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EPIDERMAL GROWTH FACTOR RECEPTOR OVEREXPRESSION IN TRIPLE NEGATIVE BREAST CANCER

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ABSTRACT

Objective: To observe the frequency of EGFR overexpression and its correlation with clinicopathological parameters in patients diagnosed with triple negative breast carcinoma.

Methodology: This Cross-sectional study was conducted at Rehman Medical Institute from June 2022 to June 2023. Biopsy specimen of 41 female patients diagnosed with triple negative breast carcinoma (TNBC) were received in histopathology department. Specimen underwent eosin and hematoxylin staining and immunohistochemistry analysis. Demographic characteristics and histopathological parameters were recorded.

Results: Mean age of the participants was 46.27 ± 3.75 years. Axillary metastasis was observed in 24(58.5%) patients while perinodal extension was observed in 05(12.2%) patients. Immunohistochemistry analysis revealed 23(56.1%) EGFR positive specimens while 18(43.9%) specimens were EGFR negative. Out of 23 patients with axillary metastasis the frequency of EGFR positive carcinomas was 17(73.9%) which was significantly greater than 06(26.1%) patients exhibiting negative EGFR expression. Frequency of EGFR positive expression was significantly increased with higher grade and stage of the tumor (p-value <0.05).

Conclusion: EGFR overexpression in Triple negative breast carcinoma is associated with poor clinicopathological indicators which leads to poor prognosis.

Keywords: Breast Neoplasms; Epidermal Growth Factor Receptor; Triple Negative Breast Cancer

INTRODUCTION

Cancer poses a great burden on the health care with an estimated 18 million new diagnosed cases in the year 2018 as per GLOBOCAN.[1] Among Asian countries Pakistan has the highest rate of patients diagnosed with breast cancer with reported incidence of 19.3%.[2] Triple negative breast carcinoma (TNBC) is one of the rare types of carcinomas which doesn't exhibit the expression of receptors commonly found in carcinoma breast including progesterone receptors, estrogen receptors, human epidermal growth factor Her2 receptors. Prevalence of TNBC is 15% and can further be divided into further histological types. The lack of hormone receptor expression and invasive nature of the cancerous cells found in this variant, limits the treatments options with a poor prognosis.[3,4]

On the basis of immunostaining and other features breast carcinomas can be divided into luminal A, luminal B, Her-2 enriched, normal, basal and claudin low types of carcinomas. TNBC can be basal or claudin low type of breast carcinomas which are further segregated into subdivisions on the basis of gene expression.[5] With the increasing prevalence of breast carcinoma diagnostic modalities have been in use with the aim to detect the pathology in the early stages. The diagnosis of breast carcinoma can be performed by radiographic screening which includes mammography, ultrasound and magnetic resonance imaging. Immunohistochemistry analysis however is a more reliable technique which can detect and help in identification of the histological pattern of the carcinoma. Identification of the type of breast neoplasm helps in prediction of behavior and aggressiveness of the carcinoma.[6]

Several growth factor receptors are expressed in the different variants of breast carcinoma however in TNBC epidermal growth factor receptor (EGFR) display a prominent role. Role of EGFR in cell division, metastasis of cancer cells and resistance against therapeutic agents can help in predicting the behavior of cells which over express or under express these receptors.[7]

Poor prognosis of neoplasms have been observed in tumors with high expression of EGFR which includes

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head and neck, lungs , endometrial and colorectal carcinomas.[8] Breast carcinomas which exhibit 15-45% EGFR expression can help for prompt diagnosis of the subtype of neoplasm with commencement of prompt treatment. The general trend is that tumors which exhibit high expression of EGFR usually exhibits invasive nature carrying a poor prognosis however, exceptions are there.[9]

Triple negative breast carcinoma being metastatic and one of the rare subtypes, carry a high risk of morbidity and mortality.[10] Although there is a growing body of research to evaluate the expression of EGFR in patients with TNBC but there are gaps in our current knowledge like relation of clinicopathological parameters and EGFR overexpression. Therefore, this study was planned to evaluate the overexpression of EGFR in patients diagnosed with TNBC and its immunopathological correlations.

METHODOLOGY

This cross sectional study was conducted in Rehman Medical Institute, Peshawar from June 2022 to June 2023 after obtaining approval from the ethical review committee of the hospital. Total 41 participants were included in the study based on sample size calculation using statistical calculator available on open epi website (available on www.openepi.com) with a 10% level of significance and 95% power of test when the reported prevalence of triple negative breast carcinoma was 12%.[11] Sampling was done using non-probability purposive technique.

Specimens of female patients obtained after lumpectomy with or without axillary lymph node dissection, simple mastectomy or modified radical mastectomy and cases without evidence of systemic metastasis diagnosed with primary breast cancer planned for tumor resection were included in the study. Patients with history of neoadjuvant chemoradiation were excluded.

Samples from 41 patients diagnosed as cases of triple negative breast carcinoma on the basis of clinical, radiological and histopathological findings were retrieved. Demographic variables of each patient were recorded.

Histopathologic examination: All samples underwent eosin and hematoxylin staining (fig.1). Tumor grading, staging and invasion of the nodes, vessels, and lymphatics was done by histopathological exam and recorded. Immunohistochemistry was performed for identification of basal and non-basal TNBC using FLEX monoclonal mouse antihuman cytokeratin 5/6 by using DAKO kit. On examination if the staining was observed in greater than 10% of the cancer cells CK5/6 expression was recorded as positive. Immunohistochemistry analysis was performed using 3-4 micrometer sections of biopsied tissues on DAKO IHC coated slides. These slides were placed inside oven for 20 mins at 70 to 80^0C followed by de-waxing using xylene and then processed with decreasing concentration of alcohol. Antigen retrieval was performed by placing the slides in solution at 99-100^0 centigrade for a period of 40 mins followed by washing with buffer solution and blocking peroxidase for another 10 minutes. Using washing buffer slides were washed and EGFR (Rabbit monoclonal antibody) was applied using a 1:200 dilutions for 20 minutes followed by washing. This was followed by a second application of secondary antibody and incubation for 20 minutes then washing and application of Diaminobenzidine (DAB) chromogen solution.

EGFR staining was recorded as no staining if the no staining or light staining in <10% of the cells was observed and recorded as positive if >10% of the cells revealed light/moderate/strong staining. After examination by consultant histopathologist all clinicopathological details were recorded and reported as per the hospital protocol.

Data recorded was analyzed by using SPSS version 23. EGFR expression was observed in all the specimens and clinicopathological correlation of neoplasm with EGFR expression was analyzed by using tests of significance. For categorical variables frequencies and percentages were calculated while mean and standard deviation were calculated for continuous variables. Chi Square test was applied on categorical variables while independent t-test was applied on continuous variables, keeping p-value of ≤0.05 as significant.

RESULTS

Total 41 participants of TNBC were included in the study with a mean age of 46.27 ± 3.75 years. 11 (26.8%) tissue specimens revealed basal type TNBC while 30 (73.2%) specimens revealed non-basal type TNBC. Axillary metastasis was observed in 24 (58.5%) patients while perinodal extension was observed in 05 (12.2%) patients. Immunohistochemistry analysis revealed 23 (56.1%) EGFR positive specimens (Figure 2) while 18 (43.9%) specimens were EGFR negative (Figure 3,4). Clinicopathological characteristics of the participants are shown in Table 1. Out of 23 patients with axillary metastasis the frequency of EGFR positive carcinomas was 17 (73.9%) which was significantly greater than 06 (26.1%) patients exhibiting negative EGFR expression. Frequency of EGFR positive expression was significantly increased with higher grade and stage of the tumor (p-value <0.05). Table 2 shows the correlation of EGFR and clinicopathological parameters.

DISCUSSION

This cross-sectional study was conducted to evaluate the overexpression of EGFR in triple negative breast carcinoma. EGFR expression was observed in 23(56.1%) participants diagnosed with TNBC while 18(43.9%) patients were EGFR negative on

Table 1: Clinicopathological characteristics of patients (n=41)

Variables		Results
Age in years (Mean ± S.D)		46.27 ± 3.75
Tumor size in cms (Mean ± S.D)		5.96 ± 1.06
Axillary metastasis n(%)	Yes	24(58.5%)
	No	17(41.5%)
N-Staging n(%)	N0	16(39%)
	N1	10(24.4%)
	N2	07(17.1%)
	N3	08(19.5%)
Perinodal extension n(%)	Present	05(12.2%)
	Absent	36(87.8%)
Grading of Tumor n(%)	Grade II	08(19.5%)
	Grade III	33(80.5%)
Surgery n(%)	Modified radical mastectomy	32(78%)
	Simple mastectomy	09(22%)
Lymphocytic invasion n(%)	Present	35(85.4%)
	Absent	06(14.6%)
Lymphovascular invasion n(%)	Present	19(46.3%)
	Absent	22(53.7%)
Type of triple negative breast carcinoma n(%)	Basal	11(26.8%)
	Non-basal	30(73.2%)
EGFR n(%)	Positive	23(56.1%)
	Negative	18(43.9%)

Table 2: Correlation of EGFR and clinicopathological parameters (n=41)

Parameters		EGFR Positive (n=23)	EGFR Negative (n=18)	p-value
Age in years (Mean ± S.D)		45.48 ± 3.38	47.28 ± 4.04	0.129
Tumor size in cms (Mean ± S.D)		5.76 ± 1.127	6.22 ± 0.94	0.171
Axillary metatstasis n(%)	Yes	17(73.9%)	06(26.1%)	0.024
	No	06(26.1%)	11(61.1%)	
N-Staging n(%)	N0	04(17.4%)	12(66.7%)	<0.01
	N1	05(21.7%)	05(27.8%)	
	N2	06(26.1%)	01(5.6%)	
	N3	08(34.8%)	00	
Grading of Tumor n(%)	Grade II	02(8.7%)	06(33.3%)	0.048
	Grade III	21(91.3%)	12(66.7%)	
Type of triple negative breast carcinoma n(%)	Basal	07(30.4%)	04(22.2%)	0.556
	Non-basal	16(69.6%)	14(77.8%)	

immunohistochemistry analysis. A significant correlation was observed in EGFR expression frequency with axillary metastasis, grade of tumor and stage of tumor. The increase in frequency of EGFR expression with the increase in invasiveness of the tumor cells predicted the poor prognosis of the disease. Similar to our study another study done by Munawar S et al concluded that a significant number of patients diagnosed with triple negative metastatic breast carcinoma revealed EGFR expression. Immunohistochemical analysis of TNBC biopsy specimens revealed the increase in frequency of EGFR expression with increase in the grade and stage of the tumor. These results were similar to our research hence EGFR overexpression can be used as a prognostic tool to predict the invasive nature of the tumor which can lead to poor prognosis.[12] Contrary to the high expression of EGFR in our study another regional study concluded that EGFR overexpression was observed in only 2.7% of the cases with TNBC which may be due to underlying genetic alterations.[13] In our study the mean age of the patients diagnosed with TNBC was 46.27 ± 3.75 years as compared to 46.26 ± 12.22 years in an observational study conducted in Pakistan. [14]

The presence of kinase inhibitors in breast carcinomas are found to be associated with better prognosis.[15] In a similar trial specimen of TNBC diagnosed patients were examined immunohistochemically for the expression of EGFR and mitogen activated protein kinases (MAPKs). Results concluded that a positive correlation between EGFR and MAPKs expression was there in TNBC. An increase in the expression of MAPKs and EGFR in breast neoplasms was associated with nodal metastasis, higher grade on histology, increased recurrence, metastasis, poor prognosis and decreased rate of survival. [16]

Triple negative breast carcinoma is rare, aggressive and metastatic. These cancerous cells can lead to debilitating consequences with multisystemic effects. The resistant nature of this variant can lead to treatment failure, and this has become a great concern in the field of medicine.[17] Treatment of breast carcinomas are usually targeted at the receptors expressed like estrogen, progesterone and Her2 receptors however the treatment options in TNBC are limited because hormonal and tarastuzumab are not effective. Tumor markers like EGFR can

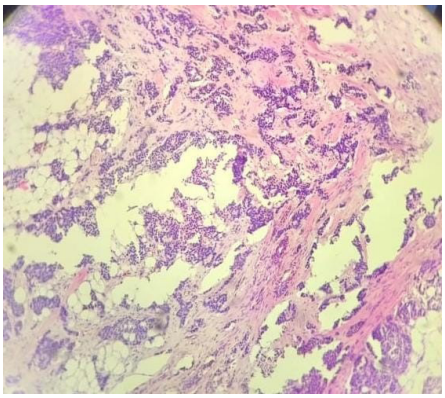
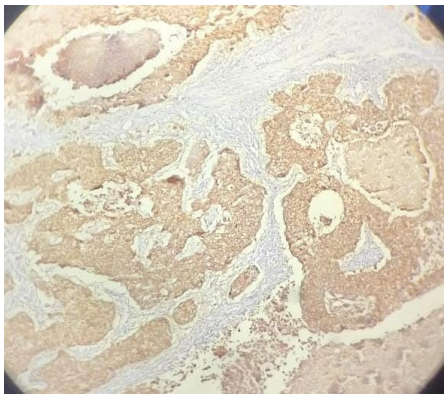
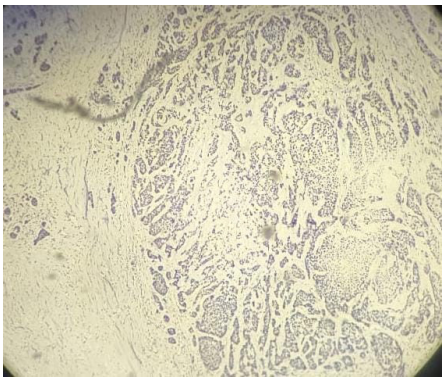
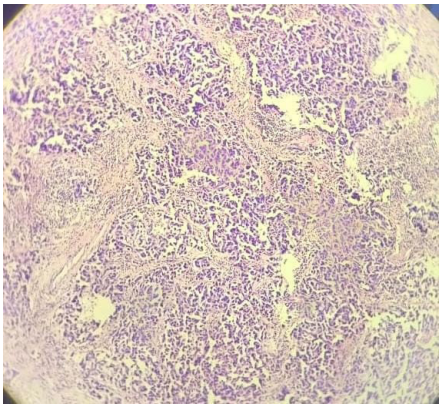

Figure 1, (x10) Triple negative breast carcinoma (hematoxylin & Eosin staining). Figure 2, (x10) Immunohistochemical staining using EGFR showing positive labelling. Figure 3, (x10) Immunohistochemical staining using EGFR showing negative labelling. Figure 4, (x10) Triple negative breast carcinoma (Hematoxylin & Eosin staining)

serve as a target for developing new hormonal/pharmacological strategies for the treatment of TNBC.[18] The area of research in TNBC is scarce but requires a serious attention attributed to the increased mortality and poor prognosis observed in TNBC. Therapies under trial for the treatment of TNBC include drug antibody conjugates, poly ADP ribose polymerase PARP inhibitors, immune checkpoint inhibitors and therapies targeted at androgen receptors however none are considered gold standard.[19]

The novel marker EGFR which is overexpressed in TNBC may serve as a useful target for targeted therapies. A recent trial has assumed that the use of therapies like chimeric antigen receptor engineered T-cells targeted at EGFR may be effective in humans as it inhibits the growth and rapid division of cancerous cells in TNBC variant.[20]

In a nutshell unlike other breast tumors where therapies targeted at estrogen and Her-2 receptors may improve the survival and prognosis of the patients,[21] TNBC do not express such receptors and therapies targeted at EGFR which are potent and effective keeping in view the high resistance to pharmacotherapy by these cancerous cells is the need of hour. Hence this study proves that EGFR overexpression in TNBC is associated with greater aggressiveness and metastatic potential of the cancer cells which predicts a poor prognosis, and this can serve as a crucial information for developing new therapies aimed at EGFR over expression.

CONCLUSIONS

EGFR overexpression in Triple negative breast carcinoma is associated with poor clinicopathological indicators which may lead to poor prognosis.

LIMITATIONS OF THE STUDY

The sample size was small which may create a discrepancy in generalized results for a larger population. All the patients included in the study belonged to the same ethnicity however the results may significantly differ if patients of different ethnicities were included.

RECOMMENDATIONS

Overexpression of EGFR in patients with triple negative breast carcinomas predicts metastatic behaviour of the cancerous cells. Further research on employing EGFR targeted therapies in these patients may prove beneficial for better prognosis.

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Author's Contribution

AS conceived the idea and designed the study. MTK, MK and IMK helped in designing the study and performed data analysis. RM, FJ and AD helped in the write up of the manuscript. All authors made substantial intellectual contributions to the study.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.