

## CORRELATION OF TUMOUR PROLIFERATIVE COMPARTMENT, STEROID RECEPTOR EXPRESSION AND GRADE IN INFILTRATING DUCT CARCINOMA OF BREAST

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### ABSTRACT

#### BACKGROUND

Hormone receptor evaluation is an important component of breast carcinoma prognostication and is essential for selection of patients for adjuvant chemotherapy. Proliferation is the key for growth and progress of all malignancies. This study is done to determine the proliferative fraction, hormone receptor expression, grade and nodal status in infiltrating duct carcinoma of breast.

#### MATERIALS AND METHODS

It was a descriptive study; 49 cases of breast lesions were studied, of which 45 cases were confirmed by histopathology as infiltrating duct carcinoma and 2 cases as fibroadenoma and 2 of normal breast tissue received in the Department of Pathology, Government Medical College, Thiruvananthapuram for one year. None of the patients had any previous therapy. Haematoxylin and Eosin stained sections were used to confirm the diagnosis of infiltrating duct carcinoma and for Bloom-Richardson grading. Immunohistochemistry was done in the serial section for the expression of ER, PR and PCNA. These were evaluated and correlated with clinical variables like grade and nodal status.

#### RESULTS

ER expression showed strong positive correlation with PR with majority of tumours being negative for ER and PR. ER/PR expression by tumour cells showed an inverse correlation with PCNA expression and grade; and higher-grade tumours showing intense PCNA and negative ER/PR expression.

#### CONCLUSION

An inverse relation occurs between proliferative fraction of tumour and ER/PR expression status in infiltrating duct carcinoma of the breast. High grade and node positive tumours show a higher proliferation fraction and lower levels of oestrogen and progesterone receptor expression. Even though hormonal stimuli play a role in breast carcinogenesis, tumour progression is paralleled by a progressive hormone independence.

#### KEYWORDS

Breast carcinoma - Oestrogen Progesterone Receptor Expression, PCNA, Grade and Nodal Status.

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#### BACKGROUND

As per the recent statistics, Breast Carcinoma tops the list of all cancers among females in India and its incidence continue to increase.<sup>1</sup> It is a heterogeneous disease with a small percentage of tumours showing a much greater biological aggressiveness than expected on the basis of their stage and grade. This observation lead to the development of more effective molecular prognostic markers, which include steroid receptors like Oestrogen and Progesterone receptors (ER and PR), HER2/neu, etc.<sup>2</sup> They are used not only for prognostication, but also selection of patients for adjuvant chemotherapy with anti-oestrogens like Tamoxifen and the recent aromatase inhibitor group of drugs. Proliferative fraction of tumours is also important in assessing

the biological aggressiveness of any malignancy.<sup>3</sup> This can be done by immunohistochemistry using proliferation specific antigens like PCNA and Ki67.

This study was a cross-sectional study designed to determine the proliferating cell fraction, oestrogen and progesterone receptor expression, grade and nodal status in infiltrating duct carcinoma of the breast.

#### MATERIALS AND METHODS

This was a descriptive study. A total of 49 cases; 45 cases of infiltrating duct carcinomas and 2 cases each of fibroadenoma and normal breast tissue confirmed by histopathology using Haematoxylin and Eosin-stained sections received in the Department of Pathology, Medical College, Thiruvananthapuram were included in the study.

#### Inclusion Criteria

Only patients diagnosed by histopathology as infiltrating duct carcinoma and received during the study period were included. The relevant clinical information like nodal status and previous treatment history of all patients included in the study were made available.

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**Exclusion Criteria**

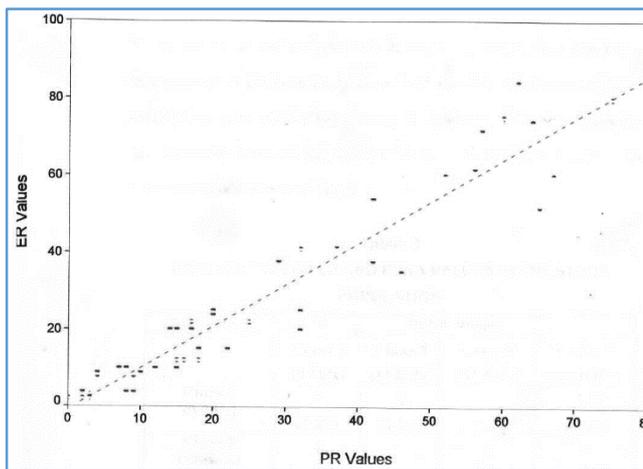
None of the patients included had any chemotherapy, hormonal therapy or radiation prior to excision. Excised tissue was fixed in formalin and processed to paraffin wax. Sections stained with haematoxylin and eosin were evaluated in each patient. A representative paraffin block with the greatest diameter of the viable tumour was selected and serial sections at 4µ were obtained for light microscopy and immunohistochemistry. H and E stained tumour sections were graded as per Modified Bloom and Richardson grading. Sections for immunohistochemistry were treated with respective primary monoclonal antibody at dilution 1:20 after antigen retrieval. The antibodies were monoclonal anti-PCNA from Dako, Monoclonal ER and PR from Novocastra.

500 cells were counted. The intensity and percentage of cells showing positivity were noted and categorised using scores. ER, PR and PCNA were found to be localised in the nucleus. The expression of ER and PR were graded based on proportion and intensity of positivity from Class 1 to Class 4 as 0 - 20%; 21 - 40%, 41 - 60% and 61 - 100% into ER 1 - 4 and PR 1 - 4. PCNA was graded from 0 - 10%, 11 - 30%, 31 - 50% and 51 - 100%, as Class 1 to Class 4 (K1-K4). The normal ductal cells showed ER-PR expression, while stromal and inflammatory cells were negative. The results were statistically correlated using Mann-Whitney Spearman correlation tests using SPSS 16 version.

**RESULTS**

**Association between ER and PR Levels-**

A strong positive correlation was observed between ER and PR levels as shown in Figure 1. Tumours with low ER reactivity also showed low PR expression, while intense expression of ER was associated with moderate and intense PR. A small fraction of tumours showed discordance in ER and PR.



**Figure 1. Scatterplot representing Oestrogen and Progesterone Receptor Status**

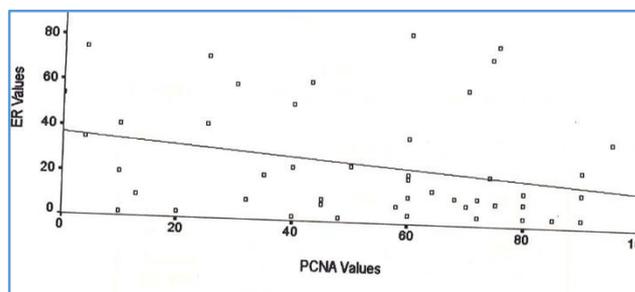
**Association between Hormone Receptors and Proliferation**

Oestrogen and Progesterone receptor negative tumours tend to have a high proliferation rate as measured by the PCNA immune reactivity. The results are outlined in Table 1. An inverse correlation between ER and PCNA was seen as in Figure 2. Even though a highly significant correlation was observed between ER and PR, PR levels were not found to correlate with PCNA expression. The majority of PR negative

tumours were PCNA positive. But 3 cases with strong PR positivity showed an intense PCNA expression. This could probably be the reason for lack of correlation between PR and PCNA expression.

PCNA	ER & PR Values							
	Class I (0-20%)		Class 2 (21-40%)		Class 3 (41-60%)		Class 4 (61-100%)	
	ER 1	PR1	ER 2	P2	ER3	P3	ER 4	P4
K 1 (0-10%)	2	2	1	1	2	3	1	-
K 2 (11-30%)	2	2	-	1	2	1	1	1
K 3 (31-50%)	6	6	2	2	1	1	1	1
K 4 (51-100%)	19	20	5	3	1	2	3	3
ER & PCNA (r= 0.279, p= 0.050)					PR & PCNA (r= 0.223, p= 0.122)			

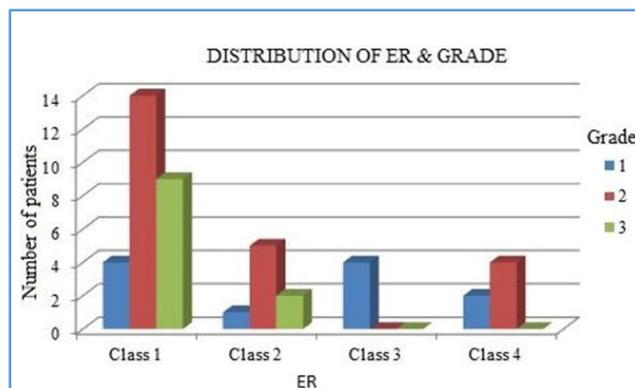
**Table 1. Steroid Receptor and PCNA Expression**



**Figure 2. Scatterplot showing Oestrogen Receptor and PCNA Expression**

**Hormone Receptor Levels, PCNA and Histopathological Grading**

Out of the 49 cases studied, 4 were benign tissue and so were taken as Grade 0; 23 tumours were of Grade 2 and 11 cases each were of Grade 1 and 3. There was an inverse correlation between ER-PR expression and the histological grade as seen in Figures 3 and 4. The correlation between PR values with grade were not as significant as that of ER. A correlation between histopathological grading and PCNA expression is depicted in Figure 5. The majority of tumours showing intense PCNA expression were of Grade 3.



**Figure 3. Bar Chart showing Correlation of ER Expression and Tumour Grade**

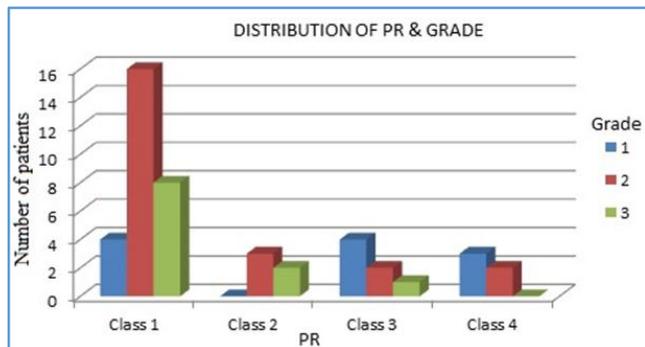


Figure 4. Bar Chart showing Correlation of PR Expression and Tumour Grade

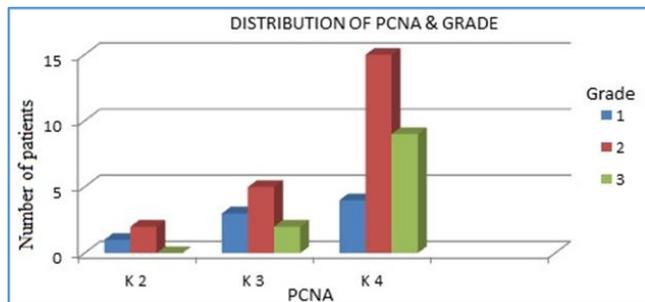


Figure 5. Bar Chart showing Correlation of PCNA and Histopathological Grade

The Grade 3 tumours expressed a weak ER/PR, but a strong PCNA expression.

**Hormone Receptors and Clinical Features**

Histopathological grading and tumour size showed a statistically significant correlation (Table 2). ER positivity was found mostly in lymph node negative patients (Table 3). None of the axillary lymph node positive patients included in our study expressed ER. Expression of ER in lymph node negative patients varied widely from tumour to tumour. Only one case of lymph node negative tumour expressed oestrogen receptor negativity, while majority of the tumours expressed mild-to-intense expression. A highly significant correlation was also observed between ER/PR expression and lymph node status. Both ER and PR were not found to correlate with tumour size.

Grade Values	Tumour Size				
	T0	T1 (0-2 cm)	T2 (2-5 cm)	T3 (6-10 cm)	T4 (> 10 cm)
Grade 0	4	-	-	-	-
Grade 1	1	2	6	1	1
Grade 2	-	1	12	7	3
Grade 3	-	1	2	4	4

Table 2. Distribution of Tumour Size and Grade

(r= 0.6266, p= 0.000)

Nodal Status	ER & PR Expression Values							
	Class 1 (0-20%)		Class 2 (21-40%)		Class 3 (41-60%)		Class 4 (61-100%)	
	ER1	P1	ER2	P2	ER3	P3	ER4	P4
Node Positive	28	27	-	1	-	-	-	-
Node Negative	1	3	8	6	6	7	6	5

Table 3. Correlation of ER, PR and Nodal Status

ER and Nodal status, r= 0.0082 and p= 0.576. PR and Nodal status, r= 0.094 and p= 0.53.

**DISCUSSION**

The major issues in the management of patients with operable breast cancer are control of local recurrence and prevention of distant metastases. Many patients die from disseminated cancer even after fully controlled local disease, which suggest that the cancer spread has occurred before local surgical treatment was given. For this reason, effective systemic adjuvant therapy is required to eliminate distant micrometastasis. The main role of the molecular prognostic markers is the selection of such patients for this adjuvant chemotherapy.

Histological status and number of the axillary lymph nodes involved remains the most important single conventional prognostic marker for patients with operable breast cancer.<sup>4</sup> Although, negative nodal status is a very favourable prognostic factor, 10 - 30% of these patients also develop distant metastasis during the disease course. This highlights the need to examine interrelationships between various prognostic factors to identify the patients with high risk of recurrence and who may benefit from adjuvant therapy. Markers of proliferative activity and steroid receptor expression help to identify patients with aggressive disease.

In concordance with earlier studies, we found that histological grade is found to increase with increasing tumour size.<sup>4</sup> The size of tumour does not correlate with steroid receptor expression, but oestrogen receptor positive tumours show less tendency for lymph node involvement compared to oestrogen negative tumours.<sup>5,6</sup> A well-differentiated or low-grade tumours expressed steroid receptors more when compared to poorly differentiated ones. As expected, the poorly differentiated tumours showed marked proliferation and hence express intense PCNA immunoreactivity.<sup>7</sup>

Oestrogen receptor is a nuclear transcription factor, which when activated by oestrogen migrate from the cytoplasm to the nucleus and play an important role in the normal growth of breast. This receptor stimulation lead to an increase in RNA synthesis and in effect cause cell proliferation by subsequent increase in production of transcription factors like cyclins and c-Myc, which act on cell cycle control points.<sup>8</sup> This increase in proliferation provides opportunities for genomic instability of the cell and initiation of malignancy.

As an attempt to clarify the role of this interaction, we analysed the relationship between hormone receptor status and cellular proliferation in infiltrating duct carcinoma of breast.<sup>7</sup> In the present study our findings of increased ER/PR expression in tumours compared to normal support, the assumption was made that hormonal stimuli contribute to the development of mammary malignancy.

ER is basically of two types, the ER $\alpha$  and ER $\beta$ , which are co-expressed in target tissues as functional homodimers or heterodimers. When oestrogens bind to homodimers the transcription of target genes are activated and reverse if it is to heterodimers. PR expression in epithelial cell is an indirect evidence of a functionally active ER.

ER and PR expression rates in this study were comparable to earlier studies in India with majority being ER/PR negative and a small percentage showing discordance in ER/PR expression.<sup>9</sup> The significance of this is that the

response to hormone therapy is best in ER/PR positive tumours and moderate with those showing ER/PR discordance. However, the absence of ER and PR immunoreactivity in several cases do not imply a true ER negativity, since there is a significant body of evidence suggesting that many human breast carcinomas produce defective forms of ER.<sup>10</sup>

Research in breast carcinoma cell lines have shown that rearrangements or deletions in the steroid receptor genes or their mRNA's that cause premature termination of translation can lead to a negative ER expression. But even then, proteins required for cell proliferation are produced within these tumour cells if exposed to oestrogen. This is demonstrated by RT, PCR and Western Blot. Thus, several ER-negative breast cancer cell lines respond to oestrogens and anti-oestrogens suggesting that ER-negative cell lines are not truly ER-negative, but ER variants. Breast cancer cells expressing such ER variants even though appear ER negative will still proliferate and that too in an uncontrollable fashion.

Several variants of ER $\alpha$  are known to exist with differences in oestrogen responsiveness due to varying ratios of wild-type to variant ER mRNA. The  $\Delta 5$  variant of ER increases ER activity. Some cell lines misclassified as ER-negative may exhibit the  $\Delta 5$  variant, which activates gene transcription in the absence of the actual hormone stimulation. The acquirement of this variant may represent a transitional state in the progression of ER positive cells to hormone independence, tamoxifen-resistance and highly proliferating ER-negative tumours.<sup>11</sup>

Our results also point to the existence of successive steps of progression from a hormone dependent level towards an autonomous growth in breast carcinomas. The demonstration of higher proliferation rates as measured by PCNA in ER negative carcinomas reinforce the hypothesis that breast carcinoma progression is paralleled by a progressive hormone independence. These properties may result directly from the emergence of constitutive ER variants and are commonly regarded as pivotal steps in the malignant progression of this cancer.<sup>12</sup> Although, recognising structural variants of ER is far beyond the scope of this study, we feel to have found enough evidence by the current study of demonstrating ER-PR expression and relating it to proliferative indices to suggest that our results fit with the aforementioned possibility.

## CONCLUSION

An inverse relation occurs between proliferative fraction of tumour picked up by PCNA and ER/PR expression status in infiltrating duct carcinoma of the breast. High grade and node positive tumours show a higher proliferation fraction and lower levels of oestrogen-progesterone receptor expression. Even though hormonal stimuli play a role in breast

carcinogenesis, tumour progression is paralleled by a progressive hormone independence.

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