Original Article

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Pathology Section

Immunohistochemical Expression of Alpha Smooth Muscle Actin in Infiltrating Ductal Carcinoma of Breast and its Association with Histopathological and Hormonal Status: A Cross-sectional Study

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ABSTRACT

Introduction: Breast carcinoma is the second most common cancer and cause of death worldwide, with a mortality rate of 15%. Among various theories regarding the pathogenesis of cancer, the tumour microenvironment is known to play an essential role in cancer development and progression.

Aim: To determine the expression of alpha Smooth Muscle Actin (α-SMA) in infiltrating ductal carcinoma of breast carcinoma and its association with histopathological parameters and hormonal receptors status.

Materials and Methods: This was a cross-sectional observational study done in Department of Pathology, RL Jalappa Hospital, a tertiary care centre, Karnataka, India from January 2018 to December 2022. A total of 100 cases of Infiltrating Ductal Carcinoma confirmed by histopathological examination were included in the study. All the Haematoxylin and Eosin (H&E)-stained slides were screened for histological type, tumour grade, and nodal metastasis and Immunohistochemistry (IHC) markers like Oestrogen Receptor (ER), Progestrone Receptor (PR), Ki67, Human Epidermal growth factor 2 neu (HER2/neu) and α-SMA were performed. The p-value (Probability that the result is accurate) of <0.05 was considered stastically significant after assuming all the rules of stastistical tests.

Results: The majority (33%) of the subjects belonged to the age group 51-60 years. 54% of the subjects were positive for ER, 52% of the subject was positive for PR, and 30% of the subjects were HER2/neu. 53% of the subjects had >14% Ki67 and 47% of the subjects had <14% Ki67. According to molecular typing, 38% was luminal A, 20% was luminal B, Triple-Negative Breast Cancer (TNBC) was present in 30%, and HER2/neu positivity was seen in 12%. According to Nottingham Prognostics Index (NPI), patients were grouped into four categories according to the NPI score: I (excellent) was 15%; II (good) was 36%, III (moderate) was 32%; and IV (poor) was 17%.

Conclusion: SMA positivity can be used as an important prognostic factor in infiltrating ductal carcinoma as its stromal expression is associated with grade of the tumour and lymph node status.

Keywords: Breast carcinoma, Hormonal receptors expression, Molecular typing

INTRODUCTION

Breast Cancer (BC) is the commonest malignancy among women globally. It has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases [1]. Epidemiological studies have shown that the global burden of BC is expected to cross almost two million by the year 2030. It is the second most common cause of cancer worldwide, following lung carcinoma, and accounts for the second most common cause of death in women, with a mortality rate of 15% [1,2]. Similar trend is noted in India as well; however, in Indian women, breast carcinoma is the most common cause of cancer death and accounts for 11.1% of all cancer death [2].

According to the World Cancer Report 2020, the most efficient intervention for BC control is early detection and rapid treatment [3]. A systematic review published in 2018 reported that BC treatment costs increased with advanced cancer stage at diagnosis. On the contrary, earlier diagnosis of BC can lower the treatment costs [4].

Among various cancer pathogenesis theories, the tumour microenvironment is well known to play an essential role in cancer development and progression [5]. Cancer-Associated Fibroblasts (CAFs) like myofibroblasts, smooth muscle cells, endothelial cells, mesenchymal cells, and immune cells form this microenvironment. Carcinoma of breast, colon, and other solid tumours shows abundant myofibroblasts in the stroma [6]. These are directly associated with the induction of angiogenesis and release of growth factors contributing to tumour progression.

Vimentin, Desmin, Paladin 41 g, α-SMA, and E-cadherin are commonly used immunohistochemical markers for detecting myofibroblasts among which α-SMA is a well-established marker of myofibroblasts. However, the role of myofibroblasts in pathogenesis and treatment resistance has not been well established [7].

Despite tailored treatments protocol for carcinoma breast, recurrences and mortality are still high. Hence, this study has been taken-up to see the expression of α-SMA in CAFs of the tumour microenvironment in infiltrating ductal carcinoma and its relationship with other histopathological factors.

MATERIALS AND METHODS

This was a cross-sectional observational study and was conducted between January 2018 to December 2022 at RL Jalappa Hospital, tertiary care centre, Kolar, Karnataka, India. The study was conducted after obtaining ethical clearance from Institutional Ethics Committee (IEC) No. DMC/KLR/IEC/665/2022-23.

Inclusion criteria: Women with infiltrating ductal carcinoma (NOS) type who underwent modified radical mastectomy and confirmed by histopathological examination were included in the study.

Exclusion criteria: Women subjected to neoadjuvant radiotherapy/ chemotherapy before radical mastectomy, recurrent tumours, women who received chemotherapy for other cancer over the past five years and diagnosed male BC patients were excluded from the study.

Study Procedure

After anonymising the patient's demographic details, clinical information, tumour size, and axillary lymph node status, the paraffin blocks and slides were collected from archives of the department for performing IHC (α-SMA). All H&E-stained slides were screened for histological type, tumour grade which was done by Nottingham-Bloom-Richardson (NBR) histologic grading system and staging done by American Joint Committee on Cancer (AJCC) staging system 8th edition [8,9].

Appropriate slides were selected for α-SMA IHC using appropriate positive control (Leiomyoma uterus) and negative control. Antigen detection in tissues and cells was detected by a multi step process using the peroxidase-antiperoxidase method. Positivity in the cytoplasm of tumour cells were considered positive and scored as follows: Score 0 - Less than 10% of the stromal cells. Score-1- 11-50% of the stromal cells. Score-2- 51-80% of the stromal cells. Score-3- >80% of stromal cells showing positivity. Intensity was scored as Score-0-no staining, Score-1-weak staining, Score-2-moderate staining and Score-3-strong staining [10].

All the slides stained with ER and PR and HER-2/neu, Ki67 (<14%- low and >14%-high) [11] were retrieved from the archives from the IHC lab, and scoring was done as per the College of American Pathologists-American Society of Clinical Oncology (CAP-ASCO) [12].

STATISTICAL ANALYSIS

Data was entered into a Microsoft Excel data sheet and analysed using Statistical Package for the Social Sciences (SPSS) version 22.0 version software. Categorical data was represented in the form of frequencies and proportions. The Chi-square test or Fischer's-exact test (for 2×2 tables only) was used as a test of significance for qualitative data. Continuous data were represented as mean and standard deviation. ANOVA was used as a significance test to identify the mean difference between more than two quantitative variables.

The p-value (Probability that the result is accurate) of <0.05 was considered statistically significant after assuming all the rules of statistical tests.

RESULTS

One hundred cases of infiltrating ductal carcinoma diagnosed on histopathology. The majority 33% of the subjects belong to the age group 51-60 years followed by the 41-50 years age group, which was 23%; less than 40 years was 20%, 61-70 year was 13%, and more than 70 years was 11%. The majority, 62%, had a tumour size between 2-5 cm, followed by 29% with a tumour size >5 cm, and only 9% of the tumour had a size <2 cm [Table/Fig-1].

On histopathology Tumour Grade-1 was present in 58% of the subjects. The extranodal extension was seen in 89% of the subjects, and 50% of the subjects had positive lymph nodes. The majority of 50% of subjects had Stage-II, Stage-III was present in 42% of the subjects, Stage-I was seen in 7%, and Stage-IV was seen in only one percent [Table/Fig-1]. A 54% of the subjects were positive for ER, 52% were positive for PR, and 30% were Her2/neu. A 53% of the subjects had >14% Ki67 and 47% of the subjects had <14% Ki67. According to molecular typing, 38% was Luminal A, 20% was Luminal B, TNBC was present in 30%, and HER2+ was seen in 12% [Table/Fig-2].

Variables		Frequency	%
Age group (years)	28-40	20	20.0
	41-50	23	23.0
	51-60	33	33.0
	61-70	13	13.0
	>70	11	11.0
Size (cms)	T1 (<2)	9	9.0
	T2 (2-5)	62	62.0
	T3 (>5)	29	29.0
Tumour grade	I	58	58.0
	II	30	30.0
	III	12	12.0
Lymph node	Negative	50	50.0
	Positive	50	50.0
Extra nodal extension	Present	89	89.0
	Absent	11	11.0
Stage	I	7	7.0%
	II	50	50.0%
	III	42	42.0%
	IV	1	1.0%

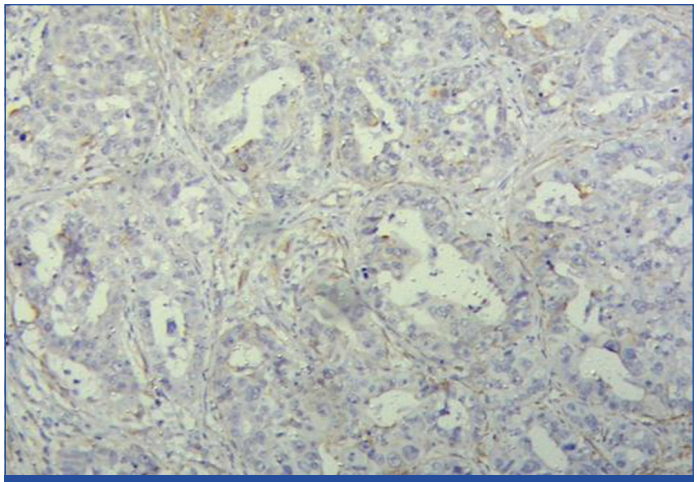
[Table/Fig-1]: Clinical and pathological features of the study subjects.

Variables		N	%
ER	Negative	46	46.0
	Positive	54	54.0
PR	Negative	48	48.0
	Positive	52	52.0
Her2/neu	Negative	70	70.0
	Positive	30	30.0
Ki67	<14 %	47	47.0
	>14%	53	53.0
Molecular typing	HER2+	12	12.0
	Luminal A	38	38.0
	Luminal B	20	20.0
	TNBC	30	30.0
Tumoural α-SMA score	Score-0	28	28.0
	Score-1	43	43.0
	Score-2	23	23.0
	Score-3	6	6.0

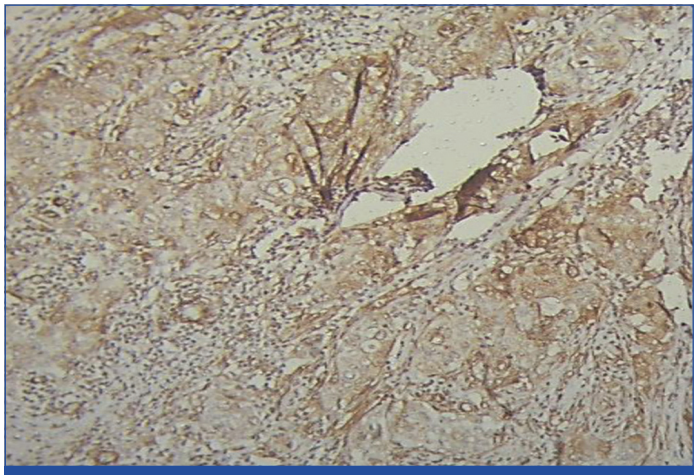
[Table/Fig-2]: Hormonal receptors expression, molecular typing and tumoural alpha SMA (α-SMA) score.

On IHC of tumoural α-SMA majority of the cases showed Score-1 (43%) followed by Score-0, Score-2 and Score-3 [Table/Fig-3-6]. Patients were grouped into four categories according to the NPI Score-1 (excellent) was seen in 15% of cases followed by Score-2 (good) in 36%, Score-3 (moderate) in 32% and Score-4 (poor) in 17% of cases, respectively.

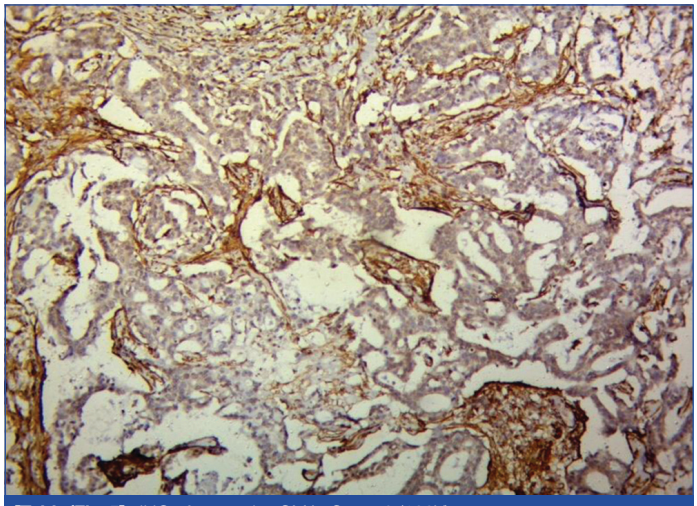
A statistically significant difference was found between tumoural α-SMA and tumour grade [Table/Fig-7]. A statistically significant difference was found between tumoural α-SMA and lymph node status of p<0.05. No statistically significant difference was found between tumoural α-SMA and hormonal receptors, molecular typing [Table/Fig-8].



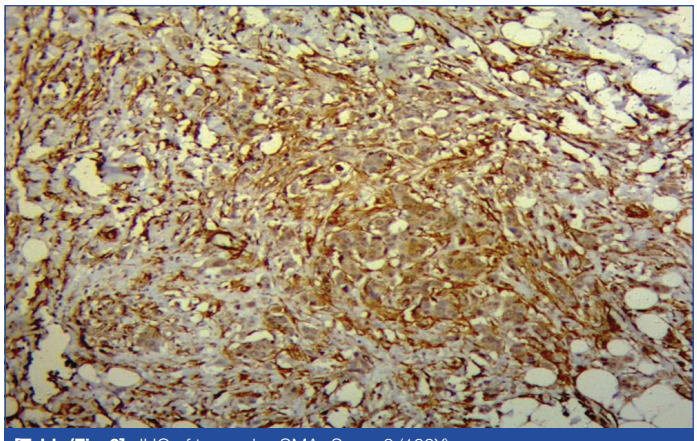
[Table/Fig-3]: IHC of tumoural alpha SMA (α-SMA) -Score 0 (100X).



[Table/Fig-4]: IHC of tumoural α-SMA -Score 1 (100X).



[Table/Fig-5]: IHC of tumoural α-SMA -Score 2 (100X).



[Table/Fig-6]: IHC of tumoural α-SMA -Score 3 (100X).

Variables		No staining N	Weak N	Moderate N	Strong N	p-value
Size (cms)	T1 (<2)	2	5	2	0	0.793
	T2 (2-5)	16	29	13	4	
	T3 (>5)	10	9	8	2	
Tumour grade	I	11	25	19	3	0.002
	II	8	16	3	3	
	III	9	2	1	0	
Lymph node	Negative	13	17	18	2	0.018
	Positive	15	26	5	4	
Stage	I	2	1	4	0	0.158
	II	12	20	13	5	
	III	13	22	6	1	
	IV	1	0	0	0	

[Table/Fig-7]: Association of tumoural alpha SMA (α-SMA) and clinicopathological features.

Variables		No staining N	Weak N	Moderate N	Strong N	p-value
ER	Negative	11	23	7	5	0.069
	Positive	17	20	16	1	
PR	Negative	10	24	9	5	0.091
	Positive	18	19	14	1	
Her2/neu	Negative	19	31	15	5	0.823
	Positive	9	12	8	1	
Ki67	<14 %	12	21	13	1	0.347
	>14%	16	22	10	5	
Molecular typing	HER2+	3	5	3	1	0.298
	Luminal A	10	15	12	1	
	Luminal B	8	7	5	0	
	TNBC	7	16	3	4	

[Table/Fig-8]: Association of tumoural alpha SMA (α-SMA) and hormonal receptors, molecular typing.

DISCUSSION

The IHC is used to characterise intracellular proteins or various cell surfaces in all tissues. Individual markers or panels of various markers can be used to characterise various tumour subtypes, confirm tissue of origin, distinguish metastatic from the primary tumour and provide additional information which may be necessary for diagnosis, prognosis, predicting response to therapy, or evaluating residual tumour post-treatment [10].

A growing list antibodies and antigen retrieval techniques will contribute to the broader utility of IHC for solving diagnostic problems or determining prognosis and response to therapy in breast pathology. The most common myoepithelial marker in breast pathology diagnosis is SMA which has been used as a sensitive marker for myoepithelial differentiation. This is because any cell with a substantial expression of actin is positive for SMA (myofibroblasts and blood vessels are positive for SMA) [10].

Fibroblast synthesises extracellular matrix, collagen fibres, glycoproteins, and glycosaminoglycans that constitute the stroma. Fibroblasts greatly influence the tumour microenvironment in malignancies by synthesising Vascular Endothelial Growth Factor A

(VEGF-A) and C-X-C Motif chemokine ligand 12 (CXCL12). Because of these properties, fibroblasts form another subpopulation of hyperactivated fibroblasts called CAF. CAF are highly heterogeneous and enhance cell migration and metabolism of epithelial tumour cells. The most commonly used immunohistochemical marker for CAF is α-SMA which detects the expression of SMA in tumour stroma [13]. SMA belongs to group of proteins that play an essential role in cell motility, structure, integrity, and wound repair. It is an actin isomer in smooth muscle cells, myofibroblasts, and blood vessels. Its expression correlates with the activation of fibroblast to myofibroblast. Contractile properties of this myofibroblast are known to be associated with increased expression of α-SMA. Carcinoma cells that transform into mesenchymal cells are also known to express α-SMA [14-16].

A study by Catteau X et al., showed a Poor disease-Free Survival (PFS) rate in the group of node-negative tumours with strong SMA stromal expression. SMA has long been used as a myoepithelial marker in breast pathology diagnosis and as a sensitive marker of myoepithelial differentiation, even if it is not specific, because any cell with a tangible expression of actin is positive for SMA (myofibroblasts and blood vessels are positive for SMA). The importance of changes in the tumoural microenvironment during tumour progression is being increasingly recognised [7].

In present study a statistically significant association between tumoural α-SMA and tumour grade was found. Also, a statistically significant association was found between tumoural α-SMA and lymph node status. No significant association was observed between tumoural α-SMA and other clinicopathological features. In a study by Catteau X et al., the myofibroblastic stromal reaction was statistically more significant in tumour Grade-2 and 3 (p-value=0.029). The strong myofibroblastic action was found in 51.5%, 61% and 24% of tumour Grades-3, 2, and 1, respectively. There was no significant relationship observed between SMA stromal expression and other clinicopathological features [7]. Julia T et al., reported that fibroblast activation protein expression in BC stroma is heterogeneous and may correlate with clinicopathologic parameters such as size, grade, axilla nodal involvement, and tumour histological types, especially TNBC. So, fibroblast activation protein may represent a potentially targetable therapy, especially for tumour's that lack targeted therapy, such as TNBC [17].

Henry LR et al., concluded that fibroblast activation protein and α-SMA expressing CAFs in the tumour margin are of importance during the early invasion and metastasis, high fibroblast activation protein expression and positive α-SMA expression in the tumour margin are correlated with small tumour size, low lymph node metastasis, and low histological grade cases. Once the invasive carcinoma is established, other factors affect clinical outcomes [18].

In present study, 54% of the subjects were positive for ER, 52% of the subjects were positive for PR, and 30% of the subjects were Her2/neu. A 53% of the subjects had >14% Ki67 and 47% of the subjects had <14% Ki67. In a study done by Dayal A et al., showed out of 80 cases, 69 (86%) cases were of infiltrating ductal carcinoma with a mean age of 53. ER and PR expression was seen in 56.9% and 35.5%, respectively. ER, PR expressing tumours tended to have lower Grade-1 with a significant p-value of 0.05. Lymph node metastases were found to be significantly associated with PR-positive status with a p-value of 0.03 [19]. According to Molecular typing, 38% were Luminal A followed by TNBC 30%, Luminal B 20% and Her2/neu positivity was seen in 12%. According to Tamiolakis D myoepithelial differentiation was determined by α-SMA expression. They concluded that infiltrating ductal carcinoma with diffuse fibrosis is associated with a myoepithelial immunophenotype of carcinoma cells [20]. In this study no statistically, significant association was found between tumoural α-SMA and hormonal receptors, molecular typing was in concordant with the study done by Catteau X et al., [7].

Limitation(s)

This was a unicentric study and there was no follow-up of the patients. The criteria for scoring of the expression of α-SMA in breast carcinoma are not well defined in the literature. Due to this the results may vary from other studies.

CONCLUSION(S)

Myofibroblasts can be an important prognostic factor for a poor clinical prognosis as stromal alpha smooth muscle expression showed association with grade and lymph node status. Thus, patients with a high level of myofibroblasts (or their marker, α-SMA) should be considered for more aggressive treatments and more frequent monitoring for the development of metastatic disease.

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