INTRODUCTION

Breast carcinoma is the most common cancer affecting females worldwide. Its incidence in Pakistan is 24.4%, making it the commonest malignancy among Pakistani females as well. Breast cancer is a heterogeneous group of diseases which comprises a spectrum of biological subtypes, natural history, clinicopathological along with molecular features, having different prognostic and therapeutic consequences. On the basis of gene expression profiling and immunohistochemistry, breast cancer is divided into five subtypes: luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER2+), basal cell-like (BCL) or 'triple-negative' phenotype (ER -, PR -, HER2- with expression of basal/myoepithelial markers), HER-2 positive tumors (ER-,PR-,HER2+), and normal breast-like group.

Triple negative breast cancer (TNBC) accounts for 10 - 20% of all breast carcinomas and is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (Her-2 neu). It has an aggressive clinical behaviour, low overall survival and is unresponsive to hormonal and anti-Her2/neu therapy. TNBC can be divided into a basal-like subtype and a normal breast-like (or unclassified) subtype. Basal-like tumours express basal markers (CK5/6 and/or EGFR). Gene expression profiling is the gold standard to identify basal-like breast cancers. However, due to high cost and technical issues, immunohistochemistry (IHC) can be applied instead.

Unlike breast tumours that are hormone receptor and/or Her2/neu positive, triple negative tumours lack a targeted therapeutics agent. The treatment of such tumours is limited to adjuvant therapy. TNBC resistant to chemotherapy is in need of effective novel therapeutic agents; several biologically targeted agents are currently being explored in this group.

The epidermal growth factor receptor (EGFR) is a cell surface receptor for the members of the epidermal growth factor family (EGF-family). Recently, it has been shown to have a potential role in triple-negative breast tumours, regarding resistance to endocrine therapies, maintenance of stem-like tumour cells, and bone metastasis. The c-KIT is a tyrosine kinase cell surface receptor. It is found to be over-expressed in BRCA-1 gene associated breast cancer, which is mostly triple negative. EGFR is considered as a potential sensible therapeutic target in TNBC. Cetuximab, which is an anti-EGFR

ABSTRACT

Objective: To determine the frequency of immunohistochemical expression of epidermal growth factor receptor (EGFR) and c-KIT in triple negative breast cancer (TNBC).

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January to December 2014.

Methodology: Fifty cases of triple negative breast cancer diagnosed on immunohistochemistry were included in the study. Patients’ gender and age were noted. Immunohistochemistry for EGFR and c-KIT were applied and the results were recorded. The data were analyzed by using computer software programme SPSS version 19. Descriptive statistics, frequencies and percentages were calculated.

Results: Out of 50 patients of TNBC, all patients were females. The ages of the patients ranged between 30 and 80 years with an average age of 48.9 years and standard deviation of ±10.0. Out of 50 cases, 29 cases (58%) were positive for EGFR while 15 cases (30%) were positive for c-KIT. Thirteen cases (26%) were positive for both EGFR and c-KIT. Nineteen cases (38%) were negative for both EGFR and c-KIT.

Conclusion: EGFR expression was observed in 58% and c-KIT expression was seen in 30% of the cases. The results of EGFR expression are almost similar while those of c-KIT are different from international studies which suggest immunohistochemical differences between the studied populations.

Key Words: c-KIT. EGFR. Triple negative breast cancer.
monoclonal antibody, has been added to chemotherapy for TNBC. A preclinical data report supports a synergistic effect of EGFR inhibition (e.g. gefitinib) with chemotherapy in patients with TNBC. It shows improved efficacy compared with either therapy alone. Another trial showed enhanced radiosensitivity by use of cetuximab in TNBC.

Tyrosine kinases include proteins like src, c-KIT and platelet derived growth factor receptor (PDGFR). Activation of src is found to be associated with the activation of EGFR pathway, which is frequently expressed in TNBC. Therefore, the need for the development of src inhibitors in breast cancer has been postulated. Dasatinib is an oral tyrosine kinase inhibitor which acts on src and c-KIT proteins. A phase II study with dasatinib in metastatic triple negative breast cancer patients showed a clinical benefit rate of 9%.

Although the immunohistochemical expression of EGFR and c-KIT in TNBC has been studied in other parts of the world, this has not been elucidated in our population. Thus, the study is aimed to investigate frequency of immunohistochemical expression of EGFR and c-KIT in triple negative breast cancer cases in the local population.

METHODOLOGY

This cross-sectional study was carried out at the Armed Forces Institute of Pathology, Rawalpindi, from January to December 2014, after taking approval from the Ethical Review Committee. Fifty cases of triple negative breast cancer were selected by consecutive, non-probability sampling. Patient data including age and gender, were, noted. Immunohistochemistry (IHC) analyses of human epidermal growth factor receptor (EGFR), and c-KIT (or CD117) were performed on formalin-fixed, paraffin-embedded triple negative breast cancer tissue. Tissue blocks were sectioned at 3µm thickness and deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. Heat induced epitope retrieval in Tris/EDTA buffer at pH 9.0 buffer was used for ready to use primary antibodies EGFR (clone by Biodiagnostic) and c-KIT (clone by Dako Corporation). EGFR and c-KIT positivity was defined as the presence of 1% or more positively stained cells in a cytoplasmic or membranous pattern. The results of immunohistochemistry were recorded.

The data was analyzed by using computer software programme SPSS version 19. Frequencies and percentages were recorded for categorical variables while mean and standard deviation were calculated for the quantitative variables.

RESULTS

A total of 50 cases of triple negative breast cancer were included in the study. All patients included in the study were females. The ages of patients ranged from 30 to 80 years with mean age of 48.9 ±10.0 years. Majority of the affected patients presented in 4th decade of life (38%, n=19). The decade-wise age distribution is shown in Figure 1. Twenty-nine cases of triple negative breast cancer (58%) showed positivity for EGFR, while 21 cases (42%) were negative. Fifteen cases of triple negative breast cancer (30%) showed positivity for c-KIT, while 35 cases (70%) were negative. Thirteen cases (26%) were positive for both EGFR and c-KIT. Nineteen cases (38%) were negative for both EGFR and c-KIT. Sixteen cases (32%) were positive for EGFR but negative for c-KIT.

DISCUSSION

The mean age in this study was 48.9 years. This is in concordance with a study conducted in United Kingdom (n=282), in which the mean age was 49.9 years. The mean age is 9.6 years younger compared to study carried out in Switzerland (n=152), 7 years younger than Turkish population (n=41), and 6.1 years younger than a study carried out in Puerto Rico (n=54). It is 3.6 years older than the study conducted in Malaysia (n=42). Comparing the age with a local study conducted at Armed Forces Institute of Pathology, Rawalpindi in 2010 (n=815), the mean age in this study is 2.7 years older. This difference may be attributable to the variability in sample size in these studies.

The expression of EGFR observed in this study was 58%, while it ranged from 51.2 to 77% in international studies. The expression of EGFR is lower as compared to a study carried out in Spain (77%, n=49/64). However, it is in concordance with the studies carried out in Turkish (51.2%, n=21/41), Malaysian (61%, n=11/18), and Indian population (58%, n=18/31). The expression of c-KIT in this study is 30%. It is close to the study conducted in China (42.1%, n=72/171), but much lower than the studies conducted in Malaysia.
and Spain, where the expression was 89% (n=16/18) and 77% (n=49/64), respectively.\textsuperscript{18,21}

As triple negative breast cancer is a clinically significant group of breast carcinoma having a poor prognosis and limited targeted treatment options, the patients with TNBC may be tested for EGFR by application of immunohistochemistry. It might prove helpful for the pathologists to determine the basal phenotype where tissue microarray is not available. Moreover, it may also be helpful for the oncologists in guiding them to predict the response to anti-EGFR therapy, if it is added to the treatment regimen of TNBC cases. However, expressions of both EGFR and c-KIT need to be studied on a larger scale to see a true picture in our Pakistani population.

**CONCLUSION**

The expression of Epidermal growth factor receptor in TNBC in this study population was 58%, while that of c-KIT is 30%. The difference in expression of c-KIT might be due to either the regional variation depending upon different genetic makeup of the population or difference in sample size.

**REFERENCES**