HER-2/neu overexpression in correlation to Vascular Endothelial Growth Factor ,grade and stage of Non other wised specified Invasive ductal carcinoma

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الخلاصة

من اجل تقييم مغزى التعبير المفرط لعامل النمو البشري(HER-2/neu) واحتمالية ترابطه مع التعبير النسيجي المناعي لعامل نمو البطانة الوعائية (VEGF) ,درجة التمايز ومرحلة الانتشار في سرطان الثدي النوع الغير معرف عند النساء.تم الحصول على 30 عينة مثبتة بالفورمالين ومطمورة بالبارافين لنساء مصابات بسرطان الثدي من النوع الغير معرف. مجموعة من 15 مصابة بورم الثدي الحميد استخدمت للمقارنة. كذلك استخدمت 15 عينة لنسيج الثدي الطبيعي كمجوعة قياسية. استخدمت طريقةLSAB لتحديد التعبير المناعي النسيجي ل(HER-2/neu) وطريقة ABCلتحديد التعبير المناعي النسيجي ل(VEGF) أظهرت ألنتائج أن التعبير النسيجي المناعي ل HER2/neu كان موجبا في(18حالة)60% من أورام الثدي الخبيثة من النوع الغير معرف و التعبير النسيجي المناعي لVEGF كان موجبا في(21حالة) 70% من أورام الثدي الخبيثة بينما لم نلاحظ أي تعبير نسيجي لـ(HER2/neuوVEGF)في نسيج الثدي الحميد و الطبيعي (P value < 0.005and <0 .005) على التوالي.لم نجد ارتباط بين التعبير النسيجي المناعي ل(HER-2/neu وVEGF)مع العمر وحجم الورم الأولي ومرحلة الخبيث (P>0.05).كما لم نجد ارتباط بين التعبير النسيجي المناعي ل(HER-2/neu وVEGF) مع خبيث الثدي موجب أو سالب العقد اللمفاوية (P<0.05).علاوة على ذلك إن التعبير المفرط ل(HER-2/neu) مرتبط بعلاقة معنوية ايجابية مع درجة تمايز الخبيث(P<0.05) بينما لم نجد ارتباط بين التعبير المفرط ل VEGF مع درجة تمايز الخبيث(P>0.05) .

إن ألتعبير ألمناعي لـHER-2/neuمرتبط بعلاقة معنوية ايجابية مع VEGF(P<0.05).أن كل من متلقي عامل النمو البشري (HER-2/neu)وعامل نمو البطانة الوعائية(VEGF)يلعب دور مهم في تولد نشأة خبيث الثدي من النوع الغير معرف ويسند الدليل عن دوره في عملية تطور وتكوين الأوعية الدموية وحياة الخلية. ويمكن ألتوجيه بأن تعطيل كل من (HER-2/neu)و(VEGF) ربما يكون هدف لإعاقة عملية تطور و تكوين الأوعية الدموية وبالتالي يطور فعالية العلاج المضاد لهذا النوع الخطير من سرطان الثدي .

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Abstract

This study aimed to assess the significance of human epidermal growth factor (HER-2/neu) protein overexpression and its possible correlation with vascular endothelial growth factor (VEGF),grade and stage in human non otherwise specified breast cancer.

The present investigation was performed over a period starting from November 2008 to January 2009 .Formalin fixed, paraffin-embedded blocks from 30 patients with breast cancer were included in this study. A group of 15 patients with benign breast lesions (fibroadenoma) was included as a comparative group and 15 normal breast tissue sections were included as control group. Labeled Streptavidin-Biotin Complex (LSAB) method was employed for immunohistochemical detection of HER-2/neu. Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of VEGF .

A total of 30 malignant cases were included.HER2/neu was considered as positive in(18 cases)60% of non otherwise specified invasive ductal carcinoma and VEGF was considered as positive in (21cases)70% with concomitant positivity of both markers in more than half (16 cases)53.3% out of 30 malignant cases . No overexpression of both markers have been noticed in normal or benign (fibroadenoma) breast tissue sections with significant difference from that of malignant cases($P<0.05$).We did not find any significant difference between overexpression of both(HER-2/neu and VEGF) in relation to age ,tumor size ,tumor stage and positive or negative lymph node breast cancer cases ($P>0.05$). However there was positive relation between HER-2/neu overexpression and the grade of tumor ($P<0.05$) ,while there was no significant difference between VEGF overexpression and the tumor grade($P>0.05$).HER-2/neu overexpression was positively correlated with VEGF immunostaining in relation to grade and stage ($P<0.05$).

Based upon the findings of this study, it can be concluded that both HER-2/neu and VEGF play an important role in the pathogenesis of non other wised specified breast cancer and supports the evidence of its role in evolution ,angiogenesis and cell survival of this aggressive tumor . This study recommended that the blocking of both HER-2/neu and VEGF may be a target for blocking the evolution and angiogenesis and hence improving the efficacy of anti-cancer therapy against this aggressive type of breast cancer.

Breast cancer is the commonest malignant tumor with more than one million new cases occurring worldwide annually[(1)]. In Iraq, where the population was exposed to high levels of depleted uranium following the first and second Gulf Wars, breast cancer is the most common malignant tumor in females[(2)]. Over the last ten years, there has been a three-fold increase in the incidence of breast cancer, with most of this increase being attributed to a particularly aggressive type of the cancer[(3)] . This may suggest that breast cancer in Iraqi women may have some biological features that need to be explored.Her-2/neu proto-oncogene amplification and or over expression is one of the most important alterations encountered in breast cancer. HER2/neu proto-oncogene is amplified and or over expressed in approximately 20-25% of invasive primary breast cancers [(4-6)]. An association have been found to exist between amplification and or over expression of HER-2/neu and advanced stage , early relapse, and reduced overall survival [(7,8)].

vascular endothelial growth factor, is believed to be a key mediator of angiogenesis which plays a central role both in local tumor growth and distant metastasis in numerous solid tumors, including breast cancer[(9)]. Activation or overexpression of HER-2/neu is associated with up-regulation of vascular endothelial growth factor (VEGF) in human breast cancer cells in vitro. Preclinical experiments indicate that increased expression of VEGF may in part mediate the biologically aggressive phenotype of HER-2/neu

overexpressing human breast cancer[10,11] . Therefore we plan to study the possibility of finding a relation between HER-2/neu and VEGF in one of the most aggressive histological types of breast cancer termed the invasive ductal carcinoma of non other wised specified as a possible explanation for the aggressive behavior of this tumor and hence whether such relation could be used as an objective tool for the early management of these patients with this aggressive type of breast cancer patients.

Materials and Methods

Thirty specimens, collected from patients with non otherwise specified breast cancer were included in this study. All the cases were collected from the teaching hospital and private laboratories in Kufa district area (located in the middle of Iraq). The age range of patients was 21 to 70 years. A group of 15 patients with benign fibroadenoma breast lesions was included as a comparative group. Labeled Streptavidin-Biotin (LSAB) method was employed for immunohistochemical detection of HER-2/neu and (ABC) method was employed for immunohistochemical detection of VEGF. All biopsies were classed, according to the modified WHO classification, into three grades: malignant grade I, malignant Grade II and malignant Grade III. The results were statistically evaluated by a Chi-squared test and correlation-regression using SSPS 17 software.

Results

A positive HER2/neu immunoexpression was detected in 18 cases(60%) of non otherwise specified invasive ductal carcinoma and VEGF was considered as positive in 21cases(70%) of cases. Furthermore there was concomitant positivity of HER-2/neu and VEGF in 16 breast cancer cases(53.3%). No positive overexpression for both HER-2/neu and VEGF has been observed in normal or benign (fibroadenoma) breast tissue sections with significant

difference from that of malignant cases (P<0.05). HER-2/neu immunohistochemical analysis in relation to grade of tumor revealed that (1 case 50% of grade I cases ,7 cases 100% of grade II cases and10 cases 47.6% of grade III) were positive for HER-2/neu with significant difference (P<0.05)(Table.1) ,while there was no significant difference between VEGF overexpression and the tumor grade(P>0.05)(Table.2).

HER-2/neu overexpression was well correlated to VEGF immunohistochemical overexpression in relation to grade and stage (r=0.981, P=0.02)(,r=0.99 ,P=0.0004)respectively .(Table.3,Figure.1).

We did not find any significant difference between overexpression of both(HER-2/neu and VEGF) in relation to age ,tumor size ,tumor stage (P>0.05). Furthermore there was(16 cases) were with positive lymph node involvement among the (24) mastectomized cases and (8 cases)were with negative lymph node involvement without significant difference between overexpression of both markers in relation to positive or negative lymph node breast cancer cases (P>0.05) (Table.1 and Table.2).

Table 1. HER-2/neu Immunohistochemical overexpression.

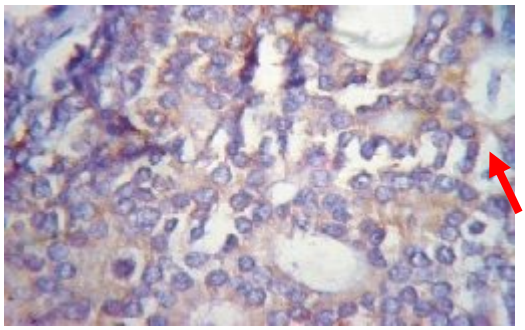
Parameters	*Total No of patients (positive)*	*c-ErbB-2 protein overexpression*		*(P value)*
		No. of patients (positive)		
Normal tissue	15	15(100%)	0 ( )	
Benign fibroadenoma	15	15(100%)	0(-)	=0.05
Malignant cases	30	15(50%)	15(50%)	
Age group(years)				
<50 years	15	5(33.3%)	10(66.7%)	= 0.05
=50 years	15	7(46.7%)	8(53.3%)	
Tumor size				
T2(2-5cm)	11	5(45.5%)	6(54.5%)	> 0.05
T3(>5cm)	19	6(31.6%)	13(68.4%)	
Grade				
I	2	1(50%)	1(50%)	
II	7	0 (-)	7 (100%)	<0.05
III	21	11 (52.4%)	10(47.6%)	
Tumor stage				
Stage I	1	1(100%)	0( )	
Stage II	7	3(42.9%)	4(57.1%)	>0.05
Stage III	15	5(33.3%)	10(66.7%)	
Stage IV	1	1(-)	1(100%)	
Axillary lymph node				
Node +ve	16	6(37.5%)	10(62.5%)	>0.05
Node -ve	8	4(50%)	4(50%)	

Table 2. VEGF Immunohistochemical overexpression.

Parameters	***Total*** *No. of patients*	***VEGF overexpression***		*P value*
		(Negative)	*(positive)*	
Normal tissue	15	15(100%)	0 (-)	<0.05
Benign fibroadenoma	15	15(100%)	0(-)	
Malignant cases	30	9(40%)	21(70%)	
Age group(years)				>0.05
<50 years	15	4(26.7%)	11(73.3%)	
≥50 years	15	5(33.3%)	10(66.7%)	
Tumor size				>0.05
T2(2-5cm)	11	5(45.5%)	6(54.5%)	
T3(>5cm)	19	4(21.1%)	15(78.9%)	
Grade				>0.05
I	2	1(50%)	1(50%)	
II	7	0 (-)	7 (100%)	
III	21	8 (30.1%)	13(61.9%)	
Tumor stage				>0.05
Stage I	1	1(100%)	0(-)	
Stage II	7	3(42.9%)	4(57.1%)	
Stage III	15	3(20%)	12(80%)	
Stage IV	1	0(-)	1(100%)	
Axillary lymph node				>0.05
Node +ve	16	3(18.7%)	13(81.3%)	
Node –ve	8	4(50%)	4(50%)	

Table 3. HER-2/neu overexpression in relation to VEGF immunohistochemical expression.

Parameters	***HER-2/neu in relation to VEGF immunohistochemical expression***					
	HER-2/neu		*VEGF*		*P value*	*R test*
	+ve	***-ve***	***+ve***	***-ve***		
Normal tissue	0(-)	15(100%)	0(-)	15(100%)		
Benign fibroadenoma	0(-)	15(100%)	0(-)	15(100%)		
Malignant cases	18(60%)	12 (40%)	21(70%)	9(30%)		
Grade					0.02	0.981
I	1(50%)	1(50%)	1(50%)	1(50%)		
II	7(100%)	0(-)	7(100%)	0(-)		
III	10(47.6%)	11(52.4%)	13(61.9%)	8(30.1%)		
Tumor stage					0.0004	0.99
Stage I	0(-)	1(100%)	0(-)	1(100%)		
Stage II	4(57.1%)	3(42.9%)	4(57.1%)	3(42.9%)		
Stage III	10(66.7%)	5(33.3%)	12(80%)	3(20%)		
Stage IV	1(100%)	0(-)	1(100%)	0(-)		



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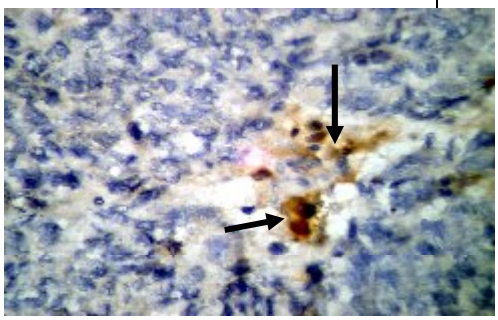

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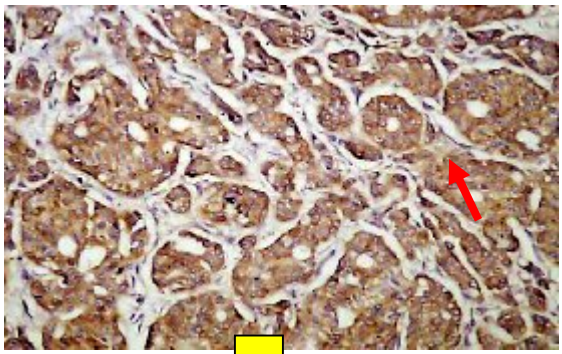

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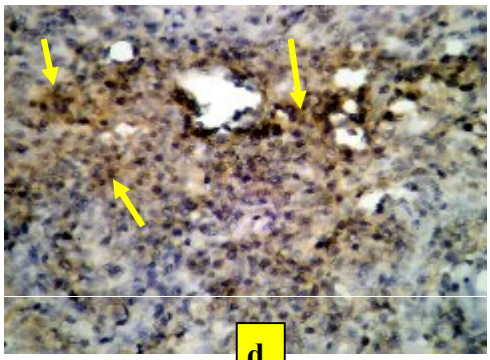

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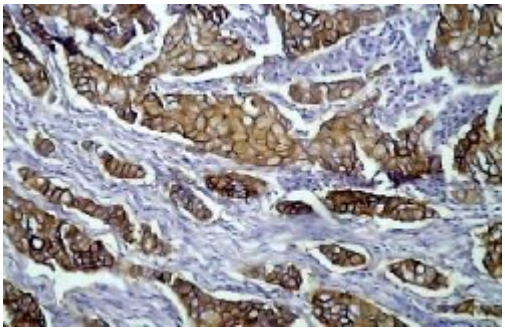

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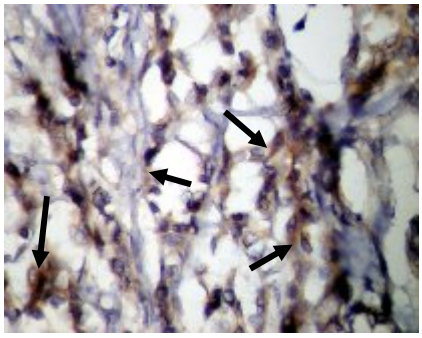

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Figure:(a) Showing HER-2/neu negative of score +1, IDC*-GI .*(X40)(b)*Showing focal score +1 for VEGF of IDC –GII.*(X40).(c)* IDC-GII showing faint to moderate complete membrane staining of score 2+.*(X10).*(d)Showing score+2,strong stain for VEGF.*(X10*).(e) Showing strong HER-2/neu positive IDC –GIII of score +3.*(X10).* (f)Showing strong cytoplasmic stain of VEGF score +3 in malignant cells infiltrating stroma and fatty tissue.*(X40).*

Discussion

HER-2/neu overexpression is associated with increased tumor progression and metastasis[12]; however, the exact mechanisms by which HER-2/neu regulates this more aggressive clinical phenotype are not fully understood. Recent studies indicate that HER-2/neu receptors play an important role in heregulin-induced angiogenesis [13]. These experimental data indicate that an important consequence of HER-2/neu signaling is increased VEGF expression. VEGF in turn is a central regulator of angiogenesis, suggesting that the aggressive phenotype of HER-2/*neu*-overexpressing breast cancers may be in part attributable to increased angiogenesis.

The current study demonstrated that there was completely absent overexpression of HER-2/neu and VEGF in normal breast tissue sections and in benign breast lesions(fibroadenoma) that has been tested in parallel with that of malignant samples . This observation was expected to us hence HER-2/neu overexpression and VEGF expression looks to be a landmark in the malignant breast tissue, and

does not play any role in the benign (fibroadenoma) breast lesions. The malignant tumor in our study was of non otherwise specified which is one of the most aggressive tumor type[10] ,HER-2/neu overexpression recorded in (18 cases)60% of malignant cases while VEGF expression was positive in (21cases) 70% of malignant cases ,with concomitant positivity of both markers in more than half of malignant cases(16 cases) 53.3%. This provides more evidence of the hypothesis that aggressive tumors seem to show significant HER-2/neu overexpression and VEGF expression and demonstrates the association between the nature of the biological expression of HER-2/neu and VEGF by the tumor and its degree of malignancy since it has been argued that nonspecific type ductal carcinomas are the most aggressive variants of breast cancer[14].

Furthermore there was significant difference between HER-2/neu overexpression and the grade of the tumor this finding is agreed with Aziz et al.,[15] . VEGF expression was positive in (50% , 100%and 61.9%) of (grade I, II and III) respectively ,but without significant difference (P>0.05) this is could be due to the small sample size of grade I and II cases (two and seven cases)respectively .Indeed HER-2/neu overexpression was not significantly associated with the tumor stage and this is agreed by Aziz et al.,[15].VEGF immunohistochemical expression was not significantly associated with the tumor stage also and this is agreed by Malcolm M et al.,[16].However in the present study ,a significant correlation between HER-2/neu overexpression and VEGF expression was demonstrated in relation to grade and stage of tumor (r=0.981, P=0.02)(,r=0.99 ,P=0.0004)respectively, this significant positive correlation between HER2/neu overexpression and VEGF immunohistochemical expression suggest that these oncogenes have an important role to play in higher histological grades and tumor progression of this aggressive tumor and this is agreed with Konenecy et al.,[17]

In summery, the current study provides clinical evidence that HER-2/neu overexpression is associated with expression of VEGF in breast cancer of non otherwise specified, suggesting that VEGF may in part mediate the aggressive phenotype of breast cancers that overexpress HER-2/neu. These data additionally support the use of combination therapies directed against both HER-2/neu and VEGF for treatment of breast cancers that contain the HER-2/neu alteration.

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