Correlation between Robinsons Cytological Grading with Elston-Ellis Nottingham Modification of Bloom Richardson Histopathology Grading for Breast Carcinoma

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Abstract

Background: In this study, we wanted to evaluate the correlation between Robinson's cytological grading with Elston-Ellis Nottingham modification of Bloom Richardson histopathology grading for breast carcinoma.

Materials and Methods: The present study is a prospective study carried out in the Department of Pathology at Maharaja Krushna Chandra Gajapati Medical College, Berhampur, Odisha from September 2018 to August 2020, with the approval of the ethical committee of this institute.

Results: 14 tumours (66.67%) were graded I on cytology and histopathology, 5 tumours (23.81%) were graded I on cytology and grade II on histopathology and 2 tumours (9.52%) were graded I on cytology and grade III on histopathology. Out of 46 cases with cytological grade II, 38 tumours (82.61%) were grade II also on histopathology, while 5 (10.87%) were downgraded to grade I and 3 (6.52%) were upgraded to grade III. Out of the 28 cases with cytological grade III, 26 (92.86%) were grade III on histopathology while 2 (7.14%) were downgraded to grade II on histopathology. The p-value was <0.05.

Conclusion: Every FNAC report should be incorporated with a cytological nuclear grade for robust clinical management.

Keywords: Robinsons Cytological Grading, Elston-Ellis Nottingham Modification of Bloom Richardson Histopathology Grading, Breast Carcinoma.

Introduction

The majority of the tumour grading systems currently employed for breast cancer combine nuclear grade, tubule formation and mitotic rate. Of various grading systems, the Nottingham method described by Elston and Ellis (also called as modified Bloom Richardson method) is a widely accepted tumour grading system and has been found to have good prognostic correlations.

It was also emphasized that the cytological grading system on FNA specimens should correspond to the grading system used in the histopathological materials. Of the different cytological grading methods corresponding to Elston and Ellis modified Bloom Richardson grading, the method described by Robinson et al was found to be useful in grading breast carcinoma in FNA.

Materials and Methods

The present study is a prospective study carried out in the Department of Pathology at Maharaja Krushna Chandra Gajapati Medical College, Berhampur, Odisha from September 2018 to August 2020, with the approval of the ethical committee of this institute.

Inclusion Criteria

1. Patients having palpable breast lumps with clinical and cytological diagnosis of breast carcinoma and subsequent histopathological follow-up.
2. Patients with informed consent.

Exclusion Criteria

1. Patients who refused to undergo FNAC of a breast lump.
2. Aspirates were inadequate or scanty.
3. Benign breast diseases/non-cancerous lesions.
4. Patients diagnosed with ductal carcinoma on cytology whose biopsy specimens were unavailable for histopathological examination.
5. Cytological smears with improper staining or air-dried artefacts.
6. Patients who were diagnosed with breast carcinoma on cytology but not confirmed on histology.
7. Patients who had a history of preoperative chemo or radiotherapy for breast carcinoma.

Statistical Analysis

Descriptive data were presented as numbers and percentages. Statistical analysis was performed using SPSS software version 17. Univariate analysis and multiple regression analysis were done to assess the significance of different cytological & histological features. Association between different grading systems was measured by a chi-square test. The correlation between cytological and histological grading systems was examined by Spearman's correlation coefficient. The sensitivity & specificity of Robinson's cytological grading method were calculated for each cytological grade. Kappa value was calculated to assess the strength of agreement between the two grading systems. The involvement of axillary lymph nodes was evaluated by employing the chi-square test. A p-value of 0.05 or less was considered to be statistically significant.

Results

Age and Gender Distribution

The age of the subjects ranged from 21 years to >80 years. The mean age of the study participants was 49.64 years with a standard deviation of 11.22. Maximum number of cases, 39 (41.05%) was in the age range of 41-50. The next common group involved was in the range of 51-60 yrs. constituting 20 cases (21.05%) and the least no. of cases 01 (1.05%) was in the age group of more than 80 years.

In the present study, 92 cases (96.84%) were females and 3 (3.16%) were males. The mean age in males was 62.0 years with a standard deviation of 11.26 years while that of females was 49.23 years with

a standard deviation of 11.05. This was found to be significant (t = 43.09, P<0.01).

Symptoms

In the present study, all the cases presented with a lump in the breast. The most common symptom was breast lump as seen in 52 cases (54.74%) followed by 18 cases (18.95%) with lump and pain, 15 cases (15.79%) had lump with ulcer and the least number of 10 cases (10.53%) presented with lump and nipple discharge.

Duration of the Presenting Symptoms

In the present study, majority of 46 cases (48.42%) presented clinically 6 months after noticing the breast lump followed by 35 cases (36.84%) who presented between 3-6 months and 14 cases (14.74%)

presented earlier than 3 months after noticing the lump.

Distribution Pattern of Side of Involvement of Breast

In the present study, 53 cases (55.79%) presented with a lump in the left breast and 42 cases (44.21%) with a lump in the right breast.

Quadrant Wise Distribution

In the present study, lumps were present in the upper outer quadrant of the breast in the majority of 37 cases (38.95%) followed by 28 cases (29.47%) in the central quadrant of the breast. The least number of cases 2 (2.11%) involved the whole of the breast.

Table 1

Robinson's Cytological grade	Number of cases	Percentage (%)
I	21	22.11
II	46	48.42
III	28	29.47
Total	95	100
Distribution of cases according to Robinson's cytological grading		
Histological grade	**Number of cases**	**Percentage (%)**
I	19	20
II	45	47.37
III	31	32.63
Total	95	100
Distribution of cases according to histological grading		

21 cases (22.11%) out of 95 cases belonged to cytological grade I which was the least among all grades. Majority i.e. 46 cases (48.42%) constituted grade II and 28 cases (29.47%) belonged to cytological grade III.

Cytological Grading

Cytological grading was done by using Robinson's system which included the following criteria: cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin and chromatin pattern.

Cell Dissociation

In the present study, cells in clusters were seen in 21 (100%) grade I tumours. Cells in clusters and singles were seen in 35 (72.92%) grade II tumours and 13 (27.08%) grade III tumours. Cells in singles were seen in 11 (42.31%) grade II tumours and 15 (57.70%) grade III tumours.

All grade I tumours showed cellular arrangement in clusters (100%). Grade II tumours showed cellular arrangement mostly in clusters and singles (72.92%) and grade III tumours showed mostly singles (57.70%).

Cell Size

In the present study, the cell size varied from small (1-2 times the size of RBC), medium (3-4 times the size of RBC) to large (5 times the size of RBC). In most of the cases, 29 (30.53%) had medium-sized cells and were seen mostly in grade II tumours, grade I tumours had small-sized cells in 19 cases (70.37%) and grade III tumours had large-sized cells in 18 cases (62.07%).

Cell Uniformity

In the present study, majority of cases, 48 (50.53%) had tumours with mild pleomorphic cells. Among which 30 cases (62.50%) belonged to grade II and 12 cases belonged to grade III. 19 cases (20%) showed monomorphic tumour cells out of which 15 cases (78.95%) belonged to grade I and 4 cases (21.05%) belonged to grade II. Pleomorphism was seen in 12 cases (42.86%) of grade II tumours and 16 cases (57.14%) of grade III tumours.

Nucleolar Features

In the present study, majority of tumours, 44 (46.32%) had noticeable nucleoli out of which 5 cases (11.36%) were grade I tumours, 28 cases (63.64%) were grade II and 11 cases (25%) were grade III. Out of 31 cases (32.63%) with prominent nucleoli, 14 cases (45.16%) belonged to grade II and 17 cases (54.84%) belonged to grade III tumours. Indistinct nucleoli were present in 16 cases (80%) of grade I tumours and 04 cases (20%) of grade II tumours.

Nuclear Margins

In the present study, majority of tumours, 45 (47.37%) had irregular grooves or folds in the nuclear margin, 31 (32.63%) showed buds or clefts and 19 (20%) had smooth margin. Out of 45 cases (47.37%) with irregular grooves or folds, majority 34 (75.56%) were grade II tumours, 7 (15.56%) were grade III and 4 (8.89%) were grade I tumours.

Nuclear Chromatin Pattern

In the present study, majority of the tumours, 48 (50.53%) had nuclei with granular chromatin, 29 (30.53%) had clumped chromatin and 18 (18.95%) had vesicular chromatin.

Distribution of Histological Grading Patterns

In the present study, the majority (45 cases) of the tumours (47.37%) were grade II, followed by 31 (32.63%) grade III tumours and 19 (20%) grade I tumours. Histological grading was done using modified Bloom Richardson's Grading. The criteria followed were tubule formation by tumour cells, variation in nuclear sizes and mitotic count per 10 hpf (0.45 mm field diameter/0.159mm2 area).

Tubule Formation

In the present study, tubule formation in 10-75% of the tumour mass was seen in 49 (51.58%) out of 95 tumours. Most (36) of them (73.47%) were grade II tumours. Tubule formation in less than 10% of tumour mass was seen in 33 cases (34.74%) out of which 26 (78.79%) were grade III tumours and 7 (21.21%) were grade II tumours. The p-value was <0.05 which was found to be significant.

Variation in Nuclear Size

In the present study, the majority of the tumours (52) had moderate variation in nuclear size (54.74%) followed by marked variation in 34 (35.79%) and mild variation in 9 (9.47%). Most (34) of the tumours with moderate variation were grade II tumours (65.38%), while marked variation was found mostly in 25 (73.53%) of grade III tumours. The p-value was <0.01 which was found to be significant.

Mitotic Count

In the present study, the majority (54) of the tumours showed a mitotic count of 6-10/10 hpf (56.84%), out of which, 39 (72.22%) were grade II tumours. This was followed by 23 tumours (24.21%) with a mitotic count of ≥11 / 10 hpf. Out of these, mostly 18 (78.26%) were grade III

tumours. Mitotic count of 0-5/10 hpf was seen in 18 tumours (18.95%) out of which 17 (94.44%) were grade I tumours. The p-value was <0.05.

Correlation between Cytological and Histological Grades

In the present study, 14 tumours were (66.67%) graded I on cytology and histopathology, 5 tumours (23.81%) were graded I on cytology and grade II on histopathology and 2 tumours (9.52%) were graded I on cytology and grade III on histopathology. Out of 46 cases with cytological grade II, 38 tumours (82.61%) were grade II on histopathology also, while 5 (10.87%) were downgraded to grade I and 3 (6.52%) were upgraded to grade III. Out of the 28 cases with cytological grade III, 26 (92.86%) were

grade III on histopathology while 2 (7.14%) were downgraded to grade II on histopathology. The p-value was <0.05.

Deep Surgical Margin

In the present study, 47.37% of the tumours showed involvement of a deep surgical margin and 44 cases out of 95 (46.36%) had lymph node involvement. 23/28 (52.27%) of grade III cases, 16/46 (16.84%) of grade II cases and 05/21 (11.36%) of grade I cases had axillary lymph node involvement. The highest involvement was noted in higher degree tumours. Axillary lymph node metastasis was found to be statistically significant with chi-square=21.1935 and P=0.000025. Thus the strength of agreement between the two grading systems was considered to be moderate.

Table 2. Comparison between cytological (RCG) and histological grading (MBR) with statistical analysis

		Histological Grade			Kappa value	P-value	Standard error	95% CI	Strength of agreement
		I	II	III					
Cytological Grade	I	14	5	2	0.698	<0.01	0.064	0.000-0.031	Moderate
	II	5	38	3					
	III	0	3	25					
	Total	19	46	30					

Table 3. Cytological and histological correlation with chi-square analysis

		Histological Grade			Chi Square	P value	95% CI
		I	II	III			
Cytological Grade	I	14	5	2	94.244	<0.01	0.000-0.031
	II	5	38	3			
	III	0	3	25			
	Total	19	46	30			

Table 4. Comparison of concordance rates between the cytological and histological grades

Cytological grade	Number of cytological grading	Histological Grading			Concordance rate (%)
		Grade I	Grade II	Grade III	
	1	14	5	2	66.66%
II	6	5	38	3	82.60%
III	8	0	3	25	89.28%
Total	5	19	46	30	
Absolute Concordance				77/95 = 81.05%	

Cytological grading was comparable to histological grading. The findings of cytological and histological grading were compared and concordance rates were calculated.

The concordance rate of grade I cases between cytology and histology was 66.66%, 82.60% for grade II and 89.28% for grade III cases. The overall concordance rate was found to be 81.05%.

The sensitivities were 73.68%, 82.61%, and 83.33% for cytological grade I, II and III tumours respectively. The specificities for cytological grade I, II and III tumours were 90.79%, 83.67% and 95.38% respectively.

Table 5. Multiple regression analysis of cytological features

Cytological features	Spearman correlation coefficient	Significance (p-value)
Cell dissociation	0.728	<0.001
Cell size	0.662	<0.001
Cell uniformity	0.628	<0.001
Nucleolus	0.638	<0.001
Nuclear margin	0.753	<0.001
Chromatin pattern	0.676	<0.001
Correlation of cytological features with cytological grade using Univariate analysis		
Cytological features	Regression coefficient	Significance (p)
Cell dissociation	12.315	0.015
Cell size	2.773	0.597
Cell uniformity	2.46	0.79
Nucleolus	2.773	0.597
Nuclear margin	8.376	0.079
Chromatin pattern	9.847	0.043

All the cytological features used for cytological grading were statistically significant using univariate analysis.

Cell dissociation and chromatin pattern as the most specific parameters (P<0.05) in determining the grades of the tumour followed by other parameters like cell uniformity (P=0.79), cell size (P=0.597), nucleolus (P=0.597) and nuclear margin(P=0.079). (Only cell dissociation and chromatin patterns are significant)

Table 6. Multiple regression analysis of histological features

Histological features	Spearman correlation coefficient	Significance (p-value)
Tubule formation	0.753	<0.001
Pleomorphism	0.678	<0.001
Mitotic figures	0.766	<0.001
Correlation of histological features with histological grade using Univariate analysis		
Histological features	Regression coefficient	Significance (p-value)
Tubule formation	35.010	<0.001
Pleomorphism	42.485	<0.001
Mitotic figures	87.319	<0.001

All the histological features are statistically significant with P<0.001 using univariate analysis. All the histological features have equal influence in histopathological grading with P<0.001 using multiple regression analysis.

Discussion

Age Incidence

In the present study, patients were distributed into the age group ranging from 21 to more than 80 years. A maximum number of cases, 41 (43.16%) constituted the pre-menopausal age group i.e. 41-50 years. Farley et al. (2000) suggested that breast carcinoma in India peaks at a younger age (40-50 years) than in western countries as a result majority of new cases are diagnosed in the pre-menopausal age group. [1]

Comparative Study of Male & Female Ratio

In the present study, 92 subjects (96.84%) with breast carcinoma were females and 3 (3.16%) were males. The mean age of females was 49.23 years and that of males was 62 years. P-value was <0.01. Meena et al. reported a lower incidence in males with a single case out of one hundred cases in their study. [2] Vivek Kathed and Mohini Kucheria in their study had a 2% incidence of male breast carcinoma [3] which is corroborative of our study.

In the present study, all 95 patients (100%) presented with a breast lump. Out of these 52 (54.74%) presented with only a lump, 18 (18.95%) with lump and pain, 10 (10.53%) with lump and nipple discharge and 15 (15.79%) with ulcer over the lump. The upper outer quadrant of the breast was most frequently involved in our study which is similar to studies done by Touhid et al. [4] Meena et al. [2] and Aparna et al.

In the present study, we found 46 cases (48.42%) of grade II tumours followed by 28 cases (29.47%) of grade III and 21 cases (22.11%) of grade I tumours which is corroborative of the studies done by Taniguchi et al. [5] Jayaram G et al. [6] Charusheela et al. [7] and Panwar et al. [8]

In the present study, histological grading was done by Elston & Ellis modification of Scarff Bloom Richardson grading. It was found that the majority of the tumours, 46 (48.42%) were grade II followed by 28 (29.47%) grade III, and 21 (22.11%) grade I. Study was done by Taniguchi et al., Jayaram G et al., Aparna et al., Charusheela et al., Chalise et al., & Panwar et al. showed similar observations. In contrast, Robinson et al., Sinha et al. and Goswami et al. observed that the majority of the tumours were grade II followed by grade I and grade III.

54 cases were cytologically graded using Robinson cytological grading method and correlated with histological specimens in Bhanvadia et al. [9] study.

We found that Robinson's cytological grading system was highly specific for grade I tumours (90.79%) and cytological grade III tumours (95.38%) while being moderately specific (83.67%) for grade II tumours. Bhanvadia et al. and Pal et al. reported a similar finding of higher specificity for cytological grade I and III tumours. This low specificity could also be due to the variation in cytological features in different areas of tumour on histology, which cannot be appreciated in cytology because of a limited area of the approach.

The present study showed that the overall k value for all grades of tumours was 0.698 indicating moderate agreement between cytological and histological grading systems. 95% CI was 0.000-0.031 and SE was 0.064.

In this study, correlation between Robinson cytological grading method and Elston and Ellis Modified Bloom Richardson grading system was done using chi-square analysis which revealed a P value less than 0.01 which was statistically significant. Therefore, it can be stated that cytological grading is comparable to histological grading to assess the tumour behaviour and prognosis and needs to be evaluated further for its usefulness as a pre-operative predictor, especially in cases undergoing neoadjuvant therapy. [10] In a study done by Robinson et al. on multiple regression analysis of various cytological

features, they found that regression coefficients for cell dissociation and nucleoli were 0.58 and 0.48 respectively. P-value was <0.001. These two features were the most important parameters in determining the cytological grades. [11]

In our study, all the cytological features were statistically significant using univariate analysis. Similarly, all the histological features were significant using univariate analysis. All the histological features had an equal influence on histopathological grading. These findings are corroborative with the study done by Chalise S et al. & junu rajan and pai.

Lymph Node Status

Pal et al. noticed 31/50 cases (62%) positivity for axillary lymph node metastasis. They found an increase in the incidence of axillary lymph node metastasis with an increase in cytological grades i.e. 3/11 cases (21.43%) for grade I tumours, 21/29 (72.41%) for grade II and all the 7 cases (100%) for grade III tumours. The association between an increase in cytological grade and axillary lymph node metastasis was found to be statistically significant with a p value<0.001. In the present study, 44 cases out of 95 (46.32%) showed axillary nodal metastasis. 5/21 cases (23.80%) of grade I tumours, 16/46 (34.78%) of grade II tumours and 23/28 (82.14%) of grade III tumours had nodal metastasis. The association between an increase in cytological grade and axillary lymph node metastasis was found to be statistically significant with a p value<0.0001 which was corroborative to the above study.

Special Types of Breast Carcinoma

There was 100% cytohistocorrelation found in the cases (medullary, mucinous, metaplastic carcinoma) which was similar to the study done by Charusheela et.al.

Conclusion

As per the present study, it could be said that this grading system will be fruitful in the prognostication of malignant breast lesions shortly. The parameters used in Robinson cytological grading are found to influence the cytological score. These grading parameters were effectively used and aptly correlated in the present study. The three components of Elston and Ellis's modification of Scarff Bloom Richardson grading were found to contribute to prognosis independently. The overall concordance on cytology and histology was 81.05%. A positive correlation was noted between cytological grade and histological grade. Keeping the values of FNAC it is concluded that every FNAC report should be incorporated with a cytological nuclear grade for robust clinical management.

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