Study of Her2/Neu Expression in Breast Carcinoma and Correlation with Various Prognostic Parameters

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ABSTRACT

Background: *Breast cancer is a heterogeneous disease with varied morphological appearances, molecular features, behavior, and response to therapy. High levels of human epidermal growth receptor 2(HER2/NEU) expressions are associated with recurrence and death in breast cancer (BC). Though it is associated with poorer survival but its main importance is as predictors of response to agents that target this transmembrane protein (transtuzumab).*
The aim of the study was to evaluate the expression pattern of HER2/NEU in invasive component of breast carcinoma and correlate with various prognostic parameters such a tumour size, histologic grade, histologic type, regional lymph node status, and proliferative marker Ki67
Study Setting & Design: *A total of 112 breast cancer cases were studied for the histomorphological features followed by immunohistochemical study in the Department of Pathology of a tertiary care hospital*
Material and methods: *The lumpectomy or mastectomy specimens were fixed on 10% neutral buffered formalin immediately after surgery in 10 fold volume. Important clinical information from patients were collected in Performa. Finally routine hematoxylin and eosin sections were examined to confirm presence of invasive cancer, ascertain histological types, Histological Bloom Richardson grade (BRG)[1] and axillary lymph node status. IHC of HER2/NEU protein was done using HER2/NEU antibody following standard procedures. The tumour was also immunostained with Anti ER, Anti PR, and Ki67 primary antibody.*
Statistical Analysis: *Data was analyzed by SPSS software*
Results and observation: *The mean age of breast cancer presentation in this study was 44.6 years with standard deviation 11.24.This IHC evaluation for HER2/NEU was positive with 3+ score in 18.75% of cases. The data of HER2/NEU expression profiles were correlated with 3 BRG histologic grades of the tumours, it showed a positive association with high histologic grade. HER2/NEU profiles were inversely correlated with ER, PR expression (p value < 0.05). The HER2/NEU expression profiles was also correlated with tumour size and a significant relationship was found (p = 0.041).HER2/NEU positive tumours were belonged to mostly IDC.HER2/NEU expression profile was correlated with proliferative index Ki67 expression in this study and found a direct relationship (p < 0.005). Lymph node status and HER2/NEU showed a significant statistical correlation (p = 0.037).*
Discussion: *The over-expression of HER2/NEU is associated with poorer prognosis, high grade features and resistant to usual chemotherapy[10]. Our study also revealed that the Patients with higher levels of HER2/NEU- overexpression or amplification had statistically significant with lower levels of ER and PR positive tumours. We also found that when Her 2 neu was positive in tumours along with ER and PR, then it was mostly high BRG and High Ki67.The new addition from our study wasHER2/NEU expression profile correlation with Proliferative marker Ki67 which revealed a positive association.*
Conclusion: *The expression pattern of HER2/NEU protein showed a strong correlation with high Ki67 expression which means these tumours are likely to have higher proliferative fractions leading to high probability of recurrence without management by herceptin therapy*

Keywords: *Her2neu, Ki67, Prognostic parameters*

BACKGROUND

Breast cancer is a heterogeneous disease with varied morphological appearances, molecular features, behavior, and response to therapy .In 2007, almost 1,78,480 women were diagnosed with invasive breast cancer, 62,030 with in situ carcinoma and over 40,000 died of the disease.

The incidence of breast cancer is increasing rapidly in India. Statistics reveal that it has overtaken cancer of the cervix. Recently Indian Council of Medical Research (ICMR) data shows that the incidence of breast cancer is higher among Indian females in the metropolitan cities of Mumbai, Chennai, and Delhi. It is estimated that 1 in 22 Indian females is likely to develop breast cancer during her lifetime in contrast to 1 in 8 in America.

HER2/NEU (c-erb-B2) gene product is a 185 KD transmenbrane glycoprotein associated with tyrosine kinase activity. High levels of human

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epidermal growth receptor 2 (HER2/NEU) expression is associated with recurrence and death in breast cancer (BC) patients. Evaluation of HER2/NEU gene status is an important prognostic and predictive biomarker in breast cancer management [1]. This over expression of this protein is seen in 15-30% cases. Though it is associated with poorer survival but its main importance is as predictors of response to agents that target this transmembrane protein (transtuzumab).[1] A study, Vaidyanathan et. al[3] results show that HER2/NEU overexpression is a marker for poor prognosis and also, patients with HER2/NEU overexpression along with positive lymphnode had worse prognosis.The combined study of two hormone receptors; estrogen and progesterone receptor and Her2neu have thus become most informative in the molecular classification of breast tumors and their clinical assessment for treatment and further outcome. Studies have proved that racial and demographic features of patients can also influence the pattern of immune expression Shet et al2009[19].To our knowledge, no such study has been published from Assam and North eastern region of this country. This facilitated us to conduct this study, which analysed the actual status of human epidermal growth factor receptor status in this population.

The aim of the study was to evaluate the expression pattern of HER2/NEU in invasive component of breast carcinoma and correlate with various prognostic parameters such a tumour size, histologic grade , histologic type, regional lymph node status, proliferative marker Ki67, Estrogen Receptor Progesterone Receptor. It was also to make an effort to look for relation with age of patient.

STUDY SETTING & DESIGN

A total of 112 breast cancer cases were studied for the histomorphological features followed by immunohistochemical study in Department of Pathology. The cases diagnosed by fine needle aspiration or core biopsy or cases with recurrence were not included in the study. The invasive breast cancer cases undergoing surgery with a curative intent like lumpectomy or mastectomy were included in the study. All surgical pathology specimens were received from Department of Surgery of the Tertiary care Hospital.

Ethical clearance: was received before start of the study from Institutional Ethics committee for Human research of the study institution

MATERIAL AND METHODS

The lumpectomy or mastectomy specimens were fixed on 10% neutral buffered formalin in a 10 fold volume immediately after surgery. Important information from patients were collected in Performa which includes name, age, menopausal status, child birth , lactation, present medical history, past history, family history of breast and ovarian cancer in blood relatives. Then after explaining about the study the consent was taken to carry out study along with the witnesses. Grossing were done with recording of weight, length, breath, and depth, color of skin flap, any scar, recent surgical incision, edema, discoloration, peau de orange, puckering, burging, ulceration. The tumour location was noted in terms of quadrant, distance from skin, nipple, muscle fascia, color consistency, borders such as circumscribed, necrosis, hemorrhage, calcification etc. Then recording and careful search for axillary, apical or any other lymph nodes were done. 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 & Elston[2] 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[details of Clones in table 1]

Details of Primary antibodies and clones

Antigen	Catalog No.	Clonality	Host Species
ER	AM272-2ME	Monoclonal (SP1)	Mouse
PR	AM328-5ME	Monoclonal (Y85)	Mouse
HER2/NEU	AM134-5ME	Monoclonal (CB11)	Mouse
Ki67	275R-18	Monoclonal (SP6)	Rabbit
Secondary AB	956D-21/22/23		

HER2/NEU and ER, PR interpretation and scoring were based on American Society of Clinical Oncology / College of American Pathologist Recommendations 2010.[6]

3+ = More than 30% invasive breast cancer cells showing strong complete homogenous membrane positive by Her2neu is interpretive as positive,

2+= More than 30% invasive breast cancer showing moderate or incomplete membrane positive Her2neu is interpretive as equivocal,

1+= Any proportion of invasive breast cancer cells showing weak or incomplete membrane positive by HER2/NEU is interpretive as 1+, clinically taken as negative.

STATISTICAL ANALYSIS:

Data was analyzed by SPSS software to find out significance of Her2neu expression with other prognostic parameters of breast cancer. Association among variables are calculated by Fishers exact test. A p- value of < 0.05 was taken as significant.

RESULTS AND OBSERVATION

The mean age of breast cancer presentation in this study was 44.6 years with standard deviation 11.24. This showed more than 66.07% women diagnosed with breast cancer were belonged to less than 50 years age group. Almost 52% of the women were premenopausal during the time of diagnosis. Only 3 cases had family history of breast cancer in first degree blood relatives. The sizes of the tumours were classified in 3 categories. The tumour size 2-5 cm^2 was represented highest [75.89%]. The various histological types found in this study were Invasive Duct Carcinoma[IDC] (59.82%), Invasive lobular carcinoma [ILC] (16.07%), medullary carcinoma (6.25%), mucinous carcinoma (5.36%) and others (12.5%). Axillary lymph node status were positive in 53% of cases and 47 were free from any infiltration. The tumours were studied for Modified Bloom Richardson Histologic Grade, Grade I constituted 16.96%, Grade II was 59.82%, and Grade III was 23.21% respectively.[Table 1]

This IHC evaluation for HER2/NEU was negative for 66.07% of cases whereas borderline or equivocal in 15%. HER2/NEU was positive with 3+ score in 18.75% of cases. This study showed that the largest category of breast cancer were negative for HER2/NEU.

The data of HER2/NEU expression profiles were correlated with 3 BRG histologic grades of the tumours. It showed an association with BRG grade III tumours showingHER2/NEU reactivity (2+, 3+ combined) 63.9% cases. When HER2/NEU profiles were correlated with ER, PR expression profiles, it derived into a inverse correlation (p value < 0.05). The HER2/NEU positive tumours have less chance of having expression of ER PR. However ER and or PR also co - expressed withHER2/NEU overexpressed tumours [22.1%] which are called as Triple positive. The borderline HER2/NEU 2+ cases were not correlated as they could not be categorized in either positive or negative category without further subjected to Fluorescent in-situ Hybridization (FISH).When the HER2/NEU expression profiles were correlated with tumour size, a statistical significance was seen (p = 0.041).HER2/NEU positive tumours were belonged to mostly IDC, one each from ILC (high grade), papillary, micro papillary, whereas it was negative in all cases of mucinous, medullary, metaplastic, small cell type. HER2/NEU pattern and age of patients did not show any significant relationship (p = 0.452). HER2/NEU expression profile was correlated with proliferative index Ki67 expression in this study and found a direct relationship (p < 0.005). HER2/NEUscore 3+ associated with a higher Ki67 index (52.4%) whereas moderate Ki67 index in 38.1% and low in only 9.5% of tumours. Lymph node status and HER2/NEU showed a significant correlation (p = 0.037). Breast cancer without lymph node involvement was negative for Her2 neu in 84.8% tumours and positive only in 15.2%.

Table 1: Characteristic features and their profile data

Characteristic parameters –		No of cases (Total No of Cases 112)	
1. Age			
SN			**Percentage**
I.	**≤50 years**	**74**	**66.07%**
II.	**>50**	**38**	**33.93%**
2. Menopausal status			
SN			**Percentage**
I.	**Pre-menopausal**	**58**	**51.79%**
II.	**Post-menopausal**	**54**	**48.21%**
3. Family history of breast cancer			
SN			**Percentage**
I.	**Present**	**03**	**2.68%**
II.	**Absent**	**109**	**97.32%**

4. Tumour size[in cm^2]

SN			Percentage
I.	≤2	15	13.39%
II.	2-5	85	75.89%
III	>5	12	10.71%

5. Histologic types

SN			Percentage
I.	Invasive duct carcinoma NOS	67	59.82%
II.	Invasive lobular carcinoma	18	16.07%
III	Medullary carcinoma	07	6.25%
IV	Mucinous carcinoma	06	5.36%
V	Others	14	12.5%

6. Histologic grades

SN			Percentage
I.	BRG I [low]	19	16.96%
II.	BRG II[moderate]	67	59.82%
III	BRG III[high]	26	23.21%

7. Axillary Node status

SN			Percentage
I.	Negative [0 node]	53	47.32%
II.	Positive [1-3]	39	34.83%
III	Positive [4 or more]	20	17.85%

8. Estrogen Receptor

SN			Percentage
I.	Positive	53	47.32%
II.	Negative	59	52.62%

9. Progesterone Receptor

SN			Percentage
I.	Positive	53	47.32%
II.	Negative	59	52.62%

10. ER and PR both

SN			Percentage
I.	Positive	52	46.43%
II.	Negative	60	53.53%

11. Her2 neu

SN			Percentage
I.	0[zero]	48	42.86%
II.	1+	26	23.21%
III	2+	17	15.18%
IV	3+ [positive]		

12. Ki67

SN			Percentage
I.	Low	19	16.96%
II.	Moderate	67	59.82%
III	High	26	23.21%

Table 2: Comparision of Her2/Neu Expression Profile as Found by Various Authors

YEAR	AUTHORS	Her2/Neu(%)
2005	Lal *et al*[18]	26.89
2007	Bhurgri *et al*[17]	24.70
2008	Azizun Niza *et al*[7]	24.00
2009	Mudduwa LK *et al*[4]	19.10
2009	Adedayo A *et al*[14]	18.00
2010	Gupta S *et al*[9]	16.70
2010	Vaidyanathan K *et al*[3]	43.20
2011	Ambroise M *et al*[15]	27.00
2012	Gupta S *et al*[16]	24.00
2011–12	Present Study	18.75

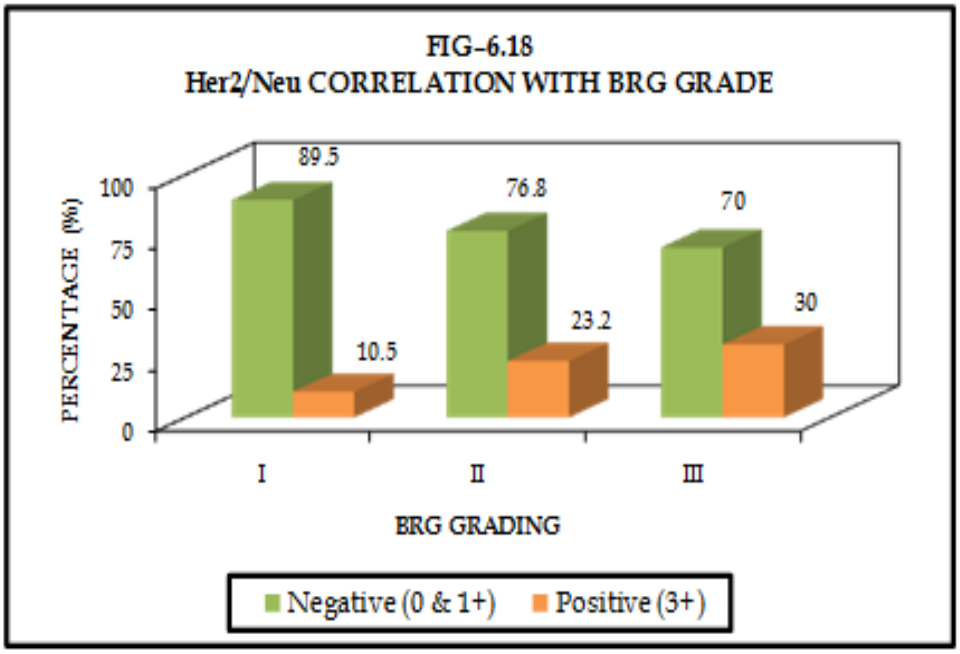

Diagram 1: Her2 neu correlation with Histologic grade

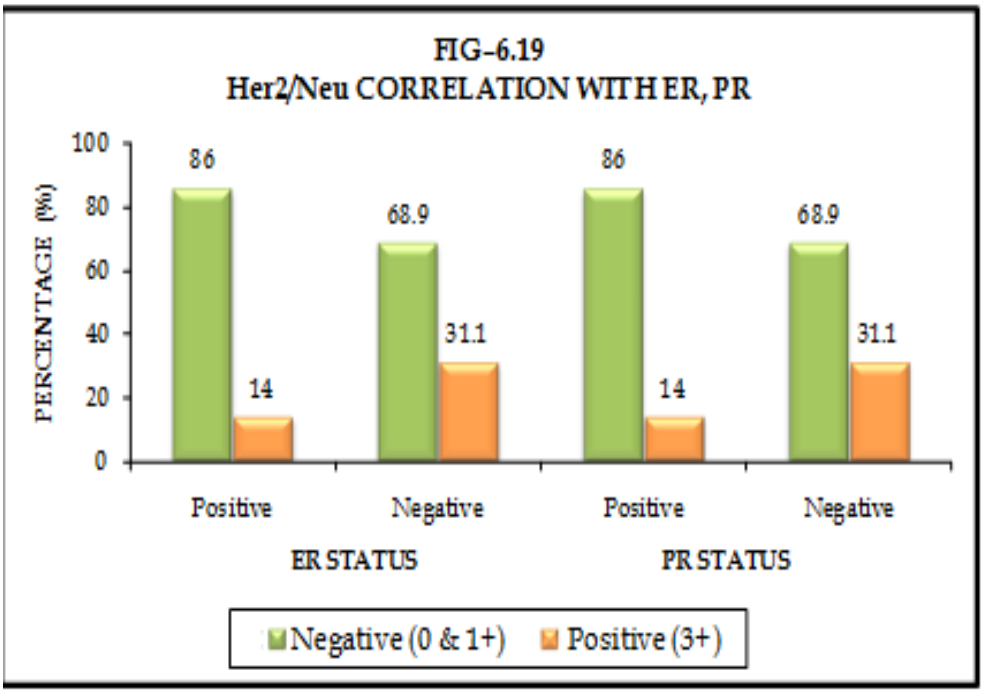

Diagram 2: Her2neu with ER, PR

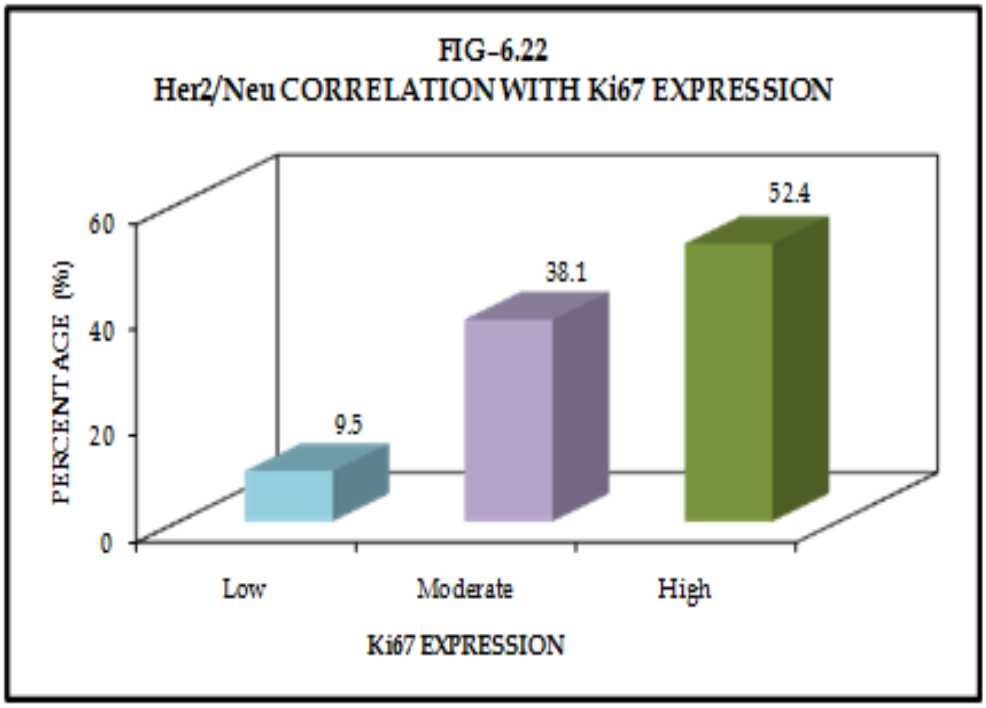

Diagram 3: Her2neu with Ki67

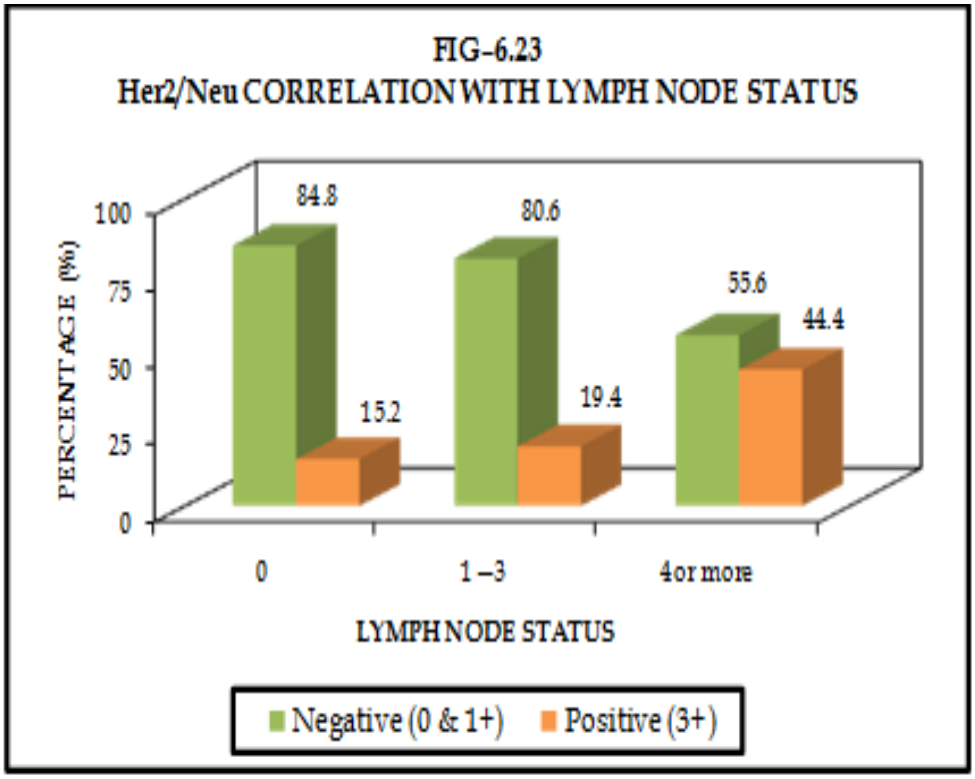

Diagram 4: Her2neu with lymph node status

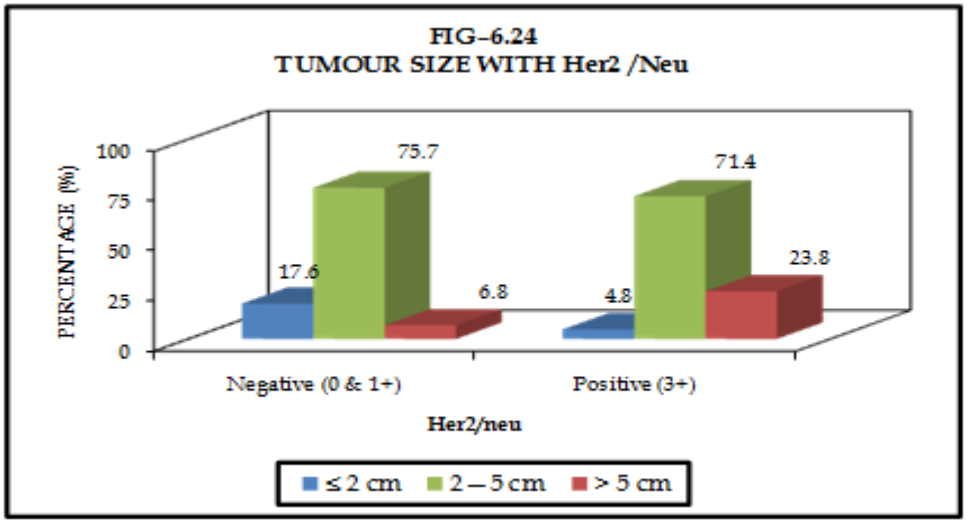

Diagram 5: Her2neu with tumour size

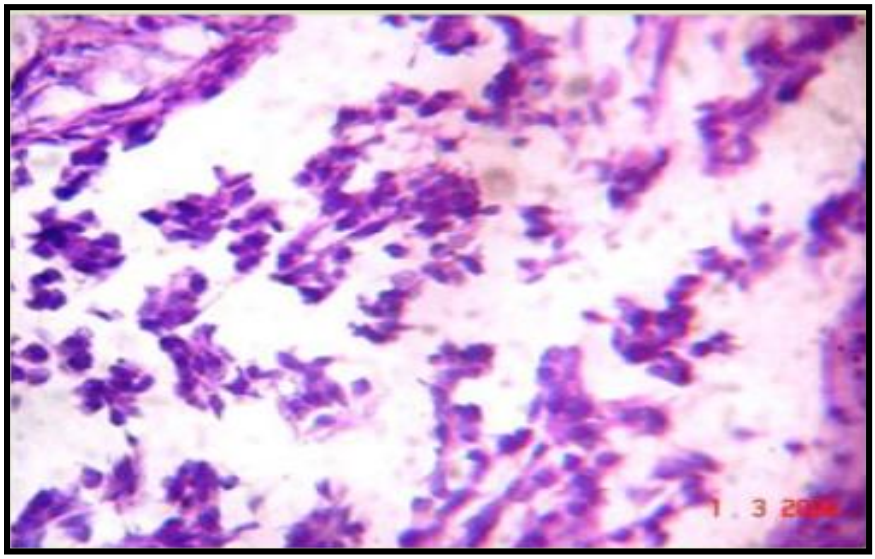

Fig. 1: Micropapillary type

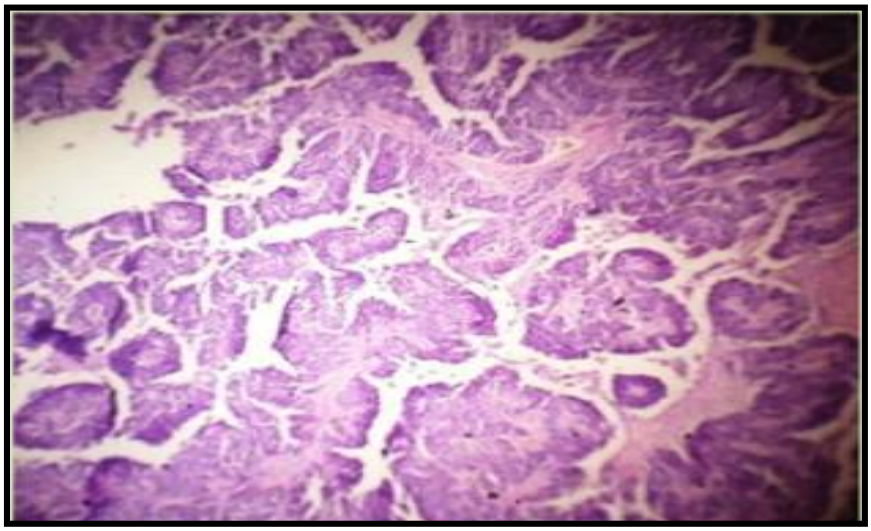

Fig. 2: Papillary type

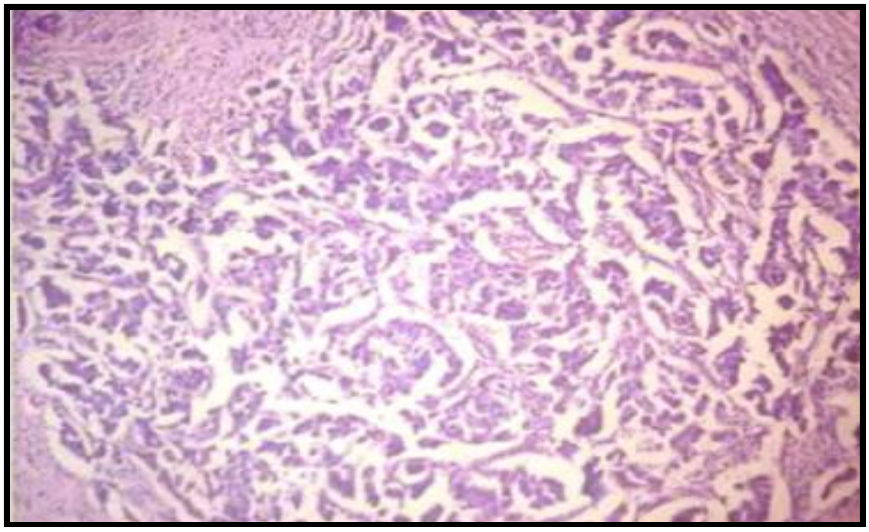

Fig. 3: Lymph node infiltration

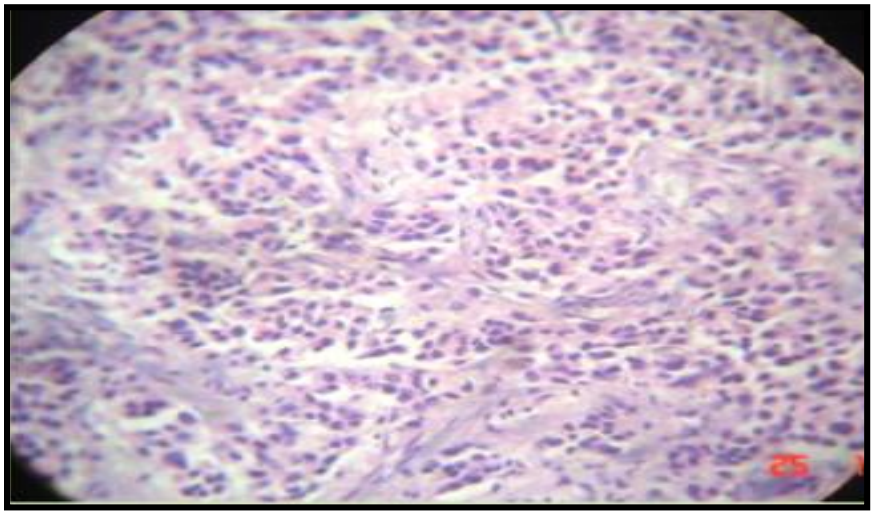

Fig. 4: IDC –NOS

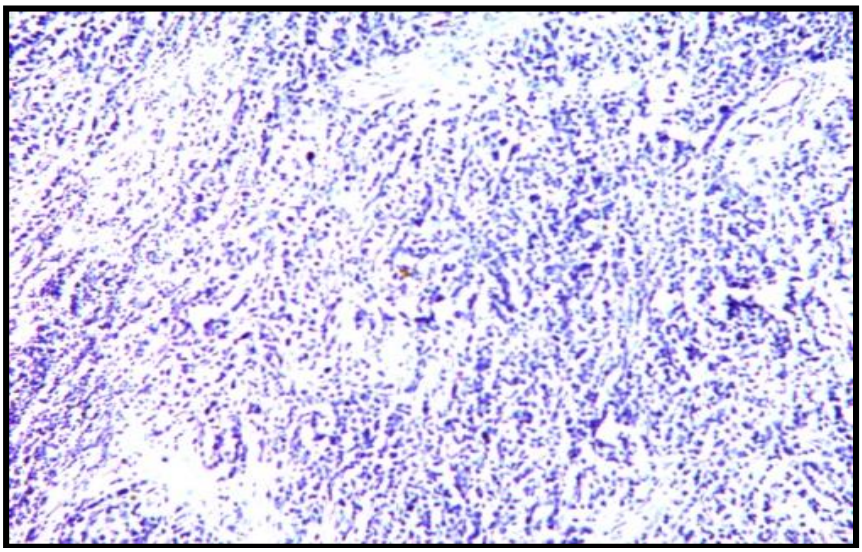

Fig. 5: ILC

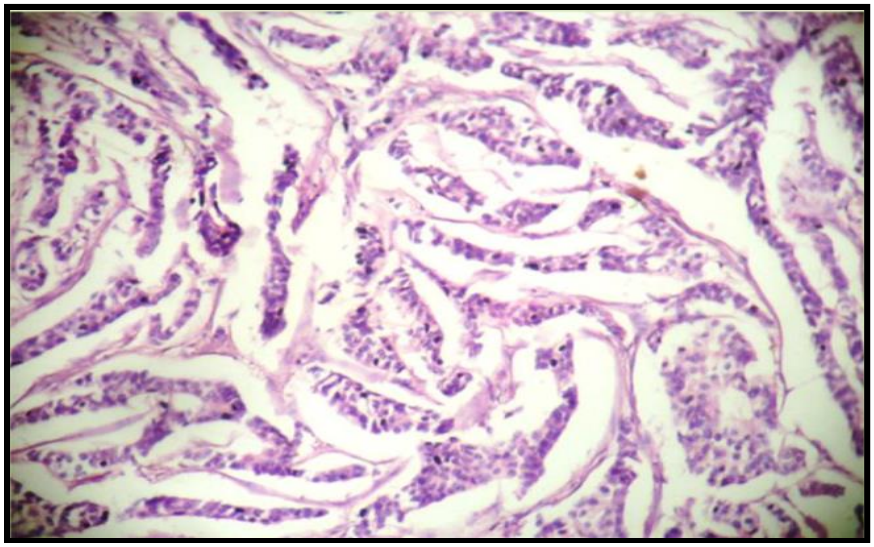

Fig. 6: ILC

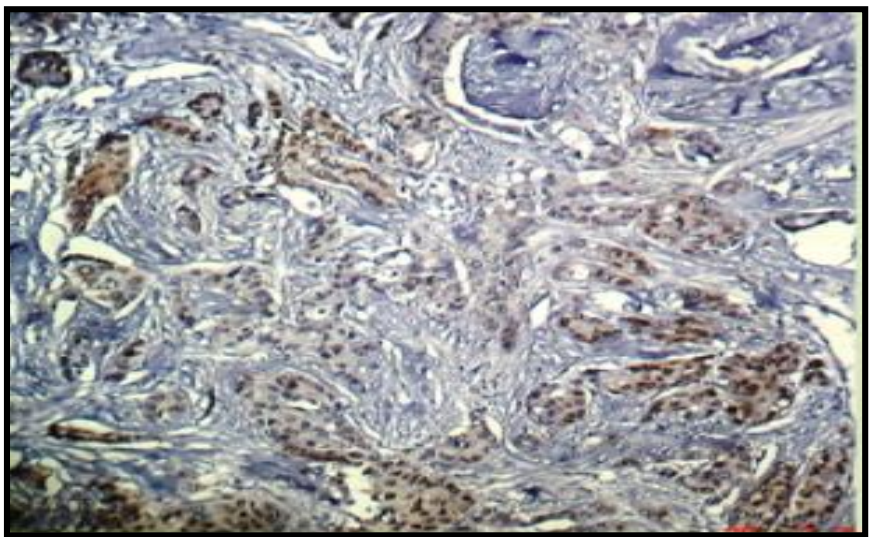

Fig. 7: ER control

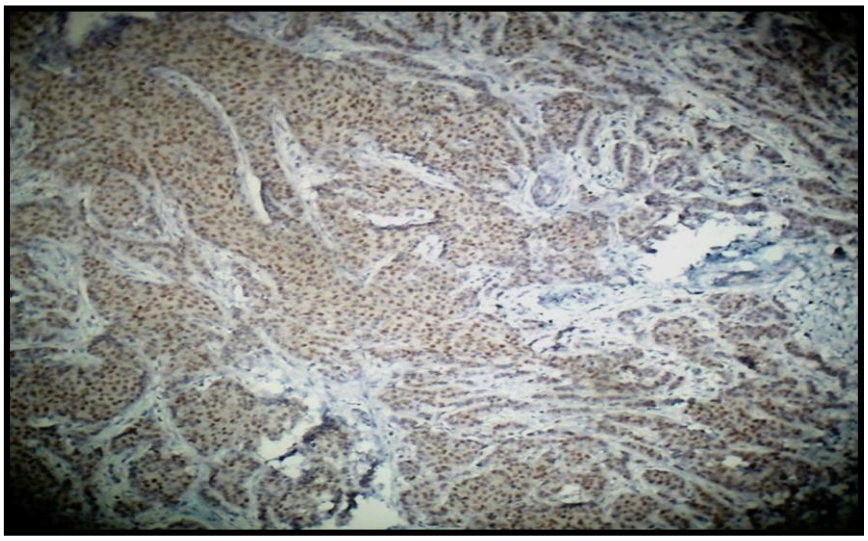

Fig. 8. ER positive

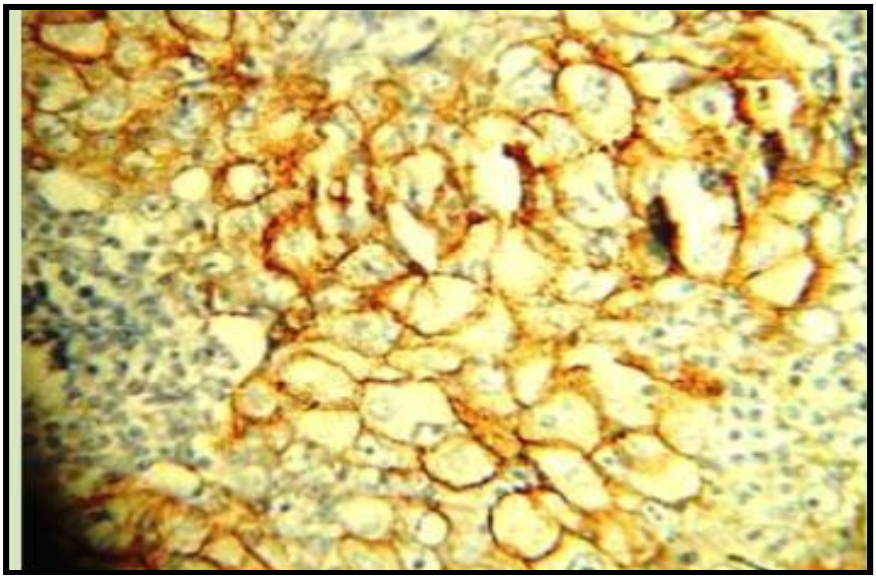

Fig. 9: Her2 control

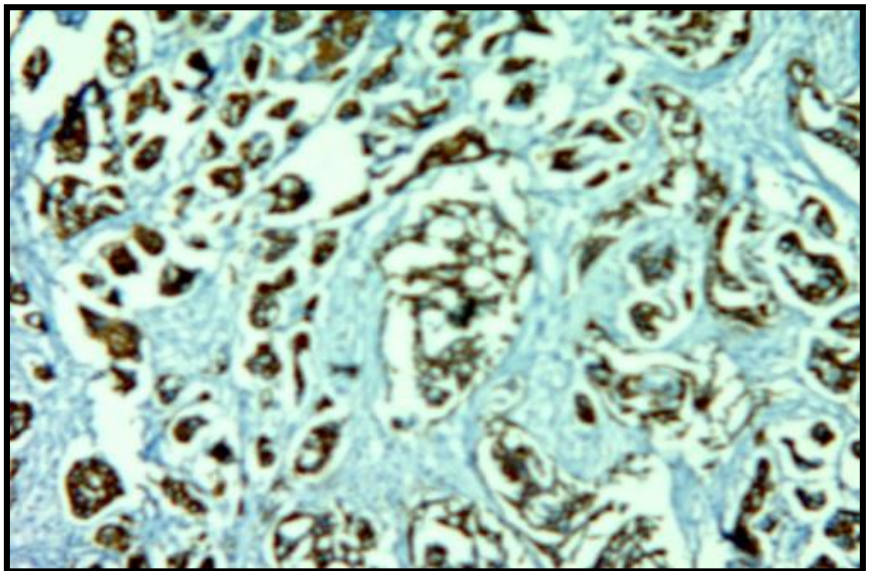

Fig. 10: Her2neu 3+ stain

DISCUSSION

The present study comprised of 112 cases of breast cancers with mean age of 44.6 years with standard deviation 11.24 which is similar to, Sandhu et al.,[20] 2010. Mean age of Indian breast cancer patients is found to be lower when compared to the Western countries with an average difference of one decade, Sandhu et al.,[20] 2010. Our study demonstrated a higher number of lymph node positive [53%] cases at the time of diagnosis. Indian and Asian studies have documented a greater percentage of breast carcinomas with lymph nodal metastasis compared to the Western figures [Sandhu et al, 201020; Vaidya Nathan et al., 2010[3]).We found HER2/NEU, were positive with 3+ score in 18.57% of cases whereas largest percentage 66.07% of invasive breast carcinoma completely negative. This finding was consistent with universally accepted HER2/NEU overexpressed in 15-30% cases[9]. This finding is almost similar to another Asian study of 19.1% done in Srilanka 2009 by Mudduwa LK *et* al[4].However the frequency of HER2/NEU positivity varies among Indian studies. A comparison of HER2/NEUexpression with other published studies [Table 2]. In a study from Bangalore, South India Vaidya Nathan et al[3]., 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[10]. The most significant aspect of this marker is availability of targeted Herceptin therapy. 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 chemo-therapy[11]. Our study also revealed that the Patients with higher levels of HER2/NEU- over-expression had a statistically significant lower levels of ER and PR positive tumour. In triple positive cases the tumours showed the biology of high histologic grade and High Ki67. The report, Vadyanathan et. al 2010 also showed significant correlation with HER2/NEU and lymph node status, tumour size and ductal carcinoma type histology[3]. Similarly Her2 neu expression was correlated in high grade tumours whereas grade I tumours were expressing higher ER&PR in our study, comparable to Azizun N et al2008[7].HER2/NEU positive tumours were belonged to mostly IDC, one each from ILC (high grade), papillary, micropapillary, whereas it was negative in all cases of mucinous, medullary, metaplastic, small cell type in our cases. So, it can be commented that the morphologic picture of invasive ductal carcinoma, not otherwise specified type having high histologic grades are likely to over expressed HER2/NEU.

The new addition from our study was HER2/NEU expression profile correlation with Proliferative marker Ki67 expression which had shown a direct relationship. HER2/NEU score 3+ associated with a higher Ki67 [52.4%] invasive component. This suggests the tumour has potential to behave aggressively. This study found no significant correlation of HER2/NEUwith histologic types and age. Besides that we experience rare histologic type (high grade) like papilary, micro papillary carcinoma also show HER2/NEU over-expression, so we should be cautious with this cases for Her2 /neu evaluation. Over-expression of HER2/NEU is a good predictor of response to Herceptin, but not a positive predictor of response to chemotherapy or overall survival[7]. HER2/NEU is also an independent negative predictor of overall survival and time to relapse in patients with lymph-node-positive in breast cancer[5,8]. Overall thses findings were comparable to Navolanic, P. M et al, 2003 where HER-2/*neu* gene protein over-expression have been associated consistently with high tumor grade, high cell proliferation rate, negative assays for nuclear protein receptors for estrogen and progesterone receptor[13].As a base line routine evaluation for treatment of breast cancer, besides ER, PR, HER2/NEU, Ki67 should be studied together for crucial information of proliferative fraction in a under resourced laboratory where advanced molecular facility still a dream as it is easily available, possible and can be highly applicable in clinical practice[12]. We examined whether there were any correlation HER2/NEU expression with age pattern of patients. Even though our cases consist of 52% of premenopausal women and the mean of the cases were 44.6 years, no significant statistical value was resulted. None of the 3 cases with family history in blood relative showed any over-expression of HER2/NEU. However there was a limitation, FISH [Fluorescent In-Situ Hybridization] was not performed in this study for 2+ borderline HER2/NEU positive tumours like Ambroise M et al 2011 nor in the other recent studies[15]. So, it is very likely that the actually HER2/NEUexpression would be slightly higher.

CONCLUSION

Our study validates the findings of previous studies which showed its direct association with poorer prognostic parameters. HER2/NEUover-expression seems to be constant [15-30%] in breast cancer unaffected by age of women population diagnosed with cancer. The expression pattern of HER2/NEU protein showed a strong correlation with high Ki67 expression which means these tumours are likely to have higher proliferative fractions leading to higher probability of recurrence without management by herceptin therapy

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