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Co-Relation of Surgical Outcome and Impact of Er, Pr, Her-2/Neu & Ki-67 Expression in Carcinoma Breast

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Abstract: ***Breast cancer is the most common cancer diagnosed in women and the commonest cause of cancer related deaths all over the world. It is heterogenous disease with variable morphology and molecular features. Hence response to their pay also varies. The global cancer burden of cancer breast is also increasing instead of development of new treatment modalities. Methodology: In this study stage 1, 2 & 3 were included and stag 4 tumor, pt received chemotherapy or radiotherapy, and recurrences were excluded. All pts followed normal protocol and subjected to MRM & followed by chemo & radiotherapy. Prognostic factors were estimated. Results_ 4 major groups were observed. ER+ve, PR+ve, HER-2/NEU +ve & Overexpression of Ki67.Worst prognosis with Tripple Negative. Poor prognosis in HER-2/NEU +ve. Recurrence rate is higher with Overexpression of Ki 67. Good prognosis in Tripple Positive.***

Keywords: ER Esotrogen receptor, PR- Progestrone receptor, Ki67 Growth factor, HER-2/NEU is protein

1. Introduction

Breast cancer is a heterogeneous disease with variable morphology, molecular features and response to therapy. Incidence of breast cancer is on the rise in India and it has overtaken cervical cancer and is now the most common cancer in women in India and accounting for 27% of all cancers in women [1, 2]. It is the leading cause of cancer related deaths with 70, 218 breast cancer related deaths as per Globocan 2012 data.[3]

There are a number of factors that determine the prognosis of a disease and response to treatment. A prognostic factor gives information on the risk of recurrence in the absence of adjuvant therapy, i.e. a prognostic factor can be used to predict the natural history of a tumor. A predictive factor gives information on the likelihood that a tumor will respond to a specific treatment. [4]

ER, PR Her-2/Neu are one of the most important and useful biomarkers currently available and their evaluation is highly recommended for best management. [5]

Estrogen is an important mitogen exerting its activity by binding to its receptor (ER) and found in 50-80% of breast cancers. [6] PR is a surrogate marker of functional ER and is as valuable in predicting the behavior of carcinoma breast. PR is expressed in 60-70 % Invasive breast cancer, higher positivity is seen in older age and postmenopausal individuals. [7]

Her-2/Neu (c-erb-b2) gene is located on 17q11 and gene product is a 185 KD trans membrane glycoprotein associated with tyrosine kinase activity, which belongs to family of epidermal growth factor receptors. Her-2/Neu is over expressed in about 20% carcinoma breast cases[8] and is associated with tumor aggressiveness, chemo resistance, recurrence, low response to tamoxifen and decreased survival[9, 10]. Ki-67 is a nuclear protein expressed during cellular proliferation particularly during the mitosis phase [11]. In early breast cancer Ki-67 is an independent factor for worse prognosis as shown by significantly shorter overall and disease free survival [12, 13]

This prospective observational study was conducted to assess the possible correlation between expression of ER, PR Her-2/Neu and Ki-67 marker with other prognostic factors like tumor size, histological grade, lymph node metastasis and their impact on overall surgical outcome in patients of operable breast cancer.

2. Aims and Objectives

- To Study ER, PR, Her-2/neu and Ki-67 reactivity pattern in patients of breast cancer.
- To study baseline characteristics of tumour (stage, grade, lymph node status) with respect to these tumour markers.
- To determine surgical outcome i.e. relapse rate in these patients with respect to various tumour markers. (Relapse rate is defined as the number of patients who develop either a local recurrence or who are diagnosed with a systemic metastasis within the study period)

3. Materials and Methods

A database in which all operable cases of breast cancer admitted in Hamidia hospital Bhopal from March 2013 to February2015 was used. We analysed data from women with primary breast cancer retrospectively and followed them up in the observation period to assess the surgical outcome.

We used the HPR, hormonal receptor markers (ER, PR, HER-2/NEU), Ki-67 status, clinical staging, that had been retrospectively reported to the database.

All newly diagnosed cases (including LABC excluding metastatic) of ca breast from March 2015 to September 2016 are included in the study. After confirmation of diagnosis by either Tru cut biopsy or final excised specimen was employed to ascertain ER, PR, HER-2/NEU and Ki-67 marker status

In this study breast cancer is classified into four groups based on IHC profile ER/PR and Her2/Neu expression, positive (+) and/or negative (-). The groups are:

ER/PR+, Her2+	**ER+/PR+, Her2+ ER-/PR+, Her2+ ER+/PR-, Her2+**
ER/PR+, Her2-	ER+/PR+, Her2- ER-/PR+, Her2- ER+/PR-, Her2-
ER/PR-, Her2+	ER-/PR-, Her2+
ER/PR-, Her2-	ER-/PR-, Her2-

Inclusion Criteria:

- Women with clinical stage I, II, III of carcinoma breast admitted in the Department of Surgery in Gandhi Medical College, Bhopal.
- They are included in study after informed consent.

Exclusion Criteria:

- Those patients of carcinoma breast with systemic metastasis (Stage IV)
- Those patients who have already received chemo/radio therapy
- Those patients who have already been operated outside
- Male breast cancer

All patients were operated by standardized protocol. Locally advanced breast cancer cases were first subjected to standard neo adjuvant chemotherapy and then taken for surgery. The mastectomy specimens were fixed on 10% neutral buffered formalin in a 10 fold volume immediately after surgery. At least 4 sections from tumour mass, one section each from skin and nipple and all palpable lymph nodes were submitted for processing besides one section from apparently normal tissue for immunohistochemistry internal control.

This was followed by standard histological processing according to laboratory standard operating procedure. Finally routine hematoxylin and eosin stain were done and sections were studied in light microscope fitted with camera connect to desktop computer.

H&E sections were examined to confirm presence of invasive cancer, ascertain histological types, Histological Bloom Richardson grade (BRG) modified by Ellis & Elston2 and axillary lymph node involvement. IHC of HER2/NEU protein was done on formalin-fixed paraffin embedded tissue blocks, using HER2/NEU antibody following standard procedures.

The tumour was also immunostained with Anti ER, Anti PR, and Ki67 primary antibody.

ER/PR Testing:

Proportion Score	
0	No nuclear staining
1	< 1% nuclei staining
2	1-10 % nuclei staining
3	11-33% nuclei staining
4	34-66 % nuclei staining
5	67-100% nuclei staining

Intensity score	
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Quick score (0-8) was calculated by adding proportion and intensity score. **Quick score >2** was considered as positive for ER & PR status

Her-2/Neu Interpretation

0	No staining observed or membrane staining in <10 % tumor cells- **Negative**
1+	A faint/barely perceptible membrane staining detected in >10% tumor cells- **Negative**
2+	A weak to moderate complete membrane staining is observed in more than 10% of tumor cells- **Negative**
3+	A strong complete membrane staining is observed in >30% tumor cells- **Positive indicate cerb B2 gene amplification**

Usually 2+ is considered as an equivocal result and it needs to be confirmed by FISG but since FISH was not available in our setup hence **only 3+ grading was considered positive for Her-2/Neu analysis** for the purpose of this study.

Ki-67 testing

Immunohistochemical staining is conducted and the proportion of the malignant cells staining positive for the nuclear antigen Ki-67 is evaluated in a quantitative and visual way using light microscopes.

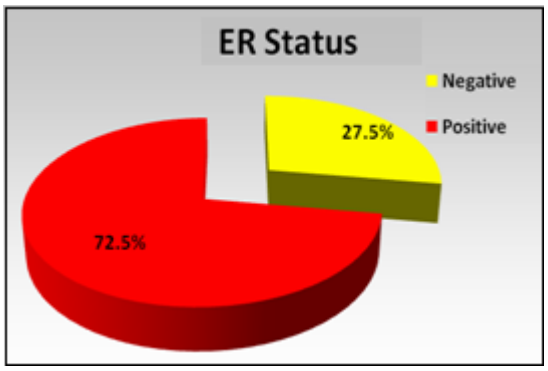
Ki-67 values are acquired as the percentage of positively marking malignant cells using the antihuman Ki-67 monoclonal antibody MIB1 which is one of the most commonly used antibodies and considered as the ‘‘gold standard’’ [13]. The Ki-67 percentage score is defined as the percentage of positively stained tumor cells among the total number of malignant cells assessed [14].

A Ki-67 cut-off point of 15 % was defined according to the experience of different pathologists as well as national and international recommendations at present [15-19].

4. Observations and Results

ER Status

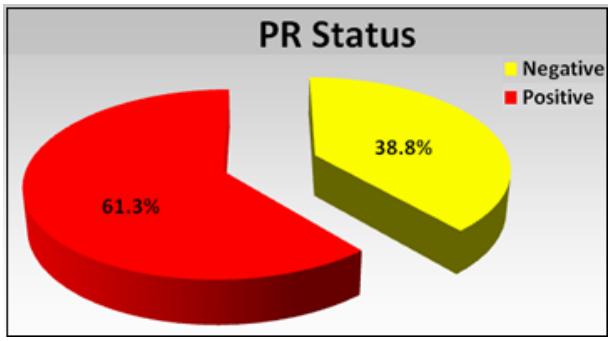
ER	*Frequency*	*%*
Negative	22	27.5%
Positive	58	72.5%
Total	80	100%



72.5% cases are tested positive for estrogen receptor while 27.5% cases are negative for estrogen receptor

PR Status

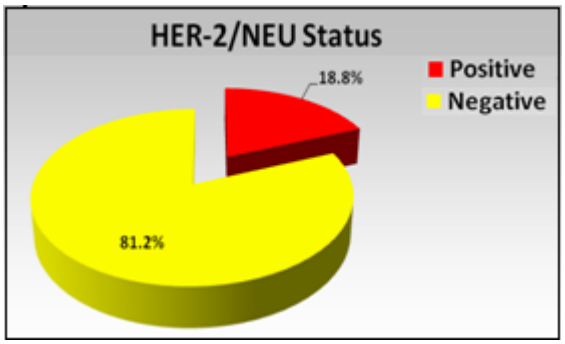
PR	*Frequency*	*%*
Negative	31	38.8%
Positive	49	61.3%
Total	80	100%



61.3% cases are found to be positive for PR while 38.3% cases are tested negative.

HER-2/NEU Status

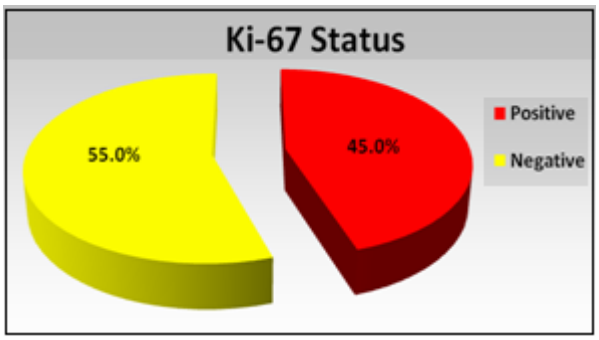
Her 2	*Frequency*	*%*
Negative	65	81.2%
Positive	15	18.8%
Total	80	100%



Majority of cases (81.3%) cases are found negative for HER-2/NEU while only 18.8 cases tested positive for it.

Ki-67 Status

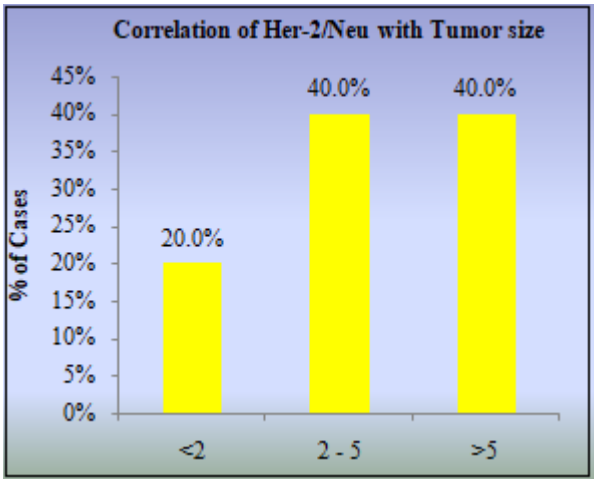
Ki-67	*Frequency*	*%*
Negative	44	55.0%
Positive	36	45.0%
Total	80	100%



45% of the tumors showed over expression of the proliferative marker Ki-67 while 55% tumors did not over express Ki-67.

Correlation of Her-2/Neu with Tumor size

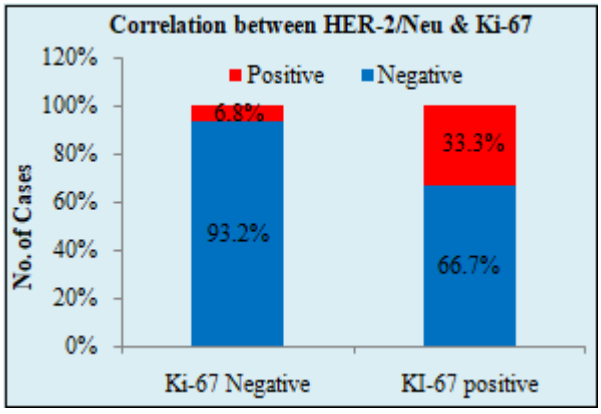
Tumor Size (cm)	Total cases	Her 2				P Value
		Negative		Positive		
		Frequency	%	Frequency	%	
<2	12	9	75.0%	3	20.0%	0.815
2 - 5	36	30	83.3%	6	40.%	
>5	32	26	81.3%	6	40.0%	
Total	80	65	100%	15	100%	



The above table shows that among the Her-2/Neu positive cases majority of tumors are >5 cm in size, however this **is not statistically significant (p=0.815)**

Correlation between HER-2/Neu& Ki-67

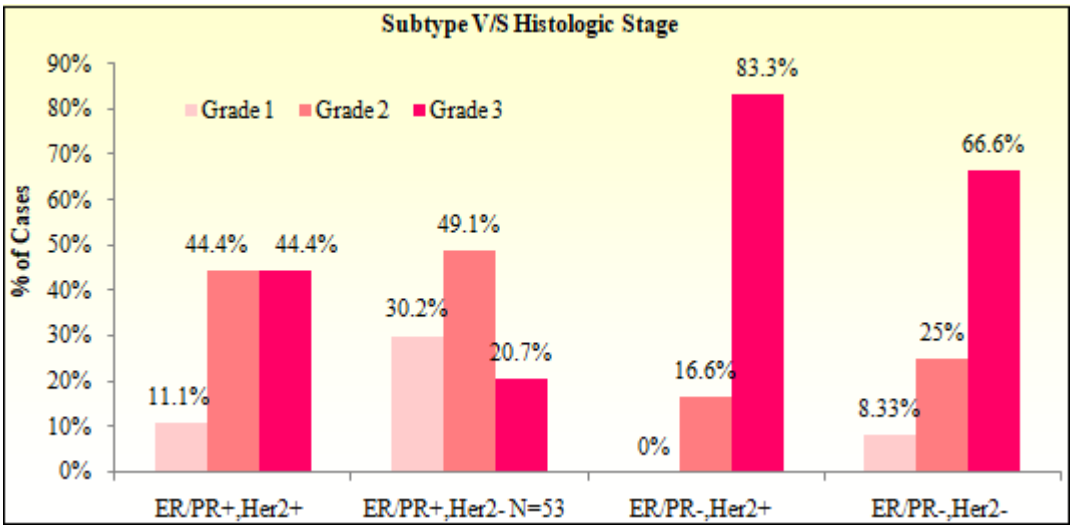
Her 2	Ki-67				P Value
	Negative		Positive		
	Frequency	%	Frequency	%	
Negative	41	93.2%	24	66.7%	0.004
Positive	3	6.8%	12	33.3%	
Total	44	100%	36	100%	



The above table shows that among the Ki-67 positive cases 33.3% cases also over expressed Her-2/Neu and this result is **statistically significant** (p=0.004)

Subtype V/S Histologic Stage

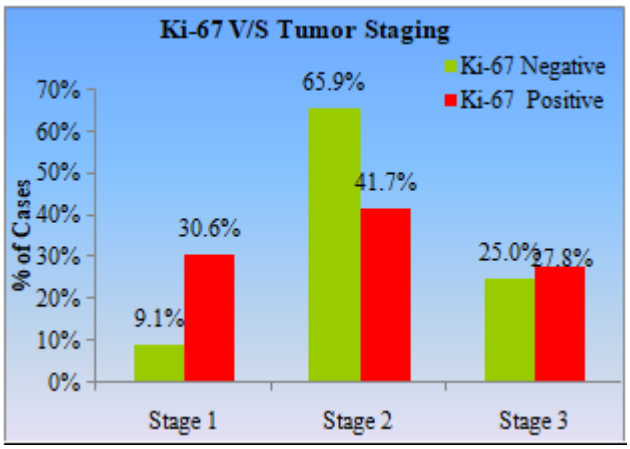
Histologic Grade	Groups				P Value
	ER/PR+ Her2 +	ER/PR+ Her2-	ER/PR- Her2+	ER/PR- Her2-	
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
Grade 1	1 (11.1%)	16 (30.2%)	0 (0.0%)	1 (8.3%)	0.008
Grade 2	4 (44.4%)	26 (49.1%)	1 (16.7%)	3 (25.0%)	
Grade 3	4 (44.4%)	11 (20.8%)	5 (83.3%)	8 (66.7%)	
Total	9 (100%)	53 (100%)	6 (100%)	12 (100%)	



The above table shows that among the triple negative tumors(ER/PR- Her2-) a majority of the fraction (66.7 %) are grade 3 tumors. Also among the Her2 overexpressing subtype (ER/PR- Her2+) majority fraction (83.3%) are grade 3 and this result is **statistically significant (p=0.008)**

Ki-67 V/S Tumor Staging

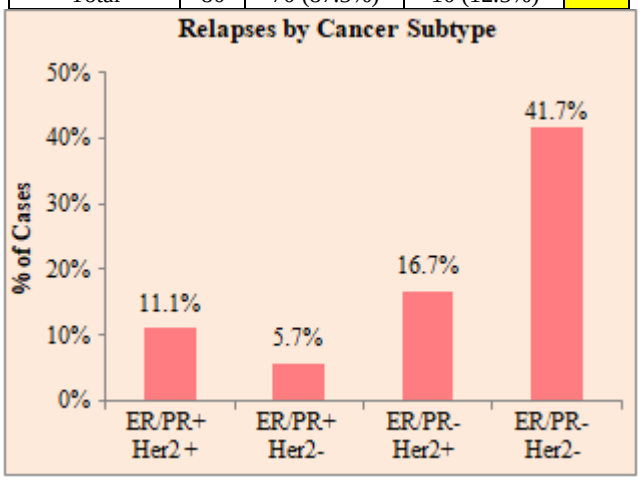
Tumor Stage	Ki-67				P Value
	Negative		Positive		
	Frequency	%	Frequency	%	
Stage 1	4	9.1%	11	30.6%	0.030
Stage 2	29	65.9%	15	41.7%	
Stage 3	11	25.0%	10	27.8%	
Total	44	100%	36	100%	



This table depicts the relationship between Ki-67 and tumor staging. It shows that both among Ki-67 positive and negative cases most common stage of presentation is stage 2(41.7% and 65.9% cases respectively). This result is also statistically significant (p=0.030)

Relapses by Cancer Subtype

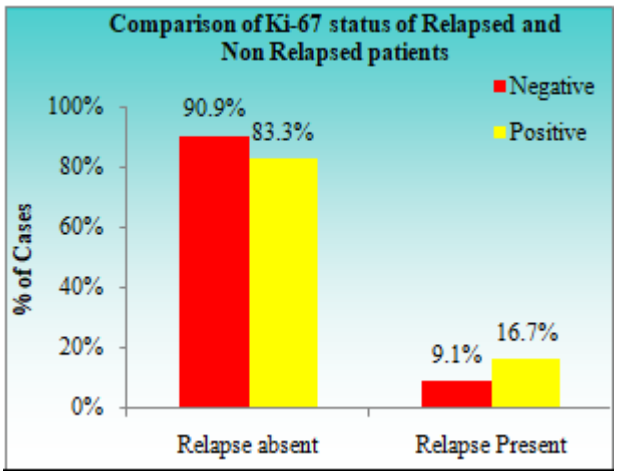
Groups	Total cases	RELAPSE		P Value
		Absent	Present	
		Frequency (%)	Frequency (%)	
ER/PR+ Her2 +	9	8 (88.9%)	1 (11.1%)	0.008
ER/PR+ Her2-	53	50 (94.3%)	3 (5.7%)	
ER/PR- Her2+	6	5 (83.3%)	1 (16.7%)	
ER/PR- Her2-	12	7 (58.3%)	5 (41.7%)	
Total	80	70 (87.5%)	10 (12.5%)	



Triple Negative subtype had the highest incidence of relapse (41.7%) while most favourable prognosis was shown by ER/PR+ Her2- subtype with a recurrence rate of 5.7%

Comparison of Ki-67 status of Relapsed and Non Relapsed patients

Ki-67	Total cases	RELAPSE		p Value
		Absent	Present	
		Frequency (%)	Frequency (%)	
Negative	44	40 (90.9%)	4 (9.1%)	0.308
Positive	36	30 (83.3%)	6 (16.7%)	
Total	80	70 (87.5%)	10 (12.5%)	



Ki-67 Positive cases had a higher incidence of relapse (16.7%) as compared to Ki-67 negative cases (9.1%). However this result is statistically insignificant (p=0.308)

5. Discussion

The use of Immuno histo chemistry in carcinoma breast has become an important part of a complete and comprehensive histopathology report. In terms of prognosis and prediction of response to treatment, in addition to histological grade and tumor sub type, hormone markers - ER/ PR and HER-2/Neu have become the cornerstone requirement for the oncologist. The present study was of 80 cases, all of which were confirmed cases of breast cancer.

ER status was found positive in 58 out of 80 cases i.e. a positivity rate of 72.5% which is similar to the result obtained. Out of 80 cases 49 cases were tested positive for PR receptor accounting for 61.2%. We found HER2/NEU, were positive with 3+ score in 18.7% of cases. This finding was consistent with universally accepted HER2/NEU overexpressed in 15-30% cases [21]. This finding is almost similar to another Asian study of 19.1% done in Srilanka 2009 by Mudduwa LK *et* al [22].However the frequency of HER2/NEU positivity varies among Indian studies. In a study from Bangalore, South India Vaidya Nathan et al [23] found a figure of 43.2% positivity by IHC in contrast to our findings. The over-expression of HER2/NEU is associated with poorer prognosis, high grade features and resistant to usual chemotherapy [24]. Amplification of this gene is associated with the rapid progression of the disease, increased metastatic potential, increased resistance to tamoxifen and better response to anthracycline-based chemotherapy [25].

Tumor subtypes in our studies classified based on ER, PR expression and HER-2 over-expression, 11.2% of cases were ER/PR+, HER-2+, 66.2% were ER/PR +, HER-2-, 7.5% were ER/PR-, HER-2+, and 15% were classified as triple negative. The most common subtype in this study was ER/PR+, HER-2-, and this finding is in agreement with Onitilo, et al, 2009 results [26] and] Elsayed M Ali et al[20].

In our study, Her-2/Neu receptor revealed a significant inverse association with hormonal receptor status. We found that ER, PR expression was increased in Her 2/neu negative tumors as compared to Her-2/neu positive tumors. Similar results were found by Mona M Rashed et al.

Triple negative breast carcinoma is characterized by lack of ER, PR and Her-2/neu expression. Our study showed 15% cases as triple negative. In HER-2/neu overexpressing subtype (ER/PR- HER+) 83.3 % tumors were grade 3 in nature (p value =.008), suggesting the higher likelihood of HER2/neu expression at higher grade tumors, thereby causing the tumors to be more invasive and more likely to relapse.

Ki67 expression in the current study was significantly higher in HER2 positive compared to HER2 negative tumors. 80% of HER2 overexpressing tumors expressed high values of Ki-67.
This correlation may be used to predict the biological behavior of breast cancer. It was also observed that higher Ki-67 values are associated with a higher risk of relapse and thus poor prognosis. Among the Ki-67 positive cases 16.7% cases developed recurrence compared to only 9.1% risk of relapse in Ki-67 negative cases. ER/PR+ HER2- subtype had the best outcome with only 3 cases out of 50 developing a relapse i.e. 5.7%(p value=0.008). Worst prognosis was for ER/PR- Her2- (triple negative group) wherein 5 patients out of 12 developing a relapse i.e. 41.7% (p value=0.008).

6. Conclusion

Our study is consistent with the findings of previous studies which studied direct association of breast cancer with poorer prognostic parameters. HER2/NUE overexpression is a poor prognostic parameter in women diagnosed with breast cancer and the expression pattern of HER2/NEU protein showed a strong correlation with Ki67 overexpression hence these tumours are likely to have higher proliferative fractions leading to higher probability of recurrence. ER/PR+ HER2- had the best prognosis whereas triple negative tumors carried the worst prognosis.

Thus evaluation of different prognostic parameters individually and in constellation can predict patient outcome and individualize treatment options for a better outcome.

7. Limitations of this study

1) In Her-2/Neu analysis 2+ is an equivocal result that needs confirmation by FISH but FISH testing was not available in our setup so 2+ grading is taken as negative, so incidence of Her-2/Neu positivity might be actually more than the results which we obtained in our study.
2) Cut-off value of Ki-67 is a matter of great debate among pathologists and to date no standard operating procedure (SOP) or generally accepted cut-off definition for Ki-67

exists [156, 157]. For this reason, both the interlaboratory and the interstudy comparability of Ki-67 are limited[29, 30]

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