Overexpression of p53 gene has an important role in induction and progression of breast carcinoma

Hussein. A. Al-hamadawi* Assad. A. Al-Janabi Adnan.w. Al-bider1.
College of Education College of Medicine College of Medicine
University of Al-Qadisiyah University of Kufa University of Al-Qadisiyah

الخلاصة: سرطان الثدي يعتبر من أكثر انواع السرطان انتشارا بين النساء، ويشكل حوالي 23% من كل انواع السرطان التي تصيب النساء في كل العالم. سرطان الثدي يعتبر مرض وراثي يحدث نتيجة لتغير في بعض الجينات التي تنظم نمو وانقسام الخلايا مثل BRCA2 , BRCA1 ,PTEN, P53, RB, K-RAS, c-MYC, HRE2. جين p53 يعتبر من الجينات الكابحة الورم و يوجد على الكروموسوم 17p13 ويتكون من (11 Exons) وهو يشفر لبروتين فوسفاتي وزنه (53-kD). P53 بروتين يلعب دور مهم في الكثير من الوظائف الخلوية مثل تنظيم دورة حياة الخلية، أصلاح DNA و الموت المبرمج للخلايا. P53جين غالبا ما يكون طافر في الكثير من انواع السرطان مثل شرطان الثدي.
الدراسة الحالية هدفت لمعرفة العلاقة بين التعبير العالي لجين P53 و علاقته بعوامل التكهن و عوامل تقدم الورم المرضية والسريرية في مرضى سرطان الثدي.
الدراسة الحالية شملت 85 عينة من الأنسجة الورميه لمرضى سرطان الثدي المثبتة بالفورمالين والمطمورة بشمع البرافين والتي استخدمت لتحليل التعبير الجيني لجين P53 بواسطة تقنية التعبير النسجي المناعي (Immunohistochemistry) ومقارنتها مع عوامل التكهن و عوامل تقدم الورم المرضية والسريرية.
نتائج الدراسة الحالية أظهرت 64.7%(55من 85) حالة من حالات سرطان الثدي كانت تمتلك تعبير جيني عالي ل P53 بينما كان 35.3% من حالات كانت سالبة بالنسبة لتعبير جيني لp53. وان التعبير الجني العالي ل p53 كان له علاقة معنوية كل من درجة ومرحلة المرض فضلا عن الفئة العمرية الأقل من 50سنة من المرضى. في حين لم يكن هنالك علاقة مع كل من جنس المريض وانتشار المرض لعقد للمفاوية وحجم وموقع الورم.
الاستنتاجات: وضحت الدراسة الحالة أن التعبير الجيني العالي لجين p53 موجودة في أكثر من نصف حالات سرطان الثدي. وان التعبير الجيني العالي لبروتينp53 له علاقة معنوية مع درجة ومرحلة المرض فضلا عن الفئة العمرية الأقل من 50سنة من المرضى.

Abstract

Breast cancer is the most common cancer in women, accounting for 23% of all female cancers around the globe. breast cancer molecular disease occurs due to the alteration in the genes that control cell growth and proliferation , such as HRE2/neu, c-MYC, K-RAS, RB, P53,PTEN,BRCA1 and BRCA2. The p53 tumor suppressor 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. p53 is the most common mutated gene in human cancers , including breast. The aim of this study was to study the correlation between the overexpression of p53 and clinicopathological parameters in breast cancer patient. The current study included 85 sample of Paraffin-embedded breast cancer tissues, which were analyzed for p53 overexpression by immunohistochemistry. We also studies correlation between p53 overexpression and clinicopathological parameters.
The results have clarified that 64.7% (55 out of 85) of cases of breast cancer had high expression of P53 immunostaining in their histological sections ,while 35.3% of cases were negative for p53 expression**.** The p53 expression was significantly correlated high grade and stage as well as age group less than 50 years ,and there was no significant correlation between p53 expression and gender of patient, lymph node status, tumor size, tumor site and histological types.
Conclusion: These results demonstrated the overexpression of p53 in more than half of breast cancer cases and that overexpression of p53 was well correlated with grade and stage as well as young age group less than 50 years breast cancer patients.

Introduction:

The breast cancer is a malignant growth of the epithelial cells that line the ducts or lobules of the breast and it is most common cancer among women in both developed and developing countries, constituting 23% of all cases worldwide (Armstrong *et al*,2000 ; parkin ,2006 ; Doaa *et al* ,2011). In Iraq,

breast cancer is the first leading cause of cancer death in Iraqi women and accounts for approximately one-third of the registered female cancers(Iraqi cancer registry,2010) .

Cancer of breast is a complex, molecular disease in which alteration occurs in the genes that control cell growth and proliferation , particularly ;the oncogenes as HRE2/neu , c-MYC and K-RAS , the ER and PR. The tumor suppresser genes as RB, P53 and PTEN ,and the breast cancer susceptibility genes as BRCA1 and BRCA2(Sledge and Miller ,2003; Ingvarsson, 2004).

The p53 tumor suppressor 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*, 2002). There is a correlation between the presence of p53 mutations and histological grade, lack of ER and/or PR expression and lack or low HRE2 expression in breast cancer patients (AL-moundhri *et al* ,2003; Ozcelik *et al* ,2007; Tsuda,2009; Pleşan *et al*, 2010). In our study we tried clarify the correlation p53 overexpression and clinicopathological factors in Iraqi breast cancer patients.

Material and methods

Patients and tissue samples.

Paraffin-embedded tissues were randomly selected from 85 breast cancer patients over period from 2012-2014. All the cases of breast cancer included in this study were collected from the private laboratories and the laboratories of AL-sadr Teaching hospital in Najaf Al-ashraf Province. 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. The slides were incubated with Monoclonal Mouse Anti-Human p53 Protein, 1 ml DAKO, Clone DO-7, Code N7001, DAKO Cytomation/Denmark A/S.produktionsvelj42.DK-2600 Glosrup, Denmark with (dilution 1:25) 20 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 **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 .

Immunostaining scoring

The scoring of immunoreactive Staining was done by calculated the percentage of immunoreactive cells per total number of malignant cells. The intensity of the staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnifications. Hence, each sample was first scanned on low magnification (10X) and at least five representative fields were assessed with a high power magnification (40X).

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). Only samples demonstrating at least 10% nuclear reactivity was considered to be p53-positive (to have an alteration in p53).

Statistical Analysis.

The Fisher's exact probability and Odds ratios (ORs) were calculated using the Statistical Package of Social Science program (SPSS for windows, version 20.0) in order to analyze the data of current study. The relationships between studied variables were considered as statistically significant at P- value ≤0.05.

The strength of the associations between studied variables was measured by calculating Odds ratios (ORs) and confidence intervals (95% CI). 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

Patients characterization :A total 85 cases of breast cancer patients were included in this study ,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 57(67.1%)cases were below the age of fifty years, while the 28(32.9%) cases were more than 51-years. Among 85cases were 78(91.8%)cases grade III and 7(8.2%) were grade II while 41.2%, 25.9% and 3.5% were III ,II and I stage respectively.

Most of our patients in this study(97.6%)were female and only 2.4% were male. We also documented 41(48.2%) of mastectomized cases were lymph node positive while negative lymph node involvement was recorded in 19 (22.4%). Invasive ductal carcinoma was (82.3%) of patients ,while invasive lobular carcinoma and medullary carcinoma were (5.9%)and (11.8%) respectively.

In this study , most of the patients' tumor included were of tumor size >5cm (67.2%). in addition, our result revealed left sided breast cancer is more than right sided breast cancer.

Immunohistochemical Analysis of p53 Protein: The results have clarified that 64.7% (55 out of 85) of cases of breast cancer were high expressing P53 immunostaining in their histological sections while 35.3% of cases were negative with p53 expression (Table.2). 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 control. (Fig.1).

This study mentioned to p53 expression increasing with high grade and stage as well as age group below than 50 years compare with low grade and stage as well as age group under than 50 years. Besides, there are not significant correlation between p53 expression and gender of patient, lymph node status, tumor size, tumor site and histological types.

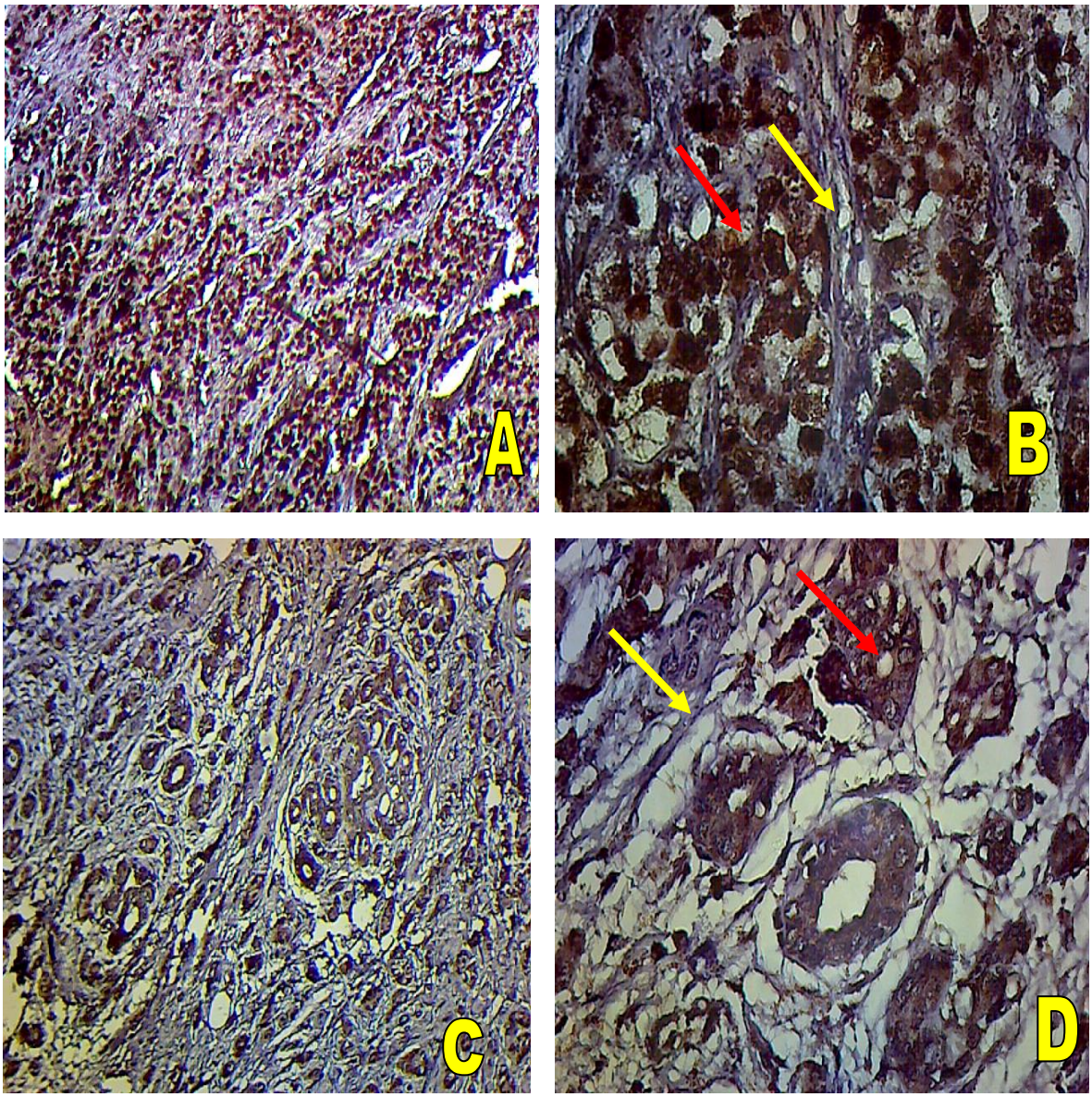
Table.1.Characteristic Clinic pathological of breast cancer patients.

Parameters		Number	Percentage	Total
Gender	Male	2	2.4%	85
	Female	83	97.6%	
Age	<50	57	67.1%	85
	≥50	28	32.9%	
Grade	I	0	0%	85
	II	7	8.2%	
	III	78	91.8%	
Stage	I	3	3.5%	85
	II	22	25.9%	
	III	35	41.2%	
	Unknown	25	29.4%	
Lymph node status	Positive	41	48.2%	85
	Negative	19	22.4%	
	Unknown	25	29.4%	
Tumor sizes	<2 cm	14	16.4%	85
	3-5 cm	14	16.4%	
	>5 cm	57	67.2%	
Histological types	Ductal	70	82.3%	85
	Lobular	5	5.9%	
	Medullary	10	11.8%	
Tumor site	Left	46	54.1%	85
	Right	**39**	**45.9%**	

Table(2).Correlation between clinicopathological parameters and p53 expression in 85 breast cancer patients.

Parameter		Total	P53 expression						
			Negative			Positive			
Gender		85	-	-/+	Total	+	++	Total	P-value=0.538
	Male	2	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	OR=
	Female	83	15 18%	15 18%	30 36%	22 27%	31 37%	53 64%	95% CI=
Age patient	>50	57	7 12.3%	6 10.5%	13 22.8%	19 33.3%	25 43.9	44 77.2%	P-value=0.001
	≥50	28	8 28.6%	9 32.2%	17 60.8%	5 17.9%	6 21.3%	11 39.2%	OR=5.231 95% CI =1.9-13.9
Histological Types	Ductal	70	12 17.1%	12 17.1%	24 34.2%	21 30.1%	25 35.7	46 65.8	P-value=0.066
	Lobular	5	3 60%	1 20%	4 80%	1 20%	0 0%	1 20%	OR= 95% CI
	medullary	10	0 0%	2 20%	2 20%	2 20%	6 60%	8 80%	
Grade	I	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	P-value=0.05
	II	7	3 42.8%	2 28.6%	5 71.4%	1 14.3%	1 14.3%	2 28.6	OR=0.18 95% CI =0.034-1.04
	III	78	12 15.4%	13 16.6%	25 32%	23 29.5%	30 38.5%	53 68%	
TNM stage	I	3	0 0%	2 66.7%	2 66.7%	1 33.3%	0 0%	1 33.3%	P-value=0.05
	II	22	6 27.3%	6 27.3%	12 54.6%	6 27.3%	4 18.1%	10 45.4%	OR= 95% CI =
	III	35	3 8.6%	6 17.1%	9 25.7%	12 34.3%	14 40%	26 74.3	
	Unknown	25	6 24%	1 4%	7 28%	5 20%	13 52%	18 72%	
Tumor site	Left	46	7 15.3%	10 21.7%	17 37%	13 28.3%	16 34.7%	29 63%	P-value=0.821
	Right	39	8 20.5%	5 12.8%	13 33.3%	11 28.2%	15 38.5%	26 66.7%	OR=.853 95% CI =.348-2.088
Lymph node status	Positive	41	7 17.1%	8 19.5%	15 36.6%	12 29.3%	14 34.1%	26 63.4%	P-value=0.488
	Negative	19	2 10.5%	6 31.5%	8 42%	7 36.8%	4 21.2%	11 58%	OR=1.26 95% CI =0.415-3.8
	Unknown	25	6 24%	1 4%	7 28%	5 20%	13 52%	18 72%	
Tumor size	≤2	14	1 7.1%	4 28.6%	5 35.7%	5 35.7%	4 28.6%	9 64.3	P-value=0.799 OR=

	3-5	14	3 21.4%	3 21.4%	6 42.8%	3 21.4%	5 35.7%	8 57.2%	95% CI =
	≥5	**57**	**11** **19.3%**	**8** **14%**	**19** **33.3%**	**16** **28.1%**	**22** **38.6**	**38** **66.7%**	



Fig(1) immunostaining for p53 in breast tissues.(A) Invasive ductal carcinoma, moderate differentiated(grade II), showing p53 expression was moderate nuclear staining.(B) Invasive ductal carcinoma, 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) .

Discussion: The p53 gene has the role of "genome guardian", that is to monitor the DNA integrity during cell division. The loss or altering of p53- protein, because of gene rearranging, can cause the unbalance of cell growth through replication errors and genetic accumulations(Ardeleanu *et al.*, 1999;Vousden and Lu, 2002; Bai and Zhu, 2006). In normal condition , p53 protein cannot be detected by Immunohistochemistry (IHC)because of the very short half-life of the protein and low levels present in normal cells. In contrast, many mutant p53 proteins are sufficiently stable to be detected by this technique, and thus immunohistochemical detection of p53 protein is synonymous with mutation(plesan *et al*,2010).

Our results revealed that overexpression of P53 protein was 64.7% (55 out of 85) of breast cancer tumors, which is a similar result to that of other researchers AL-Janabi (2004) and Hong *et al*., (2006), who reported 44.3% and 51.6% respectively of breast carcinoma had shown P53 expression but differs from that study which was carried out by Ryu jw *et al*., (2000) and Al-joudi *et al*.,(2008) who found 25.9% and 29.6% respectively of breast carcinoma cases were P53 positive. Gursan,(2001) also reported (69%) of breast cancer were overexpression to P53 protein. Such differences may reflect the variant immunohistochemical techniques applied in the various studies and to the different sample sizes.

The overexpression of p53 was reported in 77.2% of age group<50 years while that was 39.2% of age group ≥51 years , and the correlation of p53 expression and patients ages was statistically significant(p=0.001). This finding is consistent with that of Al-joudi *et al*.,(2008) and plesan *et al*, (2010) who found statistically significant correlation between P53 expression and age patient<50 years. but another study showed that higher incidence of p53 positive accumulation in the older patients 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). a possible explanation to our results most of the cases were grade III.

The present study also revealed that p53 positivity was more frequency in grade III than grade II(68% and 28.6% respectively) and in stage III than stage II and stage I (74.3% , 45.4% and 33.3% respectively) give rise to statistically significant difference(p-value=0.05)Table(4-1).Similar results were reported by Brano *et al.*, (2002); Gurkan *et al* (2004), Hassan (2008) who observed that there was significant association between p53 overexpression and grade and stage of tumor. and differs from Sheikhpour *et al*(2014) who found no statistical significant correlation between P53 and stage and grade 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% were associated with lymph node involvement whereas 29% of the cases had no lymph node involvement, with no significant difference between these two groups. This finding agrees with what was reported by Mohamed, (2006) and is against what was reported by Kourea *et al*.,(2003) that P53 expression is significantly associated with lymph node involvement , and this may be attributed to the aggressive behavior of node positive breast cancer.

The highest percentage of p53 positive cases was observed in the tumor size range >5cm(66.7%) compare to other tumor size ranges, with no significant difference between us(p>0.05). 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.

Conclusion: These results demonstrated the overexpression of p53 was found in more than half of breast cancer cases and the overexpression of p53 was well correlated to grade and stage as well as young age group below 50 years breast cancer patients.

References:

1. Armstrong, K.; Eisen, A. and Weber, B.(2000). Primary care: Assessing the risk of breast cancer. The New England J. Med. 342: 564 - 571.
2. Parkin, D.M. and Fernandez, L.M. (2006). Use of statistic to assess the global burden of breast cancer .Breast. 12(1suppl.): s70-s80.
3. Doaa, M. E.; Mohammad, A. and Tarek, B. (2011). Mutations of p53 gene in breast cancer in the Egyptian Province of Dakahliya . J. Oncol. Pharm. Practice. 17: 119–124.
4. Iraqi Cancer Board .(2010). Iraqi Cancer Registry 2008. Baghdad, Ministry of Health.16(11):1159-1164.
5. Sledge, G.W. Jr. and Miller, K.D. (2003). Exploiting the hallmarks of cancer: the future conquest of breast cancer. Eur. J. Cancer. 39:1668–75.
6. Ingvarsson., S. (2004). Genetics of breast cancer. Drugs Today. 40:991–1002.
7.Pim, D. and Banks, L. (2004). p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. *Int. J. Cancer*. 108: 196-199.
8. Zhu., Y.; Wang, J.; He, Q. and Zhang, J.Q. (2010). Association of p53 codon 72 polymorphism with prostate cancer: a meta-analysis. Mol. Biol. Rep. 27(2): 540-546.
9. Ozcelik, H.; Pinnaduwage, D.; Bull, S. B and Andrulis, I. L. (2007). Type of TP53 mutation and ERBB2 amplification affects survival in node-negative breast cancer". Breast Cancer Research and Treatment. 105(3):pp. 255–265.
10. Tsuda, H. (2009). Gene and chromosomal alterations in sporadic breast cancer: correlation with histopathological features and implications for genesis and progression. Breast Cancer. 16(3): pp. 186–201.
11. Olivier, M., Hollstein, M., and Pierre, H., (2010).TP53 mutations in human cancers: origins, consequences, and clinical use, Cold Spring Harb Perspect Biol, 2:a001008.
12. AL-moundhri, M.; Nirmala,V.; AL-mawaly, K.; Ganguly, Y.; Burney, I.; Rizvi, A. and Grant, C. (2003). Significance of p53, Bcl-2, and HER-2/neuProtein Expression in Omani Arab Females with Breast Cancer . pathology Oncology research. 9(4):226-231.
13. Plesan, D.M.; georgescu, C.V.; partana, N.; plesan, C. and Stoica, D.(2010). Immunohistochemical study of p53 and Ki67 in a group of patients with mammary carcinoma. Romanian Journal of Morphology and Embryology. 51(3):459–465.
14. Esrig D; Elmajian D; Groshen S; Freeman J A; Stein J P; Chen SC, Nichols P W; Skinner D G; Jones P A and Cote R J (1994). Accumulation of Nuclear p53 and Tumor Progression in Bladder Cancer. New Eng J Med, 331:1259-1264.
15. Esrig D, Spruck III Ch H; Nichols PW; Chaiwun B; Steven K; Groshen S; Chen S; Skinner D G; Jones PA and Cote RJ (1993). P53 nuclear protein accumulation correlates with mutations in the P53 gene, tumor grade, and stage in bladder cancer. Am J Pathol, 143(5):1389-1397.
16. Ardeleanu, C.; Comanescu, V. and Zaharia, B. (1999). Imunohistochimie, Ed. SITECH, Craiova, 191–203.
17. Vousden, K.H. and Lu, X. (2002). Live or let die: the cell's response to p53. Nat Rev Cancer. 2: 594-604,.
18. Bai, L. and Zhu, W.G. (2006). p53: Structure, Function and Therapeutic Applications. Journal of Cancer Molecules. 2(4): 141-153.
19. AL-Janabi, A.A.(2004) Immunohistochemical study of p53-onco-suppressor gene in correlation to other biochemical markers in breast cancer. Kufa Medical Journal Vol.3.No.1,214-222.
210. Hong Suk Song M.D.; Yong Rok Do M.D.; Sun Hee Kang M.D; et al. (2006)Prognostic significant of immunohistochemical expression of p53 gene product in operable Breast cancer. Cancer Res. Treat. 38(4):218-223.
21. Ryu JW, Lee MC, Jang WC, et al.(200) Detecting p53 gene mutation of breast cancer and defining differences between silver staining PCR-SSCP and immunohistochemical staining. J Korean Med Sci .15: 73-77.
22. Al-Joudi, F .S.; Iskandar.Z. and Rusli.J.(2008) .The Expression of p53 in Invasive Ductal Carcinoma of the Breast: A Study in the North-East States of Malaysia. Med J Malaysia .(63) 2:96-99.
23. Gursan N, Karakok M, Sari I, et al. (2001).The relationship between expression of p53/bcl-2

and histological criteria in breast invasive ductal carcinoma. Int-J-Cli-Pract. 55:589-590.

24. Cabel, D.C.; Raffoul, J.J.; Ge, Y.; Van Remmen, H.; Matherly, L.H. and Heydari ,A.R.(2006). Age-related loss of the DNA repair response following exposure to oxidative stress. J Gerontol A Biol Sci Med Sci. 61(5):427–34.
25. Sheikhpour,R.; Ghassemi,N.; Yaghmaei,P.; Ardekani,J. and Shiryazd.M (2014). Immunohistochemical Assessment of p53 Protein and its Correlation with Clinicopathological Characteristics in Breast Cancer Patients. Indian Journal of Science and Technology, Vol 7(4), 472–479.
26. Brano, .T.; Stankovic, N.; Savjak, D.; et al.(2002). correlation of size of the primary tumor and axillary node status with the p53 tumor suppressor gene in carcinoma of the breast. Vojnosani-Pregl. 59:29-32.
27. Gurkan,A.; Erdogan,G.; Erdogan,O.; Pestereli,E.; Ogus,M.; Karaveli ,S. and Colak.T.(2004). Expression of c-erbB-2 and p53 in Breast Carcinoma Patients: Comparison with Traditional Prognostic Factors and Survival. Journal of International Medical Research.32:455-564.
28. Hassan, A.F. (2008). Immunohistochemical Study of P53 Overexpression in Correlation to VEGF in Breast Carcinoma .Thesis. College of Medicine. Kufa University.
29. Gluck,I.; Wolf, I.; Sadetki S, et al.(2003). Prognostic characteristic in young pre-menopausal breast cancer patients compared to older patients. In: Abstract book ASCO Annual Meeting Proceedings. Abstract 3604.
30. Sidoni, A.; Cavaliere, A. and Bellezza (2003). Breast cancer in young women: clinicopathological features and biological specifity Breast .12:247-250.
31. Mohamed, . T. L. (2006).TP53 overexpression in ductal carcinoma of the breast Immunohistochemical study, A thesis Submitted to the Scientific Council of Pathology in Partial Fulfillment of the Requirement for the Degree of Felloship of the Iraqi Board for Medical Specialization in Pathology.
32. Kourea, H.P.; Koutras, A.K. and Scopa, C.D. (2003).Expression of the cell cycle regulatory proteins p34cdc2, p21waf1and p53 in node negative invasive ductal breast carcinoma. J Clin Pathol . 56:328-335.