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ER/PR Status and Apoptotic Index in Carcinoma Breast- A retroprospective study in tertiary teaching hospital in Western U.P

Kanchan Kamini[1,*], Alok Mohan[2], Kamna Gupta[3], Purva Sharma[4], Veena K. Sharma[5]

[1,4]Junior Resident, [2]Associate Professor, [3]Assistant Professor, [5]Professor & HOD, Dept. of Pathology, Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh

***Corresponding Author:**
Email: kanchanmbbs@gmail.com

Abstract

Introduction: Breast cancer is the most frequently diagnosed cancer in women and second most common cause of cancer related death in world.(1) There have been outstanding progress in breast cancer management leading to early detection and treatment of disease for the last few decades. IHC based classification of estrogen receptor/progesterone receptor status works as prognostic factor and help in choosing treatment modalities and is easily approachable.

Aim: The present study was undertaken to find out apoptotic index and ER/PR status in breast carcinoma patients and to correlate apoptotic index with ER/PR status and histological grade of breast carcinoma.

Material and Method: Sixty six patients with breast neoplasm who were underwent Modified Radical Mastectomy/lumpectomy after the diagnosis of carcinoma breast, on undergoing preliminary FNAC/ Trucut biopsy. Histopathology and IHC were applied on the MRM tissue. Apoptotic index was calculated on H&E stained section.

Results: Out of 66 cases studied, maximum cases {60cases (88.2%)} were of Invasive breast carcinoma not otherwise specified (NST). Grade II tumor showed the maximum cases(48.9%). ER positivity was 57.5%, PR positivity was 45.4%. Mean apoptotic index and grade of tumor had significant correlation whereas mean apoptotic index and ER/PR status were not correlated to each other.

Conclusion: From the present study, we came to a conclusion that apoptotic index is a useful parameter to assess the prognosis of the patient with the carcinoma breast, similar to ER/PR. Considering the cost factor, in a developing country like India where IHC is beyond the reach of every common man, we conclude that apoptotic index can also be of great help in predicting the prognosis of the patient even if ER/PR status is not known.

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Introduction

Carcinoma of the breast is the most common non-skin malignancy in women and is second only to lung cancer as a cause of cancer deaths. It is rare in women younger than age 25, but the incidence increases rapidly after age 30 and peak incidence of breast cancer is seen in 45-60 years of age.(2) Incidence of Breast cancer is about 10.4% of all cancers (both sexes counted). In India, breast cancer is the second most common cancer among women after carcinoma cervix.(3) The rising incidence of breast cancer in India is mainly attributed to westernization of the country. Apoptosis is a complex and firmly regulated process linked to cellular proliferative activity. Apoptotic Index is defined as a measure of the rate of death of cancer cells within a tumor. This is estimated by determining the proportion of dying or apoptotic cells per hundred cancerous cells.(4)

Apoptotic index is usually low in benign breast lesion. It is higher in in-situ carcinomas with a gradual increase in extent of apoptosis from grade 1 to grade 3. The apoptotic index is highest in recurrent carcinomas.(5) Immunohistochemistry (IHC) being expensive is beyond the reach of every patient while apoptotic index calculation is a simple and inexpensive method and can be done on haematoxylin and eosin (H&E) stained sections only. This study was mainly conducted with an aim to access efficacy of apoptotic index predicting the prognosis of the patient in comparison to IHC.

Material and Method

A retrospective as well as prospective study was conducted in the Department of pathology, Muzaffarnagr Medical College, Muzaffarnagr between June 2011 to July 2016 after obtaining approval from the ethical committee. The test population comprised 66 patients with breast neoplasm.

Tissue collection: The tissues of the test population submitted as MRM (Modified Radical Mastectomy)/ lumpectomy specimen were evaluated by histopathological processing and examination (HPE).The most suitable tissue block was selected for Immunohistochemical evaluation for ER, PR.

Immunohistochemistry procedure: Very thin histological sections (3µm) were taken on slides coated with Poly-L-lysine which acts as an adhesive and incubated overnight at 37˚C. Immunohistochemistry was done using peroxidise antiperoxidase (PAP) technique. ER and PR immunostainig using Rabbit/Mouse Monoclonal Antibody from Bio SB was done to demonstrate the estrogen and progesterone receptors. Positive and negative control was used with every batch of staining. Established ER/PR positive

breast carcinoma tissue served as positive control and sections from normal thyroid parenchyma were taken as negative control. Cases showing nuclear positivity were taken as positive. The result were divided into 4 categories- ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR-.

Markers: The positivity of ER and PR in tumor tissue was measured or calculated by Estrogen receptor/ Progestrone receptor assay. Quick Score method of ER/PR scoring system criteria was used in this study.

Data Analysis: Anova test and t test was applied and p-value was calculated.

Results

The age of patients ranged from 20 to 75years, with a mean age group of 49.5 years in this study. Age group of 40-49 yrs(34.84%) and > 60 yrs(33.34%) shows maximum number of cases(Table 1) and all were females. According to side affected, 40 cases (60.60%) were of left sided and 25 cases (37.88%) were of Right sided with 1 bilateral case (1.52%). Grade I tumors were seen in 19 cases (28.78%) and maximum cases were of Grade II (48.49%) and Grade III tumor were minimum (22.72%) (Table 2).

Out of the 66 cases, majority were of Invasive breast carcinoma NST (60 cases) (88.24%), followed by Invasive lobular carcinoma, Carcinoma with neuroendocrine differentiation having 2 cases of each (2.94%) and Medullary carcinoma, mucinous carcinoma having 1 case of each (1.47%).

On IHC ER/PR status were divided into 4 categories- ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR-. Maximum cases (34.85%) were of ER+/PR+ category while 31.82% cases were of category ER-/PR-Only22.73% cases were ER+/PR- & 10.60% cases were ER-/PR+. ER positivity was seen in, 38/66 (57.5%) cases and PR positivity was seen in 29/66 (45.4%) of the cases. Apoptotic index was calculated, maximum for Grade III tumor i.e. 2.53(Table 3) then for Grade II i.e. 1.46 and minimum for Grade I tumor i.e. 0.71.ANOVA test was applied to find out whether or not the means of the three groups were equal or not. The p- value was calculated which came out to be 0.00 (<0.05), which means that mean apoptotic index for at least one of the grades is significantly different from others. Mean apoptotic index was calculated for all the four immunohistochemical staining categories along with the standard deviation. The mean apoptotic index was maximum for ER+/PR- tumors i.e. 1.75±0.86 while it was minimum for ER-/PR- tumors i.e. 1.19±0.80 (Table 4). To find out whether or not the means of all the four groups are equal or not t-test was used. The p-value was calculated which came out to be <0.701 and <0.879 (<0.05) respectively, which means that mean apoptotic index is not significant.

Table 1: Age Wise Distribution

S. No	Age in years	No. of cases(N)	Percentage(%)
1.	20-29	00	00
2.	30-39	10	15.15
3.	40-49	23	34.84
4.	50-59	11	16.67
5.	≥60	22	33.34
	Total	66	100

Table 2: Grade Wise Distribution (Modified Bloom-Richardson)

S. No	Grade	No. of cases(N)	Percentage(%)
1.	Grade I	19	28.78
2.	Grade II	33	48.49
3.	Grade III	14	22.72
	Total	66	100

Table 3: Grade Vs Apoptotic Index

S. No	Grade	No. of cases(N)	Mean apoptotic index	Standard deviation
1.	I	19	0.71	0.26
2.	II	33	1.46	0.69
3.	III	14	2.53	0.93
	Total	66	4.70	1.88

Table 4: ER/PR Status Vs Apoptotic Index

S. No	ER/PR Status	No. of cases(N)	Mean apoptotic index	Standard deviation
1.	ER+/PR+	23	1.63	0.976
2	ER+/PR-	15	1.75	0.868
3.	ER-/PR+	07	1.14	0.553
4.	ER-/PR-	21	1.19	0.801
	Total	66	5.077	3.198

Discussion

IHC status is an integral part of a complete and comprehensive histopathology report of breast cancer. Hormone markers such as ER/ PR and HER-2/neu have become the mainstay requirement for the oncologist in terms of prognosis and prediction of response to treatment, in addition to histological grade and tumor sub type. The present study was undertaken with the view of correlating the histopathology of the tumor by way of tumor grade, various traditional prognostic markers and its IHC profile with respect to ER, PR hormone receptors. All the 66 cases in present study were confirmed cases of cancer.

Age ranges from 20 to 75 years, with a mean age of 49.5 years. Maximum cases were seen in the age group of 40- 49 years (34.84%) and >60 years (33.34%). No cases were seen in the 20-29 years of age

group. Similar results were published by Ghosh et al[6] (2008).

Out of 66 cases, 60 cases were of Invasive carcinoma NST type (88.24%), there were 2 cases of lobular carcinoma and carcinoma with neuroendocrine differentiation (2.94%) of each and 1 case of medullary carcinoma and mucinous carcinoma (1.47%). These findings were similar to Bhagat et al,[7] Adedayo et al[8] and Ghosh et al studies. In all the above studies it was concluded that IBC (Invasive breast carcinoma NST type) is most common type. In India, the majority of breast cancers are diagnosed at a relatively advanced stage due to the lack of awareness and absence of a breast cancer screening program.[9] Our study showed maximum case in grade II (48.49%) then grade I (28.78%) and least in grade III (22.72%). In present study ER positive cases were 57.5% while, 45.4% cases are of PR positive status. The study by Munjal et al[10] showed 41.41% positivity for ER and PR.

Mean apoptotic index was calculated for all the three grades in the study and mean apoptotic index was maximum for grade III tumors i.e. 2.53±0.93, while it was minimum for grade I tumors i.e. 0.71±0.26. ANOVA test were applied and it was concluded that grade III tumors had a significantly higher apoptotic index (p- value=0.000). Similar findings were noted in the studies done by Mustonen et al,[11] Jingxiang Huang et al.[12]

In present study, Mean apoptotic index was calculated for all the four IHC staining categories along with the standard deviation. The mean apoptotic index was maximum for ER+/PR- tumors i.e. 1.75±0.86. While it was minimum for ER-/PR+ tmors i.e. 1.14±0.55. t-test was applied and p-value > 0.05 so there was no significant correlation between apoptotic index and all the four categories of hormonal status. In the study of 116 breast cancer cases done by Jingxiang Huang et al(2003), the mean apoptotic index for ER+ tumors was 1.27±0.21 and for ER- tumors was 1.49±0.20 and the p-value was 0.879 concluding no significant correlation between apoptotic index and ER status of the tumor.

Conclusion

In the present study of 66 cases, all were females and age ranges from 20 to 75 years. Maximum cases (34.8%) were seen in the middle age & elderly age group i.e. above 40 years and mean age came out to be 49.5 years. Left sided breast tumors were more common (60.6%) than right sided breast tumors. Maximum cases were of invasive breast carcinoma NST (88.2%) followed by invasive lobular carcinoma (2.9%) as the second most common category. The most common histological grade observed was grade II (48.9%) followed by grade I(28.7%) tumors. The mean apoptotic index was maximum for grade III tumors (p-value= 0.000). IHC revealed maximum cases in ER+/PR+ category (34.8%). ER-/PR- showed 31.8% cases, 22.7% cases were of ER+/PR- and only 10.6% cases of ER-/PR+. ER positivity was seen in 57.5% tumors. Mean apoptotic index was calculated for all the four immunohistochemical staining categories. It was found that ER/PR status does not correlate significantly with apoptotic index (p-value=0.879). A significant association between grade and ER/PR status was found (p-value>0.054). A higher grade was associated with ER+/PR- tumors. Thus apoptotic index is a useful parameter to assess the prognosis of the patient with the carcinoma breast, similar to ER/PR. Considering the cost factor, in a developing country like India, where IHC is beyond the reach of every common man, apoptotic index can be of great help in predicting the prognosis of the patient even if ER/PR status is not known.

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