Immunoh is to Chemical Expression of Alpha Smooth Muscle Actin in Stromaof Infiltrating Ductal Carcinoma of Breast and its Association with Histopathologicaland Hormonal Factors

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Abstract:Background: Breast carcinoma (BC) issecond most prevalent form of cancer globally with infiltrating ductal carcinoma (IDC)being the most frequenthistologicalsubtype.Most hypotheses on cancer development emphasize the significance of the tumor microenvironment, namely the existence of cancer-associated fibroblasts, in the initiation and advancement of cancer. Myofibroblasts may be identified by employing an Immunohis to chemical marker called Alpha smooth muscle actin (α-SMA). **Objectives:** To determine the proportion of Alpha SMA in the stroma of Infiltrating ductal carcinoma of the breast and to correlate it with histopathological and hormonal expression. **Materials and Methods:** In this laboratory based observational study100 cases of IDC NOS were included. Following the immunohistochemistry analysis of Alpha SMA, the demonstration of SMA in stroma was examined in relation to various prognostic factors including age, histological grading, lymph node status, extra nodal extension, tumor staging, hormonal expression, and molecular subtype of the tumor. The study conducted descriptive statistics and evaluated the statistical significance of the difference between the two groups. The Chi-square test was used to evaluate categorical data. A p-value that is less than 0.05 was taken as statistically significant. **Results:** Overall, 51-60 age groups werethe majority of cases, accounting for 33% of the total. The majority of cases had tumor sizes ranging from 2 to 5 cm, accounting for 62% of the subjects. Additionally, Grade I tumorshave been identified in 58% of the cases. A large proportion of cases exhibit positive expression of Estrogen receptor (ER) and Progesterone receptor (PR), with corresponding percentages of 54% and 52%. Majority of the patients were classified as Luminal type A, with TNBC accounting for 38% and 30% of the cases, respectively. 12 patients had low stromal expression (score 0 and score 1) of the alpha-smooth muscle actin, whereas the remaining 88 cases showed strong expression (score 2 and score 3). The cases showed statistical significance in tumor grade, lymph node status, ER, PR, & Molecular typing. The elevated stromal expression of Alpha-SMA showed a statistically significant association with tumor grade, lymph node status, ER, PR, and molecular type. **Conclusion:** The presence of SMA positive might serve as a possible prognostic indicator, indicating a worse prognosis. This is because its stromal expression is closely associated with the histological grading of tumor and the genetic subtyping of TNBC.

Keywords: Alpha smooth muscle actin, InfiltratingDuctal Carcinoma, Hormonal expression, and Cancer associated fibroblast.

Introduction:

Recently, breast cancer has surpassed cervical carcinoma as the primary cause of malignancy in our country. The five-year overall survival rates for patients with Stages I, II, III, and IV are 95%, 92%, 70%, and 21% correspondingly. [1]The function of several biomarkers, including ER, PR, Her2 neu, Ki 67, as well as genetic variables such as BRCA mutations and p53 mutations, has been thoroughly researched and documented. However, role of tumor microenvironment in these malignancies has not received as much attention.

Tumor microenvironment consists of several cell types, including cancer-associated fibroblasts (CAFs) such as myofibroblasts, smooth muscle cells, endothelial cells, mesenchymal cells, and immune cells. These cells play an active role in promoting cancer growth. [2] The survival rate of BCiscomparatively lower in India than that in western nations. This discrepancy arises due to variables such as an earlier onset, manifestation in more advanced stages, and delayed or inadequate therapy. [3]Consequently, the early identification of breast cancer may help reduce treatment costs. [4]

The tumor microenvironment is a crucial element in the progression and advancement of cancer, and it is widely acknowledged in several hypotheses about the origins of cancer. [5]The stroma of breast, colon, and other solid tumors has a significant presence of myofibroblasts. These elements are directly linked to the stimulation of blood vessel formation and the production of substances that promote the advancement of tumors. [6] Vimentin, Desmin, Paladin 41g, Alpha SMA, and Cadherin 11 frequently serve asimmunohistochemical markers for identifying myofibroblasts. α-SMAiswidelyacknowledged and well-establishedmarker for myofibroblasts. Nevertheless, the precise contribution of myofibroblasts to the development of disease and their resistance to therapy remains unclear. [5,6]

Although there are specific treatment protocols for breast cancer, the rates of recurrence and death remain elevated. Therefore, this research aims to examine the presence of CAFs in IDCstroma and investigate its correlation with other parameters.

Objectives:

1. To determine the expression of alpha SMA in the stroma of Infiltrating ductal carcinoma of breast carcinoma.
2. To correlate the expression of alpha SMA in the stroma of Infiltrating ductal breast carcinoma with histopathological and hormonal expression.

Material and Methods

This study was conducted at the Laboratory of the Department of Pathology, using a cross-sectional observational research design. The study included all cases of patients

diagnosed with Infiltrating Ductal Breast Cancer who sought medical care at the Department of Surgery and Department of Surgical Oncology between January 2021 and November 2022.

According to research conducted by Catteau X et al, the prevalence of the condition was found to be 46%. To achieve a 95% confidence interval and 10% absolute error, a sample size of 100 was necessary for this investigation. [7]

Female patients diagnosed with IDC NOS subtype who have had modified radical mastectomy. Female individuals who have had neoadjuvant radiotherapy/chemotherapy prior to radical mastectomy, individuals with recurring tumors, those who have received chemotherapy for a different kind of cancer within the last five years, and male individuals diagnosed with breast cancer.

Study Course-Methodology:

Prior to commencing the research, the necessary ethical approval was obtained from the relevant institutional authorities. Obtaining informed permission was required for all prospective instances, whereas it was not necessary for retroactive situations. Following the normal process, the specimens were grossed and tissue pieces were extracted from the relevant locations. The tissue was treated according to the established procedure and slices stained with Haematoxylin & Eosin were examined. The demographic information of the patients in retrospective instances wereanonymised.

The Department of Pathology's archives were accessed to gather clinical information, tumordimensions, axillary lymph node status, paraffin blocks, and H&E slides. The H&E slides were examined to determine the histological type, histological grading, nodal status, extranodal extension, and hotspot areas were selected to calculate the score of tumor cells on immunohistochemistry.

Immuno Histochemical Interpretation:

Immunohistochemistry was conducted using the HRP technique to detect the presence of Alpha-SMA. The specific isoform of actin used was the N-Terminal decapeptide of the Alpha-Smooth muscle isoform, which was conjugated to KLH 1A4 Mouse by Diagnostic Biosystems. Positive and negative controls were run simultaneously.There were no research articles available in the English literature for scoring of the staining pattern of SMA. **Hence,a new scoring system was made by evaluating the intensityofthe staining of cytoplasm of stromal cells and is as follows:**

Table 1- Demonstrates IHC Scoring Used in This Study:

Score		Intensity Of Cytoplasmic Staining Of Stromal Cells
Low Expression	0	<10%
	1+	11-50%
High Expression	2+	51-80%
	3+	>80%

Score 0&1 have beenclassified as Low Expression and Score 2 & 3 have been classified as high expression.

All the slides stained with Estrogen Receptor (ER), Progesterone Receptor (PR), HER-2/neu and Ki67 were reviewed and scoring was done as per the CAP-ASCO (College of American Pathologists -American Society of Clinical Oncology).

Statistical Analysis:

The data input was performed using Microsoft Excel, and the statistical analysis was conducted by utilising the Statistical Package for the Social Sciences (SPSS) Version 22 for Windows operating system. We performed a descriptive statistical study to examine the distribution of various categorical and quantitative variables. Categorical data were summarized using frequency (n) and percentage (%), whereas quantitative variables were described using mean ± standard deviation (S.D). Findings were shown in a tabular format. The statistical significance of the difference between the two groups was assessed using a chi-square test for categorical variables. p-value<0.05 was deemed statistically significant.

Figure 1- Invasive Ductal Carcinoma Breast (A) Histological Grade 1 (B) Histological Grade 2 (C)Histological Grade 3 (H & E)-400X:

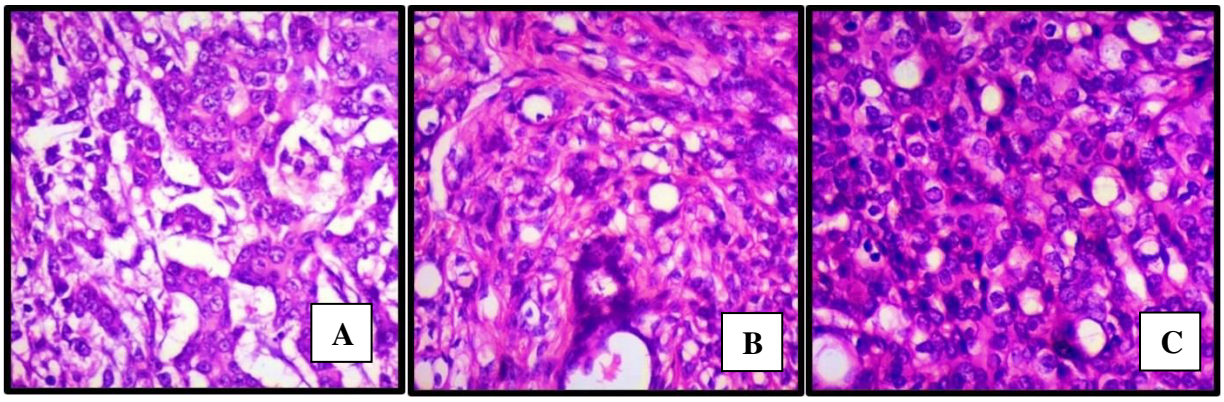


Figure 2- Alpha SMA Control-Leiomyoma-Showing Strong Cytoplasmic Positivity-400x:

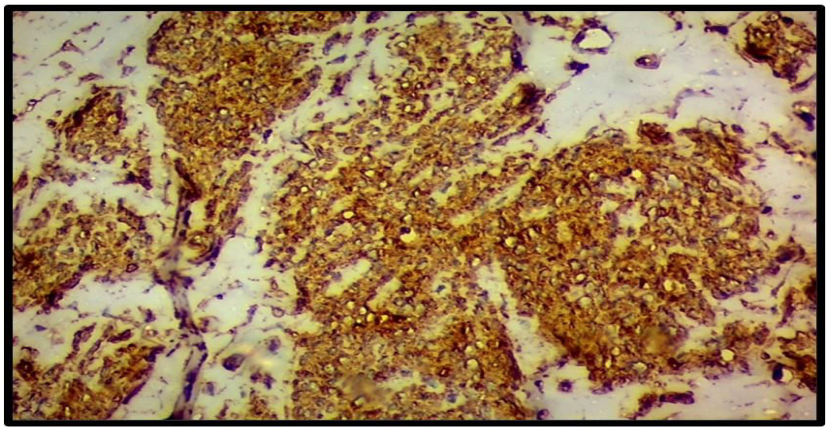
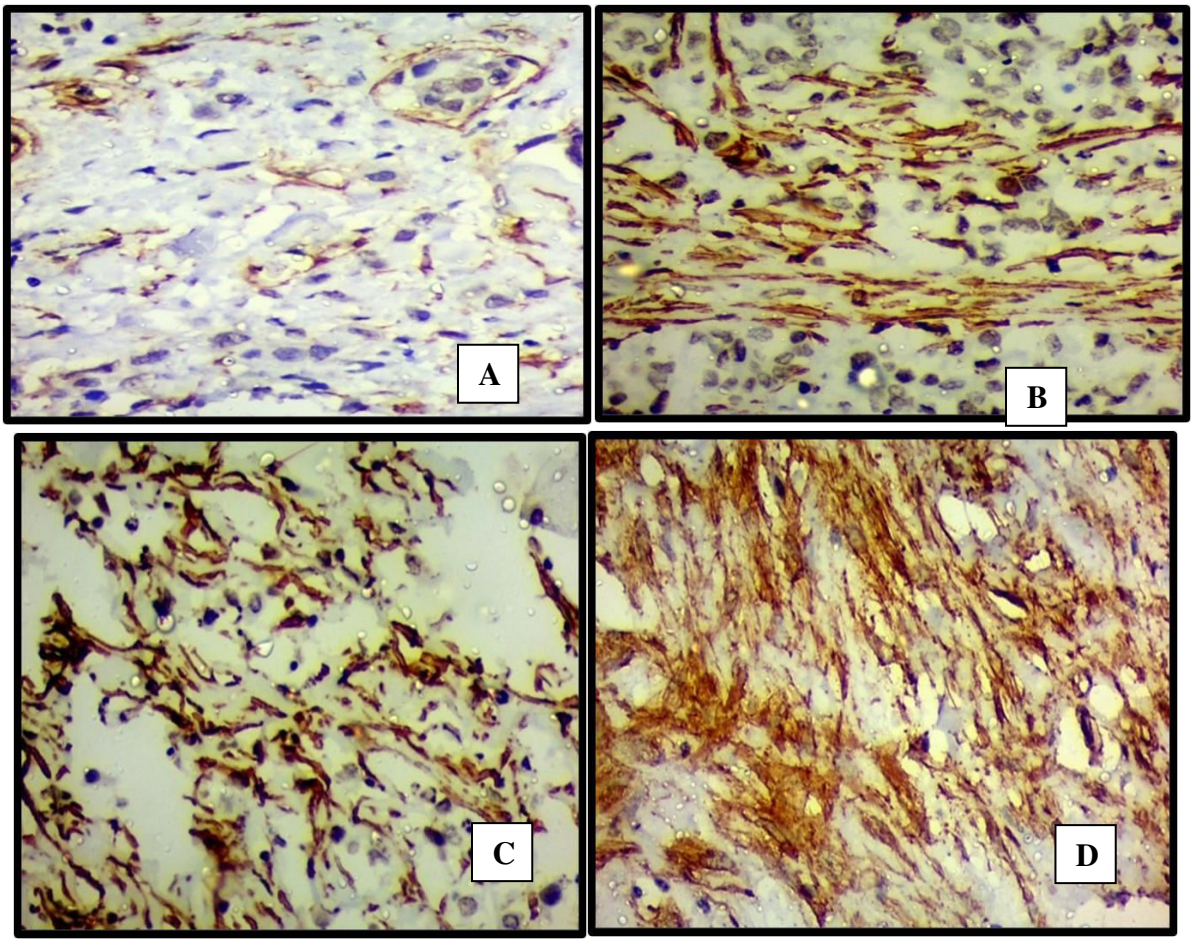


Figure 3-IHC Scoring OF Cytoplasmic StainingOf Alpha-SMA– 40



(A)Cytoplasmic positivity in stromal cells <10 % - Score 0); (B) Cytoplasmic positivity in stromal cells 11-50% Score 1; (C) Cytoplasmic positivity in stromal cells 51-80% Score 2; (D) Cytoplasmic positivity in stromal cells >80% Score 3

Results & Observations:

(A)Cytoplasmic positivity in stromal cells <10 % - Score 0); (B) Cytoplasmic positivity in stromal cells 11-50% Score 1; (C) Cytoplasmic positivity in stromal cells 51-80% Score 2; (D) Cytoplasmic positivity in stromal cells >80% Score 3

Table 2- Patients are Categorized Based on the Stromal Alpha SMA Score:

Stromal Alpha SMA Score		**Intensity Of Stromal Cells Staining**	**n**	**%**
Low Expression	**0**	No Staining	3	3%
	1	Weak Staining	9	9%
High Expression	**2**	Moderate Staining	32	32%
	3	Strong Staining	56	56%

Stromal alpha SMA staining was not seen in 3% of patients, weak staining was seen in 9%, whereas moderate and strong staining was seen in 32% and 56% of subjects respectively.

Table 3- Represents Low and High Expression of SMA was Compared with all the Histopathological (Tumor Size, Tumor Grading, Nodal Status, Extra Nodal Extension, Tumor Staging, NPI Score), And Hormonal Parameters (ER, PR, Her 2 Neu, Ki-67) and Molecular Types:

Stromal Alpha Score		**N**	**Low Expression (0&1)**	**High Expression (2&3)**
Age Group	**28-40 YEARS**	20	4	16
	41-50 YEARS	23	2	21
	51-60 YEARS	33	5	28
	61-70 YEARS	13	1	12
	>71 YEARS	11	0	11
Tumor Size (Cm)	**T1 (<2 cms)**	9	1	8
	T2 (2-5 cms)	62	5	57

	T3 (>5 cms)	29	6	23
Tumor Grading	**I**	58	6	52
	II	30	4	26
	III	12	2	10
Nodal Status	**Positive**	50	5	45
	Negative	50	7	43
Extra Nodal Extension	**Present**	11	1	10
	Absent	89	11	78
Tumor Staging	**I**	7	1	6
	II	50	7	43
	III	42	4	38
	IV	1	0	1
Npi Score	**Excellent**	15	1	14
	Good	36	4	32
	Moderate	32	5	27
	Poor	17	2	15
Er	**Positive**	54	3	51
	Negative	46	9	37
Pr	**Positive**	52	1	51
	Negative	48	11	37
Her 2 Neu	**Positive**	30	5	25
	Negative	70	7	63
Ki-67	**<14 %**	47	4	43
	>14%	53	8	45
Molecular Typing	**Luminal A**	38	3	35
	Luminal B	20	1	19
	Her2 Enriched	12	4	8
	TNBC	30	4	26

The age group with the highest number of patients was 51 to 60 years, with 33 cases, followed by the age group of 41 to 50 years, with 23 cases. The majority of the tumors measured between 2 to 5 cm (62 instances) and had a histological Grade 1 tumor (58 cases). 50% of cases had lymph node metastases, whereas 11 individuals showed extra nodal extension. The majority of cases (50 patients) were classified as TNM Stage II. The majority of the cases had a favourable NPI score, with 36 patients. The Luminal type A was the predominant molecular subtype in comparison to the others.

Table 4-Distribution Of Subjects Based On Age & Stromal Alpha SMAScore:

Stromal Alpha SMA Score		0	1	2	3	p-Value
Age Group						
28-40 yrs	n	0	4	6	10	0.20
	%	0%	44.4%	18.8%	17.9%	
41-50 yrs	n	1	1	10	11	
	%	33.3%	11.1%	31.3%	19.6%	
51-60 yrs	n	2	3	11	17	
	%	66.7%	33.3%	34.4%	30.4%	
61-70 yrs	n	0	1	1	11	
	%	0%	11.1%	3.1%	19.6%	
>71 yrs	n	0	0	4	7	
	%	0%	0%	12.5%	12.5%	

The highest percentage of patients were between ages of 51 to 60 years [33%], followed by 41 to 50 years [23%], 28 to 40 years [20%], 61 to 70 years [13%], and above 71 years [11%]. Stromal staining of Strong Alpha SMA was seen in 30.4% of individuals aged 51-60 years, followed by 19.6% in the age groups of 41-50 and 61-70 years. The correlation between age and stromal alpha SMA score was shown to be statistically insignificant, as indicated by a p-value of 0.20.

Table 5- Distribution of Subjects Based on Tumor Size, Nodal Status, Extra Nodal Extension, TNM Staging & Tumor Grading Stromal Alpha SMA Score:

Stromal Alpha SMA Score			0	1	2	3	p-Value
Tumor Size (Cm)	T1 (<2cms)	n	0	1	1	7	0.20
		%	0%	11.1%	3.1%	12.5%	
	T2 (2-5 cms)	n	2	3	25	32	
		%	66.7%	33.3%	78.1%	57.1%	
	T3	n	1	5	6	17	

	(>5 cms)	%	33.3%	55.6%	18.8%	30.4%	
Total		**n**	**3**	**9**	**32**	**56**	
		%	**100%**	**100%**	**100%**	**100%**	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-value**
Lymph Node Status	**Positive**	**n**	2	3	18	27	**0.05**
		%	66.7%	33.3%	56.3%	48.2%	
	Negative	**n**	1	6	14	29	
		%	33.3%	66.7%	43.8%	51.8%	
Total		**n**	**3**	**9**	**32**	**56**	
		%	**100%**	**100%**	**100%**	**100%**	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
Extra Nodal Extension	**Positive**	**n**	0	1	2	8	0.63
		%	0%	11.1%	6.3%	14.3%	
	Negative	**n**	3	8	30	48	
		%	100%	88.9%	93.8%	85.7%	
Total		**n**	3	9	32	56	
		%	**100%**	**100%**	**100%**	**100%**	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
Tumor Staging	**I**	**n**	0	1	2	4	**0.05**
		%	0.0%	11.1%	6.3%	7.1%	
	II	**n**	2	5	15	28	
		%	66.7%	55.6%	46.9%	50.0%	
	III	**n**	1	3	15	23	
		%	33.3%	33.3%	46.9%	41.1%	
	IV	**n**	0	0	0	1	
		%	0.0%	0.0%	0.0%	1.8%	
Total		**n**	3	9	32	56	
		%	100.0%	100.0%	100.0%	100.0%	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
Tumor Grading	**I**	**n**	1	5	24	28	
		%	33.3%	55.6%	75.0%	50.0%	
	II	**n**	2	2	6	20	
		%	66.7%	22.2%	18.8%	35.7%	
	III	**n**	0	2	2	8	

		%	0.0%	22.2%	6.3%	14.3%	**0.021**
Total		**n**	3	9	32	56	
		%	100.0%	100.0%	100.0%	100.0%	

Tumor size was less than 2 centimeters in 9% of patients, 2 to 5 centimeters in 62% of patients, and more than 5 centimeters in 29% of patients. Strong Stromal staining of Alpha SMA with a tumor size of<2 cms was 12.5%, 2 to 5 cm in 57.1% and >5 cm in 30.4% of patients. The association between tumor size and stromal alpha SMA score was not statistically significant.

Among patients with strong staining alpha SMA score, Lymph node status was positive in 48.2%. The association between Lymph node status and stromal alpha SMA score was statistically significant with a p-value of 0.05. Lymph node negativity showed more SMA which may suggest the possibility of early metastasis.

Stromal staining of alpha SMA was strong in 8 out of 11 patients with Extra nodal extension that is 73 %. The association between Extra nodal extension and stromal alpha SMA score was found to be statistically not significant.

7% of patients had a Stage I tumor, 50% had a Stage II tumor, 42% had a Stage III tumor, and 1% had a Stage IV tumor. Alpha SMA staining had high intensity in Stage I tumor, seen in 7.1% of patients. In Stage II, it was detected in 50% of patients. For Stage III and Stage IV, the staining was found in 41.1% and 1.8% of patients, respectively. The correlation between tumor stage and stromal alpha SMA score was shown to be statistically significant, with a p-value of 0.05.A higher SMA score was seen in Stage II tumors, indicating an inverse correlation between the two.

Half of the patients with Histological Grade I had a robust SMA stromal score, whereas 35.7% of subjects with Grade II & 14.3% with Grade III showed the same. The correlation between tumor grade and stromal alpha SMA score was shown to be statistically significant, with a p-value of 0.021. The majority of the participants were in Grade II and had a higher SMA score.

Table 6- Patients were Categorized by ER, PR, Her2 Neu, Ki67 Status, and Stromal Alpha SMA Score:

Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
ER	**Positive**	**n**	0	3	19	32	**0.02**
		%	0%	33.3%	59.4%	57.1%	
	Negative	**n**	3	6	13	24	
		%	100%	66.7%	40.6%	42.9%	

	Total	**n**	3	9	32	56	
		%	100%	100%	100%	100%	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
PR	**Positive**	**n**	0	1	19	32	**0.01**
		%	0%	11.1%	59.4%	57.1%	
	Negative	**n**	3	8	13	24	
		%	100%	88.9%	40.6%	42.9%	
	Total	**n**	3	9	32	56	
		%	100%	100%	100%	100%	
Stromal Alpha SMA Score			**0**	**1**	**2**	**3**	**p-Value**
Her 2 Neu	**Positive**	**n**	1	4	10	15	0.75
		%	33.3%	44.4%	31.3%	26.8%	
	Negative	**n**	2	5	22	41	
		%	66.7%	55.6%	68.8%	73.2%	
	Total	**n**	3	9	32	56	
		%	100%	100%	100%	100%	
Ki67	**<14 %**	**n**	0	4	15	28	**p-Value**
		%	0%	44.4%	46.9%	50.0%	
	>14%	**n**	3	5	17	28	0.40
		%	100%	55.6%	53.1%	50%	
	Total	**n**	3	9	32	56	
		%	100%	100%	100%	100%	

ER & PR was positive in 57.1% of patients with strong stromal alpha SMA score. ER status and PR status were found to be statistically significant with stromal alpha SMA scoreshowing a p-value of 0.02 and 0.01 correspondingly.

Her 2 neupositivity was seen in 26.8% and were showing strong alpha stromal score. There was no statisticalsignificance noted between these two.Ki67 was <14% in 50% of patients and >14% in 50% of patients and showed no statistical significance with stromal alpha SMA.

Stromal expression of SMA was more pronounced in those with ER and PR positive. Subjects that tested negative for Her2 Neu had higher levels of SMA expression compared

to those who tested positive. Stromal manifestation of SMA was more pronounced in Ki67>14%.

Table 7- Patients were Grouped Based on the Molecular Typing & Stromal Alpha SMA Score:

Stromal Alpha SMA Score			0	1	2	3	p-Value
Molecular Typing	Luminal A	n	0	3	13	22	0.043
		%	0%	33.3%	40.6%	39.3%	
	Luminal B	n	0	1	7	12	
		%	0%	11.1%	21.9%	21.4%	
	Her2 Enriched	n	1	3	3	5	
		%	33.3%	33.3%	9.4%	8.9%	
	TNBC	n	2	2	9	17	
		%	66.7%	22.2%	28.1%	30.4%	
	Total	n	3	9	32	56	
		%	100%	100%	100%	100%	

Alpha SMA score was strong amongmoleculartypeswith 39.3% of 3+ in Luminal A cases, 21.4% of Luminal B, 8.9% of HER2 NEU Enriched and 30.4% of patients with TNBC. There was a significant correlation with Molecular typing and stromal alpha SMA scorewith a p-value of 0.043. Luminal A followed by TNBC exhibited higher degree of SMA positivity.

Table 8- Distribution of Patients based on the NPI& Stromal Alpha SMA Score:

Stromal alpha SMA score			0	1	2	3	p-Value
NPI in Breast Carcinoma	Excellent	n	0	1	2	12	0.18
		%	0%	11.1%	6.3%	21.4%	
	Good	n	0	4	17	15	
		%	0%	44.4%	53.1%	26.8%	
	Moderate	n	2	3	10	17	
		%	66.7%	33.3%	31.3%	30.4%	
	Poor	n	1	1	3	12	
		%	33.3%	11.1%	9.4%	21.4%	
	Total	n	3	9	32	56	
		%	100%	100%	100%	100%	

Predicted Five-year survival rate was 96% in 21.4% with an excellent NPI score, 93% in 26.8% of patients with Good NPI score, 78% in 30.4% of moderate NPI cases, and 44% in

21.4% of patients with poor NPI score. The association between NPI and stromal alpha SMA score was found to be statistically not significant.

On statistical analysis, Alpha-SMA expression in stroma of IDC was significant with tumor grade, lymph node metastasis, stage of the tumor, NPI score, ER and PR expression and molecular subtype.

Discussion

Immunohistochemistry (IHC) is a technique used to identify and analyse proteins inside cells and on the surfaces of different types of cells in all types of tissues. Various marker proteins can be employed to demonstrate different types of tumors,confirm the source of tissue, differentiate between metastatic and primary tumors, & offer supplementary data that could be essential for prognosis, predicting management response, or assessing any remaining tumor after treatment. There is an expanding assortment of antibodies and antigen retrieval methods that enhance the overall effectiveness of immunohistochemistry in addressing diagnostic challenges and assessing prognosis and treatment response in breast pathology.

SMAhasshown to be a reliable indicator for myoepithelial cells in the diagnosis of BC. While it does not specifically indicate myoepithelial differentiation, it is a highly responsive marker. The presence of actin expression in a cell indicates a positive result for SMA (smooth muscle actin), including myofibroblasts and blood vessels. [8]The current investigation aimed to examine the expression of CAFs in stroma of infiltrating ductal carcinoma & its correlation with other parameters.

Age: The study revealed that the majority of patients belonged to the age spectrum of 51 to 60 years, constituting 33% of the overall population. Subsequently, there was a group of individuals aged between 41 and 50 years, constituting 23% of the total sample. Patients between the ages of 28 and 40 years accounted for 20% of the sample, while those between 61 and 70 years made up 13%. The smallest proportion of patients, at 11%, were beyond the age of 71.

According to study published by Yamashita M et al., the average age in the non-metastasis group was 56 years, whereas in the metastasis group it was 57.5 years.[9] In the high alpha SMA expression group, the average age was 54.4 years, whereas in the low alpha SMA expression group, the average age was 57.6 years. The p-value for this comparison was 0.37. The median age range in research by Tse GMK et al. was 47years. [10]

Tumor Size: The research found that 9% of patients had tumor sizes less than 2 centimetres, 62% had tumorsizes between 2 and 5 centimetres, and 29% had tumor sizes more than 5 centimetres.

Yamashita M et al. conducted a study which revealed that 76% of patients with strong alpha SMA expression had tumors less than 5 cm, while 24% had tumors greater than 5 cm. Conversely, among individuals with low alpha SMA expression, 88.5% had tumors measuring less than 5 cm, whereas 11.5% had tumors measuring more than 5 cm.
Their statistical analysis showed a p-value of 0.34. [9]

TNM Staging: The research revealed that individuals exhibiting a significant amount of alpha SMA staining were distributed as follows: 7.1% had Stage I, 50%, 41.1% and 1.8% were in Stage II, III and IV tumors respectively. An evident significant association was seen between dimension of the tumor and the stromal SMA score.

In their research, Yamashita M et al. reported that 36% of the participants had stage 1, 40% had stage 2, and 24% had stage 3 in terms of strong alpha SMA expression. Stage 1 accounted for 48.5% of the low alpha SMA expression, stage 2 accounted for 40%, and stage 3 accounted for 11.4%. The p-value for this distribution is 0.38. [9]

ER: Within this research, it was shown that among individuals who had a high level of alpha SMA staining, 57.1% of them tested positive for ER. The statistical analysis did not find a significant connection between the size of the tumor and the stromal alpha SMA score.
According to a study conducted by Yamashita M et al., the non-metastatic group had a positive ER rate of 65%, whereas the metastasis group had a positive ER rate of 35%. The presence of ER was seen in 64% of patients exhibiting strong alpha SMA expression, whereas it was observed in 62.8% of patients with low alpha SMA expression. The statistical analysis yielded a p-value of 0.34. [9]
PR: Among patients who had a high level of alpha SMA staining, 57.1% of them had a positive PR result.
HER 2: Among patients who had a high level of alpha SMA staining, 26.8% of them tested positive for HER 2 neu.HER 2 status was positive in 20% of the non-metastatic group and 36% in the metastasis group. In research done by Yamashita M et al., it was shown that 24% of patients with high alpha SMA expression had a positive HER 2 status, whereas 22.8% of patients with low alpha SMA expression had a positive HER 2 status. The p-value for this association was determined to be 0.91. [9]

Stromal Alpha SMA Score: According to the stromal alpha SMA score, 3% of patients did not show any staining, 9% of patients showed mild staining while 32% and 56% of subjectsexhibited moderate staining and strong stainingcorrespondingly.

Yamashita M et al. observed a substantial variation in Alpha-SMA expression, with proportion of area varied from 0.68% to 28.15%. In contrast, values in group with metastasis exhibited a substantial increase, varied between 6.5% to 28.15%, while the values in the group without metastasis ranged from 0.68% to 21.64%. The invasive BC patients were divided into two groups as high (n = 25) and low (n = 35) Alpha-SMA. A mean value of 8.48% was used as cutoff criterion. [9]

Mohamed D et al. discovered significant differences in the levels of FAP expression between the central region and outside edge of tumor. More precisely, a total of 24 individuals had a complete absence of FAP expression in the central region of their tumors. Among the 76 patients, 30 had moderate levels whereas 46 subjects showed elevated levels of stromal FAP expression. After examining the tumor margin, it was discovered that 44 cases had lack of FAP stromal expression, whereas 21 had modest, and 35 patients had a significant level of stromal FAP expression. Moreover, there were significant differences in the α-SMA expression levels at the central and the outside edge of the tumor. Out of the total of 100 occurrences, 24 cases showed no α-SMA expression in the tumorcenter, whereas the remaining 76 individuals had positive expression. Out of the total number of patients, 54 obtained a negative result for the tumor margin, whereas 46 showed positive expression. There was a direct relationship between the presence of FAP in tumorcentral area and the histological type, size, grade, and spread of cancer cells to the lymph nodes. In contrast to the tumor margin, the expression of FAP exhibited an inverse relationship with tumor development and the dissemination of cancer cells to the lymph nodes. On the other hand, a reverse correlation was seen between the size of the tumor, the grade of the tumor, and the spread of cancer cells to the lymph nodes in the surrounding area of the tumor. [11]

Julia T et al. observed that FAP expression in BC stroma is varied and may be related toclinicopathological factors such as tumordimension, histological grading, involvement of axillary lymph nodes, and particularly inTNBC. Therefore, FAP might serve as a viable treatment option, particularly for malignancies that do not have specific targeted therapies like TNBC. [12]

Henry et al. found that CAFs expressing fibroblast activation protein (FAP) and α-SMA along the edge of the tumor are highly involved in the initial invasion and spread of cancer cells to other parts of the body. The researchers discovered that there is a correlation between high FAP expression and positive α-SMA expression in the tumor margin with smaller tumor dimensions, lower nodal metastasis, and lower histological grading. Once the invasive carcinoma has formed, several factors impact the clinical outcomes. [13]

Singh A et al. conducted an Immunohistochemical experiment on tumor samples to observe the localization of alpha SMA protein in nearby stromal cells, mostly consisting of myofibroblasts. [14]
Researchers Tamiolakis D. et al. found that cancer cells with a myoepithelialimmunophenotype are associated with IDCs that had extensive fibrosis. [15]

Conclusion:

Analysis of Alpha SMA expression had been performed, these individuals would have been classified as high-risk patients and, as a result, they may have been given more aggressive treatment. SMA expression is seen due to the presence of a significant amount of stroma. The presence of this indicates a forecast of early metastasis. Myofibroblasts are a promising potential for serving as a prognostic factor. Furthermore, manipulating the activity of myofibroblasts might potentially provide a new avenue for focused therapeutic interventions. Hence, those who have a notable abundance of myofibroblasts should undergo thorough assessment for more aggressive treatment approaches and more regular monitoring to identify the onset of metastatic disease.

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