

Assessment of her2neu expression using immunohistochemistry in association with clinicopathological features and hormonal receptors in Iraqi breast cancer women patients

Noor M. Jabbar¹, Maha Fakhry Altaee^{2*} and Rana Zuhair Naji³

^{1,2} Biotechnology department, College of Science, University of Baghdad, Iraq.

³ Center health laboratory, Histopathology department, Bagdad, Iraq.

* Corresponding author: Maha Fakhry Altaee, e-mail: drmahaaltaee@yahoo.com

Received: 11 October 2015

Accepted: 12 November 2015

Online: 16 December 2015

ABSTRACT

Seventy four Iraqi breast cancer paraffin blocks were collected from patients were attended to center health laboratory, histopathology department, Bagdad, Iraq. The patients information's which included: name, age, and the pathological stage, grade, tumor size were obtained from the clinical records of the patients also relation with sex hormones was recorded. The cases which has been taken included invasive ductal and invasive lobular carcinoma type Women age were ranged from 24-80 years peak age frequency of tumor occurred in the category of more than 40 years old. Immunohistochemical expression of her-2/neu was from total 74 cases of infiltrative ductal carcinoma cases, 27(36.49%) were positive for Her-2/neu expression, 47(63.51%) were negative her-2/neu. Significant inverse relation was seen with ER and PR which were (56.76% and 34.1 % respectively) from total positive cases (27), (17) was at score 2 and (10) was at score 3.

Keywords: her-2/neu , IHC, ER, PR.

1. INTRODUCTION

Breast cancer considers the commonest type of cancer in Iraq and worldwide, Breast cancer is considered extremely heterogeneous disease. The incidence rate of this type of cancer increased in the last few years in Iraq.

According to Iraqi cancer registry In Iraq, 2014, breast cancer was the most frequent cancer among the women and accounts for approximately one-third of the registered female cancer. From total registered cases of cancer in females 10926 breast cancer incidence rate was by 3763 cases (Iraqi Cancer Registry, 2011).

Her-2/neu is a proto-oncogene located on chromosome 17q21 and encodes a 185-kD transmembrane glycoprotein, which is usually called the her-2/neu protein or receptor. Her-2/neu belongs to the human epidermal growth factor receptor family that plays a

significant role in the regulation cell growth, differentiation and survival. Her-2/neu gene is well known to be overexpressed, amplified or both indifferent human malignancies, including breast cancer. Amplification of this gene has been recorded to occur in 10-34% of breast carcinomas [1].

Approximately more than one million women are diagnosed with breast cancer each year and approximately 700.000 of these have positive (+) hormone receptors (HR)[2]. The hormone receptors are expressed proteins both in the epithelium and in breast stroma which bind to circulating hormones, mediating their cellular effects [3].

The HR best studied in breast cancer are estrogen receptor (ER) and progesterone receptors (PR). Breast cancers classified by positive immunohistochemistry (IHC) expression of ER and PR have different clinical,

pathological, and molecular characteristics [4]. It is postulated that risk factors are closely associated with breast tumors ER+ and PR+ and may involve mechanisms related to exposure to estrogen and progesterone, while etiology of breast cancer ER- and PR- should be independent of hormone exposure [5].

Immunohistochemistry (IHC) is very a wide-used biological method that combines anatomy, physiology, immunology and biochemistry. Depend on the antigen-antibody binding reaction, immunohistochemistry used to visualize distribution and localization of cellular components or specific antigen in tissue sections. Compared to other techniques that are also based on the antigen-antibody reaction like immunoprecipitation, or western-blot, immunohistochemistry provides information *in situ* which is more convincing experimental result [6].

2. MATERIALS AND METHODS

2.1 Patients

Seventy four Iraqi breast cancer paraffin blocks were collected from patients were attended to center health laboratory, histopathology department, Bagdad, Iraq. The patients information which included: name, age, and the pathological stage, grade (Nottingham Modification of Bloom-Richardson Grading Scheme was applied for grading), tumor size were obtained from the clinical records of the patients. The cases which have been taken included invasive ductal and invasive lobular carcinoma type. Hematoxylin and eosin (H and E) slides were stained for paraffin blocks for histopathological diagnosis.

Hematoxylin and Eosin (H and E) stained slides were examined and the useful area represent tumor were marked on the slides and corresponding paraffin blocks for immunohistochemistry of tumor markers her-2/neu, ER and PR

Slides prepared for immunohistochemistry staining for ER, PR, and her-2/neu/neu using antibodies, buffers and linking systems from DAKOTM (Dako, Denmark). Positive and negative control slides were used in each run of staining her-2/neu was evaluated according to HerceptTestTM Guidelines (HerceptTestTM Interpretation Manual Score 0 when membrane staining seen in <10% of the tumor cells, score 1+; weak which incomplete cell membrane staining noticed in >10% of the tumor cells, score 2+; complete and moderate cell membrane staining in >10% of the tumor cells, score 3+; complete and strong cell membrane staining in >10% of the tumor cells), score 0 and 1+ indicated normal expression of her 2 neu, score 2 consider abnormal expression intermediate cases, whereas score 3+ revealed deregulated HER-2/NEU expression strong positive

Expression status of ER and PR were assessed and evaluated and results were defined by recording a proportion score (PS) and the intensity score (IS)

according s Allred *et al.* (1998)(44). No staining = 0, Low level = 1, Medium staining = 2, Strong staining = 3, the percentage of stained cells (0% = 0, under 10% = 1, 10-50% = 2, 51-80% = 3; over 80% = 4). Many laboratories set 10% as cut off for ER positive cases. The common scoring system includes 0 (<10%), "+1" (10%-30%), "+2" (30%-80%) and "+3" (>80%). The intensity of expression of the marker was quantified using the following scores: 0=negative, 1=weakly positive, 2=moderately positive, 3=strongly positive, the same were applied for PR. Data was analyzed by using the X2-test. The results were statistically considered significant if the p value was <0.01.

3. RESULTS AND DISCUSSION

3.1 Clinicopathological characteristics of patients with breast cancer

Patient's age was ranged from (24-80) years, women < 40 were 10 cases and > 40 were 64 cases with mean age 55.39. Peak age frequency of cancer occurred in the category of more than 40 years old at percentage (86.48%) (as in the table 1) and these results was corresponded with data published by Iraqi Cancer Registry (2006, 2007, 2008, 2009)

Assessment of tumor diameter was according to TNM staging system; cases were classified into two groups; ≤ 2 cm and > 2 cm according to [7]. Six cases less than or equal to 2 cm at percentage 8.1% and 68 cases were more than or equal to 2 cm at percentage 91.1% as in (table 2) most cases were more than 2 cm. In our research we have found the tumor largest diameter percentage 92.19 for > 2 cm and 7.81 ≤ 2 cm and these results consistent with [8] that showed 90% of cases had tumor size more than 2 cm while 10% less than 2 cm. These results could be explained by poor health education and the ignorance of the importance of breast self-examination and early medical consultation.

Grading was done according to the Bloom and Richardson system and showed that 10.8 % were grade I; while 73 % were at grade II and 16.2 % were at grade III, as in (table 3). Studies revealed that women with tumors at higher grade express poor prognosis. In this study, the largest percent of cases were at grade II in (73%) followed by grade III (16.2%) and grade I (10.8). These results agreed with the [9].

The histological examination showed that the axillary lymph nodes invasion were in 51 cases (68.9%) while 23 cases (31.1%) had no lymph node tumor invasion, as in (table 4). This agreed with [9] who showed that 64.7 of women had lymph node involvement greatly agreed with our study. Axillary lymph node status considers one of the most significant prognostic factors for invasive breast carcinoma. In which the 5-year survival rate depend on invasion of the tumor to the lymph node.

Results revealed that stage 1 formed (5) cases (6.8%), followed by stage 11A (16) cases (21.6%), stage 111A was diagnosed in (23) cases (31.1%), stage 11B (21) cases (28.4%), stage 111B (8) cases (10.8%) and stage 111C (1) case (1.3%) as seen in the (table 5).

Table 1. Distribution of sample study according to the age.

Age	Number	Percentage
<40	10	13.51
> 40	64	86.48
Chi-square (χ^2)	74	13.96 **

**(P<0.01)

Table 2. Distribution of sample study according to tumor size.

Tumor size	Number	Percentage (%)
> 2 cm	68	91.9
≤ 2 cm	6	8.1
Total	74	100%
Chi-square (χ^2)	---	14.073 **

**(P<0.01)

Table 3. Distribution of sample study according to grade.

Grade	Number	Percentage (%)
I	8	10.8
II	54	73
III	12	16.2
Total	74	100%
Chi-square (χ^2)	---	12.664 **

**(P<0.01)

Table 4. Distribution of sample study according to Lymph node.

Lymph node	Number	Percentage (%)
Positive	51	68.9
Negative	23	31.1
Total	74	100%
Chi-square (χ^2)	---	10.095 **

**(P<0.01)

Table 5. Distribution of sample study according to stage.

Stage	Number	Percentage (%)
I	5	6.8
IIA	16	21.6
IIIA	23	31.1
IIB	21	28.4
IIIB	8	10.8
IIIC	1	1.3
Total	74	100%
Chi-square (χ^2)	---	11.047 **

**(P<0.01)

Table 6. Distribution of sample study according to IHC score for ER.

IHC score for ER	Number	Percentage (%)
Strong positive	15	20.3
Moderate	16	21.6
Weak positive	11	14.86
Negative	32	43.24
Total	74	100%
Chi-square (χ^2)	---	9.862 **

**(P<0.01)

3.2 Immunohistochemical expression of estrogen receptors (ER)

In current research, all the 74 cases were testing for estrogen receptor by IHC method, from the total cases 32 cases (43.84%) stained negative whereas, (42) cases 56.76% showed positive expression, In positive cases, 14.86% were weak positive, 21.6% were moderate positive and 20.3% were strong positive for ER as shown in the (table 6). Our result similar to the

study by [10]. It have been seen that, women with ER-negative tumors have very early recurrence, also poor response to endocrine therapy and survival rate decrease compared to ER-positive tumors [11].

3.3 Immunohistochemical expression of progesterone receptor (PR)

From total 74 infiltrative ductal carcinoma of breast stained for PR, 26 cases (35.1%) were positive for PR

expression. and 48 cases (65.75%) were negative for PR expression, as shown in the table (table 7). In positive cases 9.4% were strong positive, 13.5% moderate staining and 12.2% were weak staining by

immunohistochemistry. Our result agreed to great degree to the study by (10).

Table 7. Distribution of sample study according to IHC score for PR

IHC score for PR	Number	Percentage (%)
Positive	7	9.4
Moderate	10	13.5
Weak	9	12.2
Negative	48	64.9
Total	74	100%
Chi-square (χ^2)	---	12.378 **

** (P<0.01)

3.4 Immunohistochemical expression of HER-2/neu

From total 74 cases of infiltrative ductal carcinoma cases, 27 (36.49%) were completely stained cell membrane (positive for her-2/neu expression), 47 (63.51%) were negative. From 27 positive (17) cases (63%) were complete however weak or moderate membranous staining In >10% of tumor cells (score 2+) and 10 cases (37%) were strong complete membranous staining in >10 of cancerous cells (scored 3+), according to Dako scoring system as shown in the (tables(8)(9)). Negative expression showed no membrane staining as in the figure (1) Whereas Sample

photos of her-2/neu overexpression are shown in figure (2), in the photo brown stained membrane which indicate to the positive expression of her-2/neu . Our result consistence with study by [13] who reported that overexpression of her-2/neu protein found (40%) of patients. Our findings disagreed with [14] who reported a high level of the her-2/neu immunoexpression which was positive in 67.8% of breast cancer women. [15] had published that HER-2/NEU overexpression in about (28.2%) a result which is lower to that results obtained in our study.

Table 8. Distribution of sample study according to IHC score for her-2/neu.

IHC score for her-2/neu	Number	Percentage (%)
Positive	27	36.49
Negative	47	63.51
Total	74	100%
Chi-square (χ^2)	---	9.28 **

Table 9. Distribution of sample study according to positive IHC

Positive IHC	Number	Percentage (%)
Score 2	17	63
Score 3	10	37
Total	27	100
Chi-square (χ^2)	---	9.41 **

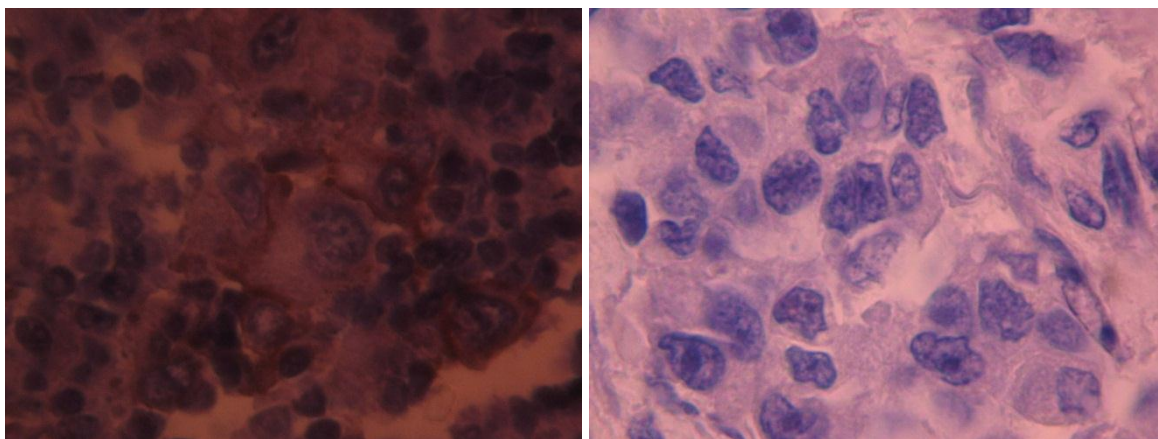
** (P<0.01).

3.5 Association of her-2/neu overexpression by IHC clinic pathological features

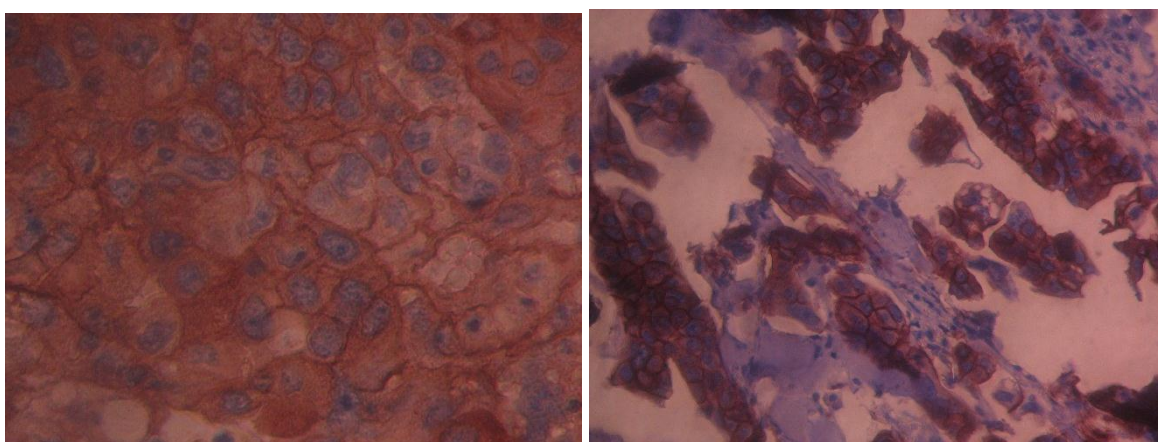
3.5.1 Association of Her-2/neu with age

There were significant association between Her-2/neu result and the age of women > 40, as shown in the (Table (10)) while there were no significant association

when age of women <40. 34.38% of cases of women with age > 40 years old were shown positive results, 65.62 % were negative for her-2/neu. Of 27 cases positive for her-2/neu 22 cases were found with women at > 40 years old.



Score 1
Score 0
Figure 1. Negative her-2/neu IDCNOS (score 1 and score 0). (100X)



Score 2
Score 3
Figure 2. Positive her-2/neu IDCNOS (score 2 and score 3). (100X)

Table 10. Association of her -2/neu overexpression with age.

Age	Total	Positive IHC	%	Negative IHC	%	Chi- square
<40 (n10)	10	5	50	5	50	0.0 NS
> 40 (n64)	64	22	34.38	42	65.62	9.61 **

** (P<0.01). NS: Non-significant

3.5.2 Association of Her-2/neu with tumor size

Our study showed significant relationship between Her-2/neu and tumor size, 66.7% of ≤2 cm group were positive as in the (Table 11) and 34% of >2 cm shown

positive results for her-2/neu. Of 27 cases positive for her-2/neu 23 cases were with tumor size more than 2 cm.

Table 11. Relationship between tumor size and score for her-2/neu (+ve and -ve).

Tumor size	Total No.	Positive		Negative		Chi-square (χ ²)
		No. 27	%	No. 47	%	
>2 cm	68	23	34.00	45	66.00	9.712 **
≤2 cm	6	4	66.7	2	33.3	9.694 **

** (P<0.01)

3.5.3 Association of her2neu overexpression with nodal status

There was significant association between her-2/neu expression and lymph node involvement, in breast

cancer (Table 12). 39.1% of lymph node negative cases were positive for her-2/neu and 35.3 % of lymph node positive cases shown positive results for her-2/neu overexpression.

Table 12. Relationship between Lymph node and score for her-2/neu (+ve & -ve).

Lymph node	Total No.	Positive		Negative		Chi-square (χ^2)
		No. 27	%	No. 47	%	
1: +	51	18	35.3	33	64.7	9.251 **
2: -	23	9	39.1	14	60.9	8.416 **

** (P<0.01)

3.5.4 Association of her-2/neu overexpression with histological grade

This study showed that a significant association between Her-2/neu and tumor grade as in the (Table 13). 37.5% of grade 1 were positive for her-2/neu, and 37.00% of grade 11, 33.33% of grade 111 were positive .Of 27 cases were positive for her -2/neu 20 cases were at Tumor grade 11.

3.5.5 Association of with pathological stage

This study revealed significant association between her-2/neu overexpression and pathological stage, except for stage 1 there was no significant association as in Table 14.

Table 13. Relationship between tumor grade and score for her-2/neu.

Grade	Total No.	Positive		Negative		Chi-square (χ^2)
		No. 27	%	No. 47	%	
I	8	3	37.5	5	62.5	9.657 **
II	54	20	37.00	34	63.00	9.683 **
III	12	4	33.3	8	66.7	10.526 **

** (P<0.01)

Table 14. Association of her-2/neu overexpression with tumor stage.

Stage	Total	Her-2/neu positive 27	%	Her-2/neu negative 47	%	Chi- square
1	5	3	60	2	40	7.258 **
11A	6	5	37.5	10	62.5	9.832 **
111A	23	7	30.4	16	69.6	11.063 **
11B	21	8	38.00	13	62.00	9.244 **
111B	8	2	25.00	6	75.00	12.371 **
111c	1	1	100	0	0	15.00 **

** (P<0.01)

Immunohistochemistry (IHC) is widely used for evaluating her- 2/neu protein expression in breast cancer [16]. It was applied in current study and it was very successful for evaluating the expression of Her-2/neu. In current study 36.49% cases were positive agreed with [17] who reported (34.3%) cases were positive for her-2/neu [13] published 40 % of positive her-2/neu very close to the result of this research. While in other studies in Iraq such as [18] showed in her study that her-2/neu overexpression was positive in 78% of breast cancer which is very high level compare to our results. In Western studies the values of positive her-2/neu ranged from 17% to 27% [19-20-21] . A study published in UK, Her-2/neu over expression was positive in 19% of cases [22] (This study found significant association between her-2/neu positive and age > 40 years old and no association , with women younger than 40 as in the (table 10).

[23] also showed association of her 2neu overexpression with older women .[24 -25] agreed with our findings [8] in his study showed that no significant correlation between her-2/neu overexpression and age. Younger women with breast cancer were reported to have higher frequency of her-2/neu but no significant association with age [26]

There was a high significant correlation between her-2/neu overexpression and histological grade. This is comparable to the results of [27] [18]. They suggested that her-2/neu over-expression was associated with a higher tumor grade. [28] suggested association of her-2/neu overexpression with poor grade. Study from Kirkuk city by [29] revealed that majority of patient were at grade 11 very consistence with our results

In this study there were significant association of her-2/neu expression with tumor size and nodal status, of 27 cases showed her-2/neu over expression 18 cases were positive for lymph node, and 23 cases were with tumor size > 2cm. These results agreed with [30] who reported 12/23 cases were positive lymph node also agreed [31] who revealed that the over expressed of her-2/neu was correlated with large tumor size and axillary lymph node involvement. In study of [32] conducted a significant relationship between her-2/neu overexpression with lymph node status and tumor size, at the same time our results disagreed with other reports in Iraq [27] they found her -2 /neu over expression not associated with age nor with tumor size.

Also in Indian study showed that no significant correlation between Her-2/neu with tumor size and nodal status [33].Whereas [34] found strong

association of her-2/neu overexpression with patients tumor size and lymph node involvement. it has been found in our study significant correlation between her-2/neu and pathological stage mostly with stage 11 this discordance with [18] and [35] from Oman who revealed no statistical correlation between Her-2/neu overexpression and histological stage this can be explained to concluded small number of cases their study. also [10].found no relation between histopathological stage and her 2neu status .however current results concordance with [8]who reported 66.66% of stage III, 45% of stage II showed her 2neu overexpression. [36] also reported association her-2/neu over expression with higher tumor grade

3.6 Correlation between the IHC markers (her-2/neu ,ER,PR)

Analysis of the Iraqi women in this study revealed that there was a significance correlation between the her-2/neu over expression and (ER ,PR expressions (P value <0.01), as in the (Table (15))

In current study there were significant inverse association between her-2/neu over expression, and hormonal status. The inverse relation between hormone receptor and HER-2/neu amplification is postulated to be due to complex interactive, signaling between ER and other growth factor signaling pathways in breast cancer cells [37].

It has been found in this research that there is an inverse correlation between ER and her-2/neu which is

statistically significant (p value <0.001) where the vast majority of ER positive cases (54.76%) were negative for Her-2/neu. Similarly the vast majority of PR positive tumors (320.7%) were Her-2/neu negative; these findings agreed with study in Kirkuk city by [29] also agreed with two studies [20][25]. This inverse correlation is one of the reasons why women who have over-expression her-2/neu may be resistant to Tamoxifen [20]. Our results confirmed the suggestion that the presence of ER and PR receptors breast cancer cell lines lead to a strong reduction of her-2/neu protein over-expression. These findings are in agreement with other reports [38], [39] and [27]. Although in other study [40], they did not find any association between hormonal receptors expression and her-2/neu over-expression which was disagreed with our results it has been suggested that The tumor with positive hormonal receptors has better prognosis and good response to hormonal therapy in contrast to those with no hormonal receptors [41]The relation between hormonal receptor and her-2/neu has been explained by hormone-dependent down regulation of Her-2/neu which involves a very complex molecular interaction. In this interaction Estrogen and its receptor are required to suppress Her-2/neu expression [42].This explained the Her-2/neu expression in high levels in women with low or absent ER expression. But this had disagreed with study in Iran [43] which claimed no significant association between hormone receptors and Her-2/neu status.

Table 15. her-2/neu overexpression by IHC association with estrogen and progesterone.

Estrogen receptor	Total	Score 1 N=12	%	Score2 N=17	%	Score3 N=10	%	Negative N=35	%	Chi-square
Positive	42	7	16.7	9	21.4	3	7.14	23	54.76	11.36 **
Negative	32	5	15.63	8	25	7	21.87	12	37.5	8.34 **
Progesterone receptor										
Positive	26	4	15.4	10	38.5	4	15.4	8	30.7	9.71 **
Negative	48	8	16.67	7	14.58	6	12.5	27	56.25	9.93 **

** (P<0.01)

4. CONCLUSION

There was significant association between her-2/neu overexpression and age of the patients, tumor size, lymph node status, and tumor grade and stage. Also a significant but reverse association between her-2/neu overexpression and ER expressions and significant relationship between her-2/neu expression with PR expressions. In general IHC as immunological method was somewhat less sensitive but in general showed good agreement with molecular methods.

5. REFERENCES

1. Citri, A.; Skaria, K. B., and Yarden, Y. (2003). The deaf and the dumb: the biology of ErbB-2 and ErbB-3. *Experimental cell research*, 284(1): 54-65.
2. Piccart-Gebhart, M. J. (2011). New Developments in Hormone Receptor-Positive Disease. *The oncologist*, 16(Supplement 1): 40-50.
3. Rosen, P. P. (1987). Adenomyoepithelioma of the breast.

Human pathology, 18(12), 1232-1237.

4. SELVARAJAN, S. (2003). C-erBB2 over-expression in invasive breast carcinoma (Doctoral dissertation).
5. Althuis, M. D.; Fergenbaum, J. H.; Garcia-Closas, M.; et al. (2004). Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiology Biomarkers & Prevention*, 13(10):1558-1568.
6. Yoo, K. Y.; Tajima, K.; Park, S. K.; et al. (2001). Postmenopausal obesity as a breast cancer risk factor according to estrogen and progesterone receptor status (Japan). *Cancer letters*, 167(1): 57-63.
7. Ramos-Vara, J. A. (2005). Technical aspects of immunohistochemistry. *Veterinary Pathology Online*, 42(4):405-426.
8. Dublin, E.; Hanby, A.; Patel, N. K., et al. (2000). Immunohistochemical expression of uPA, uPAR, and PAI-1 in breast carcinoma: fibroblastic expression has strong associations with tumor pathology. *The American journal of pathology*, 157(4):1219-1227.
9. Al-Anbari, S. S. (2009). Correlation of the clinicopathological presentations in Iraqi breast cancer patients with the findings of biofield breast cancer diagnostic system (BDS), HER-2 and Ki-67 immunohistochemical expression. A PhD

- thesis Baghdad.
10. Matloob, R.S. (2006). HER-2/neu overexpression in breast cancer: Immunohistochemical Study. A thesis Submitted to the Iraqi Board for Medical specializations.
 11. Al-Jubouri ,R. S. (2008). Immunohistochemical Assessment for Urokinase-type Plasminogen Activator System by Using Tissue Microarray Technique in Human Breast Tumors. A PhD thesis Baghdad.
 12. Lin, V. C.; Jin, R.; Tan, P. H.; et al. (2003). Progesterone induces cellular differentiation in MDA-MB-231 breast cancer cells transfected with progesterone receptor complementary DNA. *The American journal of pathology*, 162(6):1781-1787.
 13. Al-Naqqash, M. A. (2009). The Role of c-myc oncogene as a prognostic marker in breast cancer patients evaluated by immunohistochemistry and in situ hybridization. M.Sc. Thesis. College of Medicine, University of Baghdad, Iraq
 14. Hanna, W. M., and Kwok, K. (2006). Chromogenic in-situ hybridization: a viable alternative to fluorescence in-situ hybridization in the HER2 testing algorithm. *Modern Pathology*, 19(4): 481-487.
 15. AL-Dujaily, E. A.; Al-Janabi, A. A.; Pierscionek, T.; et al. (2008). High prevalence of HER-2/neu overexpression in female breast cancer among an Iraqi population exposed to depleted uranium. *Journal of carcinogenesis*, 7(1): 8.
 16. Dendukuri, N.; Khetani, K.; Mclsaac, M.; et al (2007). Testing for HER2-positive breast cancer: a systematic review and cost-effectiveness analysis. *Canadian Medical Association Journal*, 176(10): 1429-1434
 17. Jacobs, T. W., Gown, A. M.; Yaziji, H.; et al. (1999). Comparison of fluorescence in situ hybridization and immunohistochemistry for the evaluation of HER-2/neu in breast cancer. *Journal of Clinical Oncology*, 17(7):1974-1974.
 18. SELVARAJAN, S. (2003). C-erbB2 over-expression in invasive breast carcinoma (Doctoral dissertation).
 19. Elyass, T.Y.M. (2012). Molecular Study of Human Mammary Tumor Virus and Immunohistochemistry of Hormonal Receptors in women with Breast Carcinomas. M.Sc. Med.Microbiol.Thesis. College of Medicine, University of Baghdad, Iraq
 20. Taucher, S., Rudas, M.; Mader, R. M.; et al. (2003). Do we need HER - 2/neu testing for all patients with primary breast carcinoma?. *Cancer*, 98(12): 2547-2553.
 21. Huang, H. J.; Neven, P.; Drijkoningen, M.; et al. (2005). Hormone receptors do not predict the HER2/neu status in all age groups of women with an operable breast cancer. *Annals of oncology*, 16(11): 1755-1761.
 22. Lal, P.; Tan, L. K., and Chen, B. (2005). Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas. *American Journal of Clinical Pathology*, 123(4): 541-546.6
 23. Winters, Z.E.; Leek, R.D.; Bradburn, M.J.; et al. (2003). Cytoplasmic p21WAF1/CIP1 expression is correlated with HER-2/neu in breast cancer and is an independent predictor of prognosis. *Breast Cancer Res*, 5(6): 242-249.
 24. Wang, B.; Wang, X.; Zou, Y. (2015). Association between hormone receptors and HER-2/neu is age-related. *International journal of clinical and experimental pathology*, 8(7): 8472.
 25. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. correlation of HER-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. *reast J* 2005;11:278-80
 26. Prati, R.; Apple, S. K.; He, J.; et al. (2005). Histopathologic Characteristics Predicting HER - 2/neu Amplification in Breast Cancer. *The breast journal*, 11(6):433-439
 27. Panjwani, P.; Epari, S.; Karpate, A.; et al. (2010). Assessment of HER-2/neu status in breast cancer using fluorescence in situ hybridization & immunohistochemistry: Experience of a tertiary cancer referral centre in India.
 28. Ayadi, L.; Khabir, A.; Amouri, H.; et al. (2008). Correlation of HER-2 over-expression with clinico-pathological parameters in Tunisian breast carcinoma. *World J Surg Oncol*, 6(1): 112-119.
 29. Pinto, A.; Andre, S.; Pereira, T.; et al. (2001). C-erbB-2 oncoprotein overexpression identifies a subgroup of estrogen receptor positive (ER+) breast cancer patients with poor prognosis. *Annals of oncology*, 12(4): 525-533.
 30. Mahmoud, M. M. (2009). Breast Cancer in Kirkuk City, Hormone Receptors Status (Estrogen and Progesterone) and Her-2/Neu and Their Correlation with Other Pathologic Prognostic Variables.
 31. Al-Nuaimy, W. M. T.; Ahmed, A. H.; Al-Nuaimy, H. A. A. (2015). Immunohistochemical Evaluation of Triple Markers (ER, PR and HER-2/neu) in Carcinoma of the Breast in the North of Iraq.
 32. Lee, A.; Park, W. C.; Yim, H. W.; et al. (2007). Expression of c-erbB2, cyclin D1 and estrogen receptor and their clinical implications in the invasive ductal carcinoma of the breast. *Japanese journal of clinical oncology*, 37(9): 708-714
 33. Naqvi ,S.Q.H.; Jamal ,Q.;Mahmood ,R.K.; et al. (2002). Significance of HER-2/neu Oncoprotein Overexpression on node positive invasive breast cancer. *J Coll Phys Surg Pak*,12:534-7.
 34. Ambroise, M.; Ghosh, M.; Mallikarjuna, V. S.; et al. (2011). Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. *Asian Pac J Cancer Prev*, 12(3): 625-9.
 35. Ahmed, H. G.; Al-Adhraei, M. A.; Ashankyty, I. M. (2011). Association between AgNORs and Immunohistochemical Expression of ER, PR, HER2/neu, and p53 in Breast Carcinoma. *Pathology research international*, 2: 11-21.
 36. Al-Moundhri, M.; Nirmala, V.; Al-Mawaly, K.; et al. (2003). Significance of P53, Bcl2, and HER2/neu protein expression in Omani Arab females with breast cancer. *Pathol Oncol Res*, 9: 226-231.
 37. Barros, F. F. T. (2013). Biological characterisation of HER2 amplified breast cancer (Doctoral dissertation, University of Nottingham)
 38. Massarweh, S., and Schiff, R. (2006). Resistance to endocrine therapy in breast cancer: exploiting estrogen receptor/growth factor signaling crosstalk. *Endocrine-related cancer*, 13(Supplement 1): S15-S24.
 39. Almasri, N. M., and Al Hamad, M. (2005). Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. *Breast Cancer Res*, 7(5): R598-604.
 40. Ratnatunga, N and Liyanapathirana L.V.C. (2007).Hormone receptor expression and HER/2 amplification in breast carcinoma in a cohort of Sri- Lankans. *Papers*; 52(4): 133-6
 41. Anim, J. T.; John, B.; Prasad, A.; et al. (2005). Relationship between the expression of various markers and prognostic factors in breast cancer. *Acta histochemica*, 107(2): 87-93.
 42. Rosai, J. (2011) .Rosai and Ackerman's Surgical Pathology, 10th Edition
 43. Arafah M. (2010) Correlation of hormone receptors with Her 2/neu protein expression and the histological grade in invasive breast cancer in a cohort of Saudi Arabia. *Turk J Pathol* ,26:209-15.
 44. Mousavi, S. M.; Montazeri, A.; Mohagheghi, M. A.; et al. (2007). Breast cancer in Iran: an epidemiological review. *The breast journal*, 13(4): 383-391.

© 2015; AIZEON Publishers; All Rights Reserved

This is an Open Access article distributed under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.