ABSTRACT
OBJECTIVE: In this study it was intended to know the hormone receptor expression as a marker for the detection of breast cancer and correlate it with the Bloom-Richardson grading system of breast carcinoma.

BACKGROUND DATA: Breast cancer patients with tumors that are estrogen receptor (ER) positive and/or progesterone receptor (PR) positive have lower risk of mortality compared to women with ER and/or PR negative disease. However, few studies have evaluated variations in the risks of breast cancer specific mortality across ER/PR status by either demographic or clinical characteristics.

METHODS: Breast carcinoma samples from 64 patients were received in the department of Pathology, Sri Venkateswara Medical College, Tirupathi and examined for estrogen and progesterone receptors by immunohistochemical assay. Correlations with established risk factors (tumor size, lymph node status, grading) and histopathology were analyzed.

RESULTS: The estrogen and progesterone receptors determined by immunohistochemical method revealed ER+/PR+ in 32.81%, ER+/PR- in 14.06 %, ER-/PR+ in 10.94 % and ER-/PR- in 42.19% results. Invasive ductal carcinoma is the prominent type tumor. Post-menopausal women showed a higher incidence of reactivity with increasing age. T2 sized tumors were prominent than T1 and T3 group. The reactivity for steroid receptors was observed to decrease with increasing grades. Greater than 4 lymph nodes metastasis revealed ER/PR negativity.

CONCLUSION: Compared to women with ER+/PR+ tumors women with ER+/PR-, ER-/PR+ or ER-/PR- tumors have higher risks of mortality. Premenopausal women have ER-/PR- tumors.

KEY WORDS
Breast carcinoma, Estrogen receptors: Estrogen, Immunohistochemistry, Progesterone receptors: Progesterone.

1. INTRODUCTION
Breast cancer occurs in middle and elder age group people but this the most common Carcinoma in women (22 %), which is more than twice the prevalence of cancers in women at any other site. \(^1\) It ranks second among cancer deaths in adult females. \(^2\)

The incidence of breast cancer varies from 3.9/100,000 in Mozambique to as high as 101.1/100,000 in the US. \(^3 - 5\)

During the past two decades the mortality rate has been decreased significantly due to early detection of disease and the use of aggressive multimodality treatment leading to improved clinical outcomes. Further decline in the breast cancer mortality is expected as a result of the tremendous advance in the outstanding of the biology of the disease and its associated risk factors. Increasingly, women identified as being at high risk for breast cancer can take advantage of risk reducing intervention that are potentially lifesaving.

Prognosis and management of breast cancer are influenced by the classic variables such as histologic type and grade, tumor size, lymph node status, status of hormone receptors estrogen receptors(ER) and progesterone receptors (PR) of the tumor and HER-2 status. \(^6 - 9\)
ER is expressed in 70% to 95% of invasive lobular carcinomas and PR is expressed in 60% to 70% of invasive breast carcinomas. Expression of ER and/or PR generally is associated with a better outcome. Survival and response to hormone therapy are most favourable among women with tumors positive for both ER and PR, intermediate for tumors discordant on receptor status and least favorable for tumors negative for both.

Overall incidence of breast cancer is declining in the US in the last decade. However, the incidence of the biologically aggressive estrogen receptors(ER) negative, progesterone receptors(PR) negative breast cancer in women younger than 40 has been increasing in African Americans in the US, Nigerian, Chinese, Vietnamese and Taiwanese populations. Recent reports from India and Pakistan suggest an important increase in the incidence of breast cancer and specifically ER,PR negative breast cancer among the population.

We studied the distribution of hormone receptor status and its correlation with traditional prognostic parameters in 64 cases of infiltrating duct carcinomas.

2.MATERIALS AND METHODS
2.1. PATIENTS/CASE SELECTION
Unilateral, operable breast cancer patients who underwent resection of their primary tumor were received in the department of pathology for the period of 18 months. (Mean age 63 Years, age range from 41 years to 85 years). These tumours were diagnosed prior to surgery by Fine Needle Aspiration Cytology. We have selected for this study relevant clinical data including menopausal status, side of the breast involved, location of tumor within the breast and the involvement of lymph nodes were collected. All patient gave written informed consent ER, PR immunohistochemical analyses were performed at diagnostic workup.

2.2. HISTOLOGIC EXAMINATION
Histologic assessment of tumor type and grade were performed routinely on 4 to 5 um thick H & E stained sections of formalin – fixed, paraffin – embedded tumors according to the criteria outlined in the World Health Organization classification of tumors. Briefly, the nuclear grades of primary or metastatic ductal type carcinomas were designated as follows. 1. Small, regular uniform cells.2. Moderate increase in size and variability. 3. Marked variation in size and shape.

All the paraffin blocks were studied for grading according to the Scarff- Bloom-Richardson grading system of breast cancer with Nottingham modification. The tumors were histologically confirmed to be breast cancers and classified on the basis of morphology as ductal, lobular, medullary, mucinous and mixed (ductal and lobular). Infiltrating duct cell carcinoma is shown in low power and high power in figure 1 and figure 2.

2.3.IMMUNOHISTOCHEMICAL ANALYSIS
Tissue sections (4 to 5 um thick) were used for all immunohistochemical analysis. The CONFIRM anti-ER (clone 6 F11) and anti – PR(clone 16 ) Monoclonal antibodies were used for immunohistochemical analysis of ER and PR, respectively performed on
automated slide strainers according to the manufacturers instruction. The ER and PR results were screened manually and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining. At least 200 cells were counted for each tumor specimen positive and negative controls were included in each batch. ER and PR IHC stained sections are shown in figure 3 and figure 4.

3. RESULTS

All cases were subjected to Immunohistochemistry for knowing estrogen receptor and progesterone receptor status in the carcinoma of breast and to histopathological examination of this 64 cases ER/PR expression is depicted in the Pie diagram

AGE
Majority 47/64(73.4%) cases were peri and post menopausal women and the ER/PR status showed a higher incidence of reactivity with increasing age. Receptor negativity was found to prevail in the pre-menopausal women. These were 21/29 and 10/16 cases in the age groups of 51-60 years and 61-70 years respectively displaying negative hormone receptor status. Nonetheless, in the age group of 71-80 and 81-90 years, there is each one case revealed positivity for ER/PR as depicted in Table 1.

SIZE
T2 sized tumors were 41% of the total followed by T3 sized tumors 32% and the receptor status was noted to be comparatively increased in larger sized tumors than in T1 and T2 group (Table 1).

HISTOLOGICAL TYPE
Out of 64, 60 cases (93.7%) were Infiltrating Ductal Carcinoma-Not Otherwise Specified with increased ER-/PR- negativity (43.33%). 2 cases were of mucinous carcinoma, both of them were receptor positive. Single lobular carcinoma case yielded receptor negativity. Comedo carcinoma shows ER negativity and PR positivity (Table 1).

HISTOLOGICAL GRADING
27 cases (42.18%) out of 64 were grade II type of infiltrating duct cell carcinomas. The reactivity for steroid receptors was observed to decrease with increasing grades (Table 1).

LYMPHNODE STATUS
The tumor receptor negativity was more in >4 lymph node metastases than <4 lymph node metastasis depicted in table.

4. DISCUSSION

An immunohistochemical analysis of steroid receptor status was performed in 64 cases of infiltrating duct carcinomas of breast. Previous studies have shown survival advantages among women with hormone receptor positive tumor relative to women with hormone receptor negative tumors. Previous studies have shown survival advantages among women with hormone receptor positive tumor relative to women with hormone receptor negative tumors. (21, 22)

The present study shows that the proportion of both ER and PR positive tumors increases with age. The proportional increase in ER and PR positivity with age was more marked for PR than for ER. Interestingly when receptor status was examined by age, we found that this difference was driven by increased percentage of ER/PR negative disease in 40-60 years old, rather than by
younger aged women and more than 60. This correlates with Madhuri et al. (23) Low grade duct carcinoma and those with elastosis expressed steroid receptors more often. High grade infiltrating duct carcinomas were predominantly ER/PR negative in our study. The presence of necrosis and lympho vascular invasion showed an inverse relationship with ER PR non-reactivity.

In a study of Barnes et al. approximately 50% of tumors are ER+ PR, 25% ER-PR-, 20% ER+PR- and 5% ER-PR+. (24) In contrast, our study reports a high proportion of receptor negative 42.19% of cases. Desai et al. (48%) and V. Dutta et al. (66%) obtained a high incidence of steroid receptor non-reactivity in breast cancer in India which correlated with our study (25, 26). There appears to be variation in steroid receptor positivity in the Asian population. Differences in ER and PR status by race, particularly between black and white are known. Chariyalerstak et al. reported similar observations with lower rates of ER and PR reactivity in breast cases in Thailand (27). The overall positivity rate ER and PR is lower as compared to other reports, possibly because of the difference in techniques of evaluation, high tumor grades and majority being menopausal women in this study which correlates with other studies. (25)

It is also intriguing to find a higher proportion of ER and PR+(10.94 %) tumors when compared to those documented in literature. The existence of this subgroup can be explained on the basis of arte factual laboratory reporting or possibly genetic alteration, which switches on PR expression resulting in the occurrence, of a true ER-PR+ phenotype. (28) It has been documented that ER-PR+ patients have a higher frequency of soft tissue and central nervous system metastasis, a lower frequency of bony metastasis and partial responsiveness to tamoxifen treatment as compared to 80% response rate for double positive tumors (29). Moreover the S phase fraction in the ER-PR+ subgroup is considered to be significantly higher than that noted in ER+PR+ and ER+PR- subgroups. (27)

On correlation of ER/PR status with grading it has been observed that non reactivity increased with high grade tumors as compared with low grade tumors. On correlation of ER/PR status with nodal metastases it has been observed that >4 nodal involvement revealed non reactivity more than that of <4 node involvement. Our study correlates well with other studies. (25, 26)

5. CONCLUSION

A significantly high incidence of ER-PR- and ER+PR- phenotypes in the selected population of breast cancer patients was seen. These observations also suggest that breast cancers seen in the Indian population may be biologically different from that encountered in Western practice. Invasive ductal carcinoma is the most common histologic type of carcinoma reported. The population of both ER and PR positivity increases with age and high grade infiltrating duct carcinomas were predominantly ER and PR negative. The tumor receptor negativity was seen in more than 4 lymph node metastases.
TABLE 1

CORRELATION OF AGE WITH ER/PR STATUS

<table>
<thead>
<tr>
<th>Age group (No. of cases) %</th>
<th>ER+/PR+ (%)</th>
<th>ER+/PR- (%)</th>
<th>ER-/PR+ (%)</th>
<th>ER-/PR- (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-50 yrs (17)(26.56)</td>
<td>5 (29.41)</td>
<td>2 (11.76)</td>
<td>1 (5.88)</td>
<td>9 (52.94)</td>
</tr>
<tr>
<td>51-60 yrs (29)(45.31)</td>
<td>8 (27.58)</td>
<td>6 (20.68)</td>
<td>4 (13.80)</td>
<td>11 (37.93)</td>
</tr>
<tr>
<td>61-70 yrs (16)(25.00)</td>
<td>6 (37.50)</td>
<td>1 (6.25)</td>
<td>2 (12.50)</td>
<td>7 (43.75)</td>
</tr>
<tr>
<td>71-80 yrs (1)(1.56)</td>
<td>1 (100.00)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>81-90 yrs (1)(1.56)</td>
<td>1 (100.00)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SIZE VS HORMONE RECEPTOR STATUS

<table>
<thead>
<tr>
<th>Size (No. of cases)</th>
<th>ER+/PR+</th>
<th>ER+/PR-</th>
<th>ER-/PR+</th>
<th>ER-/PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2cms (17)(26.56)</td>
<td>7 (41.17)</td>
<td>3 (17.64)</td>
<td>-</td>
<td>7 (41.17)</td>
</tr>
<tr>
<td>2.5cms (26)(40.62)</td>
<td>9 (34.61)</td>
<td>2 (7.69)</td>
<td>3 (11.53)</td>
<td>12 (46.15)</td>
</tr>
<tr>
<td>&gt;5cms (21)(32.81)</td>
<td>5 (23.81)</td>
<td>4 (19.04)</td>
<td>4 (19.04)</td>
<td>8 (38.10)</td>
</tr>
</tbody>
</table>

HISTOLOGIC TYPE VS HORMONE STATUS

<table>
<thead>
<tr>
<th>Type of tumors (No. of cases)</th>
<th>ER+/PR+</th>
<th>ER+/PR-</th>
<th>ER-/PR+</th>
<th>ER-/PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDC-NOS (60)(93.75)</td>
<td>19 (31.66)</td>
<td>9 (15.00)</td>
<td>6 (10.00)</td>
<td>26 (43.33)</td>
</tr>
<tr>
<td>Mucinous (2)(3.12)</td>
<td>2 (100.00)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lobular (1)(1.56)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100.00)</td>
</tr>
<tr>
<td>Comedo (1)(1.56)</td>
<td>-</td>
<td>-</td>
<td>1 (100.00)</td>
<td>-</td>
</tr>
</tbody>
</table>
GRADE VS HORMONE RECEPTORS

<table>
<thead>
<tr>
<th>Grade (No of cases)</th>
<th>ER+/PR+</th>
<th>ER+/PR-</th>
<th>ER-/PR+</th>
<th>ER-/PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I 18(28.12%)</td>
<td>6 (33.33)</td>
<td>1 (5.55)</td>
<td>1 (5.55)</td>
<td>10 (55.56)</td>
</tr>
<tr>
<td>Grade II 27(42.18%)</td>
<td>14 (51.85)</td>
<td>5 (18.51)</td>
<td>2 (7.41)</td>
<td>6 (22.22)</td>
</tr>
<tr>
<td>Grade III 19(29.69%)</td>
<td>1 (5.26)</td>
<td>3 (15.79)</td>
<td>4 (21.05)</td>
<td>11 (57.89)</td>
</tr>
</tbody>
</table>

LYMPHNODES VS HORMONE RECEPTORS

<table>
<thead>
<tr>
<th>Lymph nodes (No.of cases)</th>
<th>ER+/PR+</th>
<th>ER+/PR-</th>
<th>ER-/PR+</th>
<th>ER-/PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 23(35.93%)</td>
<td>8 (34.78)</td>
<td>2 (8.69)</td>
<td>3 (13.04)</td>
<td>10 (43.48)</td>
</tr>
<tr>
<td>&gt;4 41(64.06%)</td>
<td>13 (31.71)</td>
<td>7 (17.07)</td>
<td>4 (9.75)</td>
<td>17 (41.46)</td>
</tr>
</tbody>
</table>

Figure -2 Section showing islands of tumor cells - Infiltrative Duct Cell Carcinoma NOS (H & E, x10)

Figure – 3 Sections showing vesicular nucleus (H & E x40)
Figure – 4  Section showing IHC – Estrogen receptor antigen stained cells represented in brown areas (x10)

Figure – 5  Sections showing IHC – Progesterone receptor stained cells represented by brown areas (x10)

6. ACKNOWLEDGMENT
The authors thank DR Sudhakar Reddy E, Professor and HOD, Department of Pathology, Sri Venkateswara Medical College, Tirupathi for providing the samples.

7. REFERENCES


