

## Comparison of Amount of Elastin Fibres with Histopathological Parameters and Elastography in Breast Carcinoma

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### ABSTRACT

**Background:** Breast carcinoma is the most frequently diagnosed cancer and the chief cause of cancer deaths among women worldwide. Cancer registry data shows that incidence of carcinoma breast ranges from 19.3 to 89.7 per 100,000 population (India) and a total of 27.5% cases of cancers in Karnataka. The normal breast tissue is comprised of two major compartments, the epithelium and the stroma. Normally elastic tissue can be seen in periductal and perivascular region of breast. Elastosis, characterized by deposition of elastic fibers in the stroma of infiltrating ductal carcinoma was first described by Cheatle and Cutler, but extensively studied by Shivas & Douglas and others. Several methods for visual grading of elastosis have been proposed but all are semi quantitative and subjective and are dependent on the experience of the observed.

**Methods:** Blocks of all 87 cases were retrieved from the histopathological section, department of Pathology of our institute. Data regarding the demographic details, clinical presentation of the cases are obtained from the medical record section of our medical record section of our college. The slides were stained with Verhoeff's van Gieson stain and were screened by two pathologists.

**Result:** Out of 87 cases studied only 1 case showed elastin fibers score 0, 18 cases showed score 1, 46 cases showed score 2 and 22 cases showed score 3. There was statistical significant correlation between Tumor stage and amount of paratumoral elastin fibers. There was no statistical correlation between Tumor grade, Tumor size, Nodes, Nottingham prognostic index and Modified tsukba scoring and paratumoral elastosis. Out of 87 cases the ERIHC was done in only 23 cases out of which 10 cases showed positivity on Allred scoring and 13 cases were negative. For Progesterone receptors out of 23 cases 9 cases were positive on Allred scoring and 14 were negative and hence the correlation could not be done. The HER2neu was done in 23 cases out of 87 cases where the positivity was seen in 07 cases and it was equivocal in 03 cases and negative in 13 cases.

**Conclusion:** Our results strongly indicate the presence of Peritumoral elastosis and the lower tumor grade. The paratumoral elastosis and lower tumor stage. Whether and how elastosis is mechanically involved in tumor development and progress requires further study.

**Keywords:** Breast Carcinoma, Elastography, Elastosis.

### Introduction

Breast carcinoma is the most frequently diagnosed cancer and the chief cause of cancer deaths among women worldwide, with an estimated 1.7 million cases and 521,900 deaths occurring in 2012.<sup>1</sup> It, alone accounts for 25% of all cancer cases and 15% of all cancer deaths among females. Cancer registry data shows that incidence of carcinoma breast ranges from 19.3 to 89.7 per 100,000 population (India) and a total of 27.5% cases of cancers in Karnataka.<sup>2</sup>

The normal breast tissue is comprised of two major compartments, the epithelium and the stroma (forms the tumor microenvironment).

The tumor microenvironment which comprises of cellular stroma (fibroblasts, myofibroblasts, endothelial cells, adipocytes, immune cells) and Extra Cellular Matrix (collagens, laminin, fibronectin, matrix metalloproteinases (MMPs) and soluble growth factors). Though stroma

is considered as passive responder to the malignant transformation. But now it is thought stromal cells, provide cues for tumorigenesis.<sup>3</sup>

Normally elastic tissue can be seen in periductal and perivascular region of breast. Elastosis, characterized by deposition of elastic fibers in the stroma of infiltrating ductal carcinoma was first described by Cheatle and Cutler, but extensively studied by Shivas & Douglas and others. Elastosis appears as eosinophilic hyaline acellular areas in sections stained with hematoxylin-eosin stain and black colour on Verhoeff's VanGieson stain.<sup>4,5</sup> Shivas and Douglas used this early observation to develop an "elastica index" on histopathology which they claimed had prognostic value in a group of patients treated by local mastectomy and radiotherapy since the patients with moderate or large amounts of elastosis had a significantly greater duration of survival.<sup>4</sup> In an analysis of prognostic

factors in breast carcinoma it was found that moderate or massive elastosis was related to a good 5-year survival rate.<sup>4</sup>

Several methods for visual grading of elastosis have been proposed but all are semi quantitative and subjective and are dependent on the experience of the observer.

Breast ultrasound elastography is an emerging sonographic imaging technique which provides information on breast lesions in addition to conventional ultrasonography (US) and mammography. Ultrasound elastography provides a non-invasive evaluation of the “stiffness” of a lesion. It increases the specificity of conventional B-mode ultrasound by more precise characterization of breast lesions. Few studies show that breast elastography has higher diagnostic accuracy compared to mammography or ultrasound alone.<sup>6</sup>

In this study we report our assessment of the of elastosis in comparison with the Histopathological parameters like TNM staging, tumor grade and Nottingham Prognostic Index and ultrasound elastography.

### Aims

1. To study expression of elastotic grade in various grades of infiltrating ductal carcinoma
2. To compare the elastotic grade with histopathological parameters
3. To compare the elastotic grade with elastography wherever possible.

### Material and Methods

It is a laboratory based observational study. Study was started after obtaining ethical clearance from our institutional ethical committee. Study was done on eighty seven cases on infiltrating ductal carcinoma.

**Inclusion criteria:** All the cases with infiltrating ductal carcinoma

**Exclusion Criteria:** Male breast carcinoma, those who underwent neoadjuvant chemotherapy

Sample size was calculated based on percentage of tumors having no / very little tissue in the malignant areas (35%) in a study done by Birgitte Bruun et al<sup>6</sup> at Denmark with considering an absolute error of 10% and 95% Confidence interval estimated sample size is eighty seven (n=87)

Blocks of all 87 cases were retrieved from the histopathological section, department of Pathology of our institute. Data regarding the demographic details, clinical presentation of the cases are obtained from the medical record section of our medical record section of our college.

All the slides that were retrieved from department were reanalyzed by the authors for nodal metastasis, tumor stage, Grade, Nottingham prognostic index and modified Tsuba score, Estrogen Receptor, Progesterone Receptor and HER2neu status. The tumor node and stage was calculated based on the TNM staging of breast carcinoma.

Sections from tumor proper without any area of necrosis for selected for elastin staining. The staining was done using Verhoeff’s van Gieson stain which is the most widely used histochemical stain for the demonstration of elastic fibers. Both paratumoral and peritumoral areas were evaluated for elastin fibers. Elastosis was graded without knowledge of other histological parameters.

The amount of elastotic stroma in the tumour were evaluated and graded in a semiquantitative manner from 0 – 3, according to Shivas & Douglas.<sup>4</sup>

Grade 0 -elastosis was absent, Grade 1- small deposits (single elastin fibrils or a thin rim of elastosis around ducts present; Grade 2 - thicker zones of elastosis; Grade 3 - large deposits dominated substantial areas of the tumor. The NPI grade is calculated using 3 parameters – Tubule formation, nuclear pleomorphism and mitotic activity.

### Results

Eighty seven cases of infiltrating ductal carcinoma were included in the study. The data such as age of the patient, tumor size, grade, nodal status, Nottingham index, modified Tsukba Score, ER, PR and HER2neu status of the patient is presented in table 1.

Out of 87 cases studied peritumoral elastosis score 1 was seen in 34 cases and score 2 was seen in 48 cases and score 3 was seen in 2 cases. There was significant correlation (p value <0.05) seen between the amount of Peritumoral elastosis and lymph node metastasis. There was no statistical correlation between Peritumoral elastosis with tumor grade, tumor size, Stage and Nottingham prognostic index. None of the cases showed zero elastin fibers, these details are represented in table 2.

Out of 87 cases studied only 1 case showed elastin fibers score 0, 18 cases showed score 1, 46 cases showed score 2 and 22 cases showed score 3. There was statistical significant correlation between Tumor stage and amount of paratumoral elastin fibers. There was no statistical correlation between Tumor grade, Tumor size, Nodes, Nottingham prognostic index and Modified tsukba scoring and paratumoral elastosis. Out of 87 cases the ER IHC was done in only 23 cases out of which 10 cases showed positivity on Allred scoring and 13 cases were negative.

For Progesterone receptors out of 23 cases 9 cases were positive on Allred scoring and 14 were negative and hence the correlation could not be done. The HER2neu was done

in 23 cases out of 87 cases where the positivity was seen in 07 cases and it was equivocal in 03 cases and negative in 13 cases.

**Table 1: The demographic data of the patients.**

Patient characteristics	n= 87	Percentage %
<b>Age</b>		
<40 years	11	12.64
40-60 years	50	57.47
>60 years	26	29.89
<b>Tumor grade</b>		
I	47	54.02
II	28	32.18
III	12	13.80
<b>Tumor Size</b>		
T1	12	13.80
T2	37	42.53
T3/T4	38	43.67
<b>Nodal state</b>		
Positive	48	55.10
Negative	33	37.90
No lymph nodes	06	07.00
<b>Tumor stage</b>		
I	04	04.69
II	45	51.72
III	38	43.67
<b>Nottingham prognostic Index</b>		
Excellent	06	6.89
Good	21	24.23
Moderate	44	50.57
Poor	16	18.39
<b>ER</b>	n=23	
Positive	10	43.47
Negative	13	56.53
<b>PR</b>	n=23	
Positive	9	39.13
Negative	14	60.87
<b>HER2neu</b>	n=23	
Positive	07	30.43
Equivocal	03	13.04
Negative	13	56.53
<b>Modified Tsukba Score</b>	n= 7	
Elastography	07	3.50

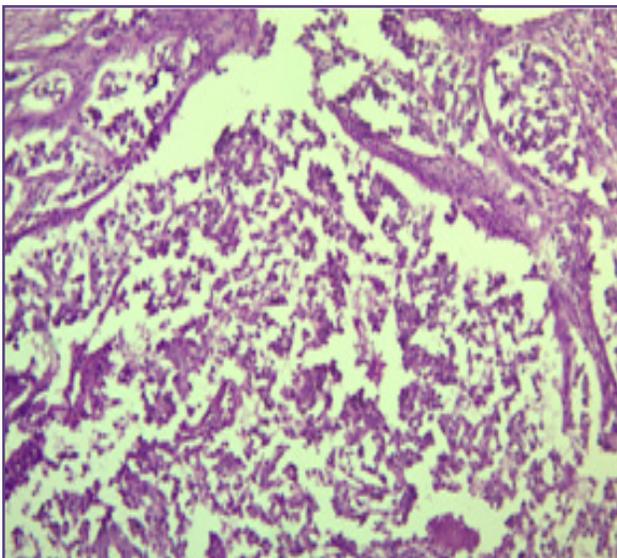
**Table 2: Comparison of Histopathological features with Peritumoral elastosis (n=87).**

PARAMETERS	PERITUMORAL ELASTIN FIBRES				p VALUE
	0	1	2	3	
Tumor grade					
I	00	16	30	1	0.493
II	00	15	12	1	
III	00	06	06	0	
Tumor size x	00	37	48	02	0.792

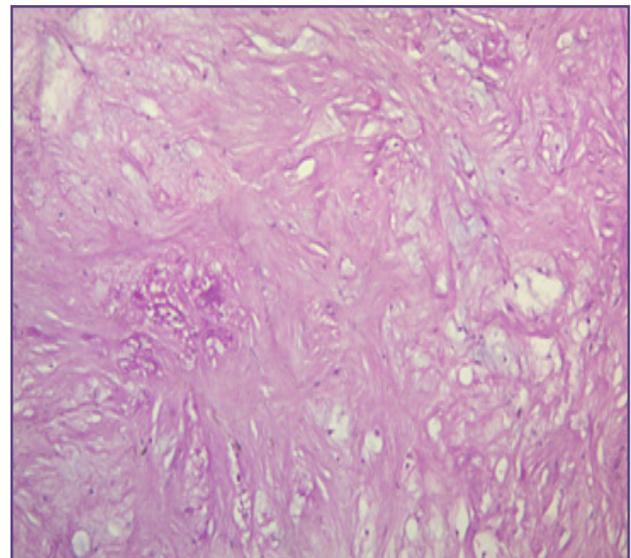
PARAMETERS	PERITUMORAL ELASTIN FIBRES				p VALUE
Tumor Nodes	00	37	48	02	<b>0.034</b>
Stage	00	37	48	02	0.068
Nottingham prognostic Index					
• Excellent	00	5	2	0	0.307
• Good	00	8	13	0	
• Moderate	00	12	27	2	
• Poor	00	10	06	0	
<b>Modified Tsukba Score</b>	00	3	3	1	0.321

**Table 3: Comparison of Histopathological features with Paratumoral elastosis.**

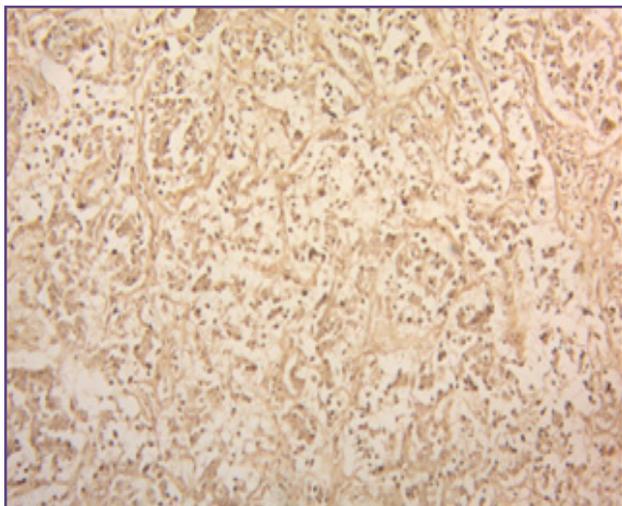
PARAMETERS	PARATUMORAL ELASTIN FIBRES				p VALUE
	0	1	2	3	
Tumor grade					0.257
I	1	06	26	14	
II	0	10	14	04	
III	0	02	06	04	
Tumor size	1	18	46	22	0.071
Tumor Nodes	1	18	46	22	0.153
Stage	1	18	46	22	<b>0.007</b>
Nottingham prognostic Index					
• Excellent	0	2	02	02	0.153
• Good	0	4	14	03	
• Moderate	0	6	24	14	
• Poor	1	6	06	03	
<b>MODIFIED TSUKBA SCORE</b>	0	2	3	2	0.321



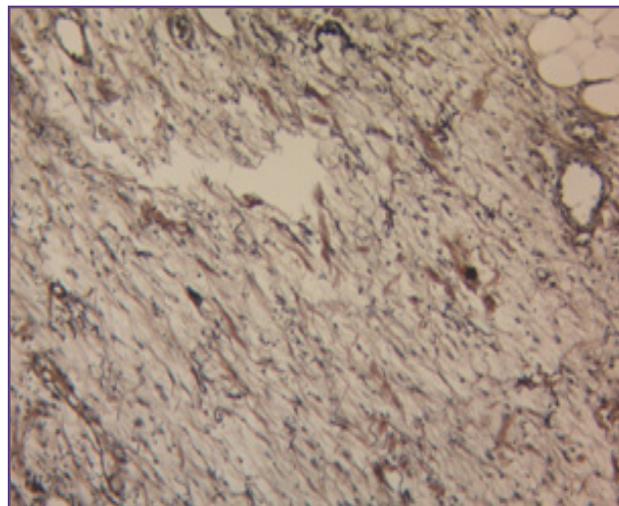
**Fig.1: H & E stain showing infiltrating ductal carcinoma breast under 10x.**



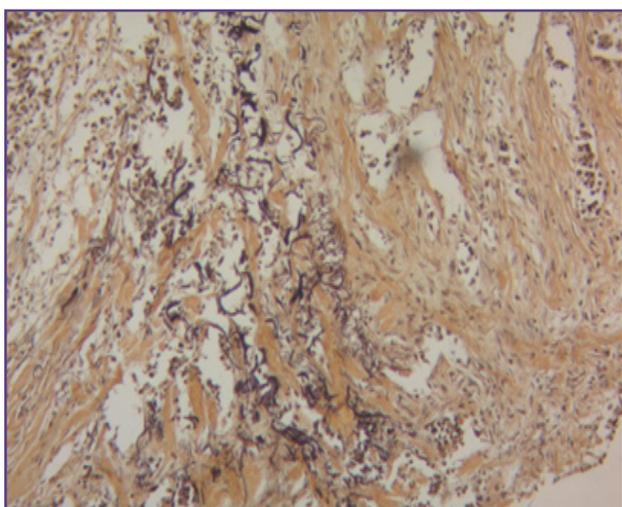
**Fig. 2: H & E stain under 10x showing paratumoral area.**



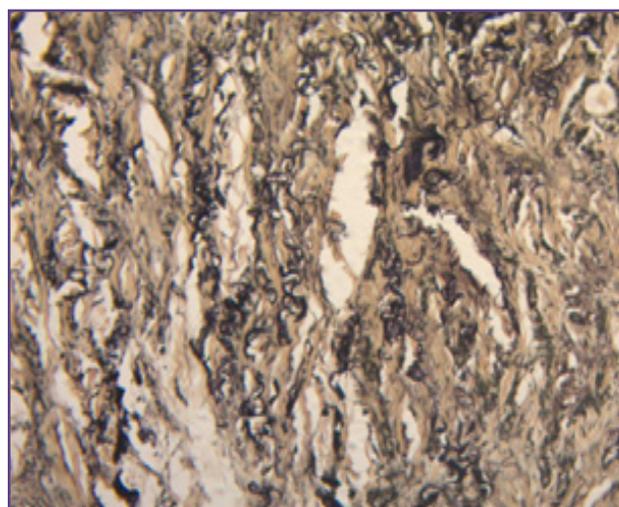
**Fig. 3: 10x power Verhoeff's van Gieson stain – Grade 0.**



**Fig. 4: 0x power Verhoeff's van Gieson stain – Grade 1.**



**Fig. 5: 10x power Verhoeff's van Gieson stain – Grade 2.**



**Fig. 6: 10x power Verhoeff's van Gieson stain – Grade 3.**

**Discussion**

The association of elastosis with carcinoma of the breast was reported as early as 1931, but it has attracted little attention. Elastosis is not unique to breast carcinoma, however. It has been also reported in carcinoma of the urethra, colon, Skin and with benign and malignant tumors of the salivary gland.<sup>7</sup> The origin of the elastotic material has been shown to be stromal cells like fibroblasts and myofibroblasts, but also the carcinoma cells. Further, a 67 kD elastin receptor (EBP/S-gal) has been reported and receptor stimulation of this receptor can affect cell proliferation, adhesion, and chemotaxis. In addition, elastin can be cleaved into small peptide fragments, which can affect different cellular processes including apoptosis, chemotaxis and metastasis.<sup>7</sup>

Study of elastosis of breast and comparison with tumor grade, and other prognostic factors was first done by Shiva's and Douglas.<sup>4</sup> Later many studies have been done to look into the prognosis of breast carcinoma based on the tumor elastosis and other parameters. Studies on comparison of both peritumoral and paratumoral elastin fibres with other histological parameters and breast elastography is a rarity. Breast cancer detection on ultrasound elastography has 86.8% sensitivity, 89.8% specificity, and 88.3% diagnostic accuracy. USG elastography measures stiffness of the lesion which is nothing but the measurement of elastin fibers which leads to stiffness of lesion.

In this study we compared the amount of elastin fibers in Peritumoral and paratumoral region with histopathological

parameters such as TNM, stage, grade, Nottingham prognostic index and USG elastography (only in seven cases).

The study of elastic fibers in relation with various histopathological parameters has shown conflicting results. Few studies have shown that the high content of elastosis shows strong correlation with other predictors of good prognosis as low histological grade, hormone receptor expression, HER 2 negative tumors and low tumor cell proliferation by the Ki67 index and improved recurrence free survival in tumors with high elastin content.<sup>7</sup>

In other few studies there was no correlation between elastosis and recurrence free survival. But the strongest relation was found between elastin and the sex steroid hormone receptors with estrogen receptors, which further indicates that the synthesis of elastin may be under regulation of estrogens.<sup>8</sup>

Peritumoral elastosis is the presence of elastin fibers just next to tumor cells and paratumoral elastosis is presence/ the amount of elastin fibers in the area away from tumor cells / in the stroma.

In this study we have tried to correlate the amount of peri and paratumoral elastic fibres with various histopathological factors. This comparison has not been discussed previously. Other studies done have considered only the amount of elastin fibers.

In our study there was correlation between tumor grade and amount of Peritumoral elastin fibers that is the higher the amount of elastin fibers the lower was the grade. The similar findings were seen in the study conducted by Chen et al<sup>7</sup>, Bindu S et al.<sup>8</sup>

In this study there was no statistical correlation between other histopathological parameters like tumor size, node, TNM, Nottingham prognostic index with Peritumoral elastosis. The same findings were seen in study done by Chen et al.<sup>7</sup>

Our study showed statistically significant correlation between paratumoral elastosis and tumor stage. The similar finding was seen in study done by Ahn et al.<sup>9</sup>

The biological significance of aggregation of large quantities of elastin fibres in breast cancer stroma and the mechanistic link between the presence of elastin and a better prognosis is explained by the theory that the angiostatic molecule endostatin has been reported to accumulate on elastin fibers and this could reduce angiogenesis and hence tumor growth and spread.<sup>16</sup> Or the effects may be related to elastin itself, the elastin receptor or elastin derived peptides.

Some elastin-related effects may be mediated by elafin, an inhibitor of elastase, which recently has been shown to be a positive prognostic factor in breast cancer.<sup>10</sup> Other recent studies have addressed the mechanical properties of tumor tissue, establishing a correlation with aggressive behavior in collagen-rich tumors.<sup>11</sup> Hypothetically, a high content of elastin may affect the mechanical properties and prognosis in the opposite direction. The elucidation of these mechanisms needs further investigation.

Out of 87 cases 7 cases were compared with ultrasonography BIRADS score, modified color score scale, mean elastographic index and E/B ratio of strain elastography. All 7 cases were given as malignancy on ultrasonography based on BIRADS score. With Elastography, the strain ratio varied from 4-5 i.e suspicious for malignancy. The modified color score has 0, 1 and 2 grades based on the colors of the lesion shows i.e grade 0 – tissue is completely green, indicating the strain is similar to adjacent normal breast tissue. Grade 1 – the lesion will show mosaic pattern where mixture of green and blue is obtained, which is taken as equivocal and Grade 2 – complete blue indicating malignancy. The principle behind this technique is resistance to deformability by an external compression. In this study all 7 cases the score was 2 indicating high likelihood for malignancy and hardening of the tissue nothing but loss of elasticity. Elastography index >2.8 is taken as malignancy and all 7 cases had elastography index >3.5. Other parameter compared was E:B ratio where ratio <1 is taken as benign and in all 7 cases the ratio was >1.05. Comparing these parameters to the elastography, out of 7 cases 5 cases had grade 1 peritumoral elastosis and grade 2 elastosis in paratumoral area indication loss of elastic fibres or less elastic fibres in the peritumoral area. 1 case showed grade 0 on peritumoral elastosis. Only 1 case showed grade 2 peritumoral and grade 3 paratumoral elastosis which was not correlating. All these cases had Nottingham prognostic index >4.5 and stage II and III. The number of cases is too small to comment on the relationship of elastography and other parameters.

## Conclusion

Our results strongly indicate the presence of Peritumoral elastosis and the lower tumor grade. The paratumoral elastosis and tumor stage. Whether and how elastosis is mechanically involved in tumor development and progress requires further study. Elastosis compared to elastography out of 7 cases 6 cases peritumoral and paratumoral grading correlated with the modified Tsukuba scoring, elastography index and E: B ratio indication there is loss of elastic fibres with the malignancy. But this requires further study to come to conclusion.

## Keywords

Peritumoral elastosis, paratumoral elastosis, Tumor grade, TNM, tumor stage, NPI, ER, PR, HER2neu and Elastography.

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