

Role of Hormone Receptors- Estrogen receptor, Progesterone Receptor and Human Epidermal Growth Factor Receptor 2/Neu (Her2/Neu) as a Prognostic and Therapeutic Tool in Breast Cancer

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ABSTRACT

Background: Breast carcinoma (BC) is the second most leading cancer which mostly occurs in younger age group. It is better to represent BC by the combined receptor expression pattern, as single receptor status alone will not help in treatment and management of tumor. This study aims to analyze the expression pattern of ER, PR and Her-2/neu hormone receptors in BC patients for evaluating their clinicopathological correlation with other factors for BC development in the Southern Assam region of India.

Methods: 60 number of breast carcinoma cases were studied from December 2016 – November 2018. Immunohistochemical analysis of all the samples were done. Data including age, gender, tumor size, histological grade and type, lymph node status were collected from the Pathology Dept. All the data were entered and analyzed in SPSS Software. ANOVA analysis and Pearson correlation test was done for determining statistical significance.

Results: The mean age was found to be 41.33 ± 9.63 . The most common type of carcinoma was found to be Invasive Breast Carcinoma (Ductal), NOS Type. Grade-II was the most prevalent type followed by Grade-III and Grade-I. PR is statistically significant with age ($p=0.001$) and gender ($p=0.002$). ER and PR are significantly positively correlated with each other ($r=0.323$ and $p=0.012$) and Her2/neu was significantly negatively correlated with ER and PR expression ($r=-0.333$, $p=0.009$ and $r=-0.274$, $p=0.034$ respectively).

Conclusion: In this present study ER/PR expressions were found to be low, while Her-2/neu over-expression was higher. So, Her-2/neu, is an important predictive and prognostic marker at a very young age.

Keywords: Her-2/neu, Prognostic marker, SPSS, Breast carcinoma, Progesterone receptor.

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INTRODUCTION

In India breast carcinoma (BC) is the second most leading cancer strongly related to age. It is mostly occurred in females of younger age group and affects a smaller number of males (less than 1 %) in comparison to female.¹ On the basis of hormone receptor status, BC can be divided into four different subtypes viz. Luminal A type (ER/PR⁺/Her2⁻); ER⁺/PR⁺/Her2⁻; ER/PR⁺/Her2⁻; ER⁺/PR⁻/Her2⁻; Luminal B type: ER⁺/PR⁺/Her2⁺; ER⁻/PR⁺/Her2⁺; ER⁺/PR⁻/Her2⁺; Non-

luminal or Her2neu type: ER⁻/PR⁻/HER2⁺ and triple negative or basal-like: ER⁻/PR⁻/Her2⁻.² It has a complex heterogenous characteristic which is controlled by multiple genetic and epigenetic alteration.^{1,3} Study of different biomarkers plays an important role as a good prognostic factor and management of BC. Other clinicopathological factors such as histological type and grade, tumor size, lymph node status

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also acts as prognostic factors. However, of all these factors, prognosis and management of BC is highly influenced by interrelationship of Estrogen Receptor (ER) and Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor-2 (Her-2/neu) expression in tumor.⁴ Earlier studies have shown that triple negative and Her-2 type BC are the two molecular BC subtypes, which are common in younger females. Moreover, this cancer type is found to be more aggressive in the younger females than the older ones.⁵ However, it is better to represent BC by the combined receptor expression pattern, as single receptor status alone will not help in treatment and management of tumor. Therefore, recent management protocol separates the triple-negative and triple-positive tumor for better treatment and diagnosis of BC.⁶

In these contexts, the present study aims to analyze the expression pattern of ER, PR and Her-2/neu hormone receptors in BC patients for evaluating their clinicopathological correlation with other factors for BC development in the Southern Assam region of India.

METHODS

In this retrospective study, we have done immunohistochemistry (IHC) technique for evaluating the level of expression of hormone receptors. The paraffin-embedded tissue sections of breast carcinoma from 60 consecutive patients who had undergone surgery at the Department of Surgery, Silchar Medical College & Hospital (SMCH) between December 2016 – November 2018 were included in the study. The histopathological diagnosis of breast carcinoma was established by standard light-microscopic evaluation of sections stained with Hematoxylin and Eosin in each case. Estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu status was also noted.

Clinical and demographic data including age, sex, tumor size, histological type, grade, lymph node status, were collected from Department of Pathology, SMCH.

Immunohistochemical (IHC) analysis: IHC of ER, PR and Her-2/neu onco-protein was performed on 4 µm thick paraffin embedded tissue sections placed on coated frosted slides. After deparaffinization and blocking of endogenous peroxidase, Her-2/neu immunostaining was performed using rabbit monoclonal antibody Her-2/neu (SP3) as primary antibody (Cell Marque, made in USA) at 1: 100 dilution and also estrogen receptor (SP1) and Progesterone Receptor (Y85). Binding of the primary antibody was checked by CRF™ Anti –polyvalent HRP Polyvalent HRP Polymer (DAB) Staining kit.⁷

HER-2/neu scoring of IHC slides was done on light microscopy as per the recommended American Society of Clinical Oncology (ASCO) guidelines.⁸ The immunostaining was read in a semiquantitative manner and graded as follows: 0, 1+, 2+ and 3+. Scores 0 and 1 were considered as negative expression while scores 2 and 3 were considered as equivocal and positive expression for HER-2/neu respectively.⁹ Sections which showed strong membrane staining of normal epithelia of breast were rejected and subjected to a repeat IHC.

Scores of 2+ were taken as equivocal cases, which were further recommended for Fluorescent in situ hybridization (FISH) analysis for Her-2 /neu amplification.⁶

Modified Bloom–Richardson–Elston grading system (also called the Nottingham system) was used for the classification of breast cancer.¹⁰

ER and PR scoring was done by using Allred Score System¹¹ which combines the percentage of positive cells and intensity of the reaction produced in most of the carcinomas. The 2 scores are then added together for a final score with scores 0-2 taken as negative and 3-8 as positive.²

Statistics:

All the data were entered and analyzed in SPSS Software [PASW Statistics 18, Release 18.0.0 (Jul 30, 2009)] and VassarStats: Website for Statistical Computation (<http://www.vassarstats.net/newcs.html>). ANOVA analysis and Pearson correlation test was done for determining statistical significance. Data were expressed as numbers and percentages.

Ethical consideration and clearance:

This study was conducted after obtaining the ethical approval from ethical committee of our institution vide letter No. SMC/13/3420 dated 11/03/2015

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RESULTS

1. Status of different clinicopathological factors in different age groups:

In our study, 60 patients of breast carcinoma were taken and categorized in four different age groups viz. <32years; 32-41years; 42-50 years and >50 years. The mean age was found to be 41.33 ± 9.63. Out of 60 patients 58 female and only 2 male patients were found and it was observed that gender is statistically significant with age (p=0.004) and most of the female patients belongs to 42-50 age group (n=22, 36.7 %) whereas both the male patient was more than 50 years old age group as shown in Table-1. The number of cases is highest in younger age group (n=22, 36.7%) and lowest in older patient (n=8, 13.3%).

The mean tumor size ranges between <4cm to >12cm with highest cases with size 8-11.9 cm. However, this was not statistically significant with age (Table-1)

The left side breast carcinoma was more common (n= 38, 68.33 %) than that of right-side carcinoma (n= 21, 35%) and we also found one case with both side carcinoma. (Table-1) Statistically significant association was not observed between location of breast carcinoma and age.

In this study we observed three types of histological grade: Grade-I, Grade-II and Grade-III. We observed that most of the cases belonged to Grade -II (n = 36, 60%) followed by Grade-III (n = 13, 21.67%) and Grade-I (n= 11, 18.33%). The result shows no statistically significance difference between histological grade with age. (Table-1)

The most common type of carcinoma was found to be Invasive Breast Carcinoma (Ductal), NOS Type (88.3%) and other types of carcinomas (n= 7, 11.67%) -includes Lobular, Papillary, Medullary, Oncocytic, Fibroadenoma breast carcinoma, Phylloid Tumor, Secretory breast carcinoma.

In present study, there were highest number of cases having less than 4 positive lymph nodes.

It was also observed that both histological type and lymph node was not statistically significant with age. (Table-1)

Table 1: Status of different clinicopathological factors among the study subjects of different age groups

Factors	Age groups (Years) [Mean Age: 41.33 ± 9.63]				Total [n = 60 (%)]	P-value
	< 32 [n = 11 (18.3%)]	32 - 41 [n = 19 (31.7%)]	42 - 50 [n = 22 (36.7%)]	> 50 [n = 8 (13.3%)]		
Gender						0.004
Male	-	-	-	2 (3.33)	2 (3.33)	
Female	11 (18.33)	19 (31.67)	22 (36.67)	6 (10.00)	58 (96.67)	
Mean Tumor Size (cm)						0.374
< 4 cm	5 (8.33)	3 (5.00)	3 (5.00)	2 (3.33)	13 (21.67)	
4-7.9 cm	3 (5.00)	5 (8.33)	6 (10.00)	1 (1.67)	15 (25.00)	
8-11.9 cm	1 (1.67)	9 (15.00)	7 (11.67)	4 (6.67)	21 (35.00)	
> 12 cm	2 (3.33)	2 (3.33)	6 (10.00)	1 (1.67)	11 (18.33)	
Location of Breast Cancer						0.740
Both	-	1 (1.67)	-	-	1 (1.67)	
Left	8 (13.33)	10 (16.67)	14 (23.33)	6 (10.00)	38 (63.33)	
Right	3 (5.00)	8 (13.33)	8 (13.33)	2 (3.33)	21 (35.00)	
Histological Grade						0.414
Grade I	1 (1.67)	3 (5.00)	6 (10.00)	1 (1.67)	11 (18.33)	
Grade II	8 (13.33)	11 (18.33)	10 (16.67)	7 (11.67)	36 (60.00)	
Grade III	2 (3.33)	5 (8.33)	6 (10.00)	-	13 (21.67)	
Histological Type						
IDC	8 (13.33)	16 (26.67)	21 (35.00)	8 (13.33)	53 (88.33)	
Others	3 (5.00)	3 (5.00)	1 (1.67)	-	7 (11.67)	
Lymph node Status §						0.188
< 4 Positive	7 (12.96)	10 (18.52)	14 (25.92)	8 (14.81)	39 (72.22)	
> 4 Positive	2 (3.70)	7 (12.96)	6 (11.11)	-	15 (27.78)	
Estrogen Receptor (ER)						0.069
Positive	1 (1.67)	7 (11.67)	3 (5.00)	4 (6.67)	15 (25.00)	
Negative	10 (16.67)	12 (20.00)	19 (31.67)	4 (6.67)	45 (75.00)	
Progesterone Receptor (PR)						0.001
Positive	3 (5.00)	-	3 (5.00)	5 (8.33)	11 (18.33)	
Negative	8 (13.33)	19 (31.67)	19 (31.67)	3 (5.00)	49 (81.67)	
HER2Neu Receptor (HR)						0.798
Positive	3 (5.00)	6 (10.00)	4 (6.67)	2 (3.33)	15 (25.00)	
Negative	8 (13.33)	13 (21.67)	18 (30.00)	6 (10.00)	45 (75.00)	
Combined Receptors Status						0.001
ER+ve PR+ve HR-ve	1 (1.67)	-	1 (1.67)	4 (6.67)	6 (10.00)	
ER+ve PR-ve HR-ve	-	7 (11.67)	2 (3.33)	-	9 (15.00)	
ER-ve PR+ve HR-ve	2 (3.33)	-	2 (3.33)	1 (1.67)	5 (8.33)	
ER-ve PR-ve HR+ve	3 (5.00)	6 (10.00)	4 (6.67)	2 (3.33)	15 (25.00)	
ER-ve PR-ve HR-ve	5 (8.33)	6 (10.00)	13 (21.67)	1 (1.67)	25 (41.67)	

Note. § In four (04) tumor samples, the Lymph node status could not be ascertained, for which the values mentioned in above table is based on 54 samples only. The BOLD values are statistically significant (p-value < 0.05)

Immunohistochemistry was done for all the samples and we found that out of 60 number of patients, 25% were ER positive and 75% were ER negative. The positivity rate of ER

was found to be more in younger age group. We also observed 18.33% were PR positive and 81.67% were PR negative and positivity rate was more in older age group. While the rate of Her-2/neu positivity was found to be 25% and 75% were Her-2/neu negative. It was noted that the over-expression of Her-2/neu was more in younger age group whereas older age group were more likely to be PR positive than younger groups (Table-1). Our study shows triple negative breast cancer (TNBC) and Her-2/neu type tumors are the two molecular subtypes which were more common in younger age groups.

In our study, expressions of different hormone receptors were statistically analyzed under different age groups, and it was observed that expression of PR is statistically significant with age (p=0.001). The Bar diagram (Fig: 1A) shows positive expression of ER and Her-2/neu found more in younger age group and expression of PR found to be more in older age group. While negative expression of ER and Her-2/neu is more in middle aged group and PR negativity was found in younger as well as middle age group.

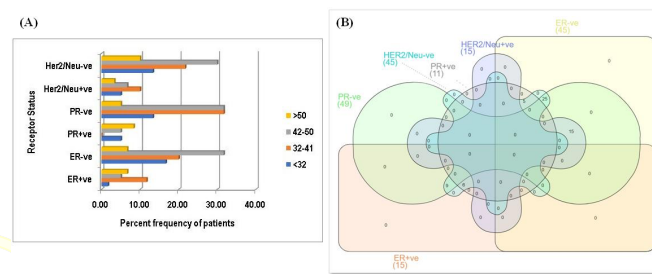


Figure 1. Distribution of hormonal receptors under study among different age groups (A), and in all possible combinations (B)

2. ANOVA analysis of hormone reactivity under different age groups:

Statistical significance difference among the cases, expression of hormone receptors and other clinicopathological factors of the breast cancer patients was determined using ANOVA. Analysis of variance outcome indicates that age is statistically significant with the expression of PR (p=0.001) and gender is also statistically significant with expression of PR (p= 0.002), all <0.05 significance level but these two factors had no significance difference with ER and Her-2/neu receptor. Except these two factors other clinicopathological factors such as tumor size, location of breast cancer, histological grade and lymph node status shows no such significant association with expression of ER, PR and Her-2/neu (Table-2).

Table 2: ANOVA table corresponding to the expressions of the hormonal receptors under study with different clinicopathological factors

Factors	Estrogen Receptor (ER)			Progesterone Receptor (PR)			Her2/Neu receptor (HR)		
	Sum of Squares	F	p-value	Sum of Squares	F	p-value	Sum of Squares	F	p-value
Age (years)	1.329	2.500	0.069	2.336	6.558	0.001	0.190	0.321	0.810
Gender	0.129	0.674	0.415	1.380	10.526	0.002	0.129	0.674	0.415
Mean Tumor Size (cm)	0.280	0.476	0.700	0.138	0.291	0.832	0.468	0.810	0.494
Location of Breast Cancer	0.873	2.397	0.100	0.305	1.003	0.373	0.096	0.245	0.784
Histological Grade	1.068	2.990	0.058	0.579	1.965	0.150	0.514	1.364	0.264
Lymph node Status	0.114	0.578	0.451	0.103	0.618	0.435	0.014	0.074	0.787

Note. The BOLD values are statistically significant (p-value < 0.05).

3. Correlation between expression of hormone receptors and clinicopathological factors:

Pearson correlation coefficient test was done to evaluate correlation between expressions of hormone receptors and different clinicopathological factors (Table-3). It was noted that ER and PR are significantly positively correlated with each other ($r=0.323$ and $p=0.012$). However, Her-2/neu was significantly negatively correlated with ER and PR expression ($r=-0.333$, $p=0.009$ and $r=-0.274$, $p=0.034$ respectively). It was also observed that PR is significantly positively correlated with gender ($r=0.392$ and $p=0.002$).

Table 3: Pearson correlation among expressions of the hormonal receptors under study with different clinicopathological factors

Receptors	Factors	Pearson correlation coefficient (r)	p-value	Remarks
Estrogen Receptor (ER)	Age (years)	0.133	0.310	
	Gender	0.107	0.415	
	Mean Tumor Size (cm)	0.019	0.887	
	Location of Breast Cancer	0.076	0.563	
	Histological Grade	0.213	0.102	
	Lymph node Status	0.213	0.122	
	Progesterone Receptor (PR)	0.323	0.012	positive
	Her2/Neu receptor (HR)	-0.333	0.009	negative
Progesterone Receptor (PR)	Age (years)	0.232	0.075	
	Gender	0.392	0.002	positive
	Mean Tumor Size (cm)	0.063	0.632	
	Location of Breast Cancer	-0.142	0.279	
	Histological Grade	0.230	0.078	
	Lymph node Status	-0.129	0.352	
	Estrogen Receptor (ER)	0.323	0.012	positive
	Her2/Neu receptor (HR)	-0.274	0.034	negative
Her2/Neu receptor (HR)	Age (years)	-0.072	0.586	
	Gender	-0.107	0.415	
	Mean Tumor Size (cm)	-0.094	0.475	
	Location of Breast Cancer	0.076	0.563	
	Histological Grade	-0.213	0.102	
	Lymph node Status	-0.096	0.490	
	Estrogen Receptor (ER)	-0.333	0.009	negative
	Progesterone Receptor (PR)	-0.274	0.034	negative

Note. The BOLD values are statistically significant (p-value < 0.05)

DISCUSSION

Breast carcinoma (BC) is a major concern and one of the leading causes of cancer related death worldwide. BC like many other types of cancer is a complex heterogeneous disease controlled by a multitude of genetic and epigenetic alterations.¹ According to WHO report of 26 march 2021, female gender is the strongest breast cancer risk factor

whereas BC occurrence rate in men is approximately 0.5-1%.¹² The treatment of breast cancer in men follows the same management as for women.¹³ In our study both male and female patients were included and we found that out of 60 there were 58 females and only 2 males in our study which is quite similar to another study.¹ In our study, the mean age of the patient is 41.33 ± 9.63 which is almost similar to another study¹⁴ and our study corroborates with the findings of another study where the age group of 41-50 has highest number of BC cases.⁶

Present study shows IDC (NOS) was most common type of BC and most tumors were of Grade-II which is similar to the data reported by many other local and international studies. Many studies reported that IDC is one of the most predominant variants.^{4,15}

In our study the positivity rate of ER, PR and Her-2/neu expression is very low in comparison to the other studies.^{1,16,17} This may be due to the fact that most of the patients in our study were of younger age group where expression of hormone receptor is lower and it increases with age i.e., in post-menopausal age group. However, the triple negative tumors found to be higher in our study and like many other reported data TNBC (Triple Negative Breast Carcinoma) is found to be over-represented in younger patients.¹⁸ Also, we analyzed that there is an over-expression of Her-2/neu in younger age groups. This shows that expression of Her-2/neu and triple negative tumors decreases with increase of age.

When it was statistically analyzed, we found the expression of PR is statistically significant with age and the positivity of ER/PR increases with age. However, the proportional increase in ER and PR positivity with age was mostly noted for PR than for ER.^{4,19} In young patients, levels of circulating estrogens are higher than that of older age groups and correspondingly they show a lower expression of hormone receptors, which is reflected in their tumors. In the Asian population there is a variation in hormone receptor positivity.¹⁹ Our studies shows that ER and PR is positively correlated with each other but inversely correlated with Her-2/neu. This is similar to the studies conducted by many authors.^{4,14}

Our study shows gender is statistically significant with age and we also found significantly positive correlation between PR expression and gender. However, this result correlates with the findings of another study which also shows significance difference in expression of PR but not ER between genders.²⁰

Unlike other reported data that shows correlation between ER expression and tumor grade, we were unable to confirm such correlation in our study.²¹ We did not find any statistically significant correlation between Her-2/neu expression and lymph node status which is in contrast to other study.¹⁴ In contrary to the other studies our study showed no correlation between ER/PR and histological grade.^{14,21} Also, we analyzed that the expression of these receptors had no significant correlation with tumor size. This finding supports the studies of many authors.^{14,22}

CONCLUSION

In this present study ER/PR expressions were found to be low, while Her-2/neu over-expression was higher. This may be because of the reason that most of the patients in our study were of younger age group and from which we can also conclude that breast carcinomas mostly occur in younger

age group which is very aggressive and has a worse clinical outcome as compared to that in the older group if detection is delayed.

So, this study shows that Her-2/neu, is an important predictive and prognostic marker at a very young age.

6. Compliance with Ethical Standards:

This work is approved by institutional ethical committee.

7. Declaration:

7.1 Consent for publication:

All the authors have given their consent to publish the article.

7.2 Availability of data and material:

All the data generated and analysed during this study are included in this published article.

7.3 Authors contribution:

Momota Naiding raised the initial research question, managed data collection. Biswadeep Choudhury refined research question. Babul Bezbaruah planned study design. Jyotika Sharma contributed in performing the experiment and writing the manuscript. Nivedita Dasgupta contributed in performing the experiment, analysing the data, writing the manuscript. Raima Das contributed in the statistical analysis and result interpretation

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8. Conflict of interest:

The authors have no conflict of interest.

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REFERENCES

- Atif N, Khalid M, Chughtai O, Asif Saad RM, AS C. Role of immunohistochemical markers in breast cancer and their correlation with grade of tumour, our experience. *Int Clin Pathol J*. 2018;6(3):141-5.
- Ansari M, Mittal A, Mehta J, Jain N. Molecular Subtypes of Breast Cancer According to Immunohistochemical Expression of Hormone Receptors in A Region of North West India: A Comparative Study with Other Regions in India and Around the Globe. *Int Arch BioMed Clin Res*. 2019;5(1):26-30.
- Jain SA, Aggrawal L, Ameta A, Nadkarni S, Goyal A, Ranjan D, et al. Study of ER, PR and HER-2/NEU reactivity pattern in the patient of Breast Cancer in northern part of India. *IOSR-JDMS*. 2014;13(2):9-19.
- Puvitha R, Shifa S. Breast Carcinoma, Receptor Status, and Her2 neu Overexpression Revisited. *Int J Sci Study*. 2016;3(10):52-8.
- AlZaman AS, Mughal SA, AlZaman YS, AlZaman ES. Correlation between hormone receptor status and age, and its prognostic implications in breast cancer patients in Bahrain. *Saudi medical journal*. 2016;37(1):37-42.
- Negi M, Kumar S, Devi S, Raina SK. Profiling estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2/neu in breast carcinoma: Study of 111 consecutive cases. *Journal of the Scientific Society*. 2018;45(1):13.
- Almasri NM, Al Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. *Breast Cancer Research*. 2005;7(5):1-7.
- Ellis I, Bartlett J, Dowsett M, Humphreys S, Jasani B, Miller K, et al. Best Practice No 176: Updated recommendations for HER2 testing in the UK. *Journal of clinical pathology*. 2004;57(3):233-7.
- Chand P, Garg A, Singla V, Rani N. Evaluation of immunohistochemical profile of breast cancer for prognostics and therapeutic use. *Nigerian Journal of Surgery*. 2018;24(2):100-6.
- Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Research*. 2010;12(4):1-12.
- Allred D, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc*. 1998;11(2):155-68.
- WHO. Breast cancer2021. Available from: <https://www.who.int/news-room/fact-sheets/detail/breast-cancer>.
- Foerster R, Foerster FG, Wulff V, Schubotz B, Baaske D, Wolfgarten M, et al. Matched-pair analysis of patients with female and male breast cancer: a comparative analysis. *BMC cancer*. 2011;11(1):1-8.
- Siadati S, Sharbatdaran M, Nikbaksh N, Ghaemian N. Correlation of ER, PR and HER-2/Neu with other prognostic factors in infiltrating ductal carcinoma of breast. *Iranian journal of pathology*. 2015;10(3):221.
- Dixon Jr J, Page D, Anderson T, Lee D, Elton R, Stewart H, et al. Long-term survivors after breast cancer. *Journal of British Surgery*. 1985;72(6):445-8.
- Fatima S, Faridi N, Gill S. Breast cancer: steroid receptors and other prognostic indicators. *Journal of the College of Physicians and Surgeons--pakistan: JCPSP*. 2005;15(4):230-3.
- Ratnatunga N, Liyanapathirana L. Hormone receptor expression and Her2/neu amplification in breast carcinoma in a cohort of Sri Lankans. *The Ceylon medical journal*. 2007;52(4):133-6.
- Aysola K, Desai A, Welch C, Xu J, Qin Y, Reddy V, et al. Triple negative breast cancer—an overview. *Hereditary genetics: current research*. 2013;2013(Suppl 2).
- Dutta V, Chopra G, Sahai K, Nema S. Hormone receptors, Her-2/Neu and chromosomal aberrations in breast cancer. *Medical Journal Armed Forces India*. 2008;64(1):11-5.
- Andres SA, Smolenkova IA, Wittliff JL. Gender-associated expression of tumor markers and a small gene set in breast carcinoma. *The Breast*. 2014;23(3):226-33.
- Yadav R, Sen R, Chauhan P. ER, PR, HER2/NEU status and relation to clinicopathological factors in breast carcinoma. *Int J Pharm Pharm Sci*. 2016;8(4):287-90.
- Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. Correlation of her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. *The breast journal*. 2005;11(4):278-80.

