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CORRELATION OF ESTROGEN RECEPTOR(ER), PROGESTERONE RECEPTOR (PR) AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR (HER-2/NEU) STATUS WITH HISTOLOGICAL TYPES, GRADES AND STAGES OF BREAST CARCINOMA

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ABSTRACT

Introduction

Breast cancer is the most common carcinoma in women. The interrelationship of ER, PR, and HER-2 has come to have an important role in the management of breast cancer.

Aims and Objectives

To study Correlation of ER, PR and HER-2-neu status with histological types, grades and stages of breast carcinoma.

Materials and Methods

47 cases of breast cancer were studied during the span of 2 ½ years in the Department of Pathology, KIMS, BBSR. ER, PR & HER-2-neu receptor expression was studied by Immuno histochemistry.

Results

Out of 47 cases 4%, 70% and 26% cases were in grade I, II and III respectively. 10.6 %, 44.7% and 44.7% cases were in stage I, II and III respectively. ER was positive in 50%, 59.4% and 23.9% cases in grade I, II and III respectively. PR was positive in 50%, 61.2% and 28.6% cases in grade I, II and III respectively. Her-2-neu was over expressed in 50%, 78.1% and 92.3% cases in grade I, II and III respectively. There was an inverse correlation between expressions of ER and PR with grade & stage of the disease. Her-2-neu correlated in a positive manner with increasing grades & stages of the disease.

Conclusions

The expression of ER and PR decreases with increasing grade and stage of breast cancer whereas Her-2-neu is overexpressed in higher stage of breast cancer and reduced expression is seen with increasing grade of tumor.

KEYWORDS: Breast Carcinoma, ER, HER-2/neu, PR

INTRODUCTION

Breast carcinoma is the most common cancer in women accounting 22% of all female cancers.[1] Its prognosis and management are greatly influenced by variables such as histological type and grade, tumor size, lymph node status, status

of hormonal receptors like estrogen receptor (ER) and progesterone receptor (PR) and, more recently, HER-2/neu status.[2, 3]

HER-2/ *neu*, also known as c- *erb* B-2 (HER-*2*), a proto-oncogene located on chromosome 17.It is amplified and/or overexpressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome.[3, 4] In contrast, ER is expressed in 70% to 95% of invasive lobular carcinomas and in 70% to 80% of invasive ductal carcinomas. PR is expressed in 60% to 70% of invasive breast carcinomas.[5, 6] Expression of ER and/or PR is generally associated with a better outcome. Survival and response to hormone therapy are most favorable among women with tumors showing positivity for both ER and PR, intermediate for tumors discordant on receptor status and least favorable for tumors negative for both.[2, 7, 8] The interrelationship of ER, PR, and HER-2 has an important role in the management of breast cancer. It has been shown that breast carcinoma patients over expressing HER-2 do not respond to Tamoxifen therapy.[3] HER-2 expression is generally inversely correlated with ER and PR expression.[9, 10]

AIMS AND OBJECTIVES

- Study of hormone receptors (ER &PR) and HER-2 receptor status in breast carcinoma
- Correlation between ER, PR and HER-2/neu status with histological types, grades and stages of breast carcinoma.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha from September 2011 to September 2013.It included 47 cases of breast carcinoma, confirmed by biopsy.

Modified radical mastectomy and lumpectomy specimens with axillary clearance of the above patients were included in the study. True cut biopsies, lumpectomy without axillary clearance, metastatic carcinoma, cell blocks and neoadjuvant treated cases without details of tumor size, node status etc were excluded from our study.

Four to five micrometer thick formalin fixed, paraffin embedded tissue sections were stained with H & E staining. Histological assessment of tumor type and grade were performed as per the criteria outlined by the WHO Classification of Tumors.[11] The microscopic grading of ductal carcinomas was done as per Nottingham Modification of Bloom-Richardson-grading system. [12] Staging was done according to AJCC and TNM system.[13,14] This was followed by Immuno histochemical study of ER, PR and HER-2-neu by Indirect Polymer two step method. ER & PR scoring was done by Quick score system.[15] For HER-2 neu semi quantitative system based on intensity of reaction product and percentage of membrane positive cells was used.[16]

OBSERVATIONS & RESULTS

47 cases were included in the study. The age range was 27-80yrs with mean age 53 yrs. Maximum number of cases was between 36-45 yrs Table 1

Out of different histological types, 44 cases (93.61%) were Infiltrating Duct Carcinoma, Not Otherwise Specified. Mucinous carcinoma, Comedo carcinoma & Invasive Lobular carcinoma each comprised of only one case (2.12%). Table 2

Out of 47 cases studied 4% tumors were in grade-I, 70% were in grade-II and 26% cases were in grade III Figure 1.Of 47 cases studied 10.6% were in stage-I, 44.7% were in stage-II & 44.7% cases were in stage-III Figure 2.

Overall ER & PR were expressed in 24 cases (51.06%) each and HER-2/neu was expressed in 34 cases (72.34%) Figure 3. Of different molecular types, 12.76% (6 cases) were Luminal A type, 36.17% (17 cases) were Luminal B type, 12.76% (6 cases) were Basal like and 38.29% (18 cases) were HER-2/neu positive tumors Table 3.

ER was positive in 50%, 59.4% and 23.9% cases of grade I, II and III tumors respectively. PR was positive in 50%, 61.2% and 28.6% cases of grade I, II and III tumors respectively. Her-2-neu was over expressed in 50%, 78.1% and 92.3% cases of grade I, II and III tumors respectively Table 4. Similarly ER was positive in 85.7%, 60% and 35% cases of stage I, II and III tumors respectively. PR was positive in 85.7%, 57.1% and 31.6% cases of stage I, II and III tumors respectively. Her-2-neu was over expressed in 33%, 54% and 86.4% cases of stage I, II and III tumors respectively. Table 5

IDC, NOS was present in 4.5%, 68.2% & 27.3% cases of grade-I, II &III tumors respectively. One case of mucinous carcinoma was in grade-I, one case of comedo carcinoma was in grade-I & one case of ILC presented in grade III Table 5. 10.6%, 40.9% & 47.7% cases of IDC, NOS were present in stage-I, stage-II & stage-III tumors respectively. Only one cases each of mucinous carcinoma, comedo carcinoma & ILC were present in stage-III, stage-II & stage-III respectively. Table 6

RESULTS OF STATISTICAL ANALYSIS

ER & PR expression show inverse correlation with increasing grades of tumor with p values of 0.028 & 0.045 respectively. Similarly ER & PR expression was lower in higher tumor stages with p values of 0.031 & 0.028 respectively. On the contrary there was over expression of HER-2/neu in higher grades and stages of tumor with p values of 0.040 & 0.012 respectively.

DISCUSSIONS

Breast cancer is a leading cause of death in women. Incidence of breast cancer is low in India compared to westerrn countries, but it is associated with poor prognosis and high mortality which may be due to late presentation at advanced stages. [17] It is the most common cancer among women of urban areas of India and has overtaken cervical cancer perhaps due to changes in lifestyle due to western influences.[18]

Important risk factors of breast cancer include age of presentation, age at menarche/menopause, parity, duration of breast-feeding, genetic, nutritional, environmental and hormone factors. But in 50% of women these are not detected. Five to ten percent of breast cancers are due to BRCA-1 and BRCA-2 gene mutation.[19]

As breast cancer is a steroid hormone–dependent tumor, stratification of patients according to hormone receptor (ER/ PR) expression & HER-2/neu status along with nodal metastasis are of great therapeutic importance.[20]

ER is located in the nucleus & is over-expressed in 70%-80% of breast cancer. PR is also known as nuclear receptor subfamily 3, group C, member 3(NR3C3), is a protein found inside cells. It is over expressed in 60-70% of invasive breast carcinoma. Survival & response to hormone therapy are most favorable among women with ER & PR positivity.

HER-2 also known as c- *erb* B-2 or neu is another prognostic factor. It is a proto-oncogene belonging to human epidermal growth factor receptor family. It is amplified in 25% of breast cancers. HER2-positive breast cancers have a more aggressive disease course, greater recurrence rate, poorer prognosis and decreased survival.[21]These tumors show

lymphoid infiltration, p53 mutation, high proliferative index, negative or lower ER/PR expression, absence of bcl-2 and present in higher grades & stages of disease.[22] It is associated with relative resistance to endocrine therapies like Tamoxifen. Trastuzumab, a humanized monoclonal antibody, in monotherapy or in combination with hormonal agents or chemotherapy is the recommended therapy in HER-2/neu tumors.[23]

The most commonly used method for estimation of steroid hormone receptors like ER & PR and HER-2 expression in breast carcinoma in routine practice is Immunohistochemistry.[22, 23, 24] Fluorescence in situ hybridization (FISH) and Chromogenic in situ hybridization (CISH)) are used to assess gene amplification study.

ER & PR positivity is expressed in immune histochemistry as brown nuclear staining whereas HER-2/neu positivity is expressed as brown cytoplasmic membrane staining. ER & PR scoring is done by Quick score system.[25] For HER-2/ neu semi quantitative system based on intensity of reaction product and percentage of membrane positive cells is used.[16]

Basing on above three molecular markers breast carcinoma is divided into five types (IHC/molecular Classification)[26]: Luminal-A, Luminal-B, Normal breast like, Basal like & HER-2/neu positive. This is the most acceptable criteria for predicting the prognosis & response of tumors to hormonal treatment.

Our study was designed to find out correlation between histological types, grades and stages of breast carcinoma with Immunohistochemical markers like ER & PR and HER-2/neu. It included 47 patients of breast carcinoma admitted to surgical wards at KIMS Hospital, BBSR from September 2011 to September 2013.

In our study Table 1 17% cases were in 25-35 years, 34% in 36-45 years, 32 % in 46-55 years, 15% in 56-65 years & 2% in 76-85 years of age. This shows highest prevalence of breast carcinoma was between the age group of 35-45 years with mean age of 53 yrs. Lobna Ayadi et al[27] showed the mean age of patients to be 51.5 years, ranging from 22 to 89 years. Azizun et al[28] reported the mean age of patients to be 48.3yrs. Kamil et al[29] found the commonest age group of breast carcinoma to be 40-49 years whereas Chandrika Rao et al[30] reported that 67% of patients were between age group of 41 and 50 years.

Out of the 47 cases included in our study Table 2, IDC NOS was the commonest type of breast carcinoma, accounting for 93.6 % (44/47). Mohamed et al [31] in their study also got similar finding as 84% (76/91) to be IDC, NOS. Similarly Fatima et al [32] & Azizun et al [21] in their study showed that majority of tumors (85.3%) were Infiltrating Duct Carcinoma. Godwin A. Ebughe et al[33] also found that IDC, NOS was the most prevalent histological type accounting for 85.2%. Our findings correlated with other authors. But Chandrika Rao et al[30]reported that 59% of the tumors were IDC, NOS, 15% were ILC, 7.9% were mixed tumors (IDC & ILC), 11 % were medullary carcinoma, 7.2% were mucinous carcinoma and 4% were invasive papillary carcinoma.

Our study showed 4% (2/47) cases in grade-I, 70% (33/47) cases in grade-II and 26% (12/47) cases in grade III Figure 1. This shows maximum number of cases presented in grade-II & grade-III. Similarly Mohamed et al [31] found that 2% were in grade-I, 46% in grade-II & 52% were in grade-III. Azizun et al[21] also reported 55.3% tumors belonging to grade II. Suvarchala S.B et al [34] showed 28.1% (18/49) cases were in grade-I, 42.2% (27/49) were in grade-II & 30% (9/49) were in grade -III. Godwin A. Ebughe et al [33] also found that grade-III to be the most common presentation accounting 66.7%. Our study correlated with the above studies.

In our study overall ER & PR expression was in 24 cases (51.06%) each and HER-2/neu was expressed in 34 cases (72.34%), Figure 3. Mohamed et al [37] reported 53% ER positivity, 23% PR positivity and 24% HER-2/neu positivity. Lobna Ayadi et al[27] showed the percentage of expression of HER-2, ER and PR were 26, 59.4, and 52.3% respectively. Ratnatunga et al[21] reported 53% cases to be ER positive, 50% to be PR positive and 15% to be HER-2/neu positive. Azizun et al [28] reported over all 33% cases to be ER positive,25.3% cases to be PR positive and 25% cases to be HER-2/neu positive.

Our study showed 12.76% (6/47) tumors were Luminal A, 36.17% (17/47) were Luminal B, 12.76% (6/47) were Basal like and 38.29% (18/47) were HER-2 positive Table 3 & Figure 4. This study showed majority of cases were HER-2 positive. Ratnatunga et al[21] found 40.3% cases to be Luminal-A type, 3.2% cases to be Luminal-B type, 28.2% cases to be Basal like and 2.4% cases to be HER-2/neu positive type. Their study showed majority of breast carcinoma were to be Luminal-A.

In our study ER was positive in 50%, 59.4% and 23.9% cases in grade I, II and III tumors respectively and negative in 50%, 40.6% and 76.9% cases of grade I, II and grade III tumors respectively. PR positivity was present in 50%, 61.2% and 28.6% cases in grade I, II and III tumors respectively and negativity in 50%, 61.2% & 71.4% cases of grade-I, grade-II & grade-II tumors respectively. Her-2-neu was over expressed in 50%, 78.1% and 92.3% cases of grade I, II and III tumors respectively & was negative in 50%,21.5% & 7.7% cases of grade-I, grade-II & grade-III tumors respectively Table 4.

Thus in our study, ER & PR were over expressed in lower grades of tumors with p values of 0.028 & 0.045 respectively. On the contrary HER-2/neu was over expressed in higher grades of tumors with p value of 0.040. There is an inverse correlation between ER, PR & HER-2/neu expression.

Priti Lal et al [35] studied 3,655 cases and showed inverse relationship between HER-2 and ER & PR receptor expression. HER-2 was overexpressed in grades II and III ductal carcinomas and correlated inversely with ER & PR expression. Ratnatunga et al[21]and Azizun et al [28] also found similar results. According to Ivković-Kapic et al[36] HER-2/neu over expression was present in 41% & 21% of grade-III & grade II tumors respectively but not detected in grade I tumors. Fatima et al [32] in 2009 and Chandrika Rao et al[30] also reported that ER and PR positivity decreased with increase in tumor size & grade. Similarly they found significant correlation between high grade tumors with necrosis and HER-2/neu over expression ($P < 0.05$).

Lobna Ayadi[27] et al showed that HER-2 over-expression was inversely related to ER expression (p value 0.002) and to PR expression (p value 0.048). This was associated with a high tumor grade with marginal significance (p value 0.072). A negative correlation was noted between ER and PR expression and tumor grade (p value 0.001) and ER and age (p value 0.002).

Our study revealed ER, PR expression was lower in higher stages of tumors with p value of 0.031 & 0.028 respectively. HER-2/neu showed over expression in higher stages of tumors with p value of 0.012. Ivković-Kapic et al[36] revealed that HER-2/neu protein over expression was present in 8% (4 of 52) T1 lesions, in 30% (11 of 37) T2 lesions, in 50% (3 of 6)T3 lesions and in 40% (2 of 5) T4 lesions with ($p<0.05$).

According to our study 68% cases of IDC, NOS were in grade-II, only one case of mucinous carcinoma in grade-I, one case of comedo carcinoma in grade-I and one case of ILC in grade-III. This shows IDC, NOS & ILC occur in higher grades of tumors where as comedo carcinoma occurs in lower grades of tumors. Similarly Kumar et al[26] & Godwin

A. Ebughe et al [33] showed that cases of IDC, NOS and ILC present in higher grades but cases of mucinous carcinoma, tubular carcinoma & comedo carcinoma present in lower grades.

CONCLUSIONS

Our study showed statistically significant correlation between HER-2/neu protein overexpression in higher histological grades, stages and reduced expression of ER & PR in increasing grades & stages of tumor. There is inverse relation between ER/PR and HER-2 expression. IDC, NOS & ILC cases presented in higher grades of tumors where as comedo carcinoma & mucinous carcinoma cases presented in lower grades of tumors.

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APPENDICES

Table 1: Distribution According to Age (n=47)

Age Group(Yr.S)	Number of Cases	% Distribution
25-35	08	17
36-45	16	34
46-55	15	32
56-65	07	15
66-75	00	00
76-85	01	02

Table 2: Distribution According to Histological Types (n=47)

Histological Type	Number of Cases	% Distribution
IDC, NOS	44	93.6
Mucinous carcinoma	01	2.1
Comedo carcinoma	01	2.1
ILC	01	2.1

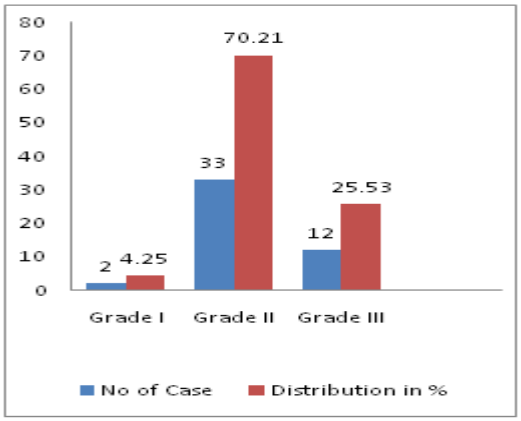

Figure 1: Distribution According to Grade (n=47)

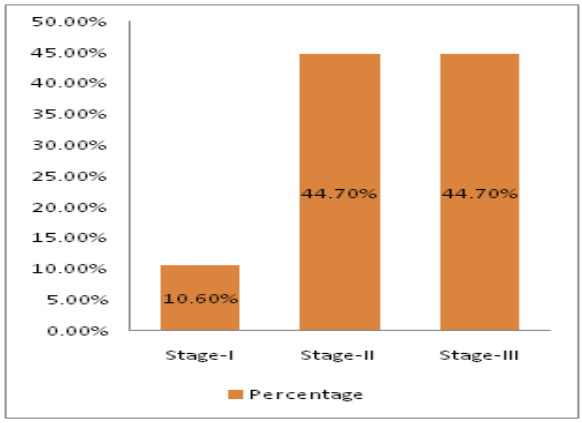

Figure 2: Distribution According to Stage (n=47)

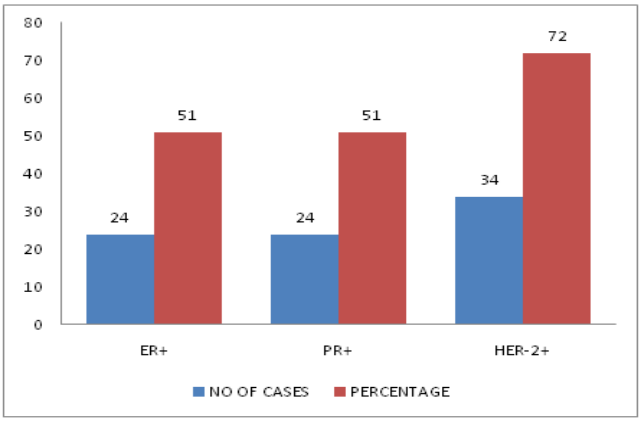

Figure 3: Overall ER, PR & HER-2/neu Positivity (n=47)

Table 3: Distribution according to Molecular Type (n=47)

Molecular Type	No of Cases	% Distribution
Luminal A	06	12.7
Luminal B	17	36.2
Basal like	06	12.8
HER-2/neu +	18	38.3

Table 4: Correlation between Grade and Receptor Expression (n=47)

Receptor	Grade-I		Grade-II		Grade-III	
	No. of Cases	% Distribution	No. of Cases	% Distribution	No. of Cases	% Distribution
ER+ve	1	50	19	59.4	3	23.1
ER-ve	1	50	13	40.6	10	76.9
PR +ve	1	50	19	61.2	4	28.6
PR -ve	1	50	12	38.7	10	71.4
HER-2/ neu +ve	1	50	25	78.1	10	92.3
HER-2/neu -ve	1	50	7	21.8	12	7.7

Table 5: Relationship between Histological Type & Grade (n=47)

Histological Type	Grade-I		Grade-II		Grade-III	
	Number	%	Number	%	Number	%
IDC,NOS	2	4.5	30	68.2	12	27.3
Mucinous carcinoma	1	100	0	0	0	0

Table 5: Contd.,

Comedo carcinoma	1	100	0	0	0	0
ILC	0	0	0	0	1	

Table 6: Correlation between Histological Types with Stage (n=47)

Histological Types	Stage-I		Stage-II		Stage-III	
	Number	%	Number	%	Number	%
IDC,NOS	5	10.6	18	40.9	21	47.7
Mucinous carcinoma	-	-	-	-	1	100
Comedo carcinoma	-	-	1	100	-	-
ILC	-	-	-	-	1	