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Original Research Article

Robinson's cytomorphological grading for breast carcinoma and its correlation with modified Bloom-Richardson histopathological grading

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ABSTRACT

Background: Rising incidence of breast cancer in India necessitates a deeper understanding of biological behaviour of tumor. Tumor grade has a pivotal role in understanding the same. Breast cytology provides a quick diagnosis and remains vital in resource limited settings. Although cytological grading systems have been in development for some time but lack sufficient evidence to validate its correlation with histopathological grading.
Methods: A prospective study over a period of 1 year, included 60 cases of invasive breast carcinoma, diagnosed on cytology (FNAC) with subsequent available histopathological examination. Robinson cytologic grading and Bloom Richardson Histologic grading was performed and correlation studied. Cytological grade was also correlated with regional node metastasis. Correlation between the two grading systems was studied using Spearman's correlation coefficient. Association between Cytological grade and axillary lymph node metastasis was analysed by Pearson's chi square test.
Results: All 60 cases were histotyped as invasive breast carcinoma, NST. Using Robinson's cytological grading, 38 cases (63.33%) were assigned grade II while 37 (61.67%) cases were graded as grade II histologically. Robinson's grading system showed an absolute concordance of 85.00% with histological grading system ($p<0.001$).
Conclusions: Cytological nuclear grade correlates precisely with the histological grade assigned to the tissue with high concordance.

Keywords: Cytology, Grade, Oncology, Prognosis

INTRODUCTION

Breast cancer is the most common malignancy in women; second only to lung cancer as a cause of cancer-specific death. The incidence of breast cancer is increasing rapidly in India.[1,2] In today's era of precision medicine, prognostic and predictive markers are of prime importance to know the biological behavior of cancer. Grade is a significant prognostic factor serving as a key component of clinical decision-making tools.

Elston's modified Bloom and Richardson method of histological grading is a widely accepted tumor grading system with good prognostic correlations.[3] For the purpose of neoadjuvant chemotherapy, biopsy with tumor grade and hormone receptor status are essential parameters, however breast lump cytology (FNAC) still remains vital in resource-limited peripheral rural centers, lacking facilities for immunohistochemistry. Cytological grading of tumors based on FNA cytology can play a pivotal role, offering a quick diagnosis through a minimally invasive technique, proving indispensable in these settings. National Cancer Institute also recommends that fine-needle aspirates can be used to provide prognostic information for patients of breast carcinoma who will undergo preoperative chemotherapy or radiotherapy.[4]

Cytological nuclear grading is practically convenient, reproducible, and efficient. Cytological grading can prove as a useful tool, especially when it's the only material available in some patients prior to neoadjuvant therapy.

Literature shows that studies in the past have assessed the nuclear grade of breast carcinoma on cytology smears but still, there is no agreement among pathologists to accept one of the cytological grading systems as the gold standard.[5-7] The present study aimed to determine whether histologic grade could be reproduced on fine needle aspiration material from breast carcinoma using a robust cytologic grading system like Robinson's cytological grading system. Also correlating this grading system with axillary lymph node status could help in assessing the aggressiveness of the disease.

METHODS

Study design and sample size

A prospective study conducted over a period of one year from January 2015 to January 2016 at department of Pathology, Guru Gobind Singh Medical college, Faridkot. All cases of invasive breast carcinoma, diagnosed on cytology (FNAC) with subsequent available histopathological examination available during the study period were enrolled. Patients with recurrent carcinoma of breast and those who had received neoadjuvant chemotherapy/radiotherapy before mastectomy were excluded. 60 cases of invasive breast carcinoma were included in the study.

Data collection

Informed consent was taken from patients and ethical approval was obtained by Institutional ethics committee. Data was collected by purposive sampling using a proforma and pertinent clinical information like age, sex, clinical presentation, and physical findings of the lump along with any other relevant investigations were recorded.

FNAC of breast lump was performed by a standard protocol using a 22-gauge needle attached to a disposable 20ml syringe, fitted onto Franzen's FNAC handle.[8] Wet smears were immediately fixed and stained with Hematoxylin& Eosin stain (H&E). Air-dried smears were stained with May-Grunwald Giemsa stain (MGG). Cytological grading was done using Robinson's Cytological grading which included 6 parameters i.e. cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin, and chromatin pattern. A score of 1 to 3 was given to each parameter and the final grade was obtained by adding up individual scores as grade I: score 6-11, grade II: score 12-14, grade III: score 15-18.

In cases where subsequent mastectomy specimens were available, grossing and tissue processing were done as per standard oncology protocols. Histological type of tumor was determined and grading was done according to Elston's modification of Bloom Richardson grading system (1991) by observing under high power (400X). Three parameters were considered: tubule formation, nuclear pleomorphism, and number of mitoses. Each parameter was scored 1-3. Final grade was obtained as grade I - score 3-5, grade II -score 6-7, and grade III - score 8-9.

Statistical analysis

SPSS software version 25.0 was used for statistical analysis. Descriptive statistics was analyzed for the nominal and ordinal variables. Correlation between Robinson's cytological grading and modified Bloom-Richardson histological grading was established using Spearman's correlation coefficient. Degree of association between Robinson's cytological grade and axillary lymph node metastasis was analyzed by Pearson's chi-square test.

RESULTS

Current study analysed 60 cases of invasive breast carcinoma. Demographic details for all cases is briefly described in Table 1.

Table 1: Demographic and clinical details of cases.

Parameter	Total cases (n=60) (%)
Age	30 to 85 years (Mean 54.3)
Sex	
Male	1 (1.6)
Female	59 (98.3)
Clinical features	
Palpable breast lump	
Laterality	60 (100)
Right	30 (50)
Left	30 (50)
Quadrant	
Upper outer	28 (46.6)
Upper inner	12 (20)
Lower outer	06 (10)
Lower inner	05 (8.3)
Periareolar/retroareolar	9 (15)
Nipple discharge	14 (23.3)
Constitutional symptoms (weight loss, loss of appetite)	27 (45)
Nipple retraction	10 (16)
Axillary lymphadenopathy	30 (50)

All 60 cases were subtyped as invasive breast carcinoma, NST. Tumor grade was evaluated in these cases using cyto-histopathological grading systems. Among the 60 cases studied, 38 (63.33%) were categorized as cytological grade II, while 12 cases (20.00%) were classified as grade I and 10 cases (16.66%) as grade III. For histological

grading, 37 cases (61.67%) were assigned grade II, followed by grade I with 12 cases (20.00%) and grade III with 11 cases (18.33%) (Figures 1 and 2). Statistical correlation was studied between cytological and histologic grading systems.

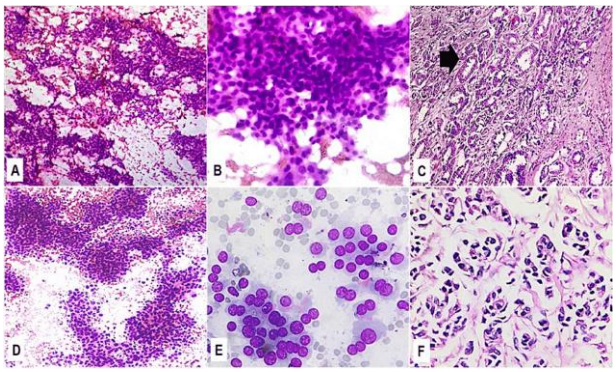

Figure 1: Cytology smears with corresponding histopathology sections showing Grade I and II cases. A) Discohesive groups and clusters of tumor cells on cytology smears. (H&E, 100X). B) Higher magnification shows mildly pleomorphic cells, with regular nuclear membrane and indistinct nucleoli: Robinson's cytological grade I (H&E, 400X). C) Corresponding histopathology section shows tumor cells with subtle nuclear atypia, arranged as tubules (>75%) (arrow): Bloom Richardson grade I (H&E, 400 X). D) Loose clusters and few singly scattered cells tumor cells (H&E, 100X). E) Nuclear size is 3-4X RBC and exhibit moderate nuclear pleomorphism, conspicuous nucleoli: Robinson's cytological grade II. (MGG, 400X). F) Corresponding histopathology section shows tumor cells in cords, trabeculae and few tubules (10-75%) exhibiting moderate nuclear atypia (H&E, 400X).

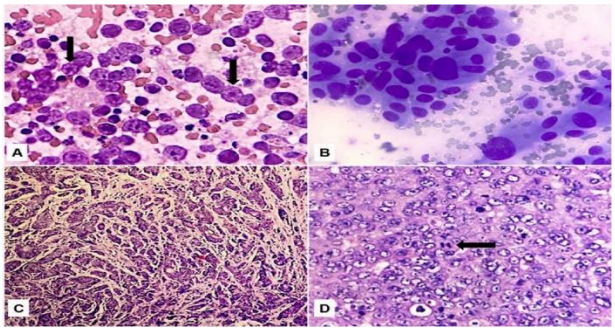

Figure 2: Cytology smears with corresponding histopathology sections for Grade III cases. A) Markedly pleomorphic isolated tumor cells with prominent nucleoli (arrow), clumped chromatin (H&E 400 X). B) Nuclear size >5X RBC size, exhibiting marked nuclear pleomorphism, nuclear budding and clumped nuclear chromatin: Robinson's Grade III. (MGG, 400X). C, D) Tumor cells in nests, trabeculae and occasional tubules (<10%), exhibiting marked nuclear anaplasia and numerous mitotic figures (arrows): grade III. (H&E, 400X).

Out of 12 cases assigned cytological grade of I, 9 corresponded with a histological grade I while the remaining 3 cases were upgraded to histological grade II. For the 38 cases classified as cytological grade II, 33 matched with histological grade II; however, 3 cases were downgraded to grade I and 2 cases were upgraded to grade III on histopathology. Among the 10 cases assigned a cytological grade III, 9 correlated with histological grade III and 1 case was downgraded to grade II on histological examination. Robinson's cytological grade showed an overall concordance rate of 85.0% with histological grade. Spearman rho correlation coefficient (ρ) suggested strong correlation between the two grading systems (Table 2).

Table 2: Correlation between cytological and histological grades (n=60 cases).

Robinson's cytological grade	Histological grade			
	No. of concordant cases between each cytological and histological grade	No. of cases in each cytological grade	Concordance rate (%)	Spearman's rho correlation coefficient (ρ)
I	09	12	75.00	ρ = 0.79 p<0.05
II	33	38	86.84	
III	09	10	90.00	
	Total = 51	Total = 60	Absolute concordance rate: 85.00	

Table 3: Robinson's cytological grading and axillary lymph node metastasis (n=60).

Cytologic grade	No. of cases with lymph node metastasis	Total no of cases for each grade
Grade I	02	12
Grade II	22	38
Grade III	09	10
Total	33	60

Out of 60 cases,33 (55.00%) exhibited axillary lymph node metastasis. Specifically, lymph node metastasis was seen in 2 out of 12 (16.66%) tumors classified as cytological grade I, 22 out of 38 (57.89%) for cytological grade II, and 9 out of 10 (90.00%) cytological grade III (Table 3). The association between increasing cytological grade and axillary lymph node metastasis was found to be statistically significant, with p-value less than 0.05.

DISCUSSION

Breast cancer is a heterogeneous disease characterized by diverse genetic and ethnic variations that may influence tumor characteristics and prognosis. Though the molecular basis of breast cancer is known to us, histologic grading of tumor is still one of the significant prognostic factors. Present study was intended to point out what can be accomplished by FNA in addition to its primary use as a tool for distinguishing malignant lesions from benign ones. Majority of cases in our study were assigned grade II both on cytological and histologic grading. Similar results were obtained by other studies in the past have also shown grade II to be the most commonly designated grade, while some studies revealed equal cases of grade I and grade II.[9-13]

When cytological and histological grades were correlated, we observed that the histological grade correlated positively with the cytological grade with an overall concordance rate of 85% which was comparatively slightly higher than seen in other Indian studies.[9,11-12,14-16] High concordance could be explained by the fact that our study used Robinson's cytological grading system which unlike other grading systems emphasized on nuclear features like nuclear pleomorphism, margins, and nucleoli. Cytological grading can also help in stratification of cases into low-grade (I) and high-grade (II and III) tumors.[9,12,16]

Grade III tumors showed maximum concordance of 90.00% followed by grade II showing 86.84% concordance while grade I tumors showed 75.00% concordance. Discordant cytological grades were observed in 9 cases (15.00%); all with one-grade difference in each case, while none of the discordant cases had a grade difference of two. Discordance between cytological and histological grades can be attributed to different staining procedures; air dried (MGG) versus wet (H&E) smears, and subjective variation in assessing nuclear size, nuclear margins (smooth/budded/folded), and chromatin clumping/ granularity.[17] Other possible reasons are difficulty in assessing tubule formation, mitotic count due to poor specimen fixation and tumor heterogeneity.

Studies in the literature have shown a variable association between the cytologic grade and the axillary lymph node metastasis. While study by Ravikumar et al, 2015 did not show any significant association between cytological grades and incidence of axillary lymph node metastasis, our study had a significant positive association between the two.[13]

All 60 cases in our study were classified as Invasive breast carcinoma, NST (no specific type). Consequently, we were unable to evaluate the validity of Robinson's cytological grading system for tumor types outside of Ductal carcinoma, which represents a limitation of this study. Additionally, small sample size is another constraint of the study.

CONCLUSION

Robinson's cytological grading system is an easy, reproducible and robust grading system, yielding results comparable to Modified Bloom Richardson histologic grading. There is a strong association between lymph nodal disease burden and increasing cytological grade, making this system an effective predictive and prognostic marker. Additionally, neo-adjuvant therapy can significantly alter the nuclear grade in histopathological resection specimens, providing additional value to cytological grade assessment before therapy.

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