ER, PR, HER2, and Ki67 Expression Patterns on the Intricate Prognostic Relevance in Breast cancers: A Cross-sectional study

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Abstract

Background: Worldwide

One of the most prevalent malignancies in women and a leading cause of death in breast carcinoma. Breast cancer death rates are predicted to be approximately 90,408 (10.6%) and new cases to be around 1,78,361 (13.5%), respectively, in India, according to Globocan 2020. The prognosis and treatment of breast cancer depend on a number of factors, including the size of the tumour and the lymph node metastases, the presence of hormone receptors, and the histological grade.

Objective: The purpose of this study is to examine the association between the human epithelial growth hormone receptor (HER2/neu) status and the oestrogen receptor (ER), progesterone receptor (PR), and progesterone receptor status in various histological grades of breast cancer. To determine the association between the proliferation status and the major prognostic variables.

Methods: From January 2020 to May 2022, a cross-sectional study was carried out at the recognised laboratory NABL (National Accreditation Board for Testing and Calibration Laboratories) at the Department of Pathology, JSS Hospital, Mysuru. In order to get thin sections from the laboratory of women who had undergone a mastectomy or core biopsy after being diagnosed with breast cancer, the study employed 100 paraffin blocks.

Results: In this study, the mean age was 54.33 (ranging between 20 and 90 years old). Among 100 cases, the majority (61%) were >50 years old, in that 67.2% were ER-positive, 50.8% were PR positive, 65.5% were HER2 negative, 59.1% were Ki-67 positive, and 61.5% were ER and PR positive. Lymph node metastases were not involved in 55% of patients. 58.1% were ER-positive, 55.5% had PR status, 74.5% showed HER2 negativity, and 58.3% were Ki-67 positive. 52.0% were ER and PR positives. 48% of patients are classified as grade II, 28% as grade III, and 8% as grade I.

Conclusion: Age-related increases in ER were seen, and they were statistically significant (P 0.05). While HER2 positive was related to tumour size, hormonal status was not (0.05). Regarding the hormonal state, the tumour grading was statistically significant. Relationships

between the tumour grade, ER PR status, and Ki-67 status were statistically significant (0.05).

Keywords: Estrogen receptor, Progesterone receptor, Ki 67, HER2, Triple negative, Breast cancer prognosis, Immunohistochemistry

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1. Introduction

One of the most prevalent cancers in women worldwide and a leading cause of death is breast carcinoma.[1] According to Globocan 2020, there are roughly 2,262,419 women breast cancer cases worldwide, which is 11.7% of all other cancer cases, and there are roughly 6,84,9966 breast cancer deaths, which is 6.9% of all other cancer cases. In India, the death rate and percentage of breast cancer is predicted to be around 90,408 cases, or 10.66%, while the number of new cases is approximately 1,78,361 cases or 13.5%. [2]

The prognosis and treatment of breast cancer depend on a number of factors, including the size of the tumour and the lymph node metastases, the presence of hormone receptors, and the histological grade. [3] Increased prevalence of risk factors such as early menstruation, later menopause, and late first delivery, as well as fewer children or null parity, oral contraceptives, unhealthy or sedentary lifestyles, an alcohol habit, and hormone therapy are all linked to higher incidence rates. There is now a difference in the incidence of breast cancer in India's urban and rural populations, with a higher number of newly reported cases among urban women. The most often employed biomarkers for evaluating breast cancer are HER2/neu, ER, PR, HER2, and Ki-67. Endocrine therapy works well on its own in patients, the cases with a high level of ER positive. [4,5.] A longer length of disease-free survival is associated with PR. Tamoxifen drug therapy resistance and survival are linked to overexpression of HER2/neu (erbB-2). It contains an epidermal growth factor-related 185-kDa transmembrane phosphoglycoprotein with tyrosine kinase activity.[6,7] Those who test negative for ER, PR, or HER2/neu have shown a poor prognosis because high-Bloom Richardson's grade tumours are more likely to develop in these cases. [8]

The purpose of this study is to investigate the association between ER, PR, and HER2/neu status in various histological grades of breast cancer, and additionally to look into how the various prognostic factors interact.

2. Methods and materials

Between 2020 and May 2022, a cross-sectional study was carried out at the NABL (National Accreditation Board for Testing and Calibration Laboratories), a recognised laboratory located in the pathology department of the JSS Hospital in Mysuru. The institutional ethics committee gave its approval to the project. 100 archival paraffin blocks from the lab that were acquired after a patient underwent a mastectomy or a core biopsy following a breast cancer diagnosis were used in the study. The JSS hospital backbone software was used to gather patient information such as age, tumour size, grade, lymph node status, oestrogen receptor (ER), progesterone receptor (PR), HER2/neu status, and Ki-67. Age 50 years or older and age 50 years or younger, tumour size 2 cm or less and tumour size 2 cm or more, Bloom-grading Richardson's I, II, or III, lymph node involvement, ER-PR positive or negative, HER2-positive or negative, and Ki-67 positive or negative were the categories in

which patients were divided into two groups. For histopathological findings, hematoxylin and eosin stains were used, and for hormonal status, immunohistochemistry was applied.

The normal protocol was followed when grading the mastectomy specimen. The Leica Histocore H embedding machine created blocks of paraffin after processing the bits in LeicaPerl's automated tissue processor. Then, a Leica 2125 rotary microtome was then used to cut a thin piece between 3 and 4 microns thick. The slides were placed on the hot plate for heat fixation after sectioning them for 5 to 10 minutes at a temperature of 65 to 70 degrees Celsius. For deparaffinization, the slides were transferred to a glass container containing decontaminated xylene at the wax melting stage. The slides were then put into absolute alcohol to get rid of the xylene. After that, the slides were washed for two to three minutes under running water to get rid of the alcohol (up until this point, both hematoxylin and eosin (H and E) and immunohistochemistry (IHC) were done in a similar manner).

H and E staining

All slides were introduced to hematoxylin for 2–5 minutes, rinsed in tap water for 1–2 minutes, and then repeatedly submerged in 1% acid alcohol. It is then kept under running water for 3 to 5 minutes for bluing (for colour development), transferred to Eosin for 30 seconds to 1 minute, then transferred to absolute alcohol for 3 changes for about 5 minutes in each glass container, and finally, the stained slides are mounted with DPX (Dibutyl phthalate Polystyrene Xylene).

Immunohistochemistry

On the poly-L-lysine slides, portions that were four microns thick were assembled. The process is then carried out as previously mentioned. The slides made of poly-L-lysine were then washed twice for two cycles lasting around three minutes each. Slides of poly-L-lysine are recovered in TRIS-EDTA (TRIS base powder 1.21 g, EDTA 0.37 g in 1000 ml distilled water, pH 9). Slides were cooked under pressure for three whistles, then the lid was opened to let the food cool before continuing to chill it. The slides were subsequently placed in PBS (phosphate buffer solution, also known as wash buffer preparation), which contained TRIS base powder. pH 7.2–7.6 is achieved by mixing 6.05 grammes of sodium chloride and 8.5 grammes of calcium chloride with 1000 ml of distilled water. The pH is then adjusted for two periods of five minutes using 50% HCL (concentrated HCl). Then, two 5-minute PBS changes were performed after 10 minutes of 3% hydrogen peroxide application. The primary antibody was then diluted and treated with the cells for an hour in a moist, dark environment. Once more, run two cycles of PBS washing for five minutes each. A target binder/enhancer was utilised for 50-20 minutes. For two five-minute shows, PBS scrubbed it once again. Using the secondary antibody HRP was incubated (horseradish protein). After then, the slides are broadcast on PBS for two 5-minute changes. The addition of DAB chromogen (DAB buffer 1 ml + DAB chromogen 50 microliters), which took 2-3 minutes to create colour. PBS was washed twice for five minutes each, Mayer's hematoxylin counterstained for three to five minutes, and then distilled water was washed. After air-dried and dehydrated with 100% alcohol for two minutes each, the prints were mounted with DPX and a cover slip. After staining expression patterns of ER, PR, HER2, Ki 67 will be observed under microscope and according to the intensity of the color as shown in figure1 scoring will be given for the next interpretation and the expression patterns are compared with the positive and negative control as shown in figure2.

3. Results

Table1: In our study majority were >50 years old (61%), 67.2% were ER positive, and 39.6% were ER negative. Considering age ≤50 years old (39%), 43.5% were ER positive, and 56.4% were ER negative. Considering tumor size majority were >2cm (53%) among 53 cases, 60.3% were ER positive, and 39.6% were ER negative. In ≤2 cm (47%), 55.3% were ER Positive, and 44.6% were ER negative. Considering lymph node metastasis 55% were not involved among them 58.1% were ER positive, 41.8% were ER negative, 45% of patients showed lymph node metastasis, among them 57.7% were ER positive and 42.2% were ER negative. Considering tumor grading majority were in Grade II (48%), among them 62.5% were ER positive, 37.5% were ER negative followed by grade III (28%) and Grade I (08%).
ER status showed statistically significant with age and tumor grading. ER status does not showed statistically significant with tumor size and lymph node metastasis.

Table2: Considering age with PR status 61% were >50 years old, among them, 50.8% were PR positive, 49.1% were PR negative. Considering tumor size majority were >2cm (53%), among them 47.1% were PR positive, and 52.8% were PR negative. 47% showed ≤2cm among them majority 57.4% were PR negative, and 42.5% were PR positive. Considering lymph nodes with no metastasis 45.4% were PR positive, 5 and 4.5 % were PR negative. 45% showed lymph node metastasis among them 44.4% were PR positive and 55.5% were PR negative. Considering tumor grading among grade II 52 % were PR positive, 48% were PR negative followed by grade III, 25% were PR positive, 75% were PR negative and among grade I, 75% was PR positive, 25% were PR negative. PR status showed statistically significant with Tumor grading. PR status does not show statistically significant with age, tumor size, or lymph node metastasis.

Table 3: Among those aged> 50 years old, 65.5% were HER 2 negative, and 34.4% were HER2 positive. Among those ≤50 years old 66.6% were HER 2 negative, and 33.3% were HER2 positive. Among tumor >2cm 71.6% were HER 2 negative, 28.3% were HER2 positive, among ≤2cm 59.5% were HER2 negative, and 44.4% were HER2 positive. Among grade II, 31.2% were HER2 positive, 68.7% were HER2 negative, and grade III showed HER2 positive and negative equally. Followed by grade I, 87.5% were HER2 negative. The presence of HER2 was associated with lymph node status and tumour grade. Age or tumour size has no statistically significant relationship with HER2 status.

Table 4: Among those aged> 50 years old, 59.1% were Ki 67 positive, and 41% were Ki 67 negative. Among those ≤50 years old 42% were Ki 67 positive, and 45.7% were Ki 67 negative. Considering tumor size majority were >2cm (56%), among them, 57.4% were Ki 67 positive, and 42.5% were Ki 67 negative. 44% showed ≤2cm among them majority 56.7% were Ki 67 positive, and 43.2% were Ki 67 negative. Considering lymph nodes with no metastasis 58.3% were Ki 67 positive, and 41.6 % were Ki 67 negative. 43% showed lymph node metastasis among them 55.5% were Ki 67 positive and 44.4% were Ki 67 negative. Considering tumor grading among grade II, 50 % were Ki 67 positive and negative followed by grade III, 62.5% were Ki 67 positive, 37.5% were Ki 67 negative and among grade I, 57.1% were Ki 67 negative, 48.8% were Ki 67 positive. 58% were ER positive among them 53.0% were Ki 67 negative, and 46.9% were Ki 67 positive. Among ER negative 71.4% were Ki 67 positive, and 28.5% were Ki 67 negative. Among 60% of ER PR positive status, 48% were Ki 67 positive, and 52% were Ki 67 negative. 40% were ER PR negative among them 70.5% were Ki 67 positive, and 29.4% were Ki 67 negative. Among HER 2 negative 58.3% were Ki 67 positive, and 41.6% were Ki 67 negative. Among HER 2 positive 55% were Ki 67 positive,

and 44.4% were Ki 67 negative.

Ki 67 showed statistically significant relationships with tumour grading, ER PR status, ER status, and PR status.

Table 5: Interrelation of Triple-Negative with Prognostic Factors

There were 15 triple negative cases out of 100.47% were 50 years old, 53% were >50 years old, 53% were <2 cm, and 47% were >2 cm. Lymph node metastasis was not involved in 60% of patients, and lymph node metastasis was involved in 40% of cases. 93% were Ki-67 positive, 7% were Ki-67 negative, and 80% were grade III, followed by grade II (20%).

Table 6: Among those aged > 50 years, 61.5% were ER-PR positive, and 38.4% were ER-PR negative. Among those 50 years old, 62.8% were ER-PR negative, and 37.1% were ER-PR positive. Given that the majority of tumours were larger than 2 cm (47%), 55.3% were ER-PR positive and 44.6% were ER-PR negative.40% showed 2 cm; among them, the majority (52.5%) were ER PR negative, and 47.5% were ER PR positive. Considering lymph node involvement, there was no metastasis in 48% of cases, among which 47.9% were ER-PR negative and 52% were ER-PR positive. Metastasis was seen in 39% of cases, among which 51.2% were ER-PR positive and 48.7% were ER-PR negative.

When it came to tumour grading, grade II had 58.1% ER PR positive and 41.8% ER PR negative, grade III had 73.0% ER PR negative and 26.9% ER PR positive, and grade I had 83.3% ER PR positive and 16.6% ER PR negative.

Considering Ki 67 positive, 58.1% were ER PR negative and 41.8% were ER PR positive, among Ki 67 negative 61.3% were ER PR positive and 43.5% were ER PR negative. 52% were HER2 negative among them 63.4% were ER PR positive, 36.5% were ER PR negative, 35% were HER2 positive among them 67.6% were ER PR negative, and 35.2% were ER PR positive. ER PR status showed statistically significant with age group, tumor grading, and HER2 status.

Table 1: Demographics and clinicopathological parameters with ER status

Categories	**Parameters**	**Total (N=100cases)**	**ER status**		**P-Value**
			Positive (N=58 cases)	**Negative (N=42 cases)**	
Age group	≤50 years	39(39%)	17(43.5%)	22(56.4%)	0.02*
	>50 years	61(61%)	41(67.2%)	20(32.7%)	
Tumor Size	≤ 2 cm	47(47%)	26(55.3%)	21(44.6%)	0.60
	>2 cm	53(53%)	32(60.3%)	21(39.6%)	
Lymph node metastasis	Present	45(45%)	26(57.7%)	19(42.2%)	0.968
	Absent	55(55%)	32(58.1%)	23(41.8%)	
Tumor grading	Grade I	08(8%)	7(87.5%)	1(12.5%)	0.000*

	Grade II	48(48%)	30(62.5%)	18(37.5%)	
	Grade III	28(28%)	9(32.1%)	19(67.8%)	

Table 2: Demographics and clinicopathological parameters with PR status

Categories	**Parameters**	**Total (N=100cases)**	**PR status**		**P-Value**
			Positive (N=45 cases)	**Negative (N=55 cases)**	
Age group	≤50 years	39(39%)	14(35.8%)	25(64.1%)	0.143
	>50 years	61(61%)	31(50.8%)	30(49.1%)	
Tumor Size	≤ 2 cm	47(47%)	20(42.5%)	27(57.4%)	0.643
	>2 cm	53(53%)	25(47.1%)	28(52.8%)	
Lymph node metastasis	Present	45(45%)	20(44.4%)	25(55.5%)	0.950
	Absent	55(55%)	25(45.4%)	30(54.5%)	
Tumor grading	Grade I	08(8%)	6(75%)	2(25%)	0.00*
	Grade II	48(48%)	25(52%)	23(48%)	
	Grade III	28(28%)	7(25%)	21(75%)	

Table 3: Demographics and clinicopathological parameters with HER2 status

Categories	**Parameters**	**Total (N=100cases)**	**HER 2 status**		**P-Value**
			Positive (N=34cases)	**Negative (N=66 cases)**	
Age group	≤50 years	39(39%)	13(33.3%)	26(66.6%)	0.910
	>50 years	61(61%)	21(34.4%)	40(65.5%)	
Tumor Size	≤ 2 cm	47(47%)	19(40.4%)	28(59.5%)	0.201
	>2 cm	53(53%)	15(28.3%)	38(71.6%)	
Lymph node metastasis	Present	45(45%)	20(44.4%)	25(55.5%)	0.046
	Absent	55(55%)	14(25.4%)	41(74.5%)	
Tumor grading	Grade I	08(8%)	01(12.5%)	7(87.5%)	0.000*
	Grade II	48(48%)	15(31.2%)	33(68.7%)	
	Grade III	28(28%)	14(50%)	14(50%)	

Table 4: Demographics and clinicopathological parameters with Ki 67 status

Categories	**Parameters**	**Total (N=84cases)**	**Ki 67 status**		**P-Value**
			Positive (N=48cases)	**Negative (N=36cases)**	

Age group	≤50 years	35(42%)	19(54.2%)	16(45.7%)	0.65
	>50 years	49(58%)	29(59.1%)	20(41%)	
Tumor Size	≤ 2 cm	37(44%)	21(56.7%)	16(43.2%)	0.949
	>2 cm	47(56%)	27(57.4%)	20(42.5%)	
Lymph node metastasis	Present	36(43%)	20(55.5%)	16(44.4%)	0.79
	Absent	48(57%)	28(58.3%)	20(41.6%)	
Tumor grading	Grade I	7(8%)	3(42.8%)	4(57.1%)	0.000*
	Grade II	40(44%)	20(50%)	20(50%)	
	Grade III	24(29%)	15(62.5%)	9(37.5%)	
ER PR status	Positive	50(60%)	24(48%)	26(52%)	0.04*
	Negative	34(40%)	24(70.5%)	10(29.4%)	
HER2 status	Positive	36(43%)	20(55.5%)	16(44.4%)	0.79
	Negative	48(57%)	28(58.3%)	20(41.6%)	
ER status	Positive	49(58%)	23(46.9%)	26(53.0%)	0.02*
	Negative	35(42%)	25(71.4%)	10(28.5%)	
PR status	Positive	38(45%)	16(42.1%)	22(57.8%)	0.08
	Negative	46(55%)	32(69.5%)	14(30.4%)	

Table 5: Comparison of triple-negative (ER, PR, HER2) with prognostic factors.

Categories	**Parameters**	**N=15 (%)**	**P-value**
Age in years	≤50 Years	7(47%)	0.82
	>50 years	8(53%)	
Tumor size in cm	≤2	8(53%)	0.82
	>2	7(47%)	
Lymph node metastasis	Present	6(40%)	0.46
	Absent	9(60%)	
Ki 67 status	Positive	14(93%)	0.01
	Negative	1(7%)	
Tumor grading	I	00(00%)	0.05
	II	03(20%)	
	III	12(80%)	

Table 6: Demographics and clinicopathological parameters with ER PR status

Categories	**Parameters**	**Total (N=87 cases)**	**ER PR status**		**P-Value**
			ER PR Positive (N=45 cases)	**ER PR Negative (N=42 cases)**	
Age group	≤50 years	35(35%)	13(37.1%)	22(62.8%)	0.02*
	>50 years	52(52%)	32(61.5%)	20(38.4%)	
Tumor Size	≤ 2 cm	40(40%)	19(47.5%)	21(52.5%)	0.46
	>2 cm	47(47%)	26(55.3%)	21(44.6%)	
Lymph node	Present	39(39%)	20(51.2%)	19(48.7%)	0.94

metastasis	Absent	48(48%)	25(52.0%)	23(47.9%)	
Tumor grading	Grade I	06(6%)	5(83.3%)	01(16.6%)	0.000*
	Grade II	43(43%)	25(58.1%)	18(41.8%)	
	Grade III	26(26%)	7(26.9%)	19(73.0%)	
Ki 67	Positive	43(43%)	18(41.8%)	25(58.1%)	0.06
	Negative	44(44%)	27(61.3%)	17(43.5%)	
HER 2	Positive	35(35%)	12(35.2%)	23(67.6%)	0.008*
	Negative	52(52%)	33(63.4%)	19(36.5%)	

Comparing age with hormonal status, the majority of our patients were >50 years of age, which is 61% of them; 67.2% were ER positive and 32.7% were ER negative. 50.8% were PR positive, 49.1% were PR negative, 34.4% were HER2 positive, 65.5% were HER2 negative, 59.1% were Ki-67 positive, and 41% showed Ki-67 negative. 61.5% were ER and PR positive, and 38.4% were ER and PR negative. 53 percent were triple negative.

4. Discussion

This study was performed on 100 cases of breast carcinoma. The average age of study participants is 54.33 years, similar to other Indian studies like Reddy G. M. et al. [9] Comparing the clinicopathological factor with ER status, the majority of our patients were >50 years of age, which is 61% in that 67.2% were ER positive and 32.7% were ER negative. 50.8% were PR positive, 49.1% were PR negative, 34.4% were HER2 positive, 65.5% were HER2 negative, 59.1% were Ki-67 positive, and 41% showed Ki-67 negative. 61.5% were ER and PR positive, and 38.4% were ER and PR negative. 53 percent were triple negative.

Comparing the interrelationship of clinicopathological factors with ER and PR status. The majority of the 87 cases (52%) were over 50 years old, with 32 ER-PR positives and 20 ER-PR negatives. Similar to Huang et al.'s study, the majority of those aged 50, or 35%, were ER-PR negative, with 22 cases, and ER-PR positive, with 13 cases, indicating a poor prognosis.[10]

According to Huang et al., the correlation between ER PR status and HER2 status is inverse in the younger age group under 50 years of age.[10] Furthermore, in the older age group, ER PR status was associated with age, tumour grading, Ki 67 status, and HER2/NEU (0.05).

There was no association between HER2 status and patient age in the present study, as observed by Ariga et al. and Prati et al. [11,12] The total number of cases with Ki 67 was 84, with 49 in the older age group (> 50 years old) and 35 in the younger age group (50 years old), where the number of Ki 67-positive cases was relatively higher, indicating a poor prognosis, as Inwald et al. observed. [13] They did not have any correlation to age, as seen by many other authors like Mohammed et al. [14]

Comparing the relationship between IHC (ER, PR, HER2, Ki67) and tumour size, the majority of tumours were >2 cm, which is 53% in that 60.3% were ER positive and 39.6% were ER negative, 47.1% were PR positive, 52.8% were PR negative, 28.3% were HER2 positive, 71.6% were HER2 negative, and the majority of 26 cases were ER PR positive as noted by Ambroise et al, similarly HER2 was negative in the majority of the cases. It did not

have any correlation with size, as noted by Ambroise et al. [8,15] The present study, which tries to show the relationship between tumour size (2 cm and >2 cm), shows that Ki 67 positivity is higher in larger tumors, indicating proliferation and a bad prognosis. Thus, there is a relationship between Ki 67 positivity and tumour size, as noted by Inwald E. C. et al. [13]

Discussing the relation of IHC (ER, PR, HER2, KI67) with Bloom Richardson's tumour grading, the majority of the cases were in Grade II, followed by Grade III and Grade I. Most of the cases in grades I and II were ER-PR positive, while most of the cases in grade III were ER-PR negative. Most cases in grades I and II were HER2-negative. Even Grade III cases were mostly HER2-negative. There is an inverse ratio of cases in Grade I and Grade II for ER, PR, and HER2/neu expression, as noted by Siadati et al. [16]

Our study was similar to those by Siadati and Inkovic et al[16, 17] as far as Grade II cases were concerned. whereas 38 cases were ER-PR positive with an inverse HER2 ratio.40 cases were HER2-negative, which provides a better prognosis as these cases will respond to chemotherapy. The majority of the Grade II cases were Ki-67 positive, indicating increased proliferation and metastasis potential. There were a good number of cases of grade III malignancy showing triple-negative. Our study differs from Siadati and Ivkovic et [16, 17] as far as Grade III cases are concerned, wherein they had an increase in HER2 expressions. In our study, the majority of the cases in Grade III showed HER2 negativity compared to HER2 positivity. As a result, Grade III has two positive ER PR results and two negative results. This finding is supported by Pinato et al. and Ferrero et al [18, 19] who found some of these cases showing similar findings. This is thought to have a better prognosis than being ER-PR positive and HER2 positive.

Thus, HER2 expression is significant and a better predictor of hormone therapy response than ER status alone. Various multivariate analyses have revealed that Ki 67 (Figure 1) has an association with grading. It has been found by Inwald et al[13] that Ki67 is an independent prognostic factor as far as disease-free survival and overall survival are concerned. Thus, the higher the grade of Ki67, the worse the prognosis. Thus, Ki67 is an important marker for prognosis, as found in our studies similar to those of Inwald et al. [13]

Considering age group and grade distribution along with hormonal status, 15 cases showed triple-negative. Of these, the age distribution was almost the same in both groups below and above 50 years of age. Of the 15 cases of triple-negative cancer, the mean age of patients was 48 years, which is comparable to the Reddy GM et al study.[9] Also, the minimum age of 25 years was similar to the studies done by Reddy GM et al. and Hasim Ishikawa et al.[20] According to the grade, 80% of triple-negative cases were grade III, 20% were grade II, and there were no cases in grade I. Similar in ratio to studies of Reddy G. M. et al., Hashmi et al. [21]

Correlating IHC (ER, PR, HER2) with lymph node status in our study, we discovered that of the 100 cases, lymph node metastasis was more common in 46 cases, while 54 cases did not have metastasis. Considering ER-PR status in conjunction with lymph node metastasis, the majority of lymph node metastasis cases (45/46) have ER-PR positivity. The ER-PR status in cases without lymph node metastasis was almost equal. This finding is significant because many researchers, including Singh et al. 22, have discovered that lymph node metastasis plays a role in recurrence and mortality in breast carcinoma patients. It is also found that endocrine therapy is very useful in those tumours that do express ER and PR positivity, as was found in our study. These findings are similar to those of Singh et al, Falck et al and

Azam et [22-24,] who found good concordance between ER-PR status and lymph node metastasis.

Comparing lymph node metastasis with HER2 status, our study is similar to the study done by Ivkovic-Kapiel et al [17], which did not find a significant association between HER2/neu expression and the presence of positive axillary lymph nodes, although it has been discussed by Sigadati et al.[16] We found the majority of the cases to be HER2/neu negative in both lymph node-positive and negative breast cancers.

Ki-67 was used to assess lymph node status in 87 samples, 72% (36/87) of which were lymph node metastases and showed Ki-67 positivity (Figure 1). This shows that Ki-67 is a marker for cell growth and has a strong link to lymph node status. Thus, Ki 67 is a prognostic parameter for disease-free survival and overall survival, Figure 2 displays the positive and negative control expression patterns of hormone status. This finding is similar to the findings of Inwald E. C. et al.[13]

In the interrelationship between ER, PR, HER2neu, and Ki 67, we observed an inverse ratio between ER/PR, which showed positivity in a good number of cases showing HER2 negativity (Figure 2). It is important, as it is known that ER and PR expression are indicators of response to hormone therapy and a better prognosis. Thus, 17 cases were ER-PR and Ki-67 positive, while 33 cases were ER-PR negative and Ki-67 positive. Also, the reverse relationship is noted between ER PR status and Ki 67 status. A similar observation was made by Siadati Setala et al. [16] In our study, 66% of cases were HER2-negative, which indirectly indicates a good prognosis. HER2 overexpression is associated with a poor prognosis. HER2 by itself is a prognostic indicator of predictive and therapeutic information, as found by Ivkovic-Kapiel et al. [17] Ki67 has an inverse proportion and correlation with ER PR, with higher rates of ER PR positivity in the tumors with the lowest proliferation, similar to studies by Inwald E. C. et al. [13] Also, we noted that in cases with low or absent ER-PR, the Ki-67 index proliferation is very high as shown in Figure 1, which exhibits the hormone status expression patterns. Our study shows a direct relationship between HER2 positivity and Ki-67 positivity. Similarly, HER2 negativity is associated with an increase in ki67 negativity(Figure 2); this is similar to studies done by Inwald E. C. et al. [13]

Limitations**:** Insufficient samples in case of core biopsy.

5. Conclusion

Breast cancer is the most common cancer in women in low- and middle-income countries and developing countries. Age, tumor size, Bloom Richardson grading, lymph node status, and immunological markers such as ER, PR, HER2, and Ki 67 are all considered prognostic factors. Age and ER PR status were statistically significant (p 0.005), as were tumor size and ER PR status, grades and all hormonal statuses, lymph node with ER PR, Ki 67 status, and Ki 67 with ER PR status. The relationship between these factors is established to help with chemotherapy. It aids in determining patients' overall survival and disease-free survival, which is critical for the patient's well-being. This helps to reduce the mortality and morbidity of breast cancer patients.

Conflicts of Interests

There is no conflict of interest.

Supplementary Material
Attached.

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Author's Contributions

Ms. Priyanka M. K. performed experiments on clinical samples, collected data, prepared charts, reviewed literature, analysed data and wrote manuscripts.
Dr. MVSST Subba Rao: Checked for plagiarism and made the necessary corrections.

Dr. Nandini N. M.: Developed the research questions, assisted me in comprehending the prognostic elements and how they interacted, reviewed the literature, and gave the go-ahead for the study.

The paper has been given the okay from both authors to be submitted in its current state.
Consent for Publication

NA

Compliance with Ethical Standards:

Funding: This work was not sponsored or supported by any grants or agencies it is Self-funded.

Declaration:
Ethics Approval: To utilise 100 paraffin-embedded patient breast cancer reported blocks, a full Institutional Ethics Committee approval (JSSMC/IEC/17112021/32NCT/2021-22, dated 18 November 2021) was acquired from the JSS medical college and Hospitals.
The ethical guidelines of the institutional and/or national research committee, the 1964 Helsinki statement and its later revisions, or equivalent ethical standards were followed in all procedures carried out in studies involving human subjects.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Figure legends

Figure 1: H & E staining of malignant tumor cells depicting ER+, PR+, HER2+, and Ki67 expression patterns, Magnification: 20x. Green arrows: expression of ER, PR, HER2, and Ki67.

Figure 2: ER positive and negative control, PR positive and negative control, HER2 Positive and negative control, Magnification:20x. Red arrows: non-expression and expression of controls.

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Figure1

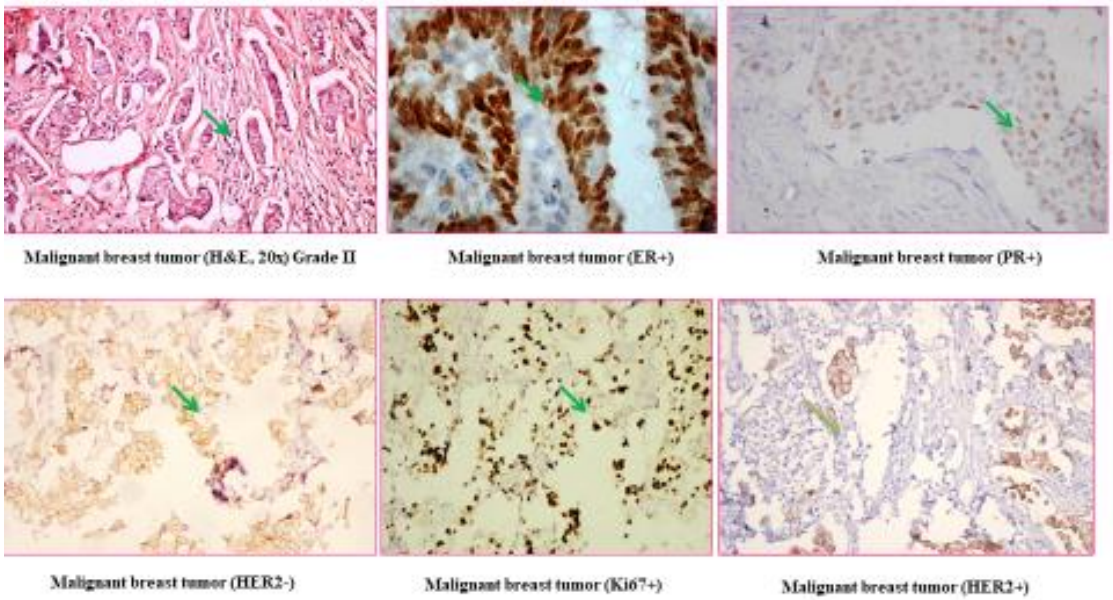

Figure 2