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ORIGINAL ARTICLE

Immunohistochemical expression of SOX10 and GATA3 in triple negative breast carcinoma and metastatic breast carcinoma.

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ABSTRACT… Objective: Determine frequency of expression of SOX10 and GATA3 in TNBC and MBC. **Study Design:** Descriptive Cross-sectional study. **Setting:** Department of Histopathology, AFIP, Rawalpindi. **Period:** November 2021 to 2022. **Methods: S**tudy conducted on sample of 121 TNBC cases including, 12 MBC cases. Tumors of any size, grade and stage were included. Biopsies with only CIS, hormone-receptor and HER2 expression, post-chemoradiotherapy and relapsed cases were excluded. Immunohistochemistry was performed using SOX10 and GATA3 antibodies on FFPE samples. Results were analyzed, interpreted and finalized by two independent histopathologists. Data analyzed using SPSS-25 and comparison made between SOX10 and GATA3. **Results:** The mean patient age was 48.45±12.978 years. 38 cases were grade-2 and 83 grade-3. SOX10 was positive in 18 cases. 4 MBC cases expressed SOX10. GATA3 was positive in 99 cases. 8 MBC cases were GATA3 positive. SOX10 was negative in GATA3 positive cases. No statistical correlation was found between age, size, and stage of tumor ($p>0.05$). Combined SOX10/GATA3 was expressed in 117 cases. Statistical correlation was established when SOX10/GATA3 compared to increasing tumor grades ($p<0.05$). **Conclusion:** Combined SOX10 & GATA3 are useful markers detected with increased frequency in TNBC and MBC and can be utilized for diagnosis of these aggressive malignancies.

Key words: ER, GATA3, HER2, Metastatic Breast Carcinoma, PR, SOX10, Triple Negative Breast Carcinoma.

INTRODUCTION

As per WHO statistics from the year 2022, 2.3 million women were diagnosed with breast carcinoma resulting in 0.67 million deaths all around the globe.[1] Breast carcinoma is the most prevalent type of malignancy in women, worldwide.[1] Pakistan has highest incidence of breast carcinoma in Asian block where every ninth woman has an increased risk lifetime risk of breast cancer.[2] TNBCs comprised 18.7% of the total breast carcinoma burden in Pakistan.[3] Breast cancer comprise vastly heterogenous disease group with diverse clinical presentation and histological features leading to variable therapeutic response to treatment modalities. This variability in treatment responses has led to vast research in its molecular classification which have different responses to targeted therapies available.[4] Breast carcinomas are molecularly classified into Luminal A, Luminal B, HER2 oncogene amplification subtype and triple negative (TNBC) subtype.[5] Although the term basal like carcinoma and TNBC are used synonymously these are not same as the latter one is defined by expression of certain specific genet set like EGFR, CK5/6 and proliferation gene clusters. The basal like breast cancer also have low expression of ER, PR and HER2 and thus not all basal like cancers are TNBCs.[6,7] Triple negative breast carcinoma (TNBC) is defined as minimal or lack of expression of ER, PR and HER2. TNBC accounts approximately 15% of all invasive breast carcinomas.[8] A major proportion of TNBCs are of invasive breast carcinoma of no special type (ductal) representing certain peculiar histological features such as high nuclear grade, central necrosis, brisk lymphocytic infiltration and increased mitoses. In addition, this molecular

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type also includes subtypes such as metaplastic carcinomas, salivary gland type neoplasms, carcinoma with apocrine features and carcinoma with medullary features.[9,10] TNBCs generally follows aggressive behavior with shorter survival duration, propensity for metastasis especially brain metastasis, relapse and poor prognosis with a five year recurrence rate of approximately 30%.[11,12] The patients with TNBCs cannot benefit from endocrine and HER2 therapy so they are treated with chemotherapy (chemotherapy sensitive) but with limited benefit, Recently novel agents have been prepared for specific subgroups with PD-L1+, germline BRCA mutated tumors and PARP inhibitors.[13]

SRY locus related HMB box gene, also known as SOX gene plays a multidimensional role in various embryological and physiological processes mainly among neural crest cells. Within this superfamily SOX10 contributes significantly in the differentiation, maintenance and migration of neural crest origin cells. Detection of SOX10 has been used for confirmation of nerve sheath derived and melanocytic tumors.[14] SOX10 is also found in salivary glands and in mammary glands and minority of malignant cases of liver, ovaries, prostate, GI tract and in different carcinomas originating from the breast.[15] It has been recently used for invasive breast carcinoma and its expression was found in TNBC including basal like breast carcinoma. Thus, raising the possibility of its use as complimentary marker for determination of TNBC and breast origin in metastatic carcinomas.[16] GATA binding protein 3 (GATA3) belongs to a family of transcription factors comprising six members. It has a critical role in the development of epithelial structures in both embryonic and adult tissues. Its expression is reported to be high in breast and urothelial tissues and its associated carcinomas.[17]

In current study, we compared the immunohistochemical expression of SOX10 and GATA3 in triple negative breast carcinoma cases and few metastatic breast carcinoma cases including nodal and extranodal metastasis. In this manner, we studied individual and combined expression of both markers establishing their utility in diagnosis of such entities, especially in cases where clear diagnosis is difficult owing to lack of expression of classical breast lineage markers.

METHODS

This was a cross-sectional study performed in department of Histopathology at Armed Forces Institute of Pathology (AFIP) Rawalpindi from November 2021 to November 2022 after approval from ethics committee [FC-HSP20-17/READ-IRB/21/1280] of Armed Forces Institute of Pathology. A total of 121 formalin fixed paraffin embedded (FFPE) tissue of cases having triple negative breast carcinoma and metastatic breast carcinoma were included in the study The sample size was close to the cases number taken by Ali S et al.[18] Patients' samples with any size, grade and stage of breast carcinoma were included. Those patients who were diagnosed with only carcinoma in situ, any degree of immunohistochemical expression of estrogen receptor, progesterone receptor, HER2 receptor, received chemotherapy, radiotherapy and relapsed patients were excluded. All patients' demographic data, tumor characteristics were confirmed at the time of sample receipt. Samples taken only as resection/lumpectomy/mastectomy (excisional) and trucut biopsy specimen (incisional) were examined. All cases were primarily stained with hematoxylin and eosin stains for confirmation of diagnosis and tumor characteristics by two histopathologists independently. After primary diagnosis staining for ER PR HER2 for establishing triple negative status and finally for SOX10 and GATA3 using Leica Bond III fully automated IHC staining system, was used. ER 6F11, PR EP2, HER2 EP3, SOX10 EP268 and GATA3 L50-823 antibody clones were used as per manufacturer's instructions. Immunohistochemical staining with subsequent dewaxing with xylene and rehydration with ethanol was performed. Antigen retrieval was done by heating the tissue sections at 100°C for 30 minutes in a citrate buffer. After keeping the sections at room temperature for 30 minutes in 5% bovine serum albumin, counter staining with hematoxylin followed by drying and mounting was done before microscopic examination.

Nuclear staining for SOX10 and GATA3 were assessed by two independent pathologists who were blinded to the clinical information of patients, for intensity and percentage of cells stained. SOX10 and GATA3 IHC stains were evaluated for the extent of invasive tumor cells with nuclear staining. Intensity of staining (SOX10 as weak=1, or strong = 2; GATA3 as weak = 1, moderate = 2 and strong = 3) and proportion score (<1%=0, 1-10% =1, 11-50%=2 and >50%=3). At least 1 % of tumor cells staining was regarded as positive. Combined scores for intensity and extent (proportion), with SOX10 scores ranging from 0 to 6 and GTA3 scores ranging from 0 to 9 were used.[19]

IBM Statistical Package for the Social Sciences version 25 is used for analysis of research data. Mean and standard deviation were calculated for quantitative variables. Percentage and frequency were used for qualitative variables like gender, grade, immunoexpression of SOX10 and GATA3 in triple negative breast carcinoma and metastatic breast carcinoma. Qualitative variables were compared using the Chi square test and a p-value of ≤0.05 was considered statistically significant.

RESULTS

This study was conducted on a sample size comprising blocks from 121 patients histologically diagnosed with invasive breast carcinoma with triple negative molecular profile, including 12 cases of metastatic breast carcinoma (nodal and extranodal metastasis). The mean age of the population was 48.45 ± 12.978 years. 118 (97.5%) patients were women and 3 (2.5%) were men. 89 (73.6%) tumors were <5 cm while 32 (26.4%) were >5 cm. 82 (67.8%) cases were Stage II, 26 (21.5%) were stage III while 13 (10.7%) were stage IV tumors. Of these, 38 (31.4%) carcinomas were declared grade 2 (moderately differentiated) and 83 (68.6%) were grade 3 (poorly differentiated). No grade 1 tumor included in this study as none presented with triple negative profile. Metastatic breast cases included comprised of 11 cases of triple negative profile and 1 case of pleomorphic lobular carcinoma of breast metastatic to bone. SOX10 was expressed in 18 (14.9%) of total cases while it was negative in 103 (85.1%) cases. SOX10 was expressed in 1 (2.63%) out of 38 Grade 2 carcinomas while it was positive in 17 (20.48%) out of 83 Grade 3 carcinomas. It was positive in 4 (33.33%) out of 12 metastatic breast carcinoma cases. GATA3 was positive in 99 (81.8%) cases while it was negative in 22 (18.2%) cases. It was expressed in 35 (92.10%) out of 38 grade 2 tumors and 64 (77.10%) out of 83 grade 3 tumors. GATA3 was positive in 8 (66.67%) out of 12 metastatic breast carcinoma cases. All cases expressing SOX10 were negative for GATA3. Combined SOX10/GATA3 expression was seen in 117 (96.69%) out of 121 total cases. These combined SOX10/GATA3 expressing tumors included those tumors expressing either SOX10, GATA3 or both. This combined expression of SOX10/GATA3 statistically imparts the role of combined use of these classic markers in diagnosis of such difficult entities thus signifying combined utility of these two novel markers for diagnosis of breast carcinoma cases. No statistically significant correlation was found between marker expression and with age of patient, tumor size, stage and grade. These statistical correlations are statistically signified in Table-I, II and III.

The association between grade of tumor and immunohistochemical expression of SOX10 and GATA3 was noted to statistically significant as p-value is 0.010 for SOX10 and 0.047 for GATA3 as highlighted in Table-II.

DISCUSSION

In the current histopathological era where immunohistochemical methods play a vital role in the diagnosis of malignant diseases TNBCs and metastatic breast cancer especially with negative hormonal and HER2 markers represent a diagnostic and therapeutic dilemma, especially if primary tumor is unknown. Another hurdle of such entities is that these hormonal receptors and proteins are used for targeted therapies and in their absence in these tumors currently available option is systemic chemotherapy leading to increased toxicity, multidrug resistance and mortality.[20] Currently these tumors are diagnosed on the basis of histology only with limited and often inconclusive diagnostic markers.[21]

SOX10						
Staining Pattern	Staining Percentage	Grade of Tumor		Total (n = 121) (%)	Chi-square	P-Value
		Grade 2 (n = 38) (%)	Grade 3 (n = 83) (%)			
Negative	<1%	37 (97.37%)	67 (80.72%)	104 (85.95%)	6.130	0.037
Patchy	1-10%	1 (2.63%)	10 (12.05%)	11 (9.09%)		
Focal	11-50%	0 (0%)	4 (4.82%)	4 (3.31%)		
Diffuse	>50%	0 (0%)	2 (2.41%)	2 (1.65%)		
Total (n = 121)		38 (31.40%)	83 (68.60%)	121 (100%)		
GATA3						
Staining Pattern	Staining Percentage	Grade of Tumor		Total (n = 121) (%)	Chi Square	P-Value
		Grade 2 (n = 38) (%)	Grade 3 (n = 83) (%)			
Negative	<1%	3 (7.89%)	17 (20.48%)	20 (16.53%)	3.177	0.370
Patchy	1-10%	4 (10.53%)	9 (10.84%)	13 (10.74%)		
Focal	11-50%	22 (57.89%)	42 (50.60%)	64 (52.89%)		
Diffuse	>50%	9 (23.68%)	15 (18.07%)	24 (19.83%)		
Total (n = 121)		38 (31.40%)	83 (68.60%)	121 (100%)		

Table-I. Frequency and statistical correlation of SOX10 and GATA3 staining pattern with grade of tumor (N=121).

Parameters		SOX10		Chi-square	P-value	GATA3		Chi-square	P-Value
		Positive	Negative			Positive	Negative		
Age (n = 121)	<50 yr	8	63	1.767	.184	61	10	1.939	.164
	>50 yr	10	40			38	12		
Grade (n = 121)	2	1	37	6.559	0.01	35	3	3.941	0.047
	3	17	66			64	19		
Stage (n = 121)	II	7	75	8.175	0.71	75	7	16.686	0.155
	III	7	19			15	11		
	IV	4	9			9	4		
Size (n = 121)	<5 cm	8	81	9.211	>0.05	80	9	14.731	>0.05
	>5 cm	10	22			19	13		
Metastatic disease (n = 12)		4	8	3.584	0.58	8	4	2.056	.152

Table-II. SOX10 and GATA3 expression with clinicopathological parameters

		SOX10 Expression		Total
		Negative	Positive	
GATA3 Expression	Negative	4 ((3.31%)	18 (14.88%)	22 (18.18%)
	Positive	99 (81.81%)	0 (0.00%)	99 (81.81%)
Total		103 (85.12%)	18 (14.88%)	121 (100%)

Table-III. Combined expression of SOX10 and GATA3 in invasive breast carcinoma and metastatic breast carcinoma. (n= 121).

GATA3 a classic marker for breast origin and it's utility is highlighted in reaching the primary origin of a tumor when utilized as diagnostic marker for breast carcinoma. However, many breast origin malignancies in particular TNBC do not express this classic marker leaving the avenue open search of accurate immunohistochemical marker or combination of markers for diagnosis such tumors. SOX10 which is routinely used for neural origin. It has been utilized for diagnostic and therapeutic purposes in TNBC and metastatic breast carcinoma.[22] Combined use of GATA3 and SOX10 in diagnosing breast origin malignancies have been studied in the recent past with promising results. However, it has not been researched sufficiently in our demographic population.

In current study, combined utility of SOX10 and GATA3 was assessed in TNBC and metastatic breast carcinoma cases. In view of the results SOX10/GATA3 when utilized in combination as a panel was found to be significantly expressed in TNBC and metastatic breast carcinoma cases with TNBC profile. However, it was also found that when used individually both markers have minimal utility in diagnosing such entities.

Tariq MU, et al in his study on the role of immunohistochemical expression of SOX10 for diagnosing TNBC and correlating with clinicopathological features deduced that SOX10 expression was observed in 58.3% of TNBC cases in particular tumors with tumor infiltrating lymphocytes (TILs) negative as compared to TILs positive. Thus, it was concluded that SOX10 has diagnostic utility in diagnosis of TNBC however it was advocated that its application is in combination with other breast specific markers. No correlation was established with the patients age, tumor size histological type, histological grade, T and N stage. In our study, SOX10 was expressed in 14.9% of TNBC cases and 33.33% of metastatic breast carcinoma cases. However, when utilized in combination with GATA3 this combined SOX10/GATA3 was expressed in 96.69% of the cases which is in line with above study.[23]

In a similar study carried out by Ali S, et al it was established that SOX10 is a reliable marker for diagnosis of TNBC and as a part of panel of immunohistochemical markers for diagnosis of tumors of unknown primary. These results are in accordance with our study.[18]

Chaiwat et al in a large cohort study comparing the expression of SOX10 with outer breast origin markers such as GATA3, mammoglobin and GCDFP15 concluded that combined SOX10/GATA3 can be used for differentiating metastatic breast from non-breast origin tumors. This study also established the role of SOX10/GATA3 combination for diagnosis of breast carcinoma especially TNBC category with metastasis.[24]

Lastly Qazi M et al., in his study emphasized the additional utility of combined use of SOX10 and GATA3 in identifying breast carcinoma cases in particular TNBC, low ER expression and with weak or patchy GATA3 expression. However, additional study was suggested for determination of extent of SOX10 utility and to improve specificity in breast cancer cases.[19]

Based on discussed studies and their correlation with our study it is pondered that there is a need for such studies to establish the role of immunohistochemical expression of SOX10 in addition to other classic markers for identification of breast cancer. Especially the combined use of SOX10 and GATA3 needs to be studied further in order to increase diagnostic utility in TNBC and metastatic breast cancer cases.

LIMITATIONS

This study was conducted in a single center, on a sample population limited to single demographic region with limited sample size. As such, the results of these findings may not be applied to the overall population in generalized consideration. Future studies in our population are required to determine the combined utility of these markers before they can be utilized in routine.

CONCLUSION

SOX10 and GATA3 in combination can serve as useful markers to establish diagnosis of breast carcinoma cases in patients lacking estrogen receptor, progesterone receptor, HER2 and metastatic breast carcinoma cases. Moreover, combined SOX10/GATA3 may serve as markers of aggressive disease. Future research should focus on diagnostic utility of these novel markers and targeted therapy modalities for such tumors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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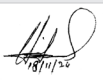
AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Muhammad Umair	Conception and study design, data collection, drafting of manuscript, interpretaion of results.	
2	Ahmed Ahson Khan	Conception and study design, data collection, drafting of manuscript, interpretaion of results.	
3	Nighat Jamal	Analysis and interpretation of data.	
4	Akhter Ali Bajwa	Drafting of manuscript and interpretation of data.	
5	Muhammad Umair Khan	Data analysis.	
6	Tabish Hassan	Data analysis.	