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Assessment of the histopathological grades of the carcinoma with nodal status, apoptotic index and mitotic index

[1]Dr Nikita Kalra, [2]Dr. Deepak Khurana

[1]Assistant Professor, Department of Biochemistry, Chintpurni Medical College, Bungal, Punjab, India

[2]Assistant Professor, Dept. of General Surgery, Chintpurni Medical College & Hospital, Bungal, Punjab, India

Correspondence: Dr Nikita Kalra

Assistant Professor, Department of Biochemistry, Chintpurni Medical College, Bungal, Punjab, India

Abstract

Introduction: Breast carcinoma is the second most common carcinoma in India after malignancy of cervix. It is a heterogeneous disease with variable biological and clinical characteristics. Pathologists have played a major role in identifying different histological and immunohistochemical markers that have a direct bearing on both the behavior and treatment of breast cancer. Hence, the present study was undertaken to assess the histopathological grades of the carcinoma with nodal status, apoptotic index and mitotic index.

Material and Methods: The study was conducted on 160 cases of carcinoma breast. Histopathological examination of the tissues obtained on surgery was made. Classification of the malignant breast tumors was done according to the modified Bloom – Richardson grading system. Mitotic index and apoptotic cells and bodies were counted among 1000 cells using 40x objective and apoptotic index was calculated in 100 cells. SPSS programme was used for calculating statistical values.

Results: Tumor size varied from 0.75 – 15 cms with majority being in 2- 5cms interval (78.8 %).Skin involvement was seen in 15 cases only.IDC (NOS) type was seen in 143 with 14 cases belonging to other categories and 3 being DCIS. 100 cases were of Grade III with only 3 cases in Grade I and rest being Grade II. Lymph nodes were recovered in 124 cases and metastasis was present in 61.9 % with majority being of Grade III.

Conclusion: Apoptotic index and mitotic index increased with increasing Grades.Apoptotic index is more in infiltrating carcinoma breast as compared to other types but does not correlate with tumor size and nodal metastasis. Maximum value of the apoptotic index was found in grade III cases.

Keywords: Breast; Carcinoma; Female; Malignancy

Introduction

Breast carcinoma is the second most common carcinoma in India after malignancy of cervix. It is estimated that every year approximately 80,000 new breast cancer cases are detected in India.[1]It is the most frequent cancer in females throughout the world with 1.05 million new cases occurring every year and represents 20 % of all malignancies among females. More than 50 % cases occur in the developed world.[2] It is the second most common neoplastic disease in females in India.[3] It is a major health problem in Punjab. It accounted for 19.11 % of malignancies in females in a study done for a period of 1974-1983.[4]The incidence increased to 22.54 % in a study done in the Dept of Pathology, Government Medical College, Amritsar in 2000 over a period of 25 years.[5] Breast cancer is a heterogeneous disease with variable biological and clinical characteristics. The racial influence in invasive breast cancer in terms of age at presentation, clinico-pathological features, and outcome of treatment has been widely reported. It has been established that breast cancer in many Asian and African countries tend to affect younger females, present in advanced stage with poorer prognostic features, and has a worse outcome when compared to their counterparts in the Western countries. There is no doubt that the lack of early detection and awareness programs contribute to advanced presentation, however, the biological aggressiveness in terms of poor differentiation, lack of steroid receptor expression, and tendency to affect younger females remains unexplained.[6] Prognostic factors in breast cancer have exploded over the past several years. Pathologists have played a major role in identifying different histological and immunohistochemical markers that have a direct bearing on both the behavior and treatment of breast cancer.[7] Hence, the present study was undertaken to assess the histopathological grades of the carcinoma with nodal status, apoptotic index and mitotic index.

Material and Methods

The study was conducted on 160 cases of carcinoma breast diagnosed in Government Medical College, Amritsar. Detailed clinical data of the patient was recorded as per proforma attached. Histopathological examination of the tissues obtained on surgery was made after the study of the size of the tumor, involvement of the overlying skin, nipple and areola with or without ulceration, retraction or dimpling along with lymph nodes number and size. The tissues were processed to prepare paraffin blocks.Blocks were cut and stained with Haematoxylin and Eosin stain and studied under light microscope for classification, histopathological

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grading,mitotic and apoptotic index.Classification of the malignant breast tumors was done according to the modified Bloom – Richardson grading system[8,9] as shown in table 1.

Table 1: Grading of breast carcinoma according to Nottingham Modification of Bloom– Richardson System[8,9]

Microscopic Grading (Nottingham Modification of Bloom Richardson System)	
Tubular formation	
1 point 2 points 3 points	Tubular formation in>75 % of the tumor Tubular formation in 10 – 75 % of the tumor Tubular formation in <10% of the tumor
Nuclear Pleomorphism	
1 point 2points 3points	1 point 2points 3points
Mitotic Count	
1 point 2 points 3 points	1 point 2 points 3 points

Tumor areas with nuclei having maximum atypia in size and shape were observed. Table 2 shows mitotic counts according to the field areas

Table 2: Mitotic Counts according to the field areas

	Leitz Ortholux	**Nikon Labophot**	**Leitz Diaplan**
Objective	X25	X40	X40
Field dm (mm)	0.59	0.44	0.63
Field area (mm^2)	0.274	0.152	0.312
Mitotic Count			
1 points	0 – 9	0 - 5	0 – 11
2 points	10 – 19	6 - 10	12 – 22
3 points	>20	>11	>23

Mitotic figures were counted only at the periphery of the tumor. 10 high power fields were counted in the same area (but not necessarily contiguous) in the fields which were filled with as much tumor as possible. Poorly preserved areas were not observed. Cells in the prophase were ignored. Table 3 shows formula by which final grade were formulated.

Table 3: Final grade was formulated by the following formula

3 – 5 points	Grade I
6 – 7 points	Grade II
8 – 9 points	Grade III

Mitotic index was calculated by counting mitotic activity among 1000 tumor cells using 40x objective and then calculating in 100 cells.Apoptotic cells and bodies were counted among 1000 cells using 40x objective and apoptotic index was calculated in 100 cells.Areas of necrosis and inflammation were excluded from the count. SPSS programme was used for calculating statistical values.

Results

Most of the cases were reported as the Infiltrating Ductal Carcinoma Breast NOS type (143) with 14 cases belonging to different categories and 3 were Intraductal Carcinoma of Breast, with Comedo pattern, papillary pattern and NOS type each (table 3).

Table 3: Showing various histopathological types of carcinoma

	No. of cases	**Percentage**
D.C.I.S.	03	1.9 %
Infiltrating Ductal Carcinoma (NOS Type)	143	89.4 %
Mucinous Carcinoma	06	3.8 %
Pleomorphic Carcinoma	01	0.6 %

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Lobular Carcinoma	01	0.6 %
Small cell type	04	2.5 %
Papillary carcinoma	01	0.6 %
Adenosquamous carcinoma	01	0.6 %

3 cases were reported as Grade I , 57 cases as Grade II and 100 cases as Grade III (table 4). Grade I comprised of 2 cases of Infiltrating Carcinoma Breast and 1 case of Intraductal Papillary Carcinoma. Grade II was seen in 2 cases of DCIS and rest were of IDC .All the Grade III cases were of IDC.

Table 4: Showing grade of carcinoma

Grade	No of cases	Percentage
I	3	1.9 %
II	57	35.6%
III	100	62.5 %
Total	160	100 %

Lymph nodes were recovered in 124 cases and metastasis was present in 99 cases while 25 cases showed reactive pathology (table 5).

Table 5: Showing status of lymph nodes

Status of lymph nodes	No of cases	Percentage
Reactive	25	15.6 %
Metastatic	99	61.9 %
Not recovered	36	22.5 %
Total	160	100 %

In Grade I lymph nodes were recovered in 2 cases of IDC and one showed secondary deposits. In Grade II all the cases which showed secondary deposits were of IDC. In Grade III lymph nodes were recovered in 81 cases and only 67 showed secondary deposits (table 6)

Table 6: Correlation of the histopathological grades of the carcinoma with nodal status

Status of lymph nodes	Grade I	Grade II	Grade III	Total
Reactive	1	10	14	25
Metastatic	1	31	67	99
Not recovered	1	16	19	36
Total	3	57	100	160

While comparing the lymph node status with the tumour size it was observed that metastasis was more in the tumours less than 5 cms(table 7).

Table 7: Correlation of tumor size with lymph node status

Status of nodes	<2 cms	2-5 cms	>5 cms	Total
Reactive	1	21	3	25
Metastatic	5	78	16	99
Not recovered	3	27	6	36
Total	9	126	25	160

Apoptotic index varied from 0.5–1.0 in grade I, 0.3–1.5 in grade II and 0.3–2.1 in grade III carcinoma breast in infiltrating carcinoma breast (table 8, figure 1 and 2). In intraductal carcinoma of the breast apoptotic index varied from 0.5–1.1. It is more in infiltrating carcinoma breast as compared to other types but does not correlate with tumor size and nodal metastasis. Maximum value of the apoptotic index was found in grade III cases.

Table 8: Correlation of the grade with apoptotic index and mitotic index

Grade	Apoptotic index	Mitotic index
I	0.5 – 1.0	0.1 – 0.6
II	0.3 – 1.5	0.1 – 1.1
III	0.3 – 2.1	0.1 – 1.2

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Variation of Apoptotic Index in various Grades

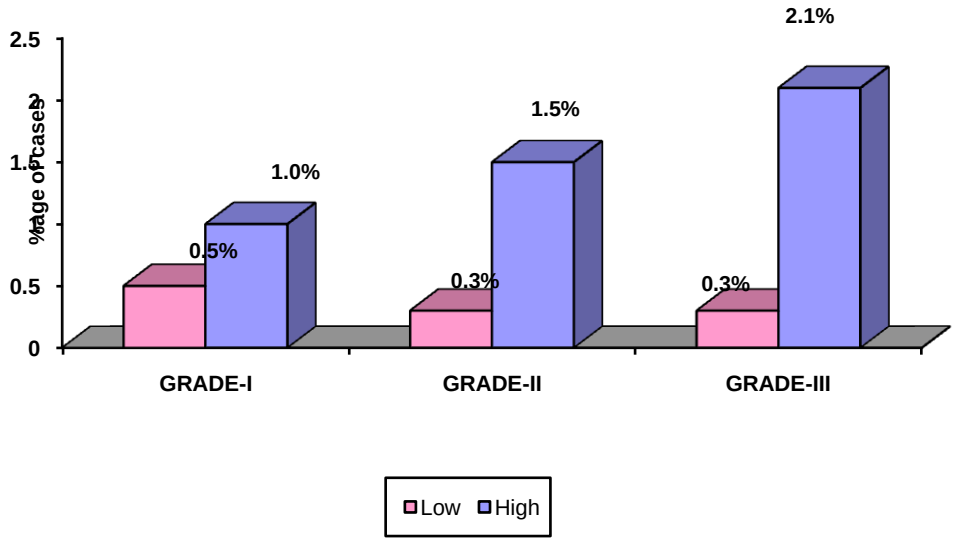

Figure 1: Variation of Apoptotic Index in various Grades

Variation of Mitotic Index in various Grades

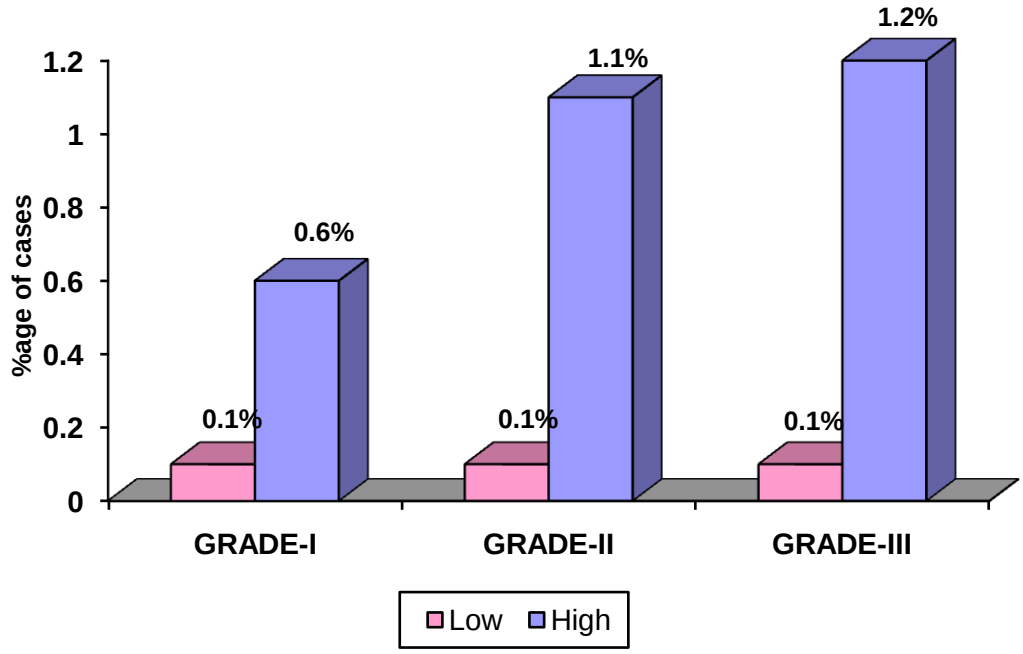

Figure 2: Variation of Mitotic Index in various Grades

Discussion

Breast carcinoma is the disease with a tremendous heterogeneity in its clinical behavior. Clinical and pathological variables such as tumor size, histological grade, histologic type, lymph node metastases, vascular space invasion, tumor cell proliferation, tumor necrosis, extent of ductal carcinoma in situ, age and pregnancy may help in predicting the prognosis and the need for adjuvant therapy. Also, the ethnic variation in carcinoma breast markers is well documented.

Mean tumor size of the cases was 13.4 cms with majority of the tumors more than 2 cms in size (94.4%) and only 5.6% cases belonged to tumor size <2 cms.

Our incidence is much higher to the findings of FatimaS et al[10] who reported about 79% of the tumors in this size range.Abd El Rehim et al[11] also reported maximum cases of carcinoma breast >1.5cm in size.The increased tumor size is a predictor of aggressive tumor behavior.[12]

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Majority of the tumors 143 (89.3%), were infiltrating ductal carcinoma NOS (not otherwise specified). One case of IDC was reported as IDC with Paget's disease of the breast.

14 cases belonged to various categories like mucinous, pleomorphic, small cell carcinoma, lobular carcinoma Papillary carcinoma and adenosquamous carcinoma of the breast.3 cases were reported as intraductal carcinoma of the breast. One case belonged to comedo carcinoma and another case was reported as Intraducatal papillary carcinoma. One case of breast carcinoma had recurrence of the disease and showed ER positivity. The incidence of IDC (NOS) was almost similar to other studies which also reported a higher proportion of this histological type like the findings of FatimaS et al[10]who reported it as 84% but are much higher to the ones reported by Di Stefano Det al[13]as 66.1% cases.

In our study Grade III carcinoma was reported in 62.5%, grade II in 35.6% and grade I in 1.9% of the cases. Mean age of patients in grade II was 49.5 while mean age of patients in Grade III was 48.09. There was no statistically significant relation between grades and age. In India another study done by Chopra R,[14] incidence of Grade II tumors was reported as 37.6% cases while grade III tumors as 51.2% cases. But it was seen in the study carriedby Tang EYet al[15]in Singapore that grade II tumors were more than 55% as compared to grade III tumors which comprised about 26% of total cases. It was further shown that the presence of grade I lesions was more in early breast cancer as compared to locally advanced breast cancers (17.3% vs 11.6%). This difference in the tumor grades may be due to lack of routine mammographic screening in our population coupled with the lack of awareness.

Out of 160 cases lymph nodes were recovered in 124 cases (77.5%) of which metastatic deposits were seen in 79.8%. The size of the tumors with nodes showing reactive pathology varied from 1 cms–10 cms whereas the size went upto 12 cms in cases with carcinomatous deposits.Mean size of the tumors with metastatic lymph nodes was 4.00 ± 0.18 while tumors with reactive lymph nodes had mean size of 3.98 ± 0.36. This is not in accordance with other studies which report increased tumor size along with the presence of the metastatic lymph nodes. As such large tumor size with metastatic lymph node is a poor predictor of survival in breast cancer patients.[12]

In grade I, 1 case each showed reactive and metastatic pathology.In grade II of the total 41 lymph nodes recovered 75.6% cases showed secondary deposits while 24.4% cases showed reactive pathology. In grade III lymph nodes were recovered in 81 cases of which 82.7% cases showed secondary deposits.Other workers have also reported the increased incidence of metastatic deposits in Grade III as compared to Grade II.[16]

Apoptotic index varied from 0.5–1.0 in grade I, 0.3–1.5 in grade II and 0.3–2.1 in grade III carcinoma breast in infiltrating carcinoma breast. In intraductal carcinoma of the breast apoptotic index varied from 0.5–1.1. It is more in infiltrating carcinoma breast as compared to other types but does not correlate with tumor size and nodal metastasis. Maximum value of the apoptotic index was found in grade III cases. These results correlate well with the findings of other research articles which explain that a high AI is related to malignant cellular features and indicates invasiveness and cell proliferation in breast cancer but it is not an independent prognostic factor and does not correlate with tumor size and nodal metastasis.[17]According to the results of a study by Merja Vakkala[18] the apoptotic index was lowest in benign breast lesions. It was higher in DCIS, and a gradual increase in the extent of apoptosis from grade I to III in situ carcinoma was seen. The apoptotic index was highest in recurrent carcinomas. Increased apoptosis was significantly associated with a high cell proliferation, and inversely associated with a positive estrogen status. A high apoptotic index (< 0.50%) was associated with a decreased survival of the patients.

Mitotic index varied from 0.1- 0.6 in grade I, 0.1–1.1 in grade II and 0.1–1.2 in grade III. A study was done to analyse the correlation between mitotic index, MIB1 score and S-phase fraction as proliferation markers in invasive breast carcinoma in a series of 257 cases. A positive correlation was found between the three markers ($p<0.001$). Univariate analysis of markers associated to disease outcome showed that MIB1, axillary node status (N) and hormone receptor status were significantly associated with overall survival and that MIB1 and SPF were associated with disease free survival, along with nodal status.[19]

Conclusion

Apoptotic index and mitotic index increased with increasing Grades. Apoptotic index is more in infiltrating carcinoma breast as compared to other types but does not correlate with tumor size and nodal metastasis. Maximum value of the apoptotic index was found in grade III cases.

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