

Research Article

Robinson's cytological grading of breast carcinoma and its correlation with Scarff Bloom Richardson's histologic grading

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Abstract

Background: Carcinoma of the breast is one of the most common cancers in women. Fine needle aspiration cytology is the most common preoperative diagnostic modality. Preoperative grading of breast carcinomas would be desirable for the selection of patients for neoadjuvant therapy. This study is aimed at to grade the Fine Needle Aspiration Cytology (FNAC) smears of breast carcinomas and to correlate with histological grading.

Methods: FNAC of 45 patients with breast cancer were studied. The cytological grading was evaluated using Robinson method (RGS) and the histological grading using Scarff-Bloom-Richardson grading system (SBR). Statistical analysis was performed using the SPSS statistical software package (SPSS) (Chicago, Illinois, USA). Correlation was established using Spearman correlation coefficient.

Results: There were 45 cases and the age of the patients ranged from 26 years to 75 years with a mean age of 53.6 years. Univariate analysis showed strong correlation. By doing multiple regression analysis of cytologic features revealed that cell size, uniformity and nucleoli were the most potent features. There was a significant association ($P < 0.000$) between the grades assigned to cytologic and histologic specimens.

Conclusion: 1) Cytological grade could be used to predict histological grade as significant relationship exist between grades assigned to cytology and histology specimens. 2) Cytologic grade may provide relevant information on the aggressiveness of ductal breast carcinoma and could be a useful parameter to take into consideration while selecting cases for neoadjuvant therapy

Keywords: Breast carcinoma, Robinson's cytological grading, Scarff Bloom Richardson's histopathological grading, Cytohistological correlation

1. Introduction

Carcinoma of the breast is the most common non-skin malignancy in women. A woman who lives up to 90 years has one eighth chance of developing breast cancer.¹ Hence, any breast lump, whether it is benign or malignant, is a source of anxiety for the patient as well as for the treating doctor.² Breast cancer is a heterogeneous disease with a wide array of histologic appearance.¹ Histologic grade has been an important prognostic indicator that can predict overall and metastasis free survival for local and regionalized breast cancer.³ Nottingham histological grading of breast carcinoma by Elston and Ellis is a widely accepted tumour grading system and has been found to have good prognostic correlation⁴

Because neoadjuvant therapy, including preoperative chemotherapy and tamoxifen, is becoming increasingly common for early breast cancer. Hence it is desirable to grade tumours before surgery so that the most appropriate medical regimen can be selected. Hence it is good to focus on grading tumours on FNAC. So grading would allow the assessment of the tumour in situ, and the morbidity associated with overtreatment of low grade tumours could be avoided.⁵ This study is aimed at to grade the fine needle aspiration cytology smears of breast carcinomas and correlate with its histological grading.

2. Materials and methods

The work constitutes the retrospective and prospective study of breast carcinomas diagnosed on FNAC in the Department of Pathology, Yenepoya Medical College Hospital from January 2009 to April 2013.

A total number of 45 cases of FNAC of breast carcinoma and corresponding histopathology were studied. Only female cases were taken for the study.

Fine needle aspiration of breast lumps was performed with 22 gauge needles which were attached to 10ml syringes. The samples which were obtained were smeared onto slides and fixed in 95% ethyl alcohol. They were stained by the Papanicolaou.⁶

The cytological grading was done by Robinson's method⁷, in which the cell dissociation, nuclear size, cell uniformity, nucleoli, nuclear margins and the chromatin patterns were studied. A value which was between 1 and 3 were given to each factor which was analysed. The scores of each of the 6 cytological features were added together to give a total score for each case. In each case, the final score ranged between 6 and 18. The scores were added and the grading was done. Grade I: 6-11, Grade II: 12-14 and Grade III: 15-18. The cytological typing was also done.

The corresponding mastectomy specimens which were received were adequately fixed in 10% formalin. Grossing was done. The tissues were processed and the blocked sections were stained with the H and E stain.

The cytological grading was evaluated using Robinson method and the histological grading using Scarff Bloom Richardson grading system.⁸ A study was undertaken to establish the correlation between histologic and cytologic grades.

2.1 Statistical Methods

The statistical analysis was done with the SPSS software. Correlation was established using Spearman correlation coefficient.

3. Results

The age incidence was assessed according to the different age groups wherein, the youngest patient presented at 26 years of age and the oldest patient was 75 years of age. The mean age of occurrence was 56.3 years. Left sided breast was predominantly involved. There were 35 (77.8%) cases of Infiltrating duct carcinoma (IDC), 2(4.4%) cases of Infiltrating lobular carcinoma (ILC), 2 cases of mixed Infiltrating ductal and lobular carcinoma, 2 case of mucinous carcinoma and 1 case each of Ductal carcinoma *in situ* (DCIS), medullary carcinoma, small cell carcinoma and tubular carcinoma.

In this study, majority of the tumours were grade I on cytology (63%); an example of which is shown in Figure: 1. The next in frequency was grade II tumours (26%) (Figure: 2) and 11 % were of cytological grade III. (Figure: 3)

In cell dissociation, score 1 was obtained in 9 cases of grade I only. Score 2 was obtained in 10 cases of grade I, 16 cases of grade II and 2 cases of grade III. Score 3 was obtained in 3 cases of grade I, 3 cases of grade II and 2 cases of grade III.

In the cytological feature cell size, score 1 was obtained in 15 cases of grade I and 1 case of grade II. Score 2 was obtained in 4 cases of grade I, 13 cases of grade II and 1 case of grade III. Score 3 was obtained in 3 cases of grade I, 5 cases of grade II and 3 cases of grade III.

In the cytological feature cell uniformity, score 1 was obtained in 15 cases of grade I and 2 cases of grade II. Score 2 was obtained in 6 cases of grade I and 12 cases of grade II. Score 3 was obtained in 1 case of grade I, 5 cases of grade II and 4 cases of grade III.

In the cytological feature nucleoli, score 1 was obtained in 16 cases of grade I and 1 case of grade II. Score 2 was obtained in 6 cases of grade I, 16 cases of grade II and 3 cases of grade III. Score 3 was obtained 2 cases of grade II and 1 case of grade III.

In the cytological feature cell nuclear margin, score 1 was obtained in 19 cases of grade I and 4 case of grade II. Score 2 was obtained in 3 cases of grade I, 13 cases of grade II and 4 cases of grade III. Score 3 was obtained in 2 cases of grade II only.

In the cytological feature chromatin, score 1 was obtained in 15 cases of grade I and 1 case of grade II and 1 case of grade III. Score 2 was obtained in 6 cases of grade I, 11 cases of grade II and 1 case of grade III. Score 3 was obtained in 1 cases of grade I, 7 cases of grade II and 2 cases of grade III. Univariate analysis (Table: 1) and Multiple regression analysis was done for this cytological features. (Table: 2)

Table 1: Univariate analysis - correlation of cytological features with cytological grade – RGS

RBS Grade	Spearman correlation coefficient-R value	p-value
Dissociation	0.425	0.004
Cell size	0.583	0
Uniformity	0.689	0
Nucleoloi	0.665	0
Nuclear margin	0.627	0
Chromatin	0.553	0

All features are significant (p valve < 0.005)

Table –2: Multiple regression analysis – RGS

Predictor	Regression coefficient	t- value	p-value
Cell Dissociation	0.153	1.873	0.069
Cell size	0.226	3.092	0.004
Uniformity	0.225	3.160	0.003
Nucleoli	0.290	2.883	0.006
Nuclear margin	0.138	1.202	0.237
Chromatin	0.208	2.513	0.016

Regression Equation Grade = -0.684 + 0.153 (Disso) + 0.226 (Size) + 0.255 (C. Uniformity) +0.29 (Nucleolus) + 0.138 (N. Margin) +0.208 (Chromatin)
Cell size, uniformity and nucleoli were the most potent features.

In this study, 15 tumours were classified as grade I (Figure: 4), 20 tumours (Figure: 5) were grade II and 9 were grade III (Figure: 6). In the topographic tubule formation, score 1 was obtained in 6 cases of grade I. Score 2 was obtained in 3 cases of grade I, 2 cases of grade II and 1 case of grade III. Score 3 was obtained in 5 cases of grade I, 20 cases of grade II and 8 cases of grade III. In the feature of nuclear pleomorphism, score 1 was obtained in 10 cases of grade I .Score 2 was obtained in 4 cases of grade I, 19 cases of grade II and 1 case of grade III. Score 3 was obtained in 3 cases of grade I, 8 cases of grade II and 3 cases of grade III. In the feature of mitosis, score 1 was obtained in 12 cases of grade I and 12 case of grade II. Score 2 was obtained in 2 cases of grade I, 10 cases of grade II and 3 cases of grade III. Score 3 was obtained in 6 cases of grade III. Univariate (Table:3) and multiple regression (Table:4) analysis was done for the topographical features.

Table -3: Univariate Analysis of SBR

Topographical features	Spearman’s correlation coefficient	
	r-value	p-value
Tubule formation	0.523	0
Nuclear Pleomorphism	0.835	0
Mitosis	0.665	0

All the features are significant statisticaly (p- valve < 0.005)

Table:4: Mutiple regression analysis of SBR

Predictors	Regression Coefficient	t-value	p-value
Tubule	0.302	5.276	0.001
Nuclear Pleomorphism	0.543	7.837	0.001
Mitosis	0.393	6.233	0.001

SBR grade = -0.623 + 0.302* tubule + 0.543 * nuclear pleomorphism + 0.393 * mitosis
All the three features of SBR grading system were statistically significant.

Comparison of cytological and histological grading was done by Spearman's Correlation Coefficient in SSPS software (Table: 5)

Table – 5: Correlation of cytological grading with histological grading

Correlation	Spearman's Correlation Coefficient	
	r- value	p-value
Cytological Grade (RGS) and Histological Grade (SBR)	0.591	0

Figure 1: Photomicrograph: Grade I duct carcinoma: Showing tumour cells with smooth vesicular nucleus, smooth nuclear margins and inconspicuous nucleoli (PAPx40)

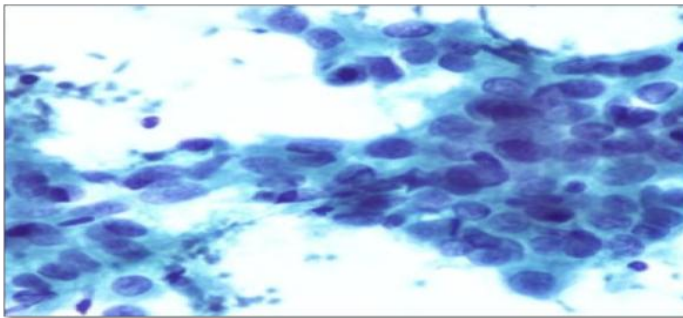


Figure 2: Photomicrograph: Grade II duct carcinoma: Nuclei are three to four times the erythrocytes, with granular nuclear chromatin and smooth contour. (PAPx40)



Figure 3: Photomicrograph: Grade III duct Carcinoma: nuclear size > 5 times RBC Pleomorphism and prominent nucleoli. (PAPX 40)

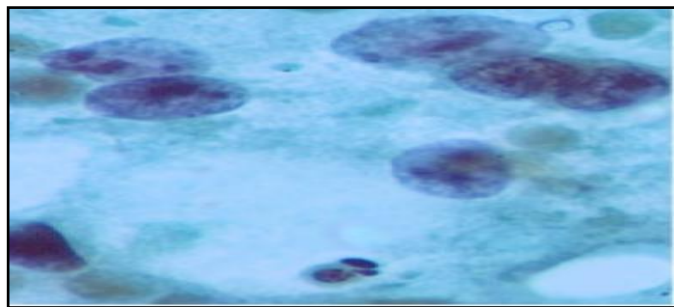


Figure 4: Photomicrograph of Grade I breast carcinoma with a tubular pattern. This was graded as a SBR score = 3 (H& E X 20)

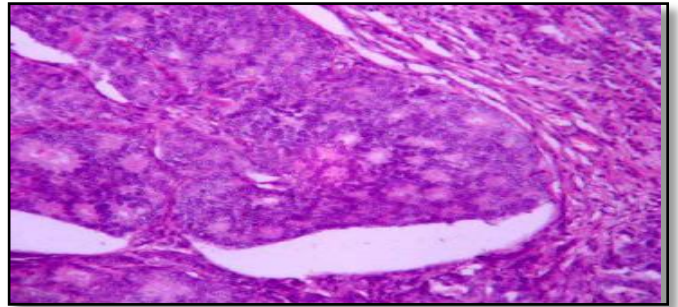


Figure 5: Photomicrograph of Grade II breast carcinoma. This was graded as a SBR score =7 (H& E X 20)

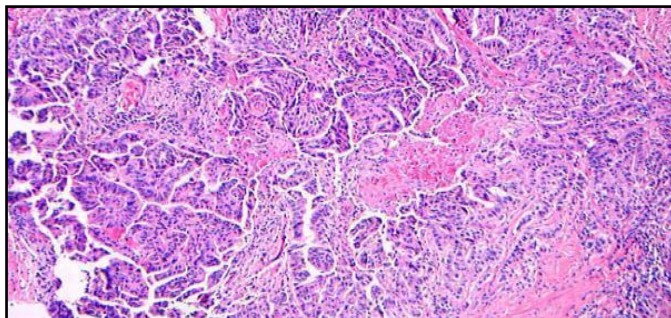
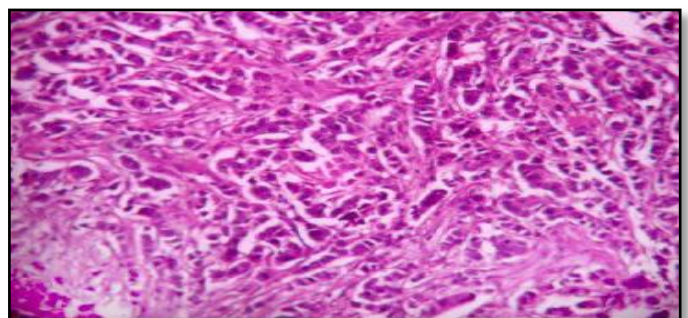


Figure 6: Photomicrograph of Grade III breast carcinoma. This was graded as a SBR score =7 (H & E X 20)



4. Discussion

The advantages of FNAC in the diagnosis breast carcinoma have been known since a long time but grading of the breast carcinoma on FNAC has been underestimated. In our study, we have attempted grading of breast carcinoma on FNAC as per the criteria proposed by Robinson and colleagues.⁷

The slide comment was given after scanning on individual cytological features. The degree of cell dissociation is an indicator of cell cohesion status and the degree of expression of E-cadherin/catenin complex.⁹⁻¹² Loss of cell cohesion appears to facilitate vascular infiltration by tumour cells, which gives rise to an increased incidence of regional LN metastasis. A number of studies have shown that the neoplasms with greater cell dissociation show a higher incidence of lymph node metastasis.^{13,14}

In our study for cell dissociation we got cell 62.2.96% (n=28) cases were given score 2 followed by 20 % (n=14) cases of score 1 and 17.8% (n=48) cases of score 3.

Cell size was assessed by comparing the size of tumour cell with adjacent red blood cell present in the aspirate. In cell size study we got 40% (n=18) of cases with score 2 followed by 35.6% (n=16) of score 1 and 24.4% (n=11) cases of score 3.

The three cellular components of cell pleomorphism were assessed separately. They are cell uniformity i.e., the similarity of cell appearance, nuclear outline and chromatin pattern. Cellular pleomorphism is useful and it is used to assess and correlate the histological pleomorphism.¹⁵ In our study we got 40% (n=18) cases with score 2 for cell uniformity followed by 22.2% (n=10) cases of score 3 and 37.8% (n=17) cases of score 1.

The presence and absence of nucleoli has been used in other grading system like Mouriquand¹⁶ and Thomas's¹⁷ grading system. Robinson *et al*⁷ found the presence and the character of nucleolus valuable in grading independently as well as in combination with the cytological features. In nucleoli, 55.6% (n=44) of cases got score 2, 37.8% (n=17) of cases got score 1 and 6.7% (n=3) got score 3.

When nuclear margins taken into consideration for scoring, 51.1% (n=23) cases were given score 1, 44.4% (n=20) cases were given score 2 and 4.4% (n=2) cases were given score 3.

Chromatin pattern differentiates the nuclei of well and poorly differentiate breast carcinoma. But this feature was not significant statistically. This might be related to subjectivity in assessment of this characteristic as well as limitation of our eye resolution in detection of subtle grades of nuclear chromasia and granularity with the light microscope.¹⁸

In the present study morphology of chromatin we got 40% (n=18) cases got score of 2, 37.8% (n=17) cases got score 1 and 22.2% (n=10) cases got score 3. Comparison of univariate and multiple regression analysis was done with the studies done by Das *et al* and Lingegowda *et al*. (Table: 6) and (Table: 7).

Table No: 6: Comparative table on correlation of cytological features with cytological grade – RGS

Cytological feature	Spearman's Correlation Coefficient					
	Present study		Lingegowda <i>et al</i> ¹⁹		Das <i>et al</i> ²⁰	
	r-value	p-value	r-value	p-value	r-value	p-value
Dissociation	0.425	0.004	0.199	0.17	0.86	0
Cell size	0.583	0	0.523	<0.01	0.97	0
Cell uniformity	0.689	0	0.567	<0.01	0.95	0
Nucleoli	0.665	0	0.725	<0.01	0.65	0
Nuclear margin	0.627	0	0.566	<0.01	0.84	0
Chromatin	0.553	0	0.736	<0.01	0.87	0

Univariate analysis of cytological features with cytologic grade showed strong correlation in our study as well as Das *et al*²⁰ and Lingegowda *et al*¹⁹.

Although cell dissociation has been studied in depth in breast tumours, it has not been accorded equal importance in tumours located in other areas. It would thus be of major interest to evaluate the importance of this correlation in tumours in which cell dissociation is a diagnostic aid; as is the case of neoplasia involving the pancreas, soft tissues, and stomach *etc.*²¹

Table -7: Comparative table on multiple regressions – RGS

Predictor	RC	p-value	RC ¹⁹	p-value	RC ²⁰	p-value	RC ⁷	p-value
Dissociation	0.153	0.069	0.20	0.004	0.804	0.000	0.055	0.0003
Cell size	0.226	0.004	0.06	0.458	-5.42	0.254	0.031	0.0335
Uniformity	0.225	0.003	0.13	0.128	1.156	0.001	0.28	0.1275
Nucleoli	0.290	0.006	0.29	0.000	0.708	0.000	0.48	0.0018
Nuclear margin	0.627	0.237	0.33	0.000	0.695	0.000	0.25	0.1971
Chromatin	0.553	0.016	0.19	0.065	0.593	0.086	0.38	0.0657

Cell size, uniformity and nucleoli were the potent features by doing multiple regression analysis of cytologic features in our study.

Robinson *et al* dissociation, nucleoli and chromatin were the influential features. In Lingegowda *et al*¹⁹ it was cell dissociation, the appearance of nucleoli and nuclear margin whereas in Das *et al*²⁰ it is cell dissociation, cell uniformity and nucleoli.

An increase in the size and the number of nucleoli is a well-known characteristic of cells engaged in growth and synthesis¹⁶. Multivariate analysis of the components of SBR revealed all the three components, that is, tubule formation, nuclear pleomorphism and mitosis contribute to prognosis independently. The results were statistically significant. Few of the recent studies have shown mitotic activity to be the most influential predictor of survival in the SBR method. Tubule formation and nuclear pleomorphism was found to have little effect on prognosis. Comparison of SBR grading was done with the Meena *et al*, Gore *et al* and Das *et al* (Table: 8)

Table – 8: Comparative table on distribution of cases –SBR grade

Histo Grade	Robinson <i>et al</i> ⁷	Meena <i>et al</i> ²¹	Gore <i>et al</i> ²²	Das <i>et al</i> ²⁰	Our study
I	9	23	6	24	15
II	29	36	38	42	20
III	14	12	14	35	9
Total	52	71	58	100	45

In all studies mentioned above including ours, a large number of patients have been placed in grade II. This is one of the limitations of SBR grading in which there is unequal distribution of patients among all the 3 grades with over 50% of patients in grade II. Furthermore, even though there is a relatively clear prognostic separation between grade I, grade III and grade II patients, often overlap of grade II was seen with grade I and grade III.²³

In our study, tubular carcinoma was grade I. Both the mucinous carcinoma was grade III. Medullary carcinomas, Small cell carcinoma as well as mixed carcinomas were grade II. Comparison of Cytohistological correlation was done with Frias *et al* and Lingegowda *et al* (Table:9)

Table – 9: Comparative table on correlation of cytologic grading with histologic grading

Grading systems – Cyto & Histo	r-value	p-value
Present study	0.591	0
Frias <i>et al</i> ²⁵	0.774	<0.0005
Lingegowda <i>et al</i> ⁶⁸	0.519	<.001

Histological grade correlated positively with Robinson cytological grade (r=0.591) in our study. It's in concordance with the Lingegowda *et al*¹⁹ and Frias *et al*.²⁴

The coefficient of correlation showed a significant and marked association ($p=0.000$) between the grades assigned to cytologic and histologic specimens in our study.

The studies conducted by Frias *et al* and Lingegowda *et al* showed p -value <0.0005 and $<.001$ respectively which are significant.

5. Conclusion

Robinson's Cytological grading of the breast carcinomas is a simple, less tedious and feasible system. It can be carried out in any laboratory. It requires no special technique, except for some expertise. Cytological grading of infiltrating duct carcinoma (NOS) as well as special varieties of breast carcinoma correlates well with histological grading. The aggressiveness of breast carcinoma can be determined by evaluating the cytological grade using FNAC. It is a useful parameter to take into consideration while selecting neoadjuvant therapy for breast carcinoma.

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