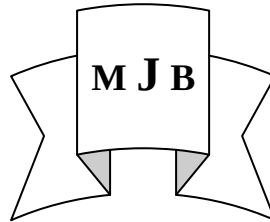


Estrogen and Progesterone Receptors Overexpression in Breast Carcinoma and Their Correlation with Ages of Patients, Histopathological Types and Grades of Tumors

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Background Carcinoma of the breast is one of the most important diseases for women, in the world .The disease is the commonest cause of death among women aged 40-50. Breast carcinoma is uncommon in women younger than age 30, thereafter, the risk steadily increases throughout life, but after menopause the upward slop of the curve almost plateaus.

The incidence of breast carcinoma is increasing in all industrialized countries, changes in diet, reproductive patterns and altered exposure to endogenous and exogenous substances with hormonal activity have been suggested as contributing to this increase.

Aim of the study: To assess estrogen and progesterone receptor over expression in breast carcinoma and its correlation with ages of patients, histopathological types & grades of tumors.

Materials and methods: This study was included 58 paraffin embedded samples from female patients with breast carcinoma were collected randomly from period of June2010- November 2012 in AL-Hilla Teaching hospital. The clinical information were collected including ages of the patients, histopathological types, & tumor grades from clinical reports of the hospital . A manual avidin biotin peroxidase complex procedure(ABC) system was used in the imunohistochemical analysis (Dako Cytomation Copenhagen, Denmark).

Results: The estrogen, progesterone receptors determined by immunohistochemical method revealed ER+/PR+ in 15.5% of cases, ER+/PR- in 3.4%, ER-/PR+ in 29.3 and ER-/PR- in 51% of cases , all ER+/PR+ are of invasive ductal carcinoma,most cases with ER+/PR+ are of premenapausal and perimenapausal age group 36.2% & 32.7% respectively and most cases with grade III are ER-/PR-

Conclusion: Present study reported high percentage of ER-/PR- cases of breast ca ,all ER+/PR+ cases are of invasive ductal ca, the population of high grade IDC were predominantly ER-/PR-

الخلاصة

سرطان الثدي يعتبر من أهم الامراض واشهرها التي تصيب النساء في العالم. ويعتبر من أهم الأمراض المؤدية الى الموت في النساء في عمر ٤٠-٥٠ سنة . سرطان الثدي غير شائع بين النساء بعمر مبكر أقل من ٣٥ سنة لذلك فإن نسبة زيادة خطورة الإصابة بهذا المرض تزداد بزيادة التقدم في السن وتقل بعد سن اليأس. أن نسبة الإصابة بسرطان الثدي تزداد في الدول المتقدمة وذلك يرجع الى عدة أسباب منها الاختلاف الغذائي،التغيرات الجنسية والهرمونية. أن الهدف من أجراء هذه الدراسة هو تقييم نسبة ظهور عوامل مثل الأستروجين والبروجسترون في سرطان الثدي وعلاقته بعمر المريضة ، نوعية النسيج المرضي ودرجة التمايز بالنسبة لسرطان الثدي.

Introduction

Carcinoma of the breast is one of the most important diseases for women, in the world. [1]. The disease is the commonest cause of

death among women aged 40-50, [2]. Breast cancer is uncommon in women younger than age 30, thereafter, the risk steadily increases throughout life,

but after menopause the upward slop of the curve almost plateaus [3].

The incidence of breast carcinoma is increasing in all industrialized countries, changes in diet , reproductive patterns and altered exposure to endogenous and exogenous substances with hormonal activity have been suggested as contributing to this increase [4].

In Iraq, breast cancer has remained the commonest tumor in women, breast cancer has remained the commonest tumor in women as it forms 15,32% of all malignant tumors and 29.06% of the registered female cancer in 2004 [5].

The most important risk factors are:
1-Age, the older the women ; the higher the risk ,less than 5% of cases are discovered before the age of 35 [6].
2-Country of birth; the incidence is high in North America and north Europe (91.4 new cases per 100,000 women per year), intermediate in southern European and Latin American countries, and low in most Asian and African countries [7] .
3-Family history and genetic predisposition; [8] .
4-menstrual and reproductive history: increased risk is correlated with early menarche, null parity, late age at first birth, and late menopause [9].
5-Previous breast diseases ; like benign proliferative breast lesion have a two folds increase in the risk of breast cancer , provided that they show no atypical epithelium , those proliferative lesions with atypical epithelial hyperplasia may increase the risk of four to five folds [10] .

Breast carcinoma development can be triggered by mutations of the signals in the network that controls cell division, and this can be associated with genetic predisposition (e.g., mutation in BRCA1 and BRCA2 genes), exposure to some environmental factors (e.g., radiation

exposure of the chest) , or both([11] , so it is an interplay between genetic changes and environmental factors [12].

Other factors associated with breast carcinoma development and progression are estrogen and progesterone exposure , normal breast cells have receptors that attach to circulating estrogen and progesterone , estrogen and progesterone bind to the receptors and may work with growth factors (e.g.,oncogenes and tumor suppressor genes) to cause cancer cell growth and proliferation [13].

There are two principal types of nuclear estrogen receptors: estrogen receptor α (ER α) encoded by a gene on chromosome 6; and estrogen receptor β (ER β), encoded by a gene on chromosome 14. Both are members of the nuclear receptor superfamily, members of which share a common structural architecture [14].

The estrogen receptor (ER) is a regulator of mammary epithelial growth, proliferation, and differentiation whose complex cellular interactions are mediated by a multitude of ligands, cofactors, and other stimuli. ER is important for normal breast development and function, but also plays a role in the development and progression of breast carcinoma [15] .

The human progesterone receptor (PR) belongs to the nuclear receptor superfamily and is encoded by a single-copy gene located on chromosome 11. PR functions as a transcription factor that modulates target gene transcription in response to progesterone [16].

The PR is an important prognostic marker in breast carcinoma, and receptor expression is routinely assessed as an integral part of disease management. Absence of PR in the primary breast tumor is associated with disease progression and may be

reflective of an aggressive tumor phenotype [17].

Materials and Methods

This study was included 58 paraffin embedded samples from female patients with breast carcinoma were collected randomly from period of June 2010- November 2012 from AL-Hilla Teaching hospital

The clinical information were collected including ages of the patients, histopathological types, & tumor grades from clinical reports of the hospital. The cases were classified according to histopathological types into invasive ductal carcinoma 55(94%), 1(1.7%) medullary carcinoma, and 2(3.44%) invasive lobular carcinoma

The tumors according to Nottingham's grading system [18] were classified into grade I, grade II and grade III.

The Avidin Biotin Peroxidase Complex procedure (ABC) was used for immunohistochemical detection of estrogen and progesterone

The criterion for positive immunoreaction is the dark brown staining of the nucleus for both estrogen and progesterone

The proportion of the staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnifications, each sample was scanned for five fields randomly with a high power magnification [19].

Qualitative assessment: Faint staining pattern, whether membranous or nuclear, that only could be detected by using higher magnification (objective 40), while strong staining pattern, easily seen by low magnification (objective 4).

Estrogen and Progesterone Receptors Scoring System [20]

Scoring is based on the examination of all tumor cells on the

slide, the Allred scoring guideline is used and includes:

1-A proportion score (PS) =estimated proportion of tumor cells with positive nuclear staining and includes five grades:

0= no cell stained.

1= > 0 to 1/100 of cells stained.

2=> 1/100 to 1/10 of cells stained.

3=> 1/10 to 1/3 of cells stained.

4=> 1/3 to 2/3 of cells stained.

5=> 2/3 to 1/1 of cells stained.

2-An intensity score (IS) =estimated average staining intensity of all positive tumor cells and includes four grades:

0=negative

1=weak

2=intermediate

3=strong

3-A total score (TS) =sum of PS and IS.

A positive result for both ER and PR is defined as $TS \geq 3$.

The results were evaluated statistically by using Microsoft excel (using chi-square test).

Results

All cases are subjected to immunohistochemistry to for knowing estrogen receptor and progesterone receptor status in the carcinoma of breast and to histopathological examination of this 58 cases.

Our study shows ER+/PR+ in 15.5% of cases, ER+/PR- in 3.4%, ER-/PR+ in 29.3 and ER-/PR- in 51% of cases.

We correlate ER/PR expression with this parameters:

1-Age: the majority of cases are in premenopausal (30-40y)36.2% and perimenopausal (41-50y)32.7%, most ER+/PR+ cases are within 30-50 years with minor expression in 51-70y while there are no expression in 71-90y. for ER-/PR+ cases the expression have decreased with age with peak expression in (30-40y) P value> 0.5 table(1) fig(1).

2- Histopathological type: all ER+/PR+ cases(9)15.5% are of invasive ductal carcinoma type (IDC) and the same for only PR+(17 cases) 29.3% and ER+(2 cases) 3.44% cases while the cases of medullary and invasive lobular carcinoma (ILC) are non reactive for estrogen and progesterone P value > 0.5 table(1) fig(2) .

3-Grade: the peak expression of estrogen & progesterone are in grade II cases (4 cases) 6.89% followed by grade I (3 cases) 5.2% and grade III (2 cases) 3.4% while the expression of PR only is increasing with age. The negative expression of ER and PR reach the peak in grade III followed by grade I and grade II consequently. P value<0.5 Table (1) Fig(3).

Table 1 Expression of estrogen and progesterone receptors vs ages of patients, histopathological types & grades of tumors

parameters	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-	Total	P value
Ages						
30-40	3(14.2%)	1(4.76%)	8(38%)	9(42.85%)	21(36.2%)	>0.5
41-50	4(21.05%)	1(11.1%)	6(31.5%)	8(42.1%)	19(32.8%)	
51-60	1(11.1%)	0	1(11.1%)	7(77.7%)	9(15.5%)	
61-70	1(11.1%)	0	2(33.3%)	3(50%)	6(10.3%)	
71-80	0	0	0	2(100%)	2(3.5%)	
81-90	0	0	0	1(100%)	1(1.7%)	
Total	9(15.5%)	2(3.4%)	17(29.3%)	30(51.7%)	58(100%)	
Hist. pathological types						
IDC	9(16.3%)	2(3.63%)	17(30.9%)	27(49%)	55(94.8%)	>0.5
Medullary	0	0	0	1(100%)	1(1.7%)	
ILC	0	0	0	2(100%)	2(3.5%)	
	9(15.5%)	2(3.5%)	17(29.3%)	30(51.7%)	58(100%)	
Grades						
Grade I	3(17.6%)	0	4(23.5%)	10(58.8%)	17(29.3%)	<0.5
Grade II	4(25%)	1(6.25%)	6(6.25%)	5(31.25%)	16(27.6%)	
Grade III	2(8%)	1(4%)	7(28%)	15(60%)	25(43.1%)	
	9(15.5%)	2(3.5%)	17(29.3%)	30(51.7%)	58(100%)	

Discussion

Immunohistochemistry is widely used in a basic research to understand the distribution and localization of biomarker in the tissue. However, it is widely used in diagnosis and treatment of cancer because specific molecular tumor markers are characteristic of particular cancer types (Boenish T 2001) [21].

The immunohistochemical staining of estrogen and progesterone was performed on 58 cases of females with breast ca which revealed the detection of ER/PR expression in 15.5% of the cases, negative detection for ER/PR expression in 51% of cases, ER+/PR- in 3.4% and ER-/PR+ in 29.3% while in suvarchala S.B et al 2011 from India [22].

ER+/PR+ cases 32.8%, ER+/PR- cases 14%, ER-/PR+ 10.9% and ER-/PR- 42.1% in which ER+/PR+ cases is twice as higher than in our study and ER-/PR+ cases is lower than in our study while the percentage of ER-/PR- cases as higher as in our study. In Desai SB and Coll V et al [23,24] show high percentage of ER/PR negative staining 48% & (66%) respectively in India, while Chariyalerstak et al [25] show lower percentage of ER/PR immunostaining in Thailand, All these results are reported in Asian population while in Mahridad Nadji 2005 et al [26].

In USA report the following results ER+/PR+ in 55% of cases, ER+/PR- in 20% of cases, ER-/PR+ 0 and ER-/PR- in 25% of the cases. So these results are completely different from our results and the results of Asian population.

These differences may be due to racial variation, and causes related to the staining technique

Regarding the correlation of ER/PR expression with age the statistical difference is not significant $P > 0.5$, peak ER/PR positive expression is

within 30-50y (perimenapausal and premenopausal age group), while in Suvarcala et al 2011(22) and Medhuri et al 2010 [27] show the peak expression of ER/PR positive in 40-60 y this difference may be due to difference in the size of the sample

In our study the same for cases with only PR expression(29.3%) (peak expression in 30-50 y) and its expression is decreasing with increasing age, the presence of these subgroup of cases may be due to artificial laboratory reporting or genetic alteration which switches on PR expression.

In Suvachala et al 2011 [22] the cases with only PR positive immunoreaction constitute 10% of cases and this expression increases with increasing age.

Correlation of ER/PR expression with histopathological type: there is no significant statistical difference $P \text{ value} > 0.5$, all the cases of positive ER/PR expression cases are of invasive ductal carcinoma and cases of medullary and invasive lobular carcinoma are negative and these results agree with Suvarchala et al 2011 [22] while in Mahridad et al 2005 [26] the same for IDC but 77% of ILC are ER+/PR+.

These results indicate there is different genetic alteration between invasive ductal carcinoma and invasive lobular carcinoma in our country.

Correlation between ER/PR expression and grades of tumor: there is significant statistical difference $P \text{ value} < 0.5$

In our study the peak expression of ER/PR in grade II followed by grade I and grade III, as in Suvarchala et al 2011 [22] the same results are reported while in Mahridad et al 2005 [26] the reverse results are obtained.

Previous studies as shown in Anderson WF et al 2001 and Fisher B

et al 2004 [28,29] have shown survival advantages among women with hormone receptor positive tumor relative to women with hormone receptor negative tumors.

So in our study there were high percentage of breast carcinoma with poor survival rate that not respond to estrogenic antagonist drugs .

Conclusions

A significantly high percentage of ER-PR- cases in our selected

population of breast cancer patients was seen. These observations also suggest that breast cancers seen in n Iraq be biologically different from that encountered in Western practice. Invasive ductal carcinoma is the most common histological type of carcinoma reported and it's the only type with ER+/PR+ .The population of high grade IDC were predominantly ER and PR negative

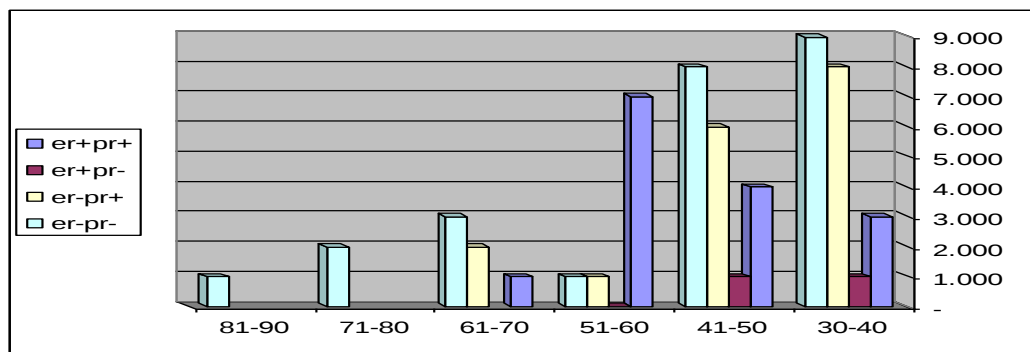


Figure 1 Relationship between ages of the patients and estrogen progesterone immunohistochemical expression

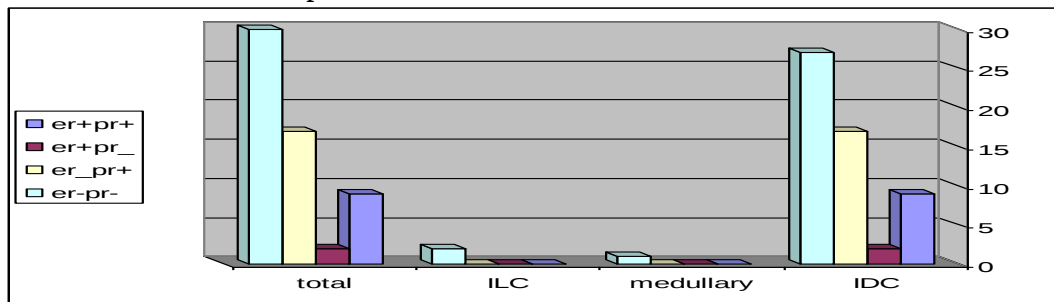


Figure 2 Relationship between histopathological type of breast ca & estrogen, progesterone immunohistochemical expression

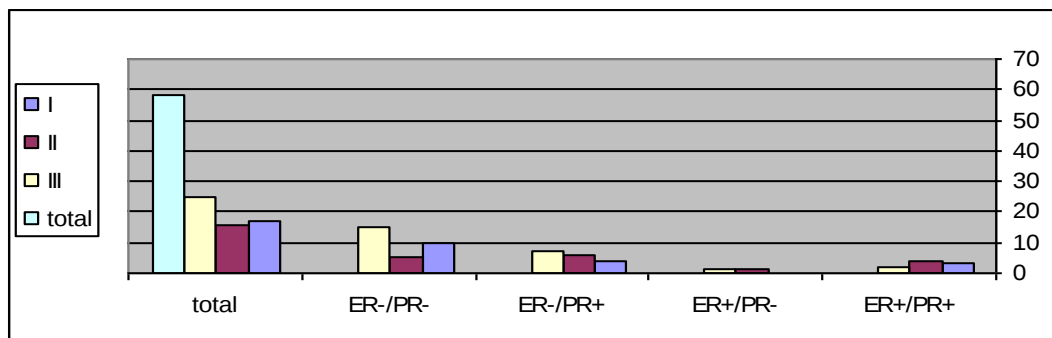


Figure 3 Relationship between grades of breast ca & estrogen, progesterone immunohistochemical expression

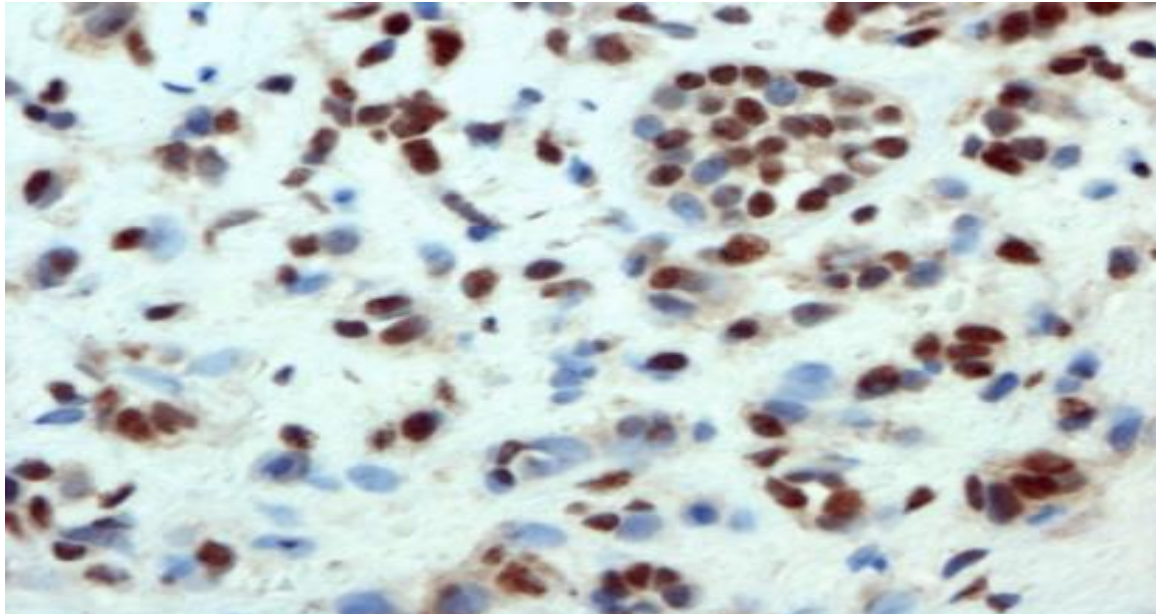


Figure 4 section shows IHC – progesterone receptor antigen stained nuclei represented in brown areas(x40)

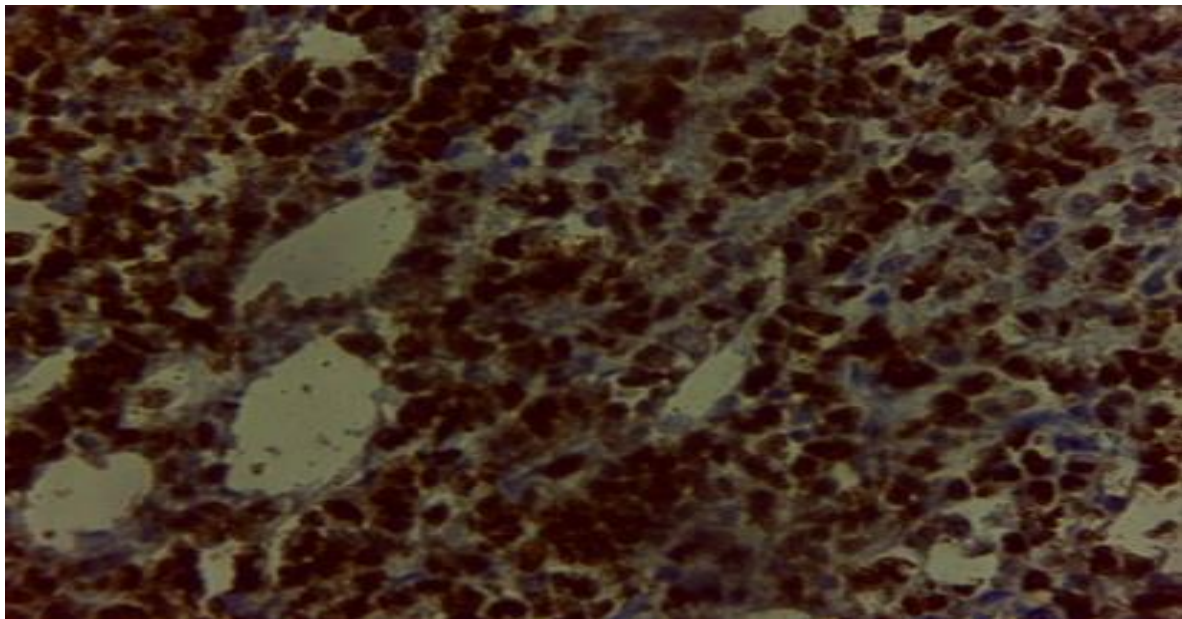


Figure 5 section showing IHC- estrogen antigen stained nuclei represented by brown areas(x 40)

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