Triple-Negative Metaplastic Breast Carcinoma: Association of Epidermal Growth Factor Receptor Expression With Prognostic Parameters and Clinical Outcome

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Abstract

Introduction

Metaplastic breast carcinoma (MBC) is one of the rare special subtypes of breast carcinoma associated with poor prognostic features compared with invasive ductal carcinoma. Moreover, therapeutic options are limited in MBC owing to frequent triple-negative profiles of these tumors. Epidermal growth factor receptor (EGFR) is a proto-oncogene that is overexpressed in many human cancers, and is a potential therapeutic target. Therefore, in this study, we evaluated the expression of EGFR in MBC by immunohistochemistry, and its association with clinicopathological and prognostic parameters.

Methods

We conducted a retrospective observational study in the Department of Histopathology at Liaquat National Hospital and Medical College, Pakistan, over a period of seven years. A total of 61 cases with a histopathological diagnosis of MBC were included in the study. All slides were reviewed by histopathologists for diagnostic confirmation. Histopathological parameters, such as tumor size, grade, and nodal metastasis, were recorded. The representative tissue blocks were also retrieved and immunohistochemical studies were performed for cytokeratin 5/6 (CK5/6), Ki67, and EGFR.

Results

The mean age of the patients was 44.48 ± 13.01 years. The mean tumor size was 5.72 ± 2.72 cm, with most of the cases belonging to tumor (T)-stage T3. Axillary metastasis was present in 57.4% cases, and the perinodal extension was present in 11.5% cases. Most tumors were grade III (85.2%), with a mean Ki67 index of 39.67% ± 20.38%. Most of the cases were nonbasal (83.6%), owing to the absent CK5/6 expression. Tumor recurrence was noted in 14.8% cases, with a median follow-up of 43 (13-83) months and median disease-free survival of 36 (12-60) months. Positive EGFR expression was noted in 52.5% cases. A significant association of EGFR expression was noted with tumor grade, mean Ki67 index, axillary metastasis, and nodal (N)-stage. Cases with positive EGFR expression were found to have higher grade (grade III), with higher Ki67 index, higher frequency of axillary metastasis, and higher N-stage. Moreover, cases with positive EGFR expression had lower disease-free survival compared to cases with negative EGFR expression.

Conclusion

We found that a significant proportion of triple-negative MBC expressed EGFR. Moreover, EGFR overexpression was associated with poor pathological parameters and lower disease-free survival. Therefore, EGFR can be considered a potential prognostic biomarker and therapeutic target in triple-negative MBC; however, the correlation between gene amplification and protein overexpression is required to better uncover the role of EGFR as a therapeutic target.

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Metaplastic breast carcinoma (MBC) is one of the rare special subtypes of breast carcinoma associated with poor prognostic features compared with invasive breast ductal carcinoma [1,2]. These tumors tend to have a large tumor size at the time of presentation, and, therefore, require neoadjuvant chemotherapy [3]. However, the response of these tumors to neoadjuvant chemotherapy is poor, with a complete pathological response rate ranging from 10% to 17% [4]. Moreover, therapeutic options are limited owing to frequent triple-negative profiles of these tumors. Triple-negative breast tumors are defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu), and, therefore, they do not respond to hormonal and Herceptin therapy. Previous studies have reported a relatively high percentage of triple-negative breast tumors in Pakistan, and a significant percentage included MBC [5,6]. Epidermal growth factor receptor (EGFR) is a proto-oncogene that is overexpressed in many human cancers, and is a potential therapeutic target [7-9]. Although molecular studies are considered the gold standard to assess EGFR amplification, immunohistochemistry (IHC) to evaluate protein overexpression is considered surrogate to gene amplification studies. Therefore, in this study, we evaluated the expression of EGFR in MBC by IHC, and its association with clinicopathological and prognostic parameters.

Materials And Methods
We conducted a retrospective observational study in the Department of Histopathology at Liaquat National Hospital and Medical College, Pakistan, over a period of seven years. The specimens included were lumpectomy with or without axillary lymph node dissection, simple mastectomy, and modified radical mastectomy. Total 61 cases with a histopathological diagnosis of MBC were included in the study. Cases with primary breast cancer without evidence of systemic metastasis undergoing upfront tumor resection were included in the study. Cases with neoadjuvant chemoradiation were excluded from the study. Cases with clinically positive axillary lymph nodes underwent axillary lymph node dissection along with breast surgery. Those with clinically negative axillary lymph nodes were first subjected to sentinel lymph node (SLN) sampling with intraoperative frozen section analysis. Cases with positive SLNs on frozen section (>2 mm tumor size) were followed by axillary lymph node dissection. All slides were reviewed by histopathologists for diagnostic confirmation. Histopathological parameters, such as tumor size, grade, and nodal metastasis, were recorded.

The representative tissue blocks were also retrieved and immunohistochemical studies were performed for cytokeratin 5/6 (CK5/6), Ki67 and EGFR. The membranous EGFR expression was evaluated as described in previous studies [10,11]. Complete membranous expression in more than 10% invasive tumor cells was taken as positive EGFR expression (Figure 1A, 1B).
Ki67 was interpreted quantitatively and reported as the average percentage of positively stained tumor cells. Moderate-to-strong cytoplasmic CK5/6 expression in more than 10% invasive cancer cells was taken as positive CK5/6 expression. CK5/6 IHC was used to differentiate between the basal and nonbasal subtypes of triple-negative tumors. CK5/6-expressing tumors were labeled as basal subtype.

ER, PR, and HER2/neu IHC was also performed to confirm the triple-negative status, and the results were interpreted as described in previous studies [12-15]. More than 1% nuclear expression of ER and PR was taken as positive ER/PR expression. For HER2/neu, strong and complete membranous expression in more than 10% invasive cancer cells was taken as positive HER2/neu IHC. Cases with equivocal HER2/neu immunohistochemical results were confirmed by fluorescence in situ hybridization (FISH) testing. Cases with positive ER, PR, or HER2/neu IHC/FISH results were excluded from the study.

Data analysis was performed using Statistical Package for Social Sciences (Version 26.0, IBM Inc., Armonk, NY, USA). Chi-square, independent t-test, and Fisher’s exact tests were used to check the association. Survival analysis was done by the Kaplan-Meier method. P-values <0.05 were considered significant.

Results

The mean age of the patients was 44.48 ± 13.01 years. The mean tumor size was 5.72 ± 2.72 cm, with most of the cases belonging to tumor (T)-stage T3. Axillary metastasis was present in 57.4% cases, and the perinodal extension was present in 11.5% cases. Most of the tumors were grade III (85.2%), with a mean Ki67 index of 39.67% ± 20.38%. Most of the cases were nonbasal (83.6%), owing to the absent CK5/6 expression. Tumor recurrence was noted in 14.8% cases, with a median follow-up of 43 (13-83) months and median disease-free survival of 36 (12-60) months. Positive EGFR expression was noted in 52.5% cases (Table 1).
| Parameter                                      | Value               |
|-----------------------------------------------|---------------------|
| Tumor size (cm); mean ± SD                   | 5.72 ± 2.72         |
| Ki67 index (%); mean ± SD                    | 39.67 ± 20.38       |
| **Ki67 index groups**                        |                     |
| ≤24%, n (%)                                   | 20 (32.8)           |
| 25%–44%, n (%)                               | 16 (26.2)           |
| >44%, n (%)                                   | 25 (41)             |
| **Disease-free survival (months); median (range)** | 36 (12–60)         |
| **Axillary metastasis**                      |                     |
| Present, n (%)                               | 35 (57.4)           |
| Absent, n (%)                                 | 26 (42.6)           |
| **N-stage**                                   |                     |
| N0, n (%)                                     | 26 (42.6)           |
| N1, n (%)                                     | 16 (26.2)           |
| N2, n (%)                                     | 9 (14.8)            |
| N3, n (%)                                     | 10 (16.4)           |
| **Perinodal extension**                      |                     |
| Present, n (%)                               | 7 (11.5)            |
| Absent, n (%)                                 | 54 (88.5)           |
| **T-stage**                                   |                     |
| T1, n (%)                                     | 5 (8.2)             |
| T2, n (%)                                     | 18 (29.5)           |
| T3, n (%)                                     | 38 (62.3)           |
| **Tumor grade**                              |                     |
| Grade II, n (%)                              | 9 (14.8)            |
| Grade III, n (%)                             | 52 (85.2)           |
| **Surgery type**                             |                     |
| Modified radical mastectomy, n (%)           | 50 (82)             |
| Simple mastectomy, n (%)                     | 11 (18)             |
| **Necrosis**                                 |                     |
| Absent, n (%)                                 | 13 (12.3)           |
| Focal, n (%)                                  | 22 (36.1)           |
| Marked, n (%)                                 | 26 (42.6)           |
| **Fibrosis**                                 |                     |
| Mild, n (%)                                   | 7 (11.5)            |
| Moderate, n (%)                               | 34 (55.7)           |
| Severe, n (%)                                 | 20 (32.8)           |
| **Lymphocytic infiltration**                 |                     |
| Absent, n (%)                                 | 5 (8.2)             |
| Moderate, n (%)                               | 39 (63.9)           |
| Severe, n (%)                                 | 17 (27.9)           |
In situ component

|                      | Present, n (%) | Absent, n (%) |
|----------------------|----------------|---------------|
|                      | 21 (34.4)      | 40 (65.6)     |

Lymphovascular invasion

|                      | Present, n (%) | Absent, n (%) |
|----------------------|----------------|---------------|
|                      | 27 (44.3)      | 34 (55.7)     |

Triple-negative subtype

|                      | Basal, n (%)   | Nonbasal, n (%) |
|----------------------|----------------|-----------------|
|                      | 10 (16.4)      | 51 (83.6)       |

Recurrence

|                      | Yes, n (%)     | No, n (%)       |
|----------------------|----------------|-----------------|
|                      | 9 (14.8)       | 52 (85.2)       |

EGFR

|                      | Positive, n (%) | Negative, n (%) |
|----------------------|-----------------|-----------------|
|                      | 32 (52.5)       | 29 (47.5)       |

**TABLE 1: Clinicopathological features of population under study**

SD, standard deviation; N, nodal; T, tumor; EGFR, epidermal growth factor receptor

Table 2 depicts the association of EGFR expression with clinicopathological features. A significant association of EGFR expression was noted with tumor grade, mean Ki67 index, axillary metastasis, and nodal (N)-stage. Cases with positive EGFR expression were found to have higher grade (grade III), with higher Ki67 index, higher frequency of axillary metastasis, and higher N-stage.
### Table 2: Association of clinicopathological features with EGFR expression

| Clinicopathological features          | Values                        | P-value |
|---------------------------------------|-------------------------------|---------|
| EGFR expression                       |                               |         |
| Positive                              | 44.72 ± 13.22                 | 0.880   |
| Negative                              | 44.21 ± 13.01                 |         |
| Age (years); mean ± SD***             |                               |         |
| ≤50 years, n (%)                      | 27 (84.4)                     | 0.001***|
| >50 years, n (%)                      | 13 (44.8)                     |         |
| Tumor size (cm); mean ± SD***         | 5.38 ± 2.31                   | 0.300   |
| Ki67 index (%); mean ± SD***          | 44.53 ± 24.30                 | 0.045***|
| Axillary metastasis*                  |                               |         |
| Present, n (%)                        | 27 (84.4)                     | <0.0001***|
| Absent, n (%)                         | 5 (15.6)                      |         |
| N-stage**                             |                               |         |
| N0, n (%)                             | 5 (15.6)                      |         |
| N1, n (%)                             | 8 (25)                        | <0.0001***|
| N2, n (%)                             | 9 (28.1)                      | 0.060   |
| N3, n (%)                             | 10 (31.3)                     | 0.010***|
| T-stage**                             |                               |         |
| T1, n (%)                             | 5 (15.6)                      |         |
| T2, n (%)                             | 7 (21.9)                      | 0.307   |
| T3, n (%)                             | 20 (62.5)                     |         |
| Tumor grade**                         |                               |         |
| Grade II, n (%)                       | 1 (3.1)                       |         |
| Grade III, n (%)                      | 31 (96.9)                     | 0.010***|
| Triple-negative subtype**            |                               |         |
| Basal, n (%)                          | 7 (21.9)                      |         |
| Nonbasal, n (%)                       | 25 (78.1)                     |         |
| Recurrence**                          |                               |         |
| Yes, n (%)                            | 7 (21.9)                      | 0.151   |
| No, n (%)                             | 25 (78.1)                     |         |

*Chi-square test was applied, **Fisher's exact test was applied, ***independent t-test was applied, ****significant at <0.05

EGFR, epidermal growth factor receptor; SD, standard deviation; N, nodal; T, tumor

Figure 2 shows the association of EGFR expression with disease-free survival. Cases with positive EGFR expression had lower disease-free survival compared to cases with negative EGFR expression.
FIGURE 2: Association of EGFR expression with disease-free survival

**Discussion**

In this study, we evaluated the prognostic significance of EGFR expression in triple-negative MBCs. We found that a significant proportion of cases of triple-negative MBC had positive EGFR expression. Moreover, EGFR expression was associated with poor prognostic parameters, such as higher tumor grade, higher mean Ki67 index, poor disease-free survival, and higher frequency of axillary metastasis.

MBCs have been reported to carry EGFR overexpression in up to 80% of cases, and approximately one-third of those cases carry EGFR gene amplification [16]. Reddy et al. reviewed triple-negative MBCs in relation to the previously linked epithelial-to-mesenchymal molecular alterations, and concluded that MBCs were aggressive tumors with poor prognostic features and overall outcome, especially in the presence of EGFR amplification [17].

Downs-Kelly et al. reported similar findings with increased local and distant recurrence with MBCs [18]. Song et al. subclassified MBCs and reported a bad outcome for most MBCs compared with other triple-negative breast cancers. They further endorsed other studies by reporting a larger tumor size, a higher percentage of ER/PR negativity, and a higher Ki67 index in MBC [19]. Similarly, Gilbert et al. reported high copy numbers of EGFR in MBC due to aneusomy and amplification and recommended further trials with targeted therapies [20].

McCart Reed et al. studied phenotypic and molecular features of MBC and reported EGFR overexpression as one of the most important negative prognostic factors [21]. However, some studies have reported a lack of relationship between EGFR overexpression and the actual presence of EGFR mutation [22,23], and it is, therefore, recommended to correlate these cases with molecular studies to select a target population for a better treatment response with EGFR inhibitors [22-24]. Gumuskaya et al. reported a positive association of membranous staining pattern of EGFR expression with EGFR gene amplification (increased gene copy number) compared to the cytoplasmic staining pattern and recommended to prioritize those patients for anti-EGFR treatment [25].

Our study had a few limitations, as it represents single-institution data. Moreover, molecular studies were not performed to assess EGFR amplification. Therefore, we advise that large-scale studies with molecular correlation should be conducted in our population to determine the prognostic significance of EGFR as a biomarker in triple-negative MBC.

**Conclusions**

We found that EGFR expression in triple-negative MBC signifies poor prognostic significance, as positive
EGFR expression was significantly associated with axillary metastasis, higher tumor grade, and higher mean Ki67 index. Moreover, EGFR-positive cases had poor disease-free survival when compared to cases with negative EGFR expression. We noted that a significant proportion of triple-negative MBC had EGFR expression; therefore, EGFR can serve as a potential therapeutic target in triple-negative MBC. However, further studies are needed to find the correlation of gene amplification with protein expression to better evaluate the therapeutic response.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Not needed issued approval N/A. IRB not needed for retrospective studies. Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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