EXPRESSION OF ESTROGEN, PROGESTERONE, AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTORS IN BREAST CANCER IN GMERS MEDICAL COLLEGE AND HOSPITAL GANDHINAGAR, INDIA

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ABSTRACT:

Background: Breast carcinoma is the most common and deadly malignancy of women globally. It is an extremely heterogeneous disease. The aim is to analyze the demographical profile of patients with breast cancer and evaluate the hormonal receptor status and their association with the grading and staging of breast cancer at the time of diagnosis in our hospital. From June 2022 to December 2023, a retrospective study was conducted in the Department of Pathology, at GMERS Medical College Gandhinagar. In this study, we found that the mean age of patients was 56.5+-7.5. Most tumors were Her2Neu negative (73.10%) followed by ER+ (54.8%), and 32.69 % of tumors were PR+. Most of the cases were in stage 2(46.1%) and stage 3(42.30%). Histological grade II was found in 50.94 % of the cases followed by grade III in 42.30% of cases at the time of diagnosis. The most common hormonal receptor expression was ER/PR+, Her2-(54.80%). Regarding, an association of hormonal receptor expression with grading and staging of tumors higher grade (III) and stage (III) were observed in Triple-negative tumors, and lower grade (II) and stage (II) tumor was found in ER/PR+, Her2- statistically significant tumors (p-value <0.05). A significant association was found between various hormonal expression statuses and stages as well as grade of the breast cancer.

Key Words: Breast, Hormone Receptor, Immunohistochemistry, ER, PR, HER2

INTRODUCTION:

Breast cancer is among the most common cancers affecting females worldwide. There are three predictive markers: Estrogen receptors, Progesterone receptors, and Her2-neu receptors have independent prognostic value in breast cancer. ER expression appears in 80-90 % of patients with breast cancer, while PR expression appears in 70-80 % of cases. Her2-neu overexpression is present in 15-20 % of cases (1,2). In India, the incidence has increased significantly, almost by 50%, between 1965 and 1985 (3). As per the Globocan data 2020, in India, Breast Cancer accounted for 13.5% (178361) of all cancer cases and 10.6%

(90408) of all deaths with a cumulative risk of 2.8% (4). When it comes to the 5-year overall survival, a study reported it to be 95% for stage I patients, 92% for stage II, 70% for stage III, and only 21% for stage IV patients (5). The survival rate of patients with breast cancer is poor in India as compared to Western countries due to earlier age at onset, late stage of disease at presentation, delayed initiation of definitive management, and inadequate/fragmented treatment (6). According to the World Cancer Report 2020, the most efficient intervention for Breast Cancer control is early detection and rapid treatment (7). A 2018 systematic review of 20 studies reported that Breast Cancer treatment costs increased with a higher stage of cancer at diagnosis. Consequently, earlier diagnosis of Breast Cancer can lower treatment costs (8).

Breast cancer is an extremely heterogeneous disease caused by interactions of both inherited and environmental risk factors which lead to progressive accumulation of genetic and epigenetic changes in breast cancer cells. Epidemiological evidence suggests that among various risk factors for Breast Cancer such as age, obesity, alcohol, estrogen exposure, and mammographic density, Family History remains the strongest risk factor. Familial forms occupy approximately 20% of all breast cancers and appear to have a distinctive pathogenesis dependent on particular susceptibility genes involved (9). Germline mutation in tumor suppressor genes (TSGs) is found to be the culprit behind most of the Familial forms. These genes include BRCA1 and BRCA2 (10).

Breast cancer is the most widely recognized threatening tumor women regularly get from the inward coating of milk conduits (ductal carcinoma) or from the Lobules (lobular carcinoma) that supply the channels with milk (11). Estrogen and progesterone receptor expressions are the greatest important and useful predictive factors currently available. Steroid receptor testing for ER and PR by immunohistochemistry is the established standard of care with almost 70–80% of the tumors in breast cancer being ER and/or PR positive (12). The frequency of positivity and the level of ER and PR increase with age, reaching their highest levels in postmenopausal women (13). Patients with breast cancer whose malignancy lacking in ER and PR do not benefit from hormonal treatment (14).

Overexpression of the HER2/neu oncogene is also an important event in breast cancer tumorigenesis. The HER2/neu receptor is a member of the epidermal growth factor receptor family of receptor tyrosine kinases, which are considered to be important mediators of cell proliferation and differentiation (15). HER2/neu positivity has been reported to be a negative predictor of response to hormonal therapy, adjuvant radiotherapy, and adjuvant chemotherapy (16). Measurement of HER-2 can be performed by either IHC or fluorescent in situ hybridization.

Expression of Hormonal Receptors

Estrogen receptor (ER) signaling plays a crucial role in mammary gland development and carcinogenesis. Approximately 78 % of invasive breast cancers are ER-positive (ER+), and this rate is projected to increase by 0.75 % each year (17), possibly owing to rising risk factors in the population such as age, obesity, and nulliparity. ER is a well-established independent prognostic and predictive marker for breast cancer as ER-targeted endocrine therapies have significantly improved the survival outcomes in patients with ER+ breast cancer and the therapeutic effects are strongly correlated with the levels of the ER expression in tumor cells (18).

ER positivity in breast cancer generally indicates a better prognosis, although its prognostic power is modified by histologic grade and tumor stage. Thus, precise assessment of ER status is pivotal for

treatment decision-making in patients with breast cancer.

As an ER-dependent gene product, progesterone receptor (PR) is typically overexpressed in ER+ tumors and thus is theoretically a surrogate marker for a functional ER signaling pathway (19). However, ER+ breast cancers are not always PR+, and a significant subset of tumors demonstrate an ER+/PR- phenotype (20).

The Her2 proto-oncogene is present in two copies in the normal breast tissue, but in its mutated form, there is an increase in the gene copy numbers, also known as Her2 gene amplification or over-activation. In its mutated form, it becomes an oncogene, inducing carcinogenicity of the breast tissue. These tumors are phenotypically very aggressive having high tumor proliferation rates,

metastasis, and mortality (21).

HER2, an epidermal growth factor receptor that is located at chromosome 17q11.2-12 encoding a tyrosine kinase that is composed of three separate regions: an extracellular region (a ligand-binding domain), a transmembrane domain, and an intracellular region (a tyrosine kinase domain). Ligand binding leads to receptor dimerization and activation of intrinsic tyrosine kinase activity. Activation of its receptors starts downstream signaling pathways which regulate various cellular functions, including cell expansion, apoptosis, angiogenesis, and motility (22).

Studies have found that trastuzumab is especially successive in the treatment of HER2-positive metastatic breast tumors (23).

Table 1: ER/PR and HER2 Scoring System and Criteria.

SCORING SYSTEM	
0	Negative for receptor
+1	Borderline
+2 to +3	Positive for receptor
CRITERIA	
0	0% Nuclear staining
+1	< 10% Nuclear Staining
+2	10% to 75% Nuclear staining
+3	>75% Nuclear staining

Table 2: HER2 NEU Scoring System and Criteria

SCORING SYSTEM	
0	Negative
+1	Negative
+2	Weak Positive
+3	Positive
CRITERIA	
0	Negative, no staining is observed, or membrane staining is <10% of the tumor cell.
+1	Negative, a faint perceptible membrane staining is detected in >10% of the tumor cells.
+2	Weak positive. A weak to moderate complete membrane staining is observed in >10% of the tumor cells.
+3	Positive. A strong complete membrane staining is observed in >10% of the tumor cells.

ER-estrogen receptor, PR-progesterone receptor, HER2-human epidermal growth factor receptor

MATERIAL AND METHOD:

This was a retrospective study conducted in the Department of Pathology, at GMERS Medical College Gandhinagar. Paraffin-embedded tissue blocks were retrieved from the archive and stained with H&E for histological confirmation and establishment of histological grade and type of tumor. Immunohistochemistry staining using a mouse-derived monoclonal antibody for hormonal receptors and HER2Neu expression was also done. The diagnosis was made according to the WHO classification of tumors. All diagnosed cases of invasive breast cancer from June 2022 to December 2023 were included in this study. A total of 104 cases were analyzed for age, sex, residence, and tumor characteristics like Stage, Histological grade, Lymph node metastasis, and Hormonal receptor status (ER, PR, HER2neu receptors).

Statistical analysis:

The differences in subjects and characteristics of tumors in this study were analyzed by using SPSS (Statistical Package for Social Sciences) version 22. In all statistical analyses, a P value < 0.05 was considered to be significant

Result:

The mean age of the patients was 56.5+/-7.5 years. Only 3 patients were of age less than 40 years (2.9 %). 39 % of the patients were in the age group of more than 60 years, 35 % of the patients were in the 50–60-year age group and 23.1 % of the patients were in the 40-50-year age group.
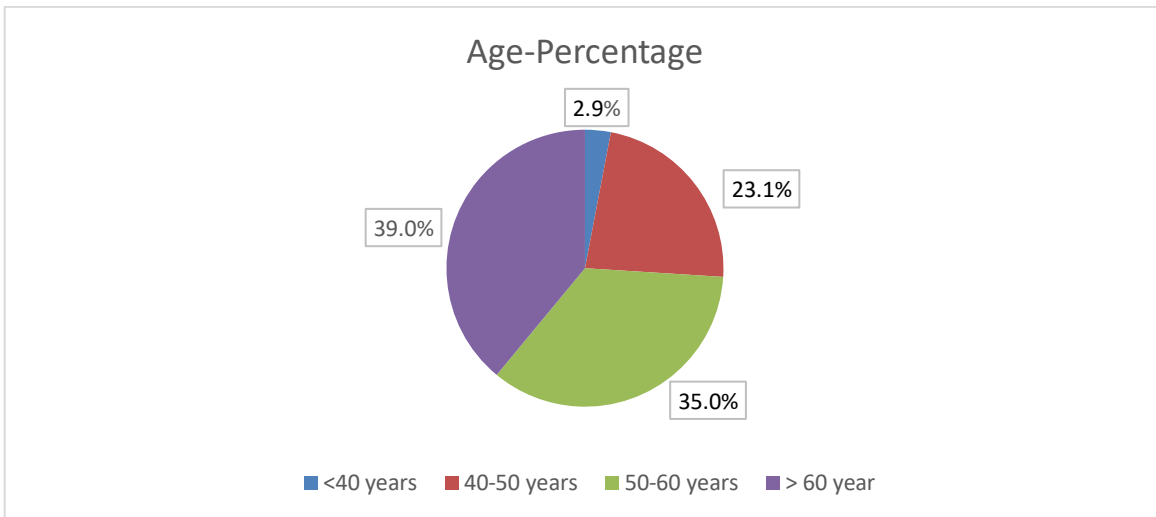
Figure 1. A pie chart showing the distribution of cases according to age.

Sex Distribution

The sex showed that 1.9 % of the patients were males and 98.1% of the patients were females, showing its major prevalence in Females.
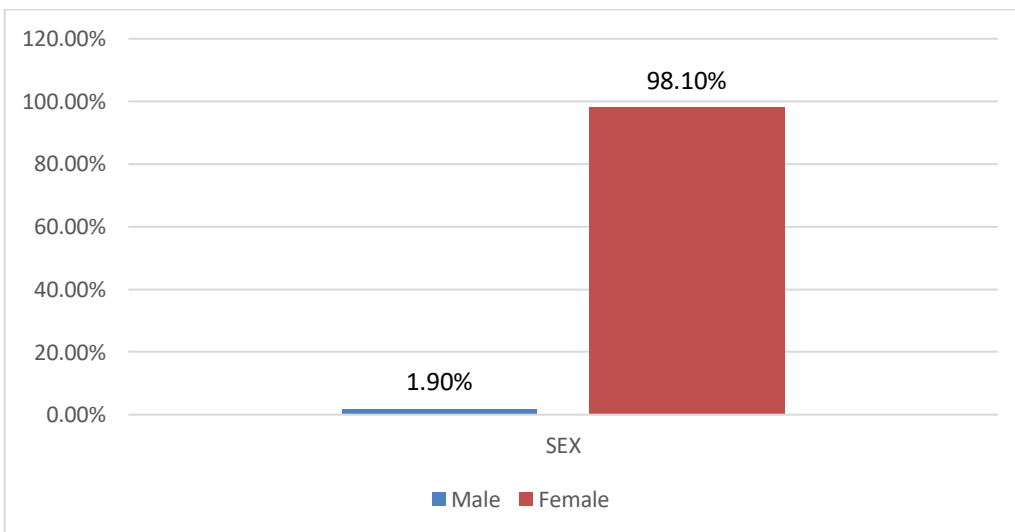

Figure 2. A bar diagram showing sex distribution.

Residence

It is shown that 41.0 % of the patients were living in rural areas while 59.0 % of the patients were living in urban areas in our city; The incidence of disease was more common in urban areas.
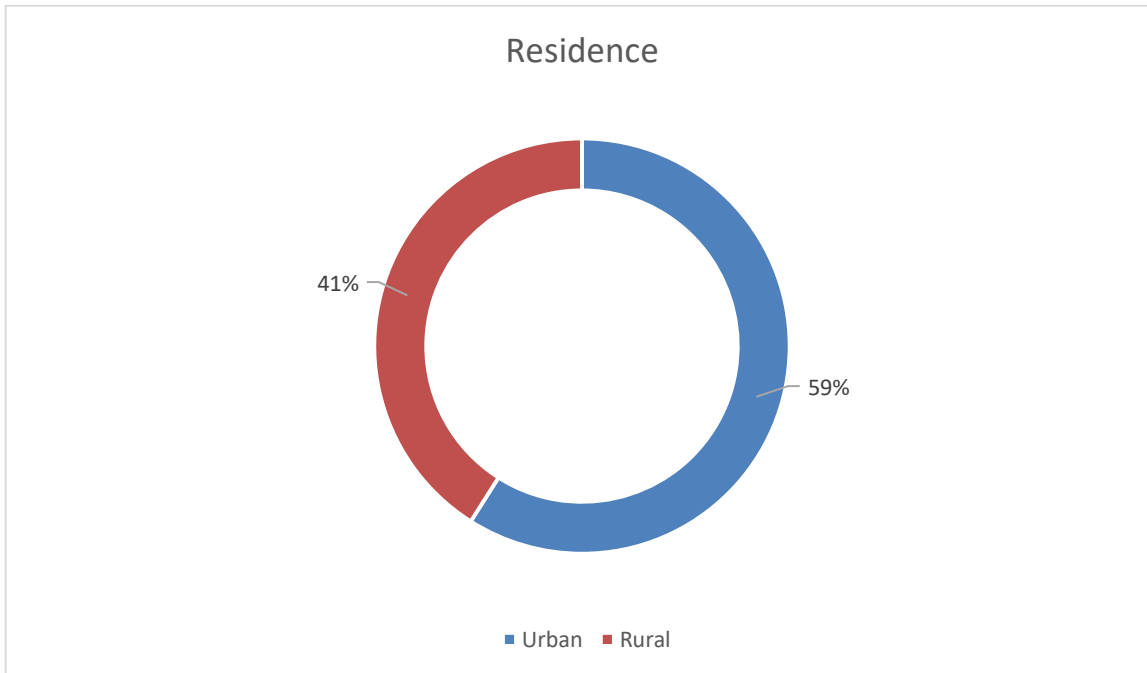

Figure 3. A bar chart showing the distribution of cases according to residence.

Histological grade:

Grading of tumors showed that 6.7% of the patients had grade I, 50.94% of the patients had grade II and 42.3% of the patients had grade III tumors.
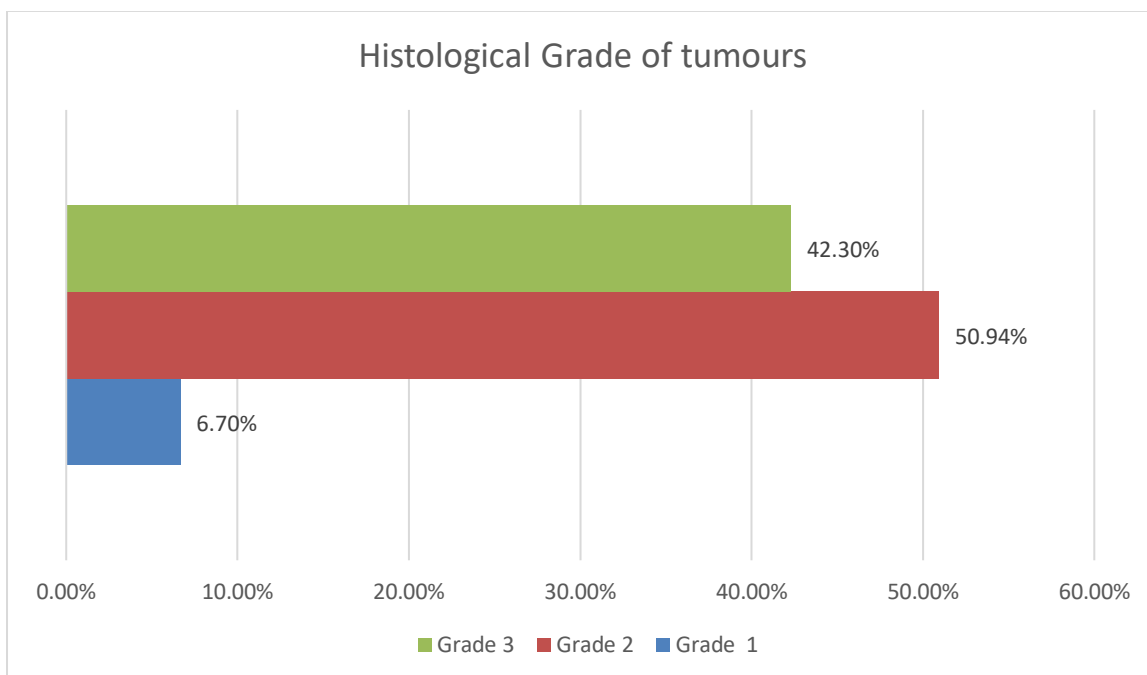

Figure4. A Bar chart showing the distribution of cases according to histological grade.

Staging of tumour

Staging of the tumor showed that 46.10% of the patients had stage II, 42.30% of the patients had stage III and 8.60% of the patients had stage I tumors. Only 4 patients had stage IV breast tumors.
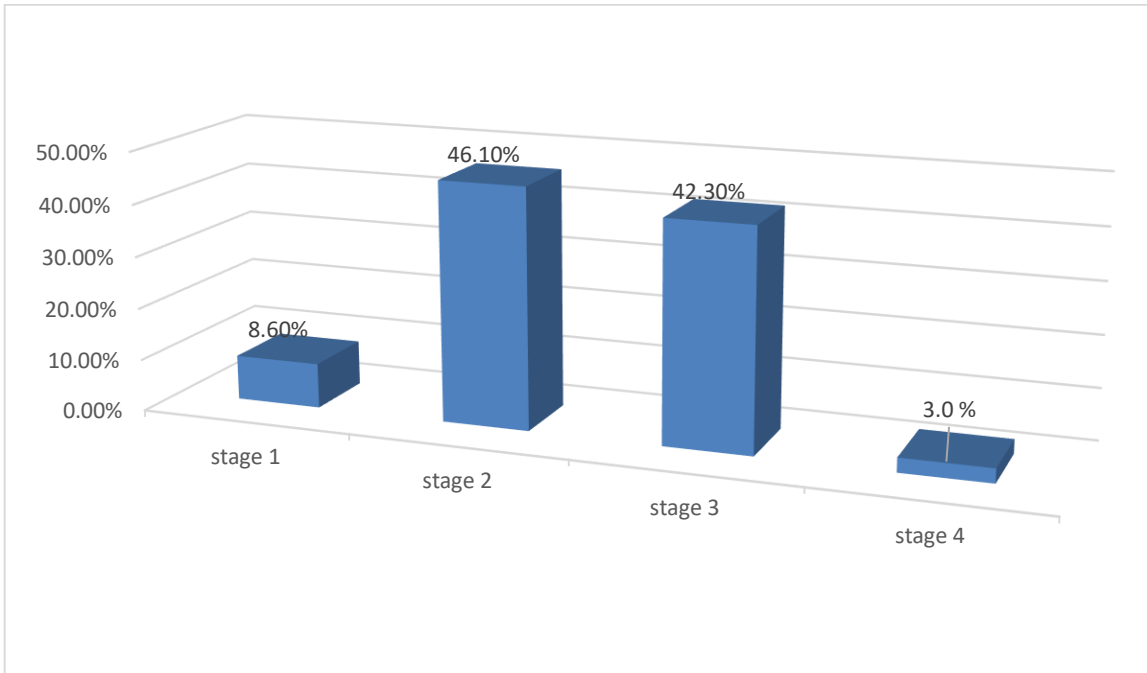

Figure 5: A Bar chart showing the distribution of cases according to the Staging of the Tumor
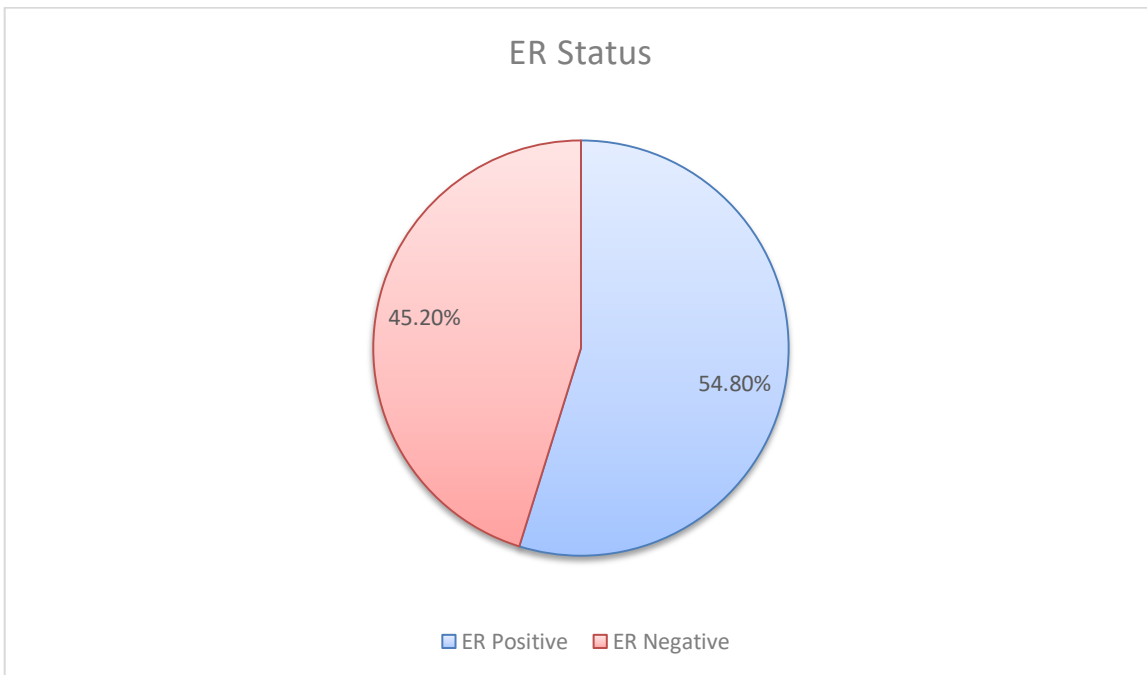
Hormonal Receptor Status:

ER Status

Figure. 6 A pie chart showing the distribution of cases according to ER status.
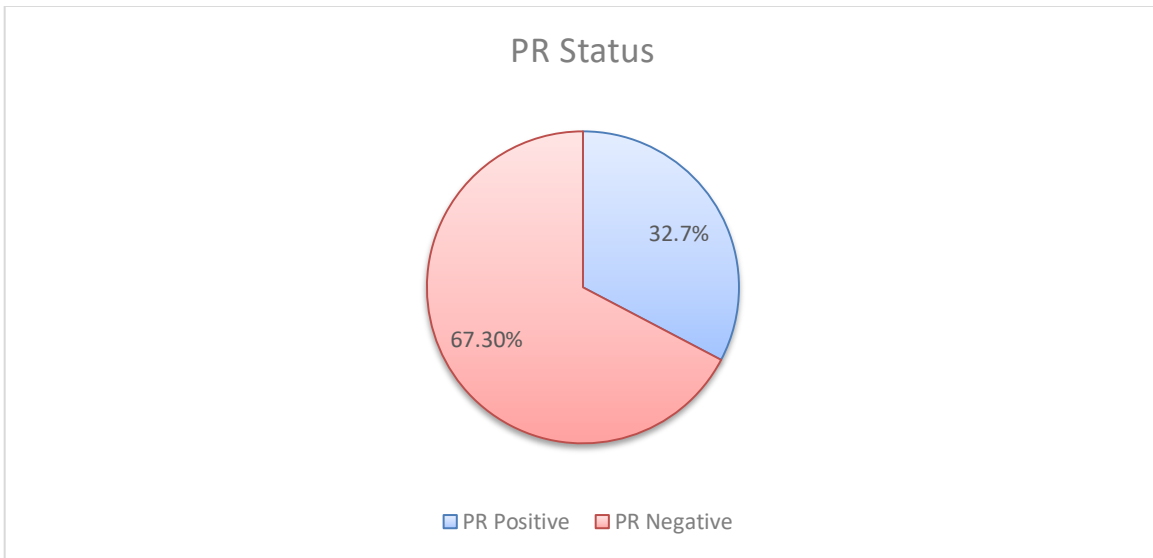
PR Status

Figure. 7 A pie chart showing the distribution of cases according to PR status
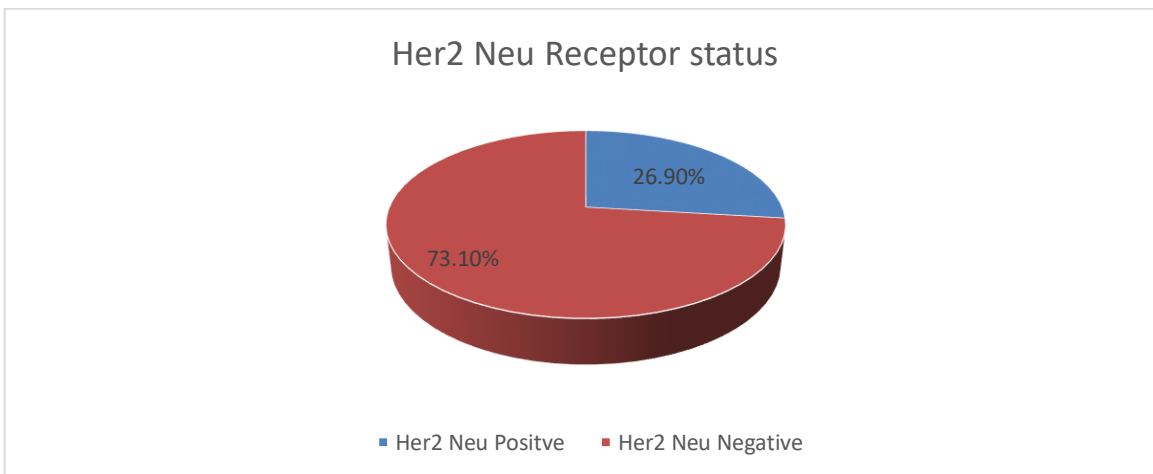
Her 2Neu Receptor status

Figure.8 A pie chart showing the distribution of cases according to Her2 Neu Receptor status.
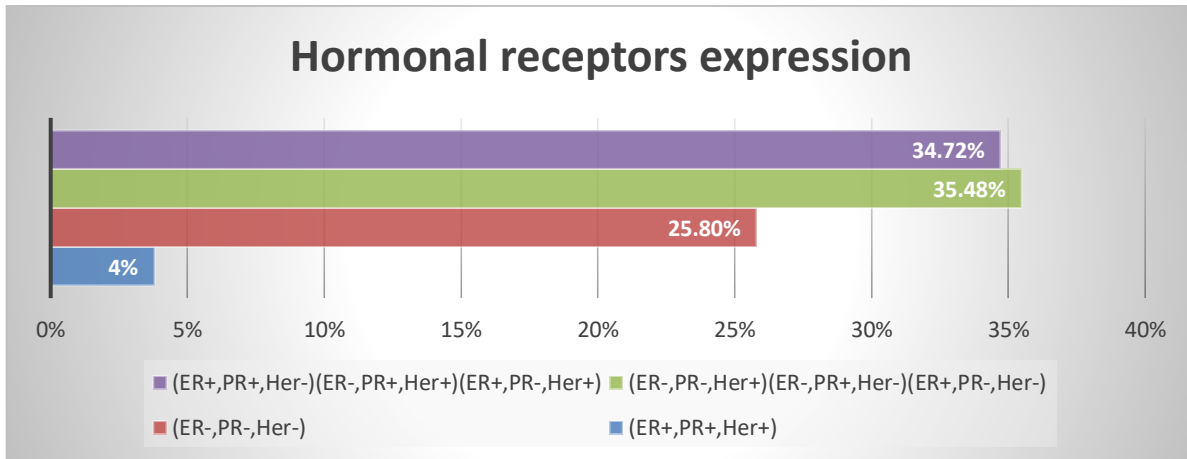
Figure. 9A bar chart showing the distribution of cases according to hormonal Expression.

Table 3: Association of staging of tumor with hormonal receptor expression

	ER/PR+,Her+	ER/PR-/Her+	ER/PR+,Her-	ER/PR-,Her-	Total	P Value
stage1	2(1.9%)	2(1.9%)	5(4.8%)	0	9	0.0001
stage2	5(4.8%)	5(4.8%)	35(33.6%)	2(1.9%)	47	
stage3	4(3.8%)	10(9.6%)	17(16.3%)	14(13.4%)	45	
stage 4	0	0	0	3(1.9%)	3	
Total	11	17	57	19	104	

Table 4: Association of grading of tumor with hormonal receptor expression

	ER/PR+,Her+	ER/PR-/Her+	ER/PR+,Her-	ER/PR-,Her-	Total	P Value
Grade 1	0	1(0.9%)	6(5.7%)	0	7	0.0018
Grade 2	6(5.7%)	6(5.7%)	37(35.57%)	4(3.8%)	53	
Grade 3	5(4.8%)	10(9.6%)	14(13.4%)	15(14.4%)	44	
	11	17	57	19	104	

Discussion

The important finding in this study is that nearly any age group may be affected by breast cancer, but it was the least in patients below 40 years (2.8%), which means this type of tumor is mostly related to prolonged periods of breast tissue exposure to the progesterone and/or estrogen hormones. These results are similar to another study performed in Kanpur which showed only 12.9 % of patients below 40 years (24).

The females are mostly affected while males may get affected but usually with early involvement of skin due to little amount of breast tissue found. Male breast cancer accounts for 1.9 % of all the cases of breast cancer in this study.

60.8% of the patients were living in urban areas; these results are similar to another study in Iran which found 69.4% of cases living in urban areas while 39.2 % of them living in rural areas (25). The most possible explanation for this difference is the lifestyle changes that occur in urban areas.

Grading of the tumor is still one of the important parameters regarding prognosis evaluation (26). Most of the cases in this study had grade II tumors (50.94%) followed by grade III tumors (42.5%) and the least were grade I tumors (6.7%), therefore that woman must be educated about breast cancer, the importance of regular breast self-examination and urgent consultation of physician in case of development of any breast symptom. This will help in early detection of the disease. These results are different from another study which found 38.3% of grade II, 35.8% of grade III, and 25.9% of grade I carcinomas (27), while they are similar to another study in Iran where the majority of the cases were in Grade II (64.2%). These differences could be the result of inter-observer differences.

Most patients are in stage II (46.10% of cases) and stage III (42.30% of cases) because of delay in diagnosis and discovery of disease. This means, that though the breast cancer is a growing tumor, it remains in the breast, or the growth gets extended to the nearby lymph nodes. However, few of them are in stage I (8.6% of cases) and rare in stage IV (3.8% of cases), which means that in rare cases, women are not diagnosed until they reach stage IV. These results are different from another study which found that most cases were in stage I (56.4% of cases), while 36.0% of cases were in stage II and 7.7% of cases in stage III (28).

In the current study, 54.8% of tumors were ER-positive, and 32.69% of tumors were PR positive which is similar to various other studies (table 6), where ER-positive tumors were the major group (29). 73.10% of tumours were Her2 neu Negative which has been seen in various other studies in the past. In the current study, Triple positive cases were only 10.57%, Triple negative were 18.2%. Most of the cases i.e 54.80% were ER/PR+, Her2-, This was the same analysis seen in the previous study (28) that showed 68.9% tumors were ER/PR+, Her2-,7.5% tumors were ER/PR-, Her2 +,10.2% tumors were triple positive and13.4% tumors were triple negative (Table 7). Statistical analysis was done with the Chi-square test (table 3,4) regarding the association of hormonal receptor expression with grading and staging of the tumor, a statistically significant association could be established between grading/staging of the tumor and hormonal receptor expression with P-value (<0.05). Analysis showed 61% of ER/PR+, Her2Neu – tumors were in stage II compared to 29% in stage III and no patient in Stage IV with a P value of 0.0001. 73.6% of the triple Negative tumors showed higher stage (stage III) and only 10.5% were in stage II, none of them were in stage I (P Value <0.0001). All the tumors in stage IV had triple negative hormonal expression. A similar association was seen with Histological Grades, where 75.4 % of the ER/PR+, Her2Neu- tumor were in stage I (10.5%) and Stage II (64.9%) compared to 24.5% in grade III (P value=0.0018). 78.9% of triple-negative tumors were showing grade III histology compared to

21.1% having grade II. No triple Negative Tumour had Grade I histology. These results were similar to a previous study by Sinha et al (30), which showed a significant association between tumor grade and hormonal receptor expression. Finally, one should be aware of the main limitation of the retrospective nature of the current study and the small sample size used in the study.

Table 6. Comparison of Hormonal expression of breast cancer in various studies.

Sr. No.	Study	No. of cases	ER Positive	PR positive	Her2Neu Positive
1	Priti Lal et al at Newyork[31]	3655	71.6%	47.4%	26.89%
2	Sughayer et al at Jordan[32]	267	50.8%	57.5%	17.5%
3	Dutt V et al[33]	75	24%	30%	57.2%
4	Present study	104	54.80%	32.69%	26.9%

Table 7. Comparison of Hormonal expression of breast cancer in various studies.

Sr. No.	Study	No of Cases	ER/PR+/Her+	ER/PR+/Her-	ER-PR-Her-	ER-PR-Her+
1	A Garcia Fernandez et al[34]	1003	**9.6%**	**72.1%**	**13.2%**	**4.8%**
2	Onitilo et al[28]	**1133**	**10.2%**	**68.9%**	**13.4%**	**7.5%**
3	Present study	**104**	**10.5%**	**54.80%**	**18.2%**	**16.3%**

CONCLUSION

I. Breast cancer has hormonal receptor characters ER, PR, and HER2 receptors.

II. Most of the cases of breast cancer were living in urban areas.

III. Majority of the cases presented with grade III tumor histology at the time of diagnosis.

IV. Stage II and III breast cancers were more frequently observed among these breast cancer cases.

V. The most common hormonal receptor expressions in breast cancer are ER/PR+, and Her2-.

VI. ER/PR+, Her2- expression has a better prognosis concerning Grade and stage of Breast Cancer.

VII. Triple Negative receptor Expression is associated with higher tumor stage and Grade. It can be termed as a Poor prognostic marker for breast cancer.

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