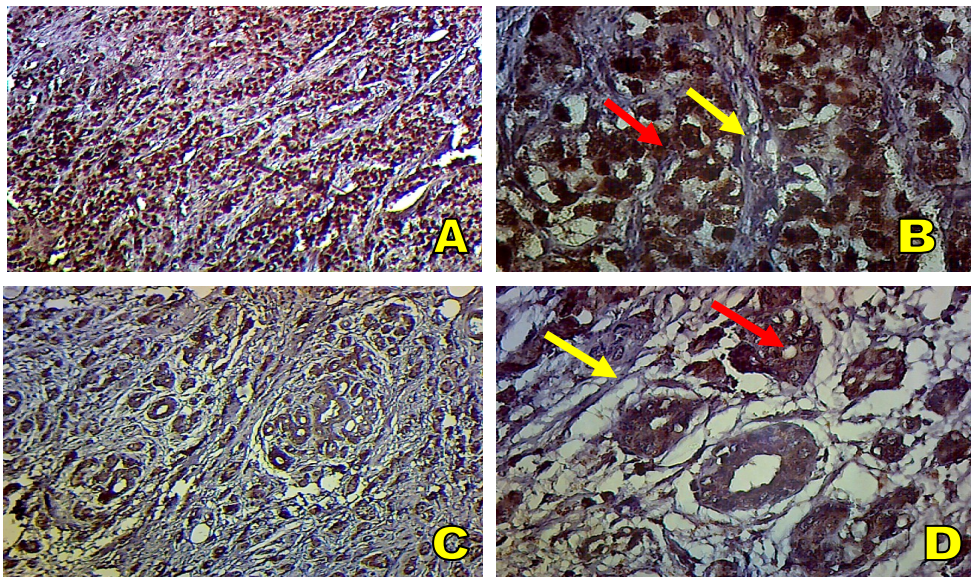


Fig.2 Immunostaining for p53 in breast tissues.(A) Invasive ductal carcinoma, moderate differentiated (grade II), showing p53 expression was moderate nuclear staining.(B) poorly differentiated (grade III), showing p53 expression was strong nuclear staining; (red arrow) (10x&40x).(Yellow arrow indicates surrounding stromal, myoepithelial cells and infiltrative lymphocytes with no nuclear p53 immunostaining)



This finding agreed with that of AL Moundhri *et al*., (2003) and Hong *et al*.,(2006) who found no significant difference of p53 expression among different tumor sizes. Our explanation to this increases in p53 positivity with larger tumor size, suggesting that it is either frequently acquired during progression of the disease or

that p53 mutations lead to a more aggressive phenotype.

The overexpression of p53 was reported in 71.4% of age group<50 years, while it was 53.7 % of age group ≥51 years, and the correlation of p53 expression and patients ages was statistically significant(p value=0.03). This finding is consistent with that of Al-joudi *et al*.,(2008) and plesan *et al*, (2010), who found significant correlation between P53 expression and age patient<50 years. In contrast, another study showed that higher incidence of p53 positive breast cancer was found in the older patients rather than young, is probably related to that the ability of cells to repair damaged DNA is reduced with age (Cabel *et al*., 2006; Sheikhpour *et al.*,2014).

The results of present study reported that 83 cases were P53 positive; 28 (33.7%) of them were positive for PTEN and 55(66.3%) cases were negative for PTEN. Seventy-six cases were PTEN negative; 55 (72.4%) were positive for P53 and 21(27.6%) cases were negative for P53(Table.2). It looks that the accumulation of p53 mutant protein in nuclei of malignant cells was increasing with loss of PTEN protein expression, with no significant correlation between these two tumor suppressor proteins (P53 and PTEN) (p=0.2, OR=1.4, CI 95=0.62-3.1) .This fact agrees with that reported by Wang *et al*,(2005) who observed the expression of PTEN protein decreased in p53-deficient cells compared with that of p53 normally expressed cells. Addition, PTEN protects wild p53 protein from degradation by Mdm2 through it restricts Mdm2 in the cytoplasm and promotes degradation but the loss PTEN function maybe cause more rapid p53 degradation by Mdm2 protein(Stambolic *et al*, 2001). Freeman *et al*., (2003) also mention to the loss PTEN expression will lead to increasing Mdm2 phposphorylation and nuclear translocation, resulting in degradation p53.

The loss function of p53 and inactivation of PTEN genes are well with high grade and stage as well as lymph node positive. The genetic alteration of p53 and PTEN genes play important role in progression of breast cancer.

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