**MANUSCRIPT (MAIN FILE)**

TITLE:-

 Histopathological spectrum of breast lesions in association with Histopathological Grade versus Estrogen receptor and Progesterone receptor status in breast cancers -A Hospital based study

**ABSTRACT**

**Background: -** A retrospective study of 2-years duration from January 2015 to December 2016 was undertaken to evaluate the histopathological spectrum of breast lesions including both the benign breast lesions and malignant breast lesions. To evaluate the Estrogen receptor and Progesterone receptor status in breast cancer cases by using immunohistochemistry and is to correlate the histopathological grade with ER, PR status of breast cancer.

**Methods:-**

 The histopathological findings of the 132 biopsied Specimens of all age groups and both **1**

Sexes were studied and most of them were lumpectomy specimens and few were mastectomy specimens.IHC markers were applied on breast cancer cases.

**Results:**

 Out of the 132 cases benign breast lesions constituted 78.78% and malignant breast lesions 21.22%.Among the benign breast lesions fibro adenoma was the commonest and among the malignant breast lesion, infiltrating ductal carcinoma was commonest. The peak incidence of benign breast lesion was in 11 to 40 years and malignant lesion in 41 to 70years. Out of 22 cases of infiltrating ductal carcinoma, 27.27% cases showed both ER, PR positivity and 63.64% cases showed both ER, PR negativity. A significant association was seen between histologic grade and ER, PR status.

**Conclusion:**

In our study fibro adenoma was the commonest benign breast lesion and infiltrating

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ductal carcinoma was the predominant malignant breast lesion. Among the breast cancers, both ER and PR negativity cases were predominant **and** low grade tumors were showing high ER, PR expression and high grade tumors were showing low ER, PR expression in our study.

**KEY WORDS**:

 Breast lesions, Fibro adenoma, infiltrating ductal carcinoma, histopathological grade, Immunohistochemistry.

**MAIN TEXT**

**INTRODUCTION**:-

 According to WHO statistics 20081, malignant breast lesions comprises 1.38 million cases (10.9% of total cancer cases).According to Indian statistics malignant breast lesion is the second most common malignancy in woman after carcinoma of cervix and is detected in 20 per1,00,000 women2, 3.Fortunately, most of the breast lesions are diagnosed as benign breast lesions4 . Main aim of our study is to evaluate the

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Histopathological spectrum of breast lesions in patient attending the NMCH, Nellore Andhra Pradesh, India,

 The spectrum of breast lesions consists of benign lesions including fibro adenoma phyllodes tumor, Gynaecomastia, Breast abscess & chronic mastitis, malignant lesions including ductal carcinoma, lobular carcinoma, colloid carcinoma and medullary carcinoma. Benign breast lesions incidence begins during the second decade of life and peaks in the fourth and fifth decades. But malignant breast lesions incidence increase after menopause5.

 **MATERIAL AND METHODS**:-

 The study was conducted in the department of pathology, NMCH, Nellore, Andhra Pradesh, India from January 2015 to December 2016.132 biopsy specimens were received. Among the 132 biopsy specimens most of biopsy specimen’s were lumpectomy specimens and few were mastectomy specimens. These biopsy specimens were fixed in 10% formalin solution for

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24 hours. The tissue was processed routinely, andparaffin embedded tissue were cut on microtome to the thickness of 4 microns.The sections were stained with Haematoxylin and Eosin stain and reported. Out of 28 cases of breast cancers, 22 cases were infiltrating ductal carcinomas which were histologically graded according to Modified Bloom-Richardson-Elston grading system. After that, breast cancer tissue block were selected for IHC evaluation (ER and PR status).

**STATISTICAL ANALYSIS:-**

* Data collected was entered in MS Excel and analyzed using SPSS-Version 22.0.Percentages and chi-square values were calculated.
* A P value of 0.05 was taken as significance.

**RESULT:-**

 132 cases of breast lesions were studied over a period of Two years from January 2015 to December 2016.The commonest presenting symptom in breast lesion was lump in the breast.

Out of 132 cases, 20 cases were males

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and 112 cases were females. Out of 20 cases of males, majority of cases were Gynaecomastia .Among 132 cases, benign breast lesions consistituted 104 cases (78.78%) and malignant breast lesions 28 cases (21.22%) (Figure 1). The youngest male (14 years) was diagnosed with gynaecomastia and the youngest female (12 years) was diagnosed with fibro adenoma breast. The ages of the cases ranged between 11 years to 80 years. The peak age of occurrence to the benign lesions was found to be in between 11-40 years, youngest case detected as fibro adenoma at 12 years of age. The peak age of occurrence of the malignant lesion was found to be in between 41-60 years, youngest case diagnosed as medullary carcinoma at 35 years of age, and oldest case diagnosed as a mucinous carcinoma at 80 years of age. (Table 1 & 2)

 Among the 104 cases of benign breast lesions, 78 cases (75%) were of fibro adenoma (Figure 2), 2 cases (1.92%) were of benign phyllodes (Figure 3), 14 cases (13.46%) were of Gynaecomastia, 4 cases (3.85%) were of breast abscess and 6 cases (5.77%) were of chronic inflammatory pathology .Among the benign breast

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lesions, fibro adenoma was the most common lesion(Table 3).

 Among the 28 cases of malignant breast lesions, 22 cases (78.57%) were of infiltrating ductal carcinoma(Figure 4), 2 cases (7.14%) were of medullary carcinoma(Figure 5),2 cases (7.14%) were of lobular carcinoma(Figure 6) and 2 cases (7.14%) were of mucinous carcinoma(Figure 7). Among the malignant breast lesions, infiltrating ductal carcinoma was the most common lesion (Table 4).

Among the 22 cases of infiltrating ductal carcinoma, the commonest grade was grade 2 accounting to 72.73% followed by grade 3 and grade 1 with 18.18% and 9.09% respectively (Table 5).

Among the 22 cases of infiltrating ductal carcinoma ,10 cases of grade 2 breast carcinoma were in age group of 41-50 years, 2 cases of grade 2 breast carcinoma were in age group of 51-60 years , 2 cases of grade 1 ,4 cases of grade 2 and 4 cases of grade 3,breast carcinoma were in age group of 61-70 years ( Table :6)

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Out of 22 cases of infiltrating ductal carcinoma, lesions were studied for the ER, PR expression. The number of tumors positive for both ER and PR (Figure 8 & 9) were 6 (27.27%).The number of tumors for ER positive and PR negative was two cases (9.09%). The number of tumors negative for both ER and PR were 14 (63.64%) (Table-7).

 Among the 22 cases of infiltrating ductal carcinoma 50% of grade1 cases were ER positive and PR positive, 25% of grade 2 and 25% grade 3 were also ER positive and PR positive. 68.75% of grade 2 and 75% of grade 3 tumors were both ER negative and PR negative (Table 8).

**Discussion:**

 The breasts are composed of specialized epithelium and stroma that may give rise to both benign and malignant lesions. The human breast contains six to ten major ductal systems. The keratinizing squamous epithelium of the overlying skin dips into the orifices at the nipple and then abruptly changes to a double layered cuboidal epithelium lining the ducts. Successive

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branching of the large ducts eventually leads to the terminal duct lobular unit. Two cell types lining the ducts and lobules are luminal epithelial cells overlaying the epithelial cells .Spectrum of breast lesions consist of benign lesions and malignant lesions. Most common benign breast lesions are fibro adenoma, phyllodes tumor, mastitis and breast abscess. Most common malignant lesion are ductal carcinoma, lobular carcinoma, tubular carcinoma, mucinous carcinoma, medullary carcinoma, papillary carcinoma and metaplastic carcinoma.

 Breast lesions are more predominance among females when compared to males and histopathological spectrum of breast lesion and their etiology varies among different countries and ethnic group 6 .Benign breast lesions are more predominant as compared to malignant breast lesion as seen throughout the world7.Risk factors for both benign breast lesions and malignant breast lesions include multiparty, low parity, low age at first child birth and late menopause, highlighting the fact towards excessive circulating estrogen8,9 .

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 In our study, benign breast lesions comprised 78.78% of the total lesions and malignant lesion 21.22%.The percentage of malignant breast lesions appears to be higher than that in the west (10%) 10 and nearer to Africa (21%) 11.

 In our study most common benign breast lesion was found to be fibro adenoma and most common malignant breast lesion was infiltrating ductal carcinoma. Similar results were noted in the other studies (Table9&Table10).

 In our study, among the benign breast lesions, incidence of fibro adenoma is 75% which is higher than the Kulkarni s study (62.32%) and Malik R study (55.0%).The incidence of benign phyllodes is 1.92% which is nearly compatible with Kulkarni S study (1.45%) and Malik R study (1.27%). The incidence of gynaecomastia is 13.46% among benign breast lesion. The incidence of Breast abscess is 3.85% which is higher than the Kulkarini S study (1.45%).Incidence of chronic inflammatory lesion is 5.77%(Table 9).

 In our study, among the malignant breast lesion, incidence of infiltrating ductal

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carcinoma is 78.57% which is lower than the Kulkarini S study (84.85%) and Malik R study (88.20%).Incidence of medullary carcinoma is 7.14% which is higher than the Malik R study (2.75%).Incidence of lobular carcinoma is 7.14% which is higher than the Malik R study (3.21%) and Kulkarini S study (3.03%).Incidence of mucinous carcinoma is 7.14% which is higher than the Malik R study(0.64%) and Kulkarini S study (3.03%). (Table 10)

 In our study incidence of benign breast lesion is 78.78% and malignant breast lesion is 21.22% which is nearly similar to U R Singh et al 2009 and Malik et al (2003) studies [Table 11].

 In our study, the number of tumors positive for both ER and PR was 27.27% which is nearly similar to Thakral etal (2016) 16 study (25.64%). In our study the number of tumors for ER positive and PR negative was 9.09% which is higher than the Thakral etal (2016) 16 study (5.98%). In our study, the number of tumors negative for both ER and PR was 63.64% which is nearly similar to Thakral etal (2016) 16 study (63.25%) (Table-12).

 In the present study majority of breast

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cancers were grade 2 (72.73%) followed by grade 3

(18.18%) and grade 1 (9.09%) which is in concordance with Azizun- Nisa et al (2008) 17 study ,Adebayo et al (2009) 18 study ,Suvarchala et al (2011) 19 study,Ambroise et al (2011) 20 study and Geethamala k et al (2014) 22 studies except for one study by Ghosh et al (2011) 21 having more of grade 3 tumors (75.4%) (Table 13).

In the present study immunohistochemistry revealed 27.27% ER + /PR + and 63.64% of ER negative/PR negative. These results were in concordance with Survachala et al (2011) 19 study (Table 14).

 In the present study significant co-relation was established between ER/PR hormone receptor status and grading of tumor. 50% of grade 1, 25% of grade 2 and 25% grade 3 tumors were ER positive /PR positive. 68.75% of grade 2 and 75% grade 3 tumors were ER negative/PR negative. Our study was in concordance with Geethamala k et al (2014) 22 study (Table 15).

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**CONCLUSION:-**

 Our study comprised 132 cases of

breast lesions. The cases presented to Narayana Medical College & Hospital, Nellore. The biopsy specimens were then evaluated histopathologically, and 22 cases were analyzed by immunohistochemistry.

 In our study the most common histopathological type of benign breast lesion was fibro adenoma (78 cases, 75% of the total benign breast lesion). The most common histopathological subtype of breast malignancy was infiltrating ductal carcinoma-NOS type (22 cases, 78.57%) of the total cases with malignant lesion. Grade 1 tumors (low grade) were showing higher ER, PR expression .Grade 3 tumors (high grade ) were showing lower ER,PR expression. Tumor grading highly correlates with the survivor rate and receptor status predicts the response to hormonal therapy.

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**FIGURE WITH LEGENDS**:-

 **Figure 1:-** Pie chart showing Spectrum of Breast lesions

**Figure2:-**Photomicroscopic picture of Fibro adenoma (H&E, 100X)

 **Figure 3:-**Photomicroscopic picture of benign phyllodes (H&E, 100X)

 **Figure 4**:- Photomicroscopic picture of Infiltrating of ductal carcinoma (H&E, 400X)

 **Figure 5:-** Photomicroscopic picture of Medullary carcinoma (H&E, 100X)

 **Figure 6**:- Photomicroscopic picture of Lobular carcinoma (H&E, 100X)

 **Figure 7:-** Photomicroscopic picture of Mucinous carcinoma (H&E, 400X)

 **Figure 8:-** Photomicroscopic picture of ER positive breast carcinoma (400X)

 **Figure 9:-** Photomicroscopic picture of PR positive breast carcinoma (400X)

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**Table -1 Age wise distribution of benign**

 **breast lesions**

P=0.000

Benign lesions were more common in the early age groups when compared to the older ages and this difference was statistically significant (p=0.000)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **s.no** | **Age** | **Fibro adenoma** | **Benign phyllodes** |  **Gynaecomastia** | **Breast****Abscess** | **Chronic inflammatory****Pathology** | **Total** |
| **1** | **11-20** | **22** | **-** | **6** | **-** | **-** | **28** |
| **2** | **21-30** | **18** | **2** | **4** | **4** | **-** | **28** |
| **3** | **31-40** | **28** | **-** | **-** | **-** | **2** | **30** |
| **4** | **41-50** | **2** | **-** | **4** | **-** | **-** | **6** |
| **5** | **51-60** | **4** | **-** | **-** | **-** | **2** | **6** |
| **6** | **61-70** | **4** | **-** | **-** | **-** | **-** | **4** |
| **7** | **71-80** | **-** |  **-** | **-** | **-** | **2** | **2** |
| **Total** | **78** |  **2** | **14** | **4** | **6** | **104** |

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|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **s.no** | **Age** | **Ductal****carcinoma** | **Lobular carcinoma** | **Medullary carcinoma** | **Mucinous****carcinoma** | **TOTAL** |
| **1** | **11-20** | **-** | **-** | **-** | **-** | **-** |
| **2** | **21-30** | **-** | **-** | **-** | **-** | **-** |
| **3** | **31-40** | **-** | **-** | **2** | **-** | **2** |
| **4** | **41-50** | **10** | **-** | **-** | **-** | **10** |
| **5** | **51-60** | **2** | **2** | **-** | **-** | **4** |
| **6** | **61-70** | **10** | **-** | **-** | **-** | **10** |
| **7** | **71-80** | **-** | **-** |  | **2** | **2** |
| **Total** | **22** | **2** | **2** | **2** | **28** |

 **Table -2 Age wise distribution of malignant breast lesions**

 Breast Carcinoma weremore common in the middle & elderly age groups when compared to the early ages groups and this difference was statistically significant (p=0.000).

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**Table: 03 Different histopathological lesions in Benign Breast lesions**

|  |  |  |
| --- | --- | --- |
| **Histopathological diagnosis** | **Number of cases**  | **Percentage of benign Breast lesions** |
| 1.Fibroadenoma | 78 | 75% |
| 2.Benign Phyllodes | 2 |  1.92% |
| 3.Gynaecomastia | 14 |  13.46% |
| 4.Breast abscess | 4 |  3.85% |
| 5.Chronic inflammatory pathology | 6 |  5.77% |
| **Total** | **104** | **100%** |

|  |  |  |
| --- | --- | --- |
| **Histopathological diagnosis** | **Number of cases**  | **Percentage of Malignant breast lesions** |
| 1.Infiltrating ductal carcinoma |  22 | 78.57% |
| 2.lobular carcinoma | 2 | 7.14% |
| 3.Medullary carcinoma | 2 | 7.14% |
| 4.Mucinous carcinoma | 2 | 7.14% |
| **Total** | **28** | **100%** |

 **Table: 4 Different histopathological lesions in Malignant Breast lesions**

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**Table no: 05 Histopathological grading of breast carcinomas**

|  |  |  |
| --- | --- | --- |
| **Grade of the tumor** |  **Number of cases** |  **percentage** |
| Grade 1 | 2 | 9.09% |
| Grade 2 | 16 | 72.73% |
| Grade 3 | 4 | 18.18% |
| **Total**  | 22 | 100% |

 **Table no: 6 Correlations between age wise distribution and grade of breast carcinomas**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **s.no** | **Age** | **Grade 1** | **Grade 2** | **Grade 3** | **Total** |
| 1 | 41-50 | -(0%) | 10(62.5%) | -(0%) | 10(45.5%) |
| 2 | 51-60 | -(0%) | 212.5% | -(0%) | 2(9%) |
| 3 | 61-70 | 2(100%) | 4(25%) | 4(100%) | 10(45.5%) |
| Total | 2(100%) | 16(100%) | 4(100%) | 22(100%) |

There is statistically significant difference among the grades when compared to the different agent age groups (p=0.042).

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 **Table no: 7 Distribution of Breast carcinomas according to ER and PR status**

|  |  |
| --- | --- |
| **ER /PR status** | **Number of cases** |
| ER+ PR+ | 6(27.27%) |
| ER+ PR- | 2 (9.09%) |
| ER- PR+ | 0 |
| ER- PR- | 14(63.64%) |

**Table no: 8 Association of IHC Hormone receptor status with grade of Breast carcinomas**

|  |  |  |  |
| --- | --- | --- | --- |
| **IHC Hormone receptor status** | **Grade 1(%)** | **Grade 2(%)** | **Grade 3(%)** |
| ER+/PR+ | 1(50%) | 4(25%) | 1(25%) |
| ER+/PR- | 1(50%) | 1(6.25%) | 0 |
| ER-/PR+ | 0 | 0 | 0 |
| ER-/PR- | 0 | 11(68.75%) | 3(75%) |
| **Total** | 2(100%) | 16(100%) | 4(100%) |

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**Table-9: Comparative study of spectrum of**

**Benign breast lesion**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **s.no** | **Benign lesions** | **Malik et al (2003)14(% benign)** | **Kulkarni S.et al (2009)15(% benign)** | **Our study****(% benign)** |
| 1 | Fibro adenoma |  55.0 |  62.32 | 75 |
| 2 | Benign phyllodes | 1.27 | 1.45 | 1.92 |
| 3 | Gynaecomastia | - | - | 13.46 |
| 4 | Breast abscess | - | 1.45 | 3.85 |
| 5 | Chronic inflammatory lesion | - | - | 5.77 |
| 6 | Fibroadenosis | 0.32 | 4.35 | - |
| 7 | Fibrocystic disease | 28.38 | 11.5 | - |
| 8 | Lactating adenoma | 0.87 | 4.35 | - |
| 9 | Chronic mastitis | 6.84 | 2.90 | - |
| 10 | Plasma cell mastitis | 1.83 | - | - |
| 11 | T.B.mastitis | 2.46 |  1.45 | - |
| 12 | Intraduct papilloma | 0.48 | 1.45 | - |
| 13 | Duct ectasia | 0.56 | 2.90 | - |
| 14 | Galactocele | - | 0.72 | - |
| 15 | Misc | 1.99 | 5.07 | - |

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**Table: 10: Comparative study of spectrum of malignant breast lesion**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **s.no** | **Malignant lesions** | **Malik et al (2003)14(%malignant)** |  **Kulkarni S.et al (2009)15 (%malignant)** | **Our study****(%malignant)** |
| 1 | Infiltrating Ductal carcinoma | 88.20 | 84.85 | 78.57 |
| 2 | Infiltrating Lobular carcinoma | 3.21 | 3.03 | 7.14 |
| 3 | Mucinous carcinoma | 0.64 | 3.03 | 7.14 |
| 4 | Medullary carcinoma | 2.57 | - | 7.14 |
| 5 | Papillary carcinoma | 0.86 | 3.03 | - |
| 6 | Squamous cell | 0.64 | - | - |
| 7 | Undifferentiated carcinoma | 0.64 | - | - |
| 8 | Non Hodgkin’s lymphoma | 0.42 | 3.03 | - |
| 9 | Intraduct carcinoma | 1.50 | 3.03 | - |
| 10 | Miscellaneous | 1.29 | - | - |

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**Table: 11 Comparisons of spectrum of Breast lesions with other studies**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.NO** | **Study group** | **Benign** | **Malignant** |
| 1 | Our study | 78.78 | 21.22 |
| 2 | UR Singh et al 200912 | 80.7 | 19.3 |
| 3 | Rasheed A.et al (2009-2011)13 | 72.97 | 27.3 |
| 4 | Malik et al (2003)14 | 80.7 | 19.30 |
| 5 | Kulkarni S.et al (2009)15 | - | - |

**Table: 12 Comparison of distribution of Breast cancer cases according to ER and PR status**

|  |  |  |
| --- | --- | --- |
| **ER/PR status** | **Thakral etal (**2016)16 | **Present study** |
| ER + PR+ | 30(25.64%) |  6(27.27%) |
| ER + PR- | 7(5.98%) |  2(9.09%) |
| ER - PR+ | 6(5.13%) |  0 |
| ER – PR- | 74(63.25%) |  14(63.64%) |

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**Table: 13 Comparative incidence of frequency of grade of Breast carcinomas**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Grade of the tumor** | **Azizun-Nisa etal** (2008)17 | **Adedayo etal (**2009)18 | **Suvarchala****etal** (2011)19 | **Ambroise** **etal** (2011)20 | **Ghosh****etal**(2011)21 | **Geethamala k****etal** (2014)22 | **Present study** |
| 1 | 6.7 | 21.2 | 28.12 | 9.4 | 0.3 | 19 | 9.09% |
| 2 | 55.3 | 38.4 | 42.18 | 57.4 | 15.9 | 54 | 72.73% |
| 3 | 38.0 | 35.9 | 29.69 | 33.3 | 75.4 | 27 | 18.18% |

**Table no: 14 Comparative incidence of frequency of hormone receptor status in Breast carcinomas**

|  |  |  |
| --- | --- | --- |
| **Hormone receptor status** | **Suvarchala etal (**2011)19 | **Present study** |
| ER+/PR+ | 32.8 | 27.27 |
| ER+/PR- | 14.0 | 9.09 |
| ER-/PR+ | 10.94 | 0 |
| ER-/PR- | 42.19 | 63.64 |

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**Table: 15 Comparison of Hormone receptor status with grade of the Breast carcinomas**

|  |  |  |
| --- | --- | --- |
| **Study** | **Grade of tumor** | **Hormone receptor positive** |
| Suvarchala etal (2011)19 | Grade 2 | 51.85% ER+/PR+ |
| Geethamala k etal (2014)22 | Grade 1Grade 2Grade 3 | 78.9% ER+/PR+64.9% ER+/PR+7.4% ER+/PR+ |
| Present study | Grade 1Grade 2Grade 3Grade 2Grade 3 | 50% ER+/PR+25% ER+/PR+25% ER+/PR+68.75% ER-/PR-75% ER-/PR- |

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**Fig No: 1 Spectrum of Breast lesions**

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 Figure 2

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 Figure 3

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 Figure 4

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 Figure 5

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 Figure 6

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 Figure 7

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 Figure 8

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 Figure 9

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