ORIGINAL ARTICLE

Expression of SOX10 in Triple Negative Breast Cancer

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ABSTRACT

Objective: To determine the frequency of SOX10 expression in patients with triple-negative breast cancer.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi, 01 Dec 2021 to 30 Sep 2022.

Materials and Methods: This study was conducted on 185 patients diagnosed with triple-negative breast cancer. Patients with any size, stage and grade of breast cancer that were negative for the presence of estrogen and progesterone receptors, as well as HER2, were included. Those who were chemo-experienced, had received radiation to the breasts or had relapsed were excluded. Immunohistochemistry was performed using staining with a SOX10 antibody on patients' tissue samples for SOX10 expression, which was quantified. Data was analyzed using SPSS 26.0, and comparison was made between patients who were SOX10 positive versus those who were SOX10 negative with regards to disease characteristics.

Results: The mean age of our sample population was 51.65 ± 9.81 years. SOX10 was positive in 138 (74.6%) cases, with patchy positivity seen in 55 (29.7%) samples, while focal and diffuse involvement was seen in 67 (36.2%) and 16 (8.7%) cases respectively. Higher SOX10 positivity was seen with advancing age, (p=0.014), larger tumour size, (p<0.001), and higher tumour grade, (p=0.003). Diffuse and focal involvement were associated with higher grade tumours, (p<0.001), while degree of SOX10 expression did not appear to have a statistically significant association with disease stage, (p=0.618).

Conclusion: SOX10 is a useful marker that can be frequently detected in triple-negative breast cancer and is associated with more aggressive disease characteristics at presentation.

Key Words: *SOX10, Triple-Negative Breast Cancer, Tumour Grade, Tumour Stage.*

Introduction

In 2022, it is estimated that more than a quarter of a million females were diagnosed with invasive breast cancer, while approximately 50,000 were diagnosed with in-situ lesions.[1] Close to 50,000 women died of breast cancer during this year.[1] In Pakistan, a staggering 1 in every 9 women is diagnosed with breast cancer in her lifetime.[2] Triple-negative breast cancer is an aggressive form which lacks the presence of estrogen, progesterone and human epidermal growth factor 2 (HER2) receptors, which are common targets for therapy.[3] Consequently, these tumours are usually high-grade at the time of presentation and, while initially responsive to chemotherapy, have high rates of recurrence, metastasis and are associated with significant morbidity and mortality.[4] This variant is fairly common and accounts for 12% of all cases of breast cancer in the United States.[5] SRY-Box Transcription Factor 10 (SOX10) is one of a series of transcription factors that performs integral functions in the embryonal development of the peripheral nervous system, neural crest, melanocytes and also plays a role in the development of the testes.[6] In addition, SOX10 is also found in salivary glands as well as in the mammary glands, and has been found to be expressed in malignancies of the liver, ovaries, prostate and the gastrointestinal tract.[7] The compound is also expressed in different carcinomas originating from the breast.[8] Measuring SOX10 expression is not only of value in diagnosing breast

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cancers that are triple-negative, but may also play a role in the prognostication of the disease as well as serve as a potential target for immunotherapy, however, this aspect requires eludication.[9,10] This study was conducted with the aim of determining what percentage of patients with triple-negative breast cancer in Pakistan have SOX10 positivity. If substantial, then this factor could be employed in the diagnosis of the disease in future. Moreover, determining the different characteristics of the disease with regards to tumour grade and disease stage, among other factors, and their association with SOX10 positivity will help to determine the effect of this factor on the prognosis expected. Lastly, SOX10 expression may serve as a future target for therapy, and it would be useful to establish a baseline presence in the Pakistani population. This research protocol was conducted to determine the frequency of SOX10 expression in patients with triple-negative breast cancer in this population.

Materials and Methods

We conducted this descriptive cross-sectional study between 01st Dec 2021 and 30th Sep 2022 in the Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi on 185 patients with triple-negative breast cancer, after obtaining informed consent. Consecutive, non-probability sampling was used to select the patients. The study permission was obtained from institutional review board. The WHO sample size calculator was used to calculate the sample size keeping a confidence level of (1-α) of 95%, an absolute precision (d) of 0.07 and an anticipated population proportion (P) of 0.619, which was the percentage of patients with triple-negative breast cancer with positive staining for SOX10, from Ali et al.[11]

All patients diagnosed with any stage and grade of breast cancer that was negative for the presence of estrogen and progesterone receptors, as well as HER2, were included in the study. Patients who have received prior chemotherapy, radiation or have a recurrent breast carcinoma, were excluded.

All patients' demographic data, as well as tumour characteristics, including type and stage, were documented at the time of sample receipt. Only samples collected via excision, resection or core biopsy were examined. The diagnosis of breast cancer, its tumour grade, and its triple-negative status were re-examined via light microscopy. Immunohistochemistry was performed on the tissue samples, with xylene-based de-waxing and alcohol rehydration. A SOX10 antibody was applied to the tissue samples to determine whether the antigen was being expressed and, if present, its presence as a percentage and as a pattern were documented as displayed in Table-I.

Table I : SOX10 Degree of Histological Involvement Interpretation

Expression	Interpretation
<1% cells in tumor cells	Negative
1 – 10% cells in tumor cells	Patchy positive
11 – 70% cells in tumor cells	Focal positive
>70% cells in tumor cells	Diffuse positive

Data was analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows version 26, IBM Corp; Armonk, USA). Mean and standard deviation was calculated for quantitative variables specifically patient age at diagnosis, primary tumour size in largest diameter and percentage of SOX10 present. Qualitative variables like tumour stage, tumour grade and pattern of SOX10 presence were recorded in terms of frequency and percentage. Patients were divided into two groups: one with positive SOX10 staining, and the other with those who were negative, and the various patient and disease characteristics were compared across these groups. Quantitative variables were compared using the independent samples *t*-test while the chi square test was used for qualitative variables and a *p*-value of ≤ 0.05 was considered significant.

Results

This study was conducted on samples from 185 patients diagnosed with triple-negative breast cancer. The mean age of the population was 51.65 ± 9.81 years. The average size of the primary lesion as measured in its largest diameter for the entire sample was 3.28 ± 1.17 cm. A total of 12 (6.5%) patients had tumour grade I lesions, while 127 (68.6%) and 46 (24.9%) had grade II and III lesions, respectively. Stage I disease was seen in 25 (13.5%) cases, Stage II in 72 (38.9%) patients, while Stage III and IV disease was seen in 61 (33.0%) and 27 (14.6%) cases, respectively. The mean percentage of SOX10 positive cells per sample were 26.32 ± 29.04% for the whole sample. Samples were negative for SOX10 in

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47 (25.4%) cases, patchy positivity was seen in 55 (29.7%) samples, while focal and diffuse involvement was seen in 67 (36.2%) and 16 (8.7%) cases, respectively. Higher SOX10 positivity was seen with advancing age, (p=0.014), larger tumour size, (p<0.001), and higher tumour grade, (p=0.003), while disease stage did not appear to be statistically significantly associated with SOX10 positivity, (p=0.206). Table-I shows the difference characteristics of each patients distributed according to SOX10 status.

Table II: Patient Characteristics According to SOX10 Status

Variable	SOX10 Positive (n=138)	SOX10 Negative (n=47)	*p*-value
Age at Diagnosis (years)	52.71 ± 9.05	48.64 ± 11.34	0.014
Tumour Maximum Diameter (cm)	3.62 ± 0.99	2.28 ± 1.04	<0.001
Tumour Grade			
Grade I	4 (2.9%)	8 (17.0%)	0.003
Grade II	98 (71.0%)	29 (61.7%)	
Grade III	36 (26.1%)	10 (21.3%)	
Disease Stage			
Stage I	16 (11.6%)	9 (19.1%)	0.206
Stage II	58 (42.0%)	14 (29.8%)	
Stage III	42 (30.4%)	19 (40.4%)	
Stage IV	22 (16.0%)	5 (10.7%)	

Table-II displays the distribution of patients according to the pattern of SOX10 involvement. Diffuse and focal involvement were associated with higher grade tumours, (p<0.001). There did not appear to be a statistically significant association of SOX10 positivity with disease stage, (p=0.618).

Table III : Patient Characteristics According to Pattern of SOX10

Variable	Negative (n=47)	Patchy (n=55)	Focal (n=67)	Diffuse (n=16)	*p*-value
Tumour Grade					
Grade I	8 (17.0%)	4 (7.2%)	-	-	<0.001
Grade II	29 (61.7%)	44 (80.0%)	48 (71.6%)	6 (37.5%)	
Grade III	10 (21.3%)	7 (12.8%)	19 (28.4%)	10 (62.5%)	
Disease Stage					
Stage I	9 (19.2%)	6 (10.9%)	7 (10.4%)	3 (18.8%)	0.618
Stage II	14 (29.8%)	24 (43.6%)	28 (41.8%)	6 (37.4%)	
Stage III	19 (40.4%)	17 (30.9%)	22 (32.8%)	3 (18.8%)	
Stage IV	5 (10.6%)	8 (14.6%)	10 (14.9%)	4 (25.0%)	

Discussion

Triple-negative breast cancer represents a diagnostic and therapeutic dilemma, in that, it lacks the usual protein markers used for diagnosis, which is a particularly difficult hurdle to overcome if the focus of primary disease is unknown.[12,13] In addition, such proteins are used for targeted therapy and their absence results in the limitation of therapeutic options.[14,15] While a number of different modalities are used in conjunction with one another to establish the presence of triple-negative breast cancer, definitive diagnoses are established only on histology, even in such cases.[12] SOX10 may represent a biomarker that can be used to both diagnose these cases and potentially serve as a target for drug therapy in such patients.[15-16]

SOX10 was positive in 138 (74.6%) cases of triple-negative breast cancer, in the current study. Ali et al noted that 61.9% cases of triple-negative breast cancer were positive for the expression of SOX10 in their study.[11] Yoon et al reported a much higher positivity of 85.7% for SOX10 in this form of breast cancer in their study,[17] while Qazi et al reported a figure that was similar to ours: 74.0%.[18] However, the reported frequency of SOX10 positivity was not uniformly high across all studies: Jamidi et al noted that only 31.3% of triple-negative breast cancers had SOX10 positivity in their study sample.[19] We believe this variability in results may attributable to ethnic differences between the populations studied.

Samples were negative for SOX10 in 25.4% cases in our study, patchy positivity was seen in 29.7% samples, while focal and diffuse involvement was seen in 36.2% and 8.7% cases, respectively. Ali et al noted similar frequencies for the a fore mentioned patterns in their study: 33.0% samples were SOX10 negative, 15.0% had patchy positivity, 37.0% of the samples were focal positivity while 20.0% showed diffuse positivity.[11] Thus, the majority of samples show patchy or focal positivity in literature.

The mean age of women suffering from triple-negative breast cancer was 51.65 ± 9.81 years in our study sample. Higher SOX10 positivity was seen with advancing age, (p=0.014). Ali et al reported a slightly lower mean age of 44.31 ± 12.31 years in their patients,[11] and reported that advancing age was not associated with a higher frequency of SOX10 positivity, (p=0.290). Conversely, Jamidi et al

reported that SOX10 was seen at a significantly higher frequency in younger patients as opposed to older ones, (p=0.001), which was at odds with our study.[19] While these may also be attributable to differences in populations originating from a variety of geographical locations, the literature on the subject is conflicted and a more detailed review of the subject would be advisable before reaching concrete conclusions.

The average maximum diameter of the primary breast cancer lesions for our entire sample was 3.28 ± 1.17 cm. SOX10 positivity was associated with larger tumour sizes, (p<0.001). Jamidi et al noted that there was no association between SOX10 positivity and larger tumour size at diagnosis, (p=0.231),[19] a finding that was echoed by Ali et al, (p=0.33).[11] Conversely, Kriegsmann et al noted that SOX10 positivity was associated with smaller size lesions at the time of diagnosis, (p=0.039).[20] We believe that there are a number of confounding factors which had led to this variability in results, chief among which is the institution of screening programs for the early detection of breast cancer resulting patients being diagnosed at times when the primary tumour size was still small, as opposed to our populations where screening programs do not exist and patients present late.

In this study, 6.5% of patients had tumour grade I lesions, while 68.6% and 24.9% had grade II and III lesions, respectively. Patients with SOX10 positivity had a higher tumour grade at presentation, (p=0.003). SOX10 expression was associated with higher grade tumours at diagnosis, (p<0.001), in Qazi et al,[18] a finding that was reported by Klaric et al where 93.2% of cases with grade III lesions were positive as opposed to only 70.1% with grade II or I lesions, (p<0.001).[21] Saunus et al studied a number of biomarkers and their association with different disease characteristics in patients with triple-negative breast cancer and echoed our findings, reporting that tumour grade was higher in SOX10 positive patients at the time of diagnosis.[22]

In our study, Stage I disease was seen in 13.5% of cases, Stage II in 38.9% patients, while Stage III and IV disease was seen in 33.0% and 14.6% cases, respectively. The stage of the disease at presentation did not appear to have a significant relationship with SOX10 positivity, (p=0.206). Ali et al, Jamidi et al and Klaric et al all reported that SOX10 expression did not appear to be associated with a more advanced disease stage at the time of diagnosis, (p=0.619, p=0.295 and p=0.257, respectively),[11,19,21] however, studies such as Liu et al have noted that SOX10 expression was associated with a higher frequency of lymph node involvement at presentation in their study.[23] While SOX10 has been reported to be definitively associated with higher stage malignancy at presentation in other neoplasms originating from other organs,[24,25] its effect in breast cancer needs further elucidation before concrete conclusions can be drawn.

Study Limitations

We conducted this study in a single center, on a population derived military personnel and their families, the results of which may not be generalizable to the population at-large. Additionally, the study was focused on triple-negative breast cancer exclusively, whether SOX10 occurs at the same or different frequencies in non-triple negative breast cancer in the Pakistani population requires further study. Lastly, this study only established association: further study is required to determine the diagnostic validity of the marker before it can be used routinely in the diagnosis of these forms of breast cancer.

Conclusion

SOX10 can serve as a useful marker to establish a diagnosis of breast cancer in patients whose disease lacks receptors for estrogen, progesterone, and human epidermal growth factor. Moreover, it may also have a role in serving as a marker for more aggressive disease. Future research should focus on determining the diagnostic validity of SOX10 in predicting the presence of breast cancer and the presence of aggressive disease, as well as the development of effective drugs to target the compound in the hopes of providing an effective treatment modality in such cases.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

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

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