RESEARCH PAPER

Epidermal growth factor receptor overexpression in invasive breast carcinoma: a correlation with clinicopathological parameters

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Abstract

Background: **e**pidermal Growth Factor Receptor (EGFR) is a transmembranous glycoprotein belonging to the Erb B family receptor of tyrosine kinases. EGFR overexpression is associated with the development of a wide variety of tumors. It's believed that EGFR overexpression in primary breast carcinoma has been linked to aggressive Basal-like phenotype and reflects poor prognosis.

Aim of the study: the study aims to provide a correlation between immunohistochemical EGFR overexpression in invasive breast carcinoma with the patient's age, histological type, tumor grade and molecular subtype

Patients and method: this cross-sectional study was carried out in Basrah. The data was collected in the period from October 2020 through November 2021. Tissue sections submitted to routine heamatoxylin and eosin stain. Then immunohistochemical staining for molecular classification and EGFR marker was performed by using primary monoclonal anti-EGFR antibody.

Results: fifty three cases of invasive breast carcinoma were collected with a mean age of 56.32. Fifty cases were diagnosed as invasive ductal carcinoma. Forty nine cases were graded as grade 2. Nineteen cases (35%) were classified as Luminal A, (32%) were classified as Luminal B1 and (13.2%) as Luminal B2. Basal-like and HER2 overexpression subtypes constitute (9.4%) of cases each. Twenty eight (52.83%) cases showed EGFR overexpression with a mean age of 55.86.

Conclusion: EGFR overexpression showed no significant relationship with age, molecular subtypes and histological type of breast carcinoma. The relation between EGFR overexpression and tumor grade couldn't be assessed. The immunohistochemical technique alone is not sufficient for the proper detection of EGFR overexpression.

Key words: Breast Cancer, Molecular classification, EGFR, IHC

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Introduction

Breast carcinoma is the most frequent cancer among women worldwide. It accounts for about 23% of all female malignancies and constitutes the second leading cause of death among all types of malignancies in women (15% of all cancer deaths) after lung cancer (26% of all cancer deaths). According to the WHO, breast carcinoma incidence rate is increasing in developing countries, as a result of urbanization, increased life duration, and sedentary lifestyles.[1] A profound increase in the understanding and clinical management of breast carcinoma has occurred over the past two decades, which has led to significant progress in prevention, early detection, and personalized breast cancer therapy. Because of this, many studies focus on analyzing morphological and, mainly, molecular patterns of breast cancer, to group these tumors into classes or entities to assist in clinical management. [2] Four clinically

relevant molecular subtypes of breast cancer have been identified by gene expression profile studies: Luminal A, Luminal B, Human Epidermal Growth factor 2(HER2) overexpression and Basal-like. The main genes that lead to the segregation of these subtypes are genes responsible for the expression of estrogen receptors (ER), progesterone receptors (PR), HER2 and cell proliferation regulator (Ki-67). Immunohistochemical panel for these markers has been regarded efficient, significant and cost-effective method for molecular classification in clinical practice.[3] Epidermal growth factor receptor (EGFR), a member of the ErbB family of receptor tyrosine kinases, has specific relevance in tumerogenesis of the breast. It is a transmembranous receptor which activates tyrosine phosphorylation by downstream signalling. [4] Efforts were made to understand the role of EGFR in tumerogenesis and study its correlation with other parameters. This is important to evaluate EGFR as a valid predictive biomarker, keeping in mind the limited and disappointing clinical outcome of anti-EGFR therapy in breast cancer.[5] Some studies report a significant relationship between EGFR and basal-like subtype.[6,7] And other studies suggest an inverse relationship between EGFR overexpression and ER-positive luminal subtypes in invasive breast carcinoma.[8,9,10] This study aims to provide a correlation between immunohistochemical EGFR overexpression in invasive breast carcinoma and with patient's age, histological type, tumor grade and molecular subtype.

Patient and method

This is a cross-sectional study, carried out in Basra. The data was collected from Al-Sadr Teaching Hospital and private laboratories during the period from October 2020 through November 2021. The cases included in the study were those newly diagnosed invasive breast carcinoma. Tissue biopsies from mastectomy, lumpectomy, and true-cut needle specimens were collected. Formalin fixed, paraffin embedded, Three to five micrometres thickness sections were obtained and stained with routine hematoxylin and eosin stains. Additional sections using positively charged slides were provided for immunohistochemical staining for ER, PR, HER2, Ki67 and EGFR. Staining for estrogen-alpha (clone EP1), progesterone, Her 2, Ki67 and EGFR (Dako Denmark A/S) was performed by BOND-MAX fully automated IHC strainer, from Leica Biosystems at Bayan group private laboratory. The stained sections were examined by two histopathologists to determine the histological type and grade of breast carcinoma as well as the molecular subtyping of the cases into Luminal A, Luminal B1, Luminal B2, Basal-like and HER2 overexpression.[2] Regarding immunohistochemistry (IHC) stains, only definite positive or negative results were included, however, for EGFR staining, a positive result was given for any percentage of definite membranous and/or cytoplasmic staining[11], whether it is focally or extensively stained. The results were tabulated and analyzed using SPSS for Windows, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Independent-sample t-test was used to investigate the significance of any statistical differences in quantitative data. The $\chi 2$-test was applied to investigate the association between qualitative data. P-values less than 0.05 were considered to be statistically significant.

Results

A total of fifty three cases were included in the study, all diagnosed as invasive breast carcinoma. Fifty two of the cases (98.1%) were female and one case was male (1.9%). The Mean age was 56.32 years (ranging from 29 to 76 years).

Histological types and grade

Fifty cases (94.3%) were diagnosed as invasive ductal carcinoma of not otherwise specified type (NOS). Two cases were diagnosed as Invasive lobular carcinoma (3.7%) and one case was diagnosed as invasive papillary carcinoma. Forty nine (98%) of the cases were grade 2 and one case (2%) was grade 3. None of the cases was of grade 1. Nineteen (35.8%) of the cases were classified as Luminal A, (32.1%) were Luminal B1, (13.2%) luminal B2 (Figure 6,7), (9.4%) were Basal like and (9.4%) showed HER2 overexpression. (Table-1)

Table 1. Distribution of cases according to molecular subtype

Molecular subtype	Frequency	Percent
Luminal A	19	35.8
Luminal B1	17	32.2
Luminal B2	7	13.2
Basal Like	5	9.4
Her2 overexpression	5	9.4
Total	53	100.0

EGFR overexpression

Twenty eight cases (52.83%) showed positive expression of EGFR and twenty five cases (47.13%) were stained negatively. (Figure-1)

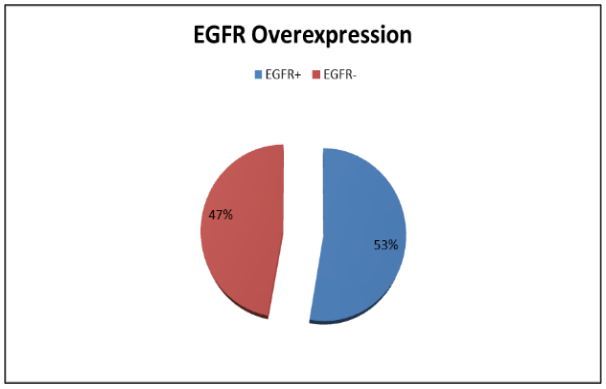

Fig 1. Distribution of EGFR overexpression among the studied group

Relationship of EGFR overexpression with Age:

The mean age of cases with EGFR overexpression was 55.86 years. The mean age of EGFR-negative cases was 56.86 years. The relationship was statistically not significant. (P value 0.761). (Table-2)

Table 2. The relationship of EGFR overexpression with age

EGFR	No.	Mean age	P-value
Positive	28	55.86 ± 11.28	0.761
Negative	25	56.84 ± 12.15	

*** T-Test**

The relationship between EGFR overexpression and histological type:

Twenty seven out of 28 EGFR positive cases (96.4%) were of Invasive ductal carcinoma NOS type. The only case of Invasive Papillary carcinoma was EGFR positive (3.6%). None of the Invasive lobular carcinoma cases overexpressed EGFR. The relationship between EGFR overexpression and histological types is not statistically significant. (P value = 0.22) (Table-3)

Table 3. The relationship between EGFR overexpression and histological type

Histological type	EGFR		Total	P value
	Positive No.(%)	Negative No.(%)		
Invasive Ductal Carcinoma NST	27(96.4)	23(92.0)	50(94.3)	
				0.22*
Invasive lobular Carcinoma	0(0.0)	2(8.0)	2(3.8)	
Invasive papillary Carcinoma	1(3.6)	0(0.0)	1(1.9)	
Total	28(100)	25(100)	53(100)	

*** Fisher's Exact Test**

The relationship between EGFR overexpression and Luminal subtypes (ER-positive):

Thirteen out of nineteen cases (68.4%) of Luminal A class were positively stained with EGFR. About fifty three percent of Luminal B1 and 42.9% of Luminal B2 were positively stained with EGFR. No significant correlation was found between EGFR overexpression and Luminal subtypes. (P value= 0.46) (Table 4 & Figure-2)

Table 4. The relationship between EGFR overexpression and Luminal subtypes of breast carcinoma

Molecular subtype	EGFR		Total	P value
	Positive No. (%)	Negative No. (%)		
Luminal A	13(68.4)	6(31.6)	19(100.0)	P= 0.462 (NS)
Luminal B1	9(52.9)	8(47.1)	17(100.0)	
Luminal B2	3(42.9)	4(57.1)	7(100.0)	
Total	25	18	43	

Relationship between EGFR overexpression and Hormone negative molecular subtypes (HER2 overexpression and Basal-like):

Sixty percent of HER2 overexpression subtypes were positive for EGFR, while none of the Basal-like subtype cases (N = 5) were stained with EGFR. No significant relationship was found between EGFR overexpression and the hormone-negative subtypes. (P value = 0.167) (Table-5).

Table 5. The relationship between EGFR overexpression and Hormone negative molecular subtypes

Molecular subtype	EGFR		Total	P value
	Positive No.(%)	Negative No. (%)		
				P = 0.167
Basal Like	0(0.0)	5(100.0)	5(100.0)	(NS)
Her2 overexpression	3(60.0)	2(40.0)	5(100.0)	

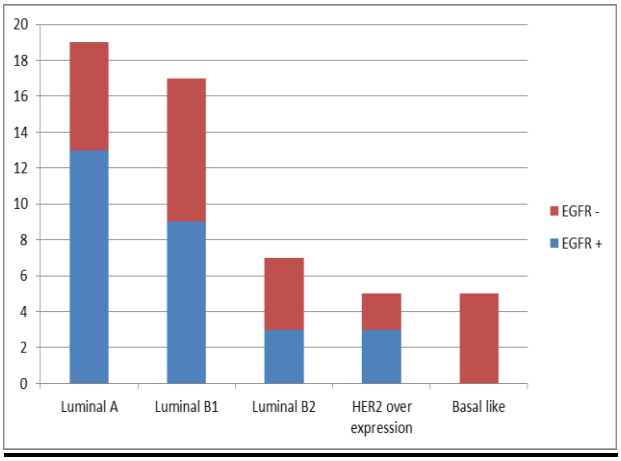

Fig 2. Molecular subtypes and EGFR overexpression

Discussion

Breast carcinoma has a heterogeneous behavior. Several pathological and clinical parameters should be considered to predict prognosis and response to therapy. The role of biomarkers like ER, PR, and HER2 provide both prognostic and predictive value.[12] Members of the EGFR family have been linked to breast carcinoma aetiology and are important therapeutic targets.[12] Efforts were made to understand the role of EGFR in tumerogenesis and study its correlation with other parameters. This is important to evaluate EGFR as a valid predictive biomarker, keeping in mind the limited and disappointing clinical outcome of anti-EGFR therapy in breast cancer.[5] In the current study, the mean age was 56.32 years. This was slightly higher than other studies in Iraq which reveals a mean age at diagnosis of about 50-54 years.[13-15] The predominant histological type in this study is invasive ductal carcinoma NST type (94%); this is in line with other studies carried out in Basrah,[16] Ninewa[17] and Saudi Arabia.[18] In the present study, almost all cases were of grade 2 followed by grade 3. This is in agreement with other local and national studies that emphasized grade 2 carcinoma as the most common histological grade in Iraq.[14,19,20] In this study, only one case

was of grade 3 and none of them was of grade 1. This may be attributed to the small sample size due to time limit. Regarding molecular classification the leading subtype in the present study was Luminal A, each of the luminal B1 and Luminal B2 is considered as a distinct subtype, taking into account the differences in treatment and prognosis of each one.[21] This is in agreement with other studies that emphasize that the Luminal A subtype is the commonest.[22-24] A study in Baghdad in which both luminal B1 and Luminal B2 were combined as one subtype mentioned as Luminal B which is reported as the most common subtype.[47] In the current study, about half of the cases showed EGFR overexpression. This result is in line with a study conducted in Baghdad.[42] But compared with other international studies is considerably elevated. For instance, a study carried out in Greece, using IHC staining, found out only (11.3%) of IDC cases were EGFR positive. [48] Another study, using ligand binding assay, stated a ratio of (18%) of EGFR-positive cases in IDC9. Hwangbo et al, from Korea, found EGFR overexpression in (17.1%) using IHC, from which (22%) showed EGFR gene amplification with high polysomy by using FISH techniques.[6] The high percentage of EGFR positivity in the present study compared with the mentioned studies may be due to variations in method and technique used. IHC staining of EGFR is not a functional assay and has inconsistent results.[49] Ligand binding assay is a functional assay but it requires a frozen section; Antigen detection is less altered in frozen tissue.[49] Detection of EGFR gene amplification by using FISH is more accurate. [6] Furthermore, most studies used formalin fixation of tissue, and the detection of antigens might be affected by formalin over-fixation. This may explain the variation in the detection yield. This variation can also be due to the absence of a standard international score regarding the evaluation of EGFR expression by IHC, leading to an appreciated interobserver variation. Indeed, in the present study, a positive result was given for any percentage of definite membranous and/or cytoplasmic staining whether it is focally, moderately or extensively positive. This may explain the high percentage of EGFR-positive cases in the present study. In the current study, no significant relationship was observed between EGFR and the age of the patient, while other studies reported EGFR overexpression association with younger age group. [6,9] It seems possible that these results are due to the small sample size. Limited cases were involved due to the time shortage of the study. EGFR's relation with histological type was inconclusive. The vast majority of cases included in this study were diagnosed as IDC NST type while two cases were diagnosed as lobular and one case was diagnosed as papillary carcinoma. Again small sample size may be responsible for this inconclusive relation between EGFR and histological type. Similarly, the relationship of EGFR and tumor grade was inconclusive since all but one case, was grade 2 carcinoma. The current study revealed a higher percentage, but not significant relation, of EGFR overexpression in luminal A and HER2 over expression molecular subtypes and negative EGFR overexpression in all cases of the Basal-like subtype. These results are not consistent with other studies which suggested an inverse relationship between EGFR overexpression and ER-positive Luminal subtypes of invasive breast carcinoma.[8-10] Other studies indicate no association between HER2 and EGFR overexpression supporting our findings.[9,10] In the current study, all cases of basal-like subtype were negative for EGFR expression. This is in agreement with a study from Pakistan that showed no significant relationship between EGFR and Basal-like

subtype[11]. However, other studies reported a significant relationship between EGFR overexpression and Basal-like subtype.[6,7] The lack of significant association between EGFR overexpression and molecular subtypes in the current study might be due to the small sample size which is due to the shortage of time of the study. Furthermore, our sample lacks specific histological types with a high probability of being basal like as medullary and metaplastic carcinomas, or carcinoma with BRCA1 mutation. Similarly, few cases with young age and high-grade tumors were included.

In conclusion, EGFR overexpression showed no significant relationship with age or molecular subtypes of breast carcinoma. The accuracy of the IHC technique has failed in precisely detecting EGFR overexpression. It is recommended to pair the mentioned technique with other confirmatory techniques to increase detection yield.

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الإفراط في التعبير عن مستقبلات عامل نمو البشرة Epidermal growth factor receptor في سرطان الثدي الغازي: علاقة مع المعلمات السريرية المرضية

الخلاصة: يعتبر مستقبل عامل نمو البشرة EGFR احد اعضاء عائلة EGF ومن مجموعة مستقبلات ErbB .فرط التعبير لعامل نمو البشرة يرتبط بتطور العديد من السرطانات. هناك اعتقاد بارتباط فرط التعبير لعامل نمو البشرة مع الصنف الشبيه القاعدي شديد العدوانية .

الهدف من الدراسة:هدف البحث لدراسة ارتباط فرط التعبير لمستقبل عامل نمو البشرة في الأصناف الخلوية لسرطان الثدي الغازي و تقييم علاقته بأعمار المرضى و الأنواع النسيجية و مرتبة الورم.

طرق الدراسة :تم إجراء دراسة عرضية مقطعية في مدينة البصرة. جُمعت المعلومات في الفترة من تشرين الاول ٢٠٢٠ الى نهاية تشرين الثاني ٢٠٢١. تم جمع العينات النسيجية للثدي ثم تم تحضير المقاطع النسيجية و صبغت بصبغة الهيماتوكسيلين والايوسين. ومن ثم تم اجراء فحص الكيمياء النسيجية المناعية لمعرفة التصنيف الخلوي لاورام الثدي و تقييم فرط تعبير مستقبل عامل نمو البشرة.

النتائج: شملت الدراسة ثلاثة وخمسون حالة مرضية لسرطان الثدي الغازي بمتوسط العمر ٥٦,٣٢ سنة. تم تشخيص خمسون حالة لسرطان الثدي الغازي غير محدد النوع. تسع و اربعون حالة كانت بالمرتبة الثانية. خمسة عشر (٣٥٪) حالة مرضية صنفت لصنف Luminal A, سبعة عشر(٣٢٪) حالة صنفت كـLuminal B1 و سبع حالات (١٣,٢٪) صنفت ك .Luminal B2 الاصناف HER2 overexpression و basal like شكلتا ٩,٤ % من الحالات (خمسة حالة مرضية لكل صنف).ثمانية و عشرون حالة اظهرت فرط التعبير لمستقبل عامل نمو البشرة مع متوسط العمر ٥٥,٨٦ سنة.

الاستنتاج: لم تظهر النتائج علاقة لفرط التعبير لمستقبل عامل نمو البشرة مع عمر المريض او الاصناف الخلوية لسرطان الثدي . ان تقنية الكيمياء النسيجية المناعية تفتقر للدقة في الكشف عن فرط التعبير لمستقبل عامل نمو البشرة. يوصى باستخدام تقنيات تحقق اخرى لزيادة دقة الكشف.

الكلمات المفتاحية: سرطان الثدى، التنصيف الخلوى، مستقبل عامل نمو البشرة، فحص المناعى النسيجى.