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ORIGINAL ARTICLE

Prognostic Markers Related to Triple-Negative Breast Cancer

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ABSTRACT

Background: The triple-negative tumor is a high-risk tumor as a targeted therapy to these proteins is not possible with this type of cancer.

Objective: To investigate the prognostic factors that can help in treatment selection in the triple-negative phenotype breast tumors

Study design: A cross-sectional study

Place and duration: This study was conducted at People's University of Medical and Health Sciences Nawabshah from Nov 2021 to Nov 2022

Methodology: The present study examined numerous cases of invasive carcinoma of the breast. The patients visited for follow-up for a long time and they were examined by tissue microarray. The series was stained through concurrent immune-histochemical prognostic panels. This was done to specify the subgroups of different types of breast cancer and for the identification of prognostic markers as well as the aggressive behavior of the tumors.

Results: In the beginning, a total of 280 cases were included in the study, out of which 45 (16.07%) patients had triple-negative breast cancer. Most of these cancers were grade 3 carcinomas. A strong association was seen with pushing margins, development of recurrence, large size, poorer Nottingham Prognostic Index, and distant metastasis. Moreover, the association was also seen with loss of expression of E-cadherin and androgen receptors, basal phenotype, p53, EGFR, and P-cadherin. The size of the tumor, androgen receptors, and lymph node staging were the most valuable prognostic markers. Androgen receptor and size had prognostic significance in the tumor subgroup with lymph node-positive tumors. On the other hand, the basal phenotype was the only prognostic marker in the subgroup with lymph node-negative tumors. Some other parameters considered in the present study are histological grade, size of the tumor, age of the patient, and vascular invasion.

Conclusion: The most significant markers identified were basal phenotype and androgen receptor. Also, tumor size and the status of the lymph node are quite significant in low-risk and high-risk patients while selecting surgical or non-surgical treatment for the triple-negative tumors

Keywords: Triple-negative, breast tumor, prognostic markers, androgen receptors

INTRODUCTION

Various types of tumors come under the umbrella of breast carcinomas and their response to the therapy is also different for each type [1]. The incidence of breast cancer has increased, however, the rate of mortality due to breast cancer has declined because of different types of treatments [2]. Certain prognostic markers identify the outcome of the treatment of the disease and also the aggressive behavior of the tumor [3]. The estrogen receptor is a significant determinant of diagnosis and treatment outcome because it is 70-75% expressed in breast carcinoma of invasive nature [4]. Progesterone receptors are expressed highly in patients with estrogen receptor expression and rare in those tumors which do not have estrogen receptor expression [5].

In 15-25% of the patients with breast cancer, human epidermal growth factor receptor 2 (HER2) is expressed and the mode of treatment is determined by its expression. Overexpression of HER2 is seen in the earliest stage of carcinogenesis [6]. The Ki-67 proteins are another prognostic marker of breast cancer. They provide information regarding the proliferation of carcinogenic cells and reflect the tumor's aggression and the response to the therapy [7]. E-cadherin is important in the epithelial-mesenchymal transition (EMT), hence when the expression of E-cadherin is lost, the risk of metastasis increases. It is also associated with TNM staging, the status of lymph nodes, and the size of the tumor [8]. Circulating circular RNAs were recently identified to be significant in the carcinogenesis of breast tumors in processes such as proliferation, and apoptosis and also increase the metastatic potential [9].

A mutation in the P53 gene has been observed in many cancers such as osteosarcoma, brain tumor, leukemia, adrenocortical carcinomas, and also in breast cancers [10]. Some other important prognostic markers for breast cancer are Mib1, MicroRNA, Tumor-associated Macrophages, and inflammation-based models such as the lymphocyte-to-monocyte ratio and the platelets-to-lymphocytes ratio [11].

The present study aims to investigate the prognostic factors that can help in treatment selection in the triple-negative phenotype breast tumors.

METHODOLOGY

In the present study, a total of 280 patients having invasive breast carcinoma were observed. Patients were asked to come for a long follow-up visit and they were treated uniformly. A wide range of biomarkers was studied. Patients were assessed for the characteristics of their tumors and a detailed history of all the patients was collected. The information that was collected from the patients included distant recurrence, local recurrence, regional recurrence, and therapy given for the tumor. The calculation of the Nottingham Prognostic Index (NPI) was done following this equation [12]:

NPI ¼ 0.2 tumor size (cm) þ grade (1–3) þ lymph node score (1–3)

This index is helpful in the prediction of the probability of survival of a patient suffering from an invasive breast tumor. It has three subsets according to the chances of survival and dying; poor (>5.4), moderate (3.41–5.4), and good (3.4) prognostic groups [13].

The median Overall Survival (OS) was 73 months. The event-free survival of the patients was 66 months. Recurrence of the disease was found in 51 (18.2%) cases, distant metastasis was seen in 31 (11.07%) patients, and 28 (10%) patients died during the follow-up period. The mean NPI was 4.2 with a range of 2.1-8.5. Hormonal therapy was provided to 98 (35%) patients. Chemotherapy was given to 47 (16.78%) patients.

Tissue microarrays were prepared for breast cancer and the tissues were stained immunohistochemically for estrogen, progesterone, EGFR, HER2, p53, androgen receptor, basal cytokeratin, E-cadherin, and P-cadherin. Table 1 describes the data regarding these markers. Two cores were taken and evaluated from the tumor tissue and the ones with invasive malignant cells were considered in the study. Scoring was done individually and the mean was collected for both readings. The scoring of immune-histochemical was performed blindly. The data was collected and statistical analysis was done in the IBM SPSS version 26.

RESULTS

A total of 280 patients with invasive breast cancer were included in the present study to check for 3 markers i.e. estrogen, progesterone, and HER2 receptors. Out of these 280, 45 (16.07%) were detected to have triple-negative breast tumors. These were considered regardless of the expression of basal cytokeratin and EGFR. The median age of the patients was 50.1 years with a range of 24-69 years. 81% of patients had ductal carcinoma. The range of NPI in these patients was 2.2-7.5 with a mean of 4.7. 11 (24.44%) received hormonal therapy and 25 (55.56%) received chemotherapy. The median OS was 54 months. The event-free survival had a median of 49 months. The features of non-triple-negative tumors have been compared with triple-negative tumors in table 2.

Table 1: Dilution, Pretreatment, Source, and cut-off values of the antibodies

Antibody	Pretreatment	Dilution	Source	Cut-off values
HER-2(cerbB-2)	None	1:250	DakoCytomation	<10% (neg)
PR[clone PgR 636]		1:100	DakoCytomation	0 % (neg)
ER[clone 1D5]	Microwave	1:80	DakoCytomation	0 % (neg)
p53[clone DO7]	Microwave	1/50	Novocstra	>5% (pos)
EGFR[clone EGFR.113]	Microwave	1:10	Novocstra	<10% (neg)
CK5/6[cloneD5/16134]	Microwave	1:100	BoehringerBiochemica	≥10% (pos)
CK14[cloneLL002]		1:100		
AR[cloneF39.4.1]	Microwave	1:30	Biogenex	0% (neg)
Anti-P-cadherin[clone56]		1/200	BD Biosciences	≥5% (pos)
Anti-E-cadherin[cloneHECD-1]	Microwave	1:100	Zymed	≥100

Table 2: Comparison of features of non-triple negative tumors with triple-negative tumors

Variables/features	Total number of patients N (%)	Non-triple negative tumors N (%)	Triple-negative tumors N (%)	P value
Grade of the tumor				<0.0001
1				
2	42	39	3	
3	85	73	12	
	153	123	30	
Size of tumor				<0.001
<1.5 cm	82	68	14	
>1.5 cm	198	167	31	
Lymph node status				0.88
Negative	202	166	36	
Positive	78	69	9	
Diabetes mellitus	31 (11.07%)	23 (9.795)	8 (17.78%)	0.001
Hypertension	51 (18.21%)	25 (10.6%)	27 (60%)	<0.0001

An association between large-size and triple-negative tumors can be seen in the table. However, there was no such association with the status of lymph nodes. Most of the patients present in the triple-negative group had grade 3 tumors. Diabetes was not very common in triple-negative tumor patients, whereas, hypertension had a strong association with the disease.

DISCUSSION

The present study included a total of 280 patients including 45 patients who had triple-negative breast tumors. Similar studies have been performed in the past to assess the outcome of treatment for such a disease. Some researchers have studied different prognostic markers individually. One such study was conducted by Haroon et al in which they studied the Ki67 expression in breast cancer patients and its correlation with disease outcomes. They included 194 patients with breast tumors and found that higher levels of Ki67 have a 30% better prognosis. They also suggested categorizing patients based on their Ki67 profile before starting adjuvant treatment [14]. Similarly, Sharif et al conducted a study to check the effect of HER2 and its association with prognosis. They included 535 patients with ductal carcinoma in their study. They concluded that the PR, ER, and HER2 expressions have a positive association with lymph node metastasis. They also observed that the expression of HER2 cannot predict the expression of other hormone receptors [15]. Sharif et al also studied the association of steroid hormone receptors with the prognostic markers of breast cancer and observed that PR and ER have an inverse correlation with HER2 and a direct association with the grade of tumor, metastasis, and age of the patient [16].

In a study by Sharif et al, they studied the predominance of ER and PR expression in male breast cancer compared to female breast cancer. They found that men have more PR and ER expression as compared to that women. The p-53 and HER2 in men have a higher prognostic role and are more significant when it comes to choosing the treatment modality [17]. Tasneem et al conducted a similar study to the present study. They analyzed the stage of cancer and prognostic markers in a retrospective study. According to their study, the prognosis mainly depends on size, grade, and lymph node involvement. Higher the variables, the poorer the prognosis of the disease and the outcomes of the treatment [18]. The present study focuses more on the triple-negative disease and the results are not comparable to a single study as most of the studies have data related to individual markers. Nonetheless, it can be seen that the results of some studies are comparable to the present study and the significance of the expression of receptors is great.

CONCLUSION

The expression of hormonal receptors (ER and PR) and HER2 is important concerning the prognosis of the disease and the outcomes after treatment with hormones or chemotherapy. Other

prognostic markers are also significant in a triple-negative tumor. Special attention should be given to androgen receptors as well as basal cytokeratin in triple-negative tumors.

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