ORIGINAL ARTICLE

Role of Diagnostic Immunohistochemistry in correlation of ER,PR,HER- 2/neu status and Modified Scarff Bloom Richardson Histological Grading in Breast Carcinoma

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Abstract:

Introduction: Breast carcinoma is the most common malignant tumour among women. Annually about one million women are diagnosed with breast cancer worldwide. There is an increased trend in the detection of breast carcinoma, which can be attributed to increased mammographic screening. But the mortality has decreased due to early screening which detects the tumour at an early curable stage and also by means of better effective treatment modalities. The currently used histologic grading system is known as the Elston-Ellis modification of Scarff-Bloom-Richardson grading system. There is a significant correlation between histologic grade and frequency of recurrence, disease free survival and overall length of survival regardless of clinical stage in invasive breast carcinoma. *Aim and Objective:* To correlate histological grading by modified Scarff-Bloom-Richardson grading system with biomarkers ER, PR and HER2/neu status in invasive breast carcinoma using diagnostic immunohistochemistry. To assess the proportion of different grade of invasive breast carcinoma by modified Scarff-Bloom-Richardson grading system. To study ER, PR and HER-2/ Neu reactivity in the patient of breast carcinoma. *Material and Methods:* An observational, prospective study of 45 cases was done during June 2020 to August 2021 in the Department of Pathology, Govt. Medical College, Bhavnagar. Approval was taken from Institutional Ethical Committee before commencement of the present study, the EC approval number is 961/2020. In every case the standard grossing protocol of the modified radical mastectomy specimen was followed according to CAP guidelines for invasive ductal cell carcinoma of breast. After a detailed specimen description, representative tissue bits were taken. These tissue bits were subjected for routine processing in an Automatic Tissue Processor Electra by Yorco TM and embedded in paraffin wax. Three to four micron thick sections were taken from paraffin embedded blocks using Lieca RM 2125 RT microtome and were stained using Hematoxylin and Eosin (H andamp; E) for histopathological study. *Results:* The predominant histologic type was Invasive Ductal Carcinoma, No Special Type (IDC, NST) consisting of 27 cases (60%) followed by 8 cases (18%) with Invasive lobular carcinoma. There were 10 cases of IDC SPECIAL TYPE. Majority of tumours belonged to Modified Bloom Richardson Grade 2 (33.3%) from IDC NST histological subtype. As per molecular classification, most of tumours found to be Luminal Type A belonged to Grade 2 (62%). Majority of IDC, NST (27 cases, 60 %) were Luminal A type. A significant correlation was found between histological subtypes and molecular classification of invasive breast carcinoma. P value (0.0037). *Conclusion:* The ER, PR and HER2 status correlate well with histopathological grading. The inter-relationship between ER, PR and HER2 has come to have an important role in the management of the breast cancer. Hence, immunohistochemical assessment of ER, PR and HER2 status along with histopathological grading should be incorporated as a routine investigation to guide the clinicians in making correct choice of treatment to increase the disease-free survival for the patient.

Keywords: Breast carcinoma, Scarff-Bloom-Richardson grading, ER, PR, HER-2/NEU

Introduction:

Breast carcinoma is the most common malignant tumour among women. Annually about one million women are diagnosed with breast cancer worldwide [1]. Clinical presentation of breast carcinoma includes palpable mass, pain in breast, redness and swelling (inflammatory breast carcinoma) over breast, skin retraction, nipple inversion, nipple discharge. All the symptoms of breast cancer may also be caused by benign breast disease therefore, evaluation with imaging and histological sampling with core biopsy or FNA are indicated to establish a definitive diagnosis.

Mammography, targeted ultrasonography and MRI are non-invasive screening diagnostic method [2].

Almost all breast malignancies are Invasive ductal carcinoma (>95%). In the most clinically useful classification system, breast cancers are divided based on the expression of hormone receptors - estrogen receptor (ER) and progesterone receptor (PR) - and the expression of the human epidermal growth factor receptor 2 (HER2, also known as ERBB2).

A classification system based on gene expression profiling divides breast cancers into four major types [3]: Luminal A. The majority are lower-grade ER-positive cancers that are HER2negative. Luminal B. The majority are higher-grade ER-positive cancers that may be HER2positive. HER2 overexpression. Basal (Triple negative).

The currently used histologic grading system is known as the Elston-Ellis modification of Scarff-Bloom-Richardson grading system. There is a significant correlation between histologic grade and frequency of recurrence, disease free survival, and overall length of survival regardless of clinical stage in invasive breast carcinoma [3].

Aim and Objective:

To correlate histological grading by modified Scarff-Bloom-Richardson grading system with biomarkers ER, PR and HER2/neu status in invasive breast carcinoma using diagnostic immunohistochemistry. To assess the proportion of different grade of invasive breast carcinoma by modified Scarff-Bloom-Richardson grading system. To study ER, PR and HER-2/ Neu reactivity in the patient of breast carcinoma.

Material and Methods:

An observational, prospective study of 45 cases was done from June 2020 to August 2021 in the Department of Pathology, Govt. Medical College, Bhavnagar. Approval was taken from Institutional Ethical Committee before commencement of the present study and EC approval number is 961/2020.

The patients who had undergone modified radical mastectomy for carcinoma breast, diagnosed by Tru-cut Biopsy/Open biopsy as well as by FNAC were

included in the study. Inadequately preserved specimens with handling artifacts, post-chemotherapy/radiotherapy specimens, in situ ductal/in situ Lobular Carcinoma, and all other non-mammary gland neoplasms were excluded from study.

All specimens included in study were fixed in 10% neutral buffered formalin and grossing done as per CAP guideline. Tissue bits were subjected for routine processing in an Automatic Tissue Processor Electra by Yorco TM and embedded in paraffin wax. Three to four micron thick sections were taken from paraffin embedded blocks using Lieca RM 2125 RT microtome and were stained using Hematoxylin and Eosin (H andamp; E) for histopathological study. Reporting done by 2 pathologists. The tumours were classified according to WHO classification system.2Nottingham modification of Bloom and Richardson grading system was used for tumour grading. Lymph node staging was done according to pTNM from the 8th edition, AJCC Staging Manual [4].

Additional sections were cut from the paraffin block of tumour tissue for immunohistochemistry (IHC) to detect ER, PR and HER2/neu overexpression. Antigen Retrieval was done by microwaving in EZ-Retriever system V.3 of Biogenex using EZ-AR3 solution- first for 10 minutes at 90℃followed by 15 minutes at 95℃.

In this study breast cancer was classified into four groups based on IHC profile ER/PR and Her2/neu expression, positive (+) and/or negative (-) as discussed above. The collected data was entered in Microsoft Office Excel and then analysed statistically. The p value of < 0.05 was considered statistically significant.

Results:

In present study, age range of presentation was 31-80 years with mean age of 52.8 ±18.87 years.

In present study, the predominant histologic type was Invasive Ductal Carcinoma, no special type (IDC, NST) consisting of 27 cases (60%) followed by 8 cases (18%) with Invasive Lobular Carcinoma. There were 10 cases of IDC special type.

The most common histologic type in which positivity for ER, PR, HER2 were noted was Invasive Ductal Carcinoma (NST).

Seventeen out of 23 (73%) ER positive cases, 17 out of 23 (73%) PR positive cases and 12 out of 12 (100%) HER2 positive cases belonged to IDC NST3 (13%) cases of Invasive Lobular Carcinoma were positive for both ER and PR receptors. Thirteen(13%) cases of IDC Special type were positive for both ER and PR receptors [3]. cases of IDC NST were equivocal for HER-2.

In present study, majority of tumours belonged to Modified Bloom Richardson Grade 2 - 15 (33.3%) and were from IDC NST histological subtype - 27 (60%).

In present study majority of tumours showing ER positivity belonged to IDC NST and were of Grade 2 - 19 (42.2%). Majority of tumours showing PR positivity belonged to IDC NST and were of Grade 2 - 19 (42.2%). Majority of tumours showing HER2 positivity belonged to IDC NST and were of Grade 2 - 07 (15.5%).

A significant correlation was found between histological subtypes and molecular classification of Invasive Breast Carcinoma. (P value = 0.0037).

In the present study, most of tumours were found to be Luminal Type A (55.5%) and most of tumours belonging to Grade 2 (62.2%).

Discussion:

The use of IHC has become the integral part of a complete and comprehensive histopathology report. In terms of prognosis and prediction to the response to treatment of breast cancer, in addition to tumour subtypes and histological grade, hormonal markers that is ER, PR and HER2 status have become the mainstay requirement.

The present study was undertaken in the view of correlating the histopathology of the tumour by the way of various traditional prognostic markers, grade and its molecular subtypes using IHC with respect to ER, PR and HER [2].

In the event of an equivocal IHC result, reflex testing to FISH is recommended but due to unavailability of FISH technique at the Institution such cases could not be further analysed.

In the present study, the age range of presentation was 31-80 years with mean age 52.8±18.87 years and similar observations made by Azizun-Nisa, Ayadi L and Mudduwa [5,6,7].

Histological grading was done using Elston and Ellis (Modified Scarff- Bloom-Richardson Scoring system). The present study showed maximum cases (62.2%) belonging to tumour grade II and these observations were similar to other studies showed in table 01. Grade I and grade III tumours were variable in these studies. The result was contradictory to the study done by Ghosh J who showed maximum cases (75.4%) belonging to tumour grade III [8].

In present study invasive ductal cell carcinoma- no special type (IDC- NST) constituted majority (60%) of the cases and this was in agreement with other studies wherein IDC-NST was the commonly encountered breast carcinoma variant. (Table 02).

Immunohistochemical studied of breast carcinoma cases for ER status showed that 73.3% cases were ER positive and 26.6% cases were negative for ER. Table 03 summarizes findings reported in present study and compares it with other studies.

Immunohistochemical studies of breast carcinoma cases for PR status showed that 73.3% cases were PR positive and 26.6% cases were negative for PR. Table 04 summarizes findings reported in present study and compares it with other studies.

Table No.1: Comparative analysis of Tumour Grade on Histopathological Examination with various studies

Authors	Tumour Grades		
	Grade I (%)	Grade II (%)	Grade III (%)
Ghosh J[8]	0.3	15.9	75.4
Moradi[9]	10.5	58.6	30.8
Ayadi L[6]	11.0	63.2	25.8
M Rashed[10]	10.0	54.0	36.0
Patnayak R[11]	3.9	60.9	35.2
Present study	22.2	62.2	15.5

Majority of our cases showed negative HER2 status (73.3%) and these findings similar to the studies done by Ayadi L, Huang HJ, Shukla A et al, Bhagat Vasudha M, Munjal K, Patnayak R and Ahmad HG who demonstrated majority of the cases having negative HER2 status. However, these findings were

contradictory to the study done by A Spitale that reported HER2 positivity in majority of the cases (85.3%) [6,11,12,13,14,15,16,17].

In our study, 3 cases showed equivocal findings for HER-2/neu but could not be analysed further due to unavailability of FISH technique at the Institution.

Comparative analysis of molecular types of breast carcinoma with various studies tabulated in table number 05. In present study, the findings were consistent with the studies done by Onitilo A et al, Huang HJ and Shukla A et al, in that ER and PR expression generally inversely correlated with HER2 overexpression [12,17,18]. However, there is a substantial number of HER2 positive tumours that are still expressed ER and or PR. In our study they comprise 17.0% of total cases (Table 05).

Table No. 5: Comparative Analysis of Molecular Types of Breast Carcinoma with various studies

Authors	Luminal A (%)	Luminal B (%)	Her 2 enriched (%)	Triple Negative (%)
Onitilo AA[18]	68.9	10.2	7.5	13.4
Shukla A et al[12]	26.66	10.0	24.44	32.22
Saroj D[19]	21.6	2.2	17.2	41.04
Huang HJ[17]	66.4	30.9	45.6	13.8
A Spitale[16]	73.2	13.8	5.6	7.4
Present study	27.4	17.0	11.0	15.0

Table No. 2: Comparative analysis of most common Histological Type with various studies

Authors	Most common subtype	Percentage
Ghosh J[8]	IDC (NST)	95.4
Azizun Nisa[5]	IDC (NST)	85.3
Shukla A[12]	IDC (NST)	89.02
Bhagat V[13]	IDC (NST)	94.82
Ayadi L[6]	IDC (NST)	83.8
Munjal K[14]	IDC (NST)	95.0
Present Study	IDC (NST)	60.0

Table No. 3: Comparative analysis of ER Status with various studies

Authors	ER Positive (%)	ER Negative (%)
Patnayak R[11]	47.6	52.4
Ahmad HG[15]	43.8	56.2
A Spitale[16]	85.3	14.7
Shukla A et al[12]	49.1	50.89
Bhagat Vasudha M[13]	48.27	51.73
Munjal K[14]	41.1	58.9
Present Study	73.33	26.6

Table No. 4: Comparative Analysis of PR Status with various studies

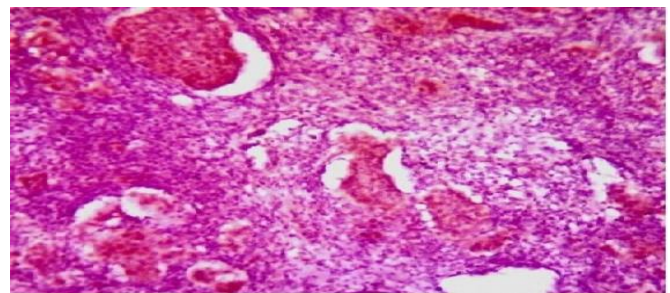| Authors	PR Positive (%)	PR Negative (%)
Patnayak R[11]	48.8	51.2
Ayadi L[6]	52.3	47.1
Huang HJ[17]	64.2	35.8
Shukla A et al[12]	43.75	56.25
Bhagat Vasudha M[13]	37.93	62.04
Munjal K[14]	41.1	58.9
Present Study	73.33	26.6

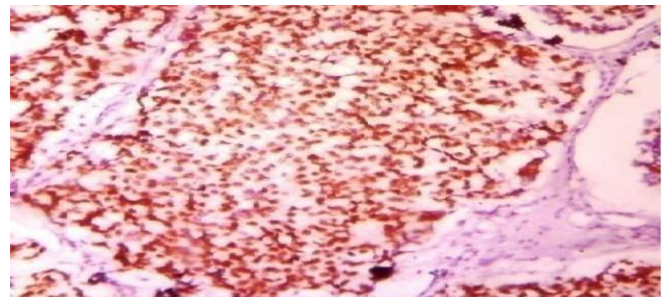Figure No.1: IDC with ER Positivity

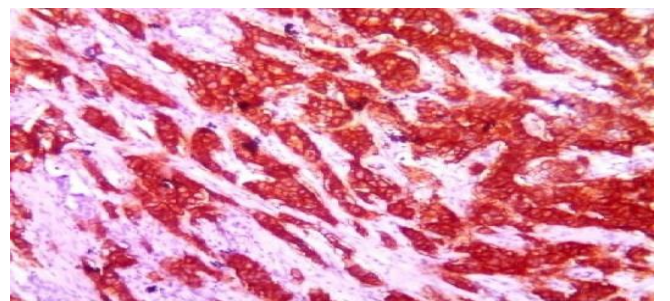Figure No.2: IDC with PR Positivity

Figure 03: IDC with HER2 Positivity
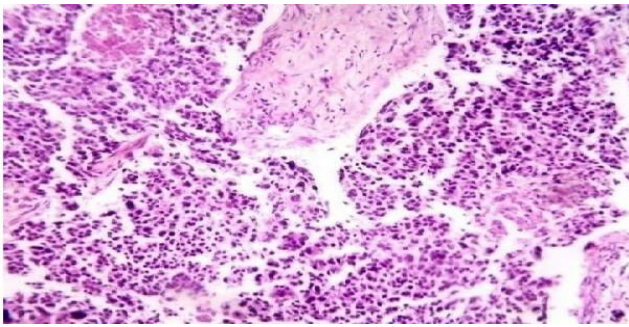
Figure No. 4: IDC Histopathology
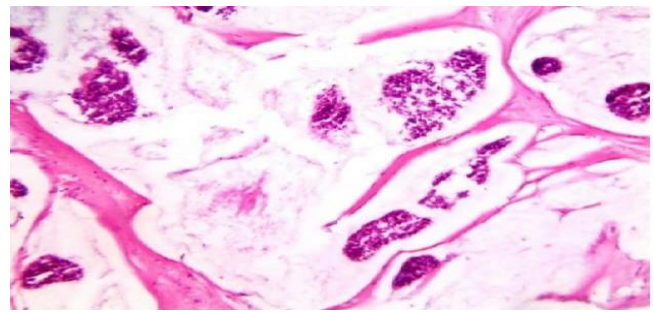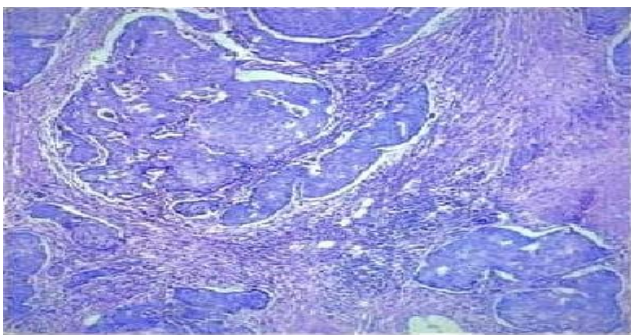

Figure No. 6: Mucinous Breast carcinoma

Conclusion:

The ER, PR and HER2 status correlate well with histopathological grading. The inter-relationship between ER, PR and HER2 has come to have an important role in the management of the breast cancer. Hence, immunohistochemical assessment of ER, PR and HER2 status along with histopathological grading should be incorporated as a routine investigation to guide the clinicians in making correct choice of treatment to increase disease free survival for the patient.

Conflict of Interest - Nil
Sources of Support - Nil

Figure No.5: IDC with Medullary Chang

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