Correlative Study of Cytological Features in Grading of Invasive Breast Carcinoma

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Abstract

Context: Fine-needle aspiration cytology (FNAC) is a proven diagnostic technique for establishing the benign or malignant character of breast lesions. Several cytological grading systems have been proposed for grading of carcinoma breast, with results similar to histologic grades. Aims: This study sought to evaluate the prognostic value of FNAC in invasive ductal carcinoma of breast by correlating it with histological grade. Settings and Design: Tertiary care hospital, retrospective analytical study. Patients and Methods: One hundred and fifty cases of breast carcinoma that underwent modified radical mastectomy consequent to an FNAC diagnosis were included in the study. Robinson’s grading system and Elston–Ellis modification of Scarff–Bloom–Richardson grading system were used to assign cytologic and histologic grades, respectively. Statistical Analysis: The cytological grades were correlated with the histological grades using \( \chi^2 \)-test and Spearman’s rank correlation coefficient. The individual features of the cytological grades were correlated with the histological grades using Kappa coefficient and \( \chi^2 \)-test. Values were considered significant at \( P < 0.05 \). Results: A statistically significant association was observed between cytologic and histologic grades \((r = 0.97; P < 0.01)\) with sensitivity and specificity, respectively, of 100% and 93.95% for cytological grade 1, 100% and 100% for cytological grade 2 and 100% and 100% for cytological grade 3. Also, a positive correlation was found between each feature of the cytologic grade and the histologic grade \((P < 0.05)\). Among these, a better correlation was demonstrated by cytological features like cell uniformity \((\text{Kappa coefficient} = 0.50)\) and appearance of nucleoli \((\text{Kappa coefficient} = 0.52)\). Conclusions: Robinson’s cytologic grading system is a reliable grading method on FNAC smears of cases of carcinoma breast. It correlates well with Elston–Ellis modification of Scarff–Bloom–Richardson grade in invasive ductal carcinoma of breast.

Keywords: Elston–Ellis modification of Scarff–Bloom–Richardson grading system, fine-needle aspiration cytology, invasive duct carcinoma, Robinson’s grading system

Introduction

Breast carcinoma is the most common malignancy and the leading cause of death from cancer among women. Prognosis of invasive ductal carcinoma of breast is determined by tumor size, nodal status, metastasis, tumor type, histological grade, presence of estrogen and progesterone receptors, Her 2 neu amplification status, and cell proliferation markers. Of these, the histological grade assumes importance as it is a widely evaluated parameter on pathological specimens and can be used in isolation to evaluate the prognosis of a tumor.

The most widely used system for histological grading of breast cancer has been the Elston–Ellis modification of Scarff–Bloom–Richardson grading system. This system is based on architectural and cytological features. Both these features have been found to correlate with prognosis and are being used together worldwide to achieve uniformity and standardization of reporting.

Fine-needle aspiration cytology (FNAC) is a proven diagnostic technique in clinical practice, but its use is limited to establishing the benign or malignant character of a given lesion and where possible to provide a morphological diagnosis. The cytopathological grading of breast carcinoma is not commonly attempted. Robinson and several other authors have shown that this method can provide additional information about intrinsic features of a tumor and have proposed cytological grading systems with results similar to histologic grades.

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Nuclear grade, evaluated in histologic grading systems, is based on cytological criteria and its application can intuitively be adapted to cytological material.

Standard of care for carcinoma breast is up-front breast conservation surgery (BCS) or neo-adjuvant chemotherapy followed by BCS. The recommended diagnostic modality is guided-core needle biopsy for tissue diagnosis and prognostic and predictive marker assessment prior to instituting therapy. However, facilities for needle biopsy are not widely available. In resource-constrained healthcare centers, FNAC diagnosis and tumor grading offer a viable alternative for garnering adequate pathological information before instituting therapy.

This study is a correlative analysis of features of a cytological grading system with that of a standardized histological grading system for breast carcinoma to identify the most correlative components of the cytological grading system.

**Patients and Methods**

A total of 150 cases of invasive ductal carcinoma of breast, where preoperative FNAC evaluation followed by subsequent resection were performed, were studied retrospectively. Cases receiving neoadjuvant therapy were excluded from the study.

Cytological grades were assigned on wet-fixed Papanicolaou-stained smears using the Robinson’s system [Table 1].[4] The grades assigned were based on the sum of the numerical score for each individual parameter of the six cytological features, giving a total score range between 6 and 18. A summation score of 6 to 11 was graded as 1, 12 to 14 as 2, and 15 to 18 as 3. Nuclear size was measured by comparison with adjacent red blood cells (RBCs).

Hematoxylin and eosin-stained slides of primary tumor were then analyzed for histological grade by the Elston–Ellis modification of Scarff–Bloom–Richardson grading system.[3] Statistical analysis was performed using the statistical software package SPSS, version 14.0. The cytological grades were correlated with the histological grades using Kappa coefficient and \( \chi^2 \)-test. Values were considered significant at \( P < 0.05 \).

**Results**

The study group spanned across all three cytological and histological grades, with adequate distribution of cases in each grade [Table 2; Figure 1].

Of 57 cases graded as 1 on cytology, 51 were subsequently found to be grade 1 on histology, while the remaining turned out to be grade 2. All cases graded as 2 and 3 on cytology were also found to be grade 2 and 3, respectively, on histology. Hence, 144 of 150 cases showed the same grade and only six cases differed in their cytologic and histologic grades, establishing a highly positive and significant correlation (\( P < 0.01 \), Spearman’s rank correlation coefficient = 0.97) between the cytological grade and the subsequent histological grade [Table 2]. The sensitivity and specificity, respectively, were 100% (confidence interval of 93.02–100.00%) and 93.95% (with confidence interval of 87.27–97.74%) for cytological grade 1, 100% (confidence interval of 93.02–100.00%) and 100% (confidence interval of 96.23 to 100.00%) for cytological grade 2, and 100% (confidence interval of 90.97–100.00%) and 100% (confidence interval of 96.73–100.00%) for cytological grade 3.

In addition, each feature of Robinson’s cytological grading system showed a statistically significant correlation (\( P < 0.05 \)) with the histological grade [Table 3]. Among these, cell

**Table 1: Robinson’s grading system**

| Features       | Score 1                  | Score 2                  | Score 3                  |
|----------------|--------------------------|--------------------------|--------------------------|
| Dissociation   | Cell in clusters         | Single, with cell clusters | Mostly single cells      |
| Nuclear size   | 1–2×RBC size             | 3–4×RBC size             | ≥5×RBC size              |
| Cell uniformity| Monomorphic              | Mildly pleomorphic           | Highly pleomorphic        |
| Nucleoli       | Indistinct               | Noticeable               | Prominent/abnormal        |
| Nuclear margins| Smooth                   | Folds                    | Clefts/buds              |
| Chromatin      | Vesicular                | Granular                 | Clumped and cleared       |

Grade 1: 6-11; Grade 2: 12-14; Grade 3: 15-18

**Figure 1:** Correlation of cytological grades with histological grades. (a) Cytological Grade 1 (Papanicolaou stain, x400). (b) Histological Grade 1 (H and E, x100). (c) Cytological Grade 2 (Papanicolaou stain, x400). (d) Histological Grade 2 (H and E, x100). (e) Cytological Grade 3 (Papanicolaou stain, x400). (f) Histological Grade 3 (H and E, x400)
uniformity (Kappa coefficient = 0.50) and appearance of nucleoli (Kappa coefficient = 0.52) showed a better correlation with histological grade. All tumors assigned a score of 1 for cell uniformity on cytology were also found to be grade 1 on histology. In addition, although only 39% of histological grade 1 tumors were assigned a cell uniformity score of 1, 73% of histological grade 2 tumors and 95% of histological grade 3 tumors were assigned scores 2 and 3 respectively for cell uniformity. Similarly, the appearance of nucleoli, too, was found to be important. While 78% of the histological grade 1 tumors were assigned a score of 1 for appearance of nucleoli, 57% of histological grade 2 tumors and 74% of grade 3 tumors were assigned scores of 2 and 3, respectively, for cytological assessment of nucleoli. None of the histological grade 1 tumors had a cytological nucleolar score of 3, just as none of the histological grade 3 tumors had a cytological nucleolar score of 1. This stresses the importance of cell uniformity and appearance of nucleoli in predicting the histological score.

**DISCUSSION**

Histopathological grading using Elston–Ellis modification of Scarff–Bloom–Richardson grading system is the gold standard for evaluation of grade of carcinoma breast. A number of studies have confirmed the prognostic value of histological grade in invasive ductal carcinoma of breast.[2,3] Cytological studies, on fine-needle aspirated breast carcinoma material, have developed a number of scoring systems with results similar to those obtained from histological sections.[4-10]

In our study, we compared each component of Robinson’s cytological grading system and the overall grade achieved by this grading system for breast carcinoma with histological grading using Elston–Ellis modification of Scarff–Bloom–Richardson grading system.

The study has shown an agreement between cytological and histological grades in 144 of the 150 cases establishing a significant correlation ($P < 0.01$, Spearman’s rank correlation coefficient = 0.97) between the two grading systems. In six cases, a discrepancy was observed, where cases assigned grade 1 on cytology were assigned grade 2 on histology. This may have resulted due to the inability of the cytological grading system to objectively assess the degree of tubule formation and mitotic index, both of which are components of the histological grade. Robinson’s cytological grading system relies on cell dissociation as a surrogate for tubule formation, as a means to assess the degree of differentiation of a carcinoma. In well-differentiated carcinomas, cohesive clusters on smears reflect tubular structures, whereas a widespread distribution of isolated malignant cells reflects diffuse infiltration of breast tissue by malignant cells.

Cellular pleomorphism, on the contrary, is a prominent feature of both cytological and histological grading systems, thus contributing to the similarity in the corresponding grades.

| Table 2: Correlation of the cytological grade with the histological grade |
|---|---|---|---|---|---|---|
| Grades | Histological 1 | Histological 2 | Histological 3 | Total | $P$ | Spearman rank correlation coefficient |
| Cytological 1 | 51 | 6 | - | 57 | <0.01 | 0.97 |
| Cytological 2 | - | 54 | - | 54 | | |
| Cytological 3 | - | - | 39 | 39 | | |
| Total | 51 | 60 | 39 | 150 | | |

| Table 3: Correlation of cytological features with histological grade |
|---|---|---|---|---|---|---|
| Cytological feature scores | Histological grade 1 | Histological grade 2 | Histological grade 3 | $P$ | Kappa coefficient |
| Cell dissociation 1 | 28 (55%) | 07 (12%) | 00 (0%) | <0.05 | 0.34 (Fair) |
| Cell dissociation 2 | 20 (39%) | 46 (76%) | 26 (67%) | | |
| Cell dissociation 3 | 03 (06%) | 07 (12%) | 13 (33%) | | |
| Nuclear size 1 | 14 (27%) | 00 (0%) | 00 (0%) | <0.05 | 0.23 (Fair) |
| Nuclear size 2 | 34 (67%) | 18 (30%) | 00 (0%) | | |
| Nuclear size 3 | 03 (06%) | 42 (70%) | 39 (100%) | | |
| Cell uniformity 1 | 20 (39%) | 00 (0%) | 00 (0%) | <0.05 | 0.50 (Moderate) |
| Cell uniformity 2 | 29 (56%) | 44 (73%) | 02 (05%) | | |
| Cell uniformity 3 | 02 (05%) | 16 (27%) | 37 (95%) | | |
| Nucleoli 1 | 40 (78%) | 14 (23%) | 00 (0%) | <0.05 | 0.52 (Moderate) |
| Nucleoli 2 | 11 (22%) | 34 (57%) | 10 (26%) | | |
| Nucleoli 3 | 00 (0%) | 12 (20%) | 29 (74%) | | |
| Nuclear margin 1 | 22 (43%) | 05 (08%) | 00 (0%) | <0.05 | 0.29 (Fair) |
| Nuclear margin 2 | 29 (57%) | 55 (92%) | 32 (82%) | | |
| Nuclear margin 3 | 00 (0%) | 00 (0%) | 07 (18%) | | |
| Chromatin pattern 1 | 21 (41%) | 07 (12%) | 00 (0%) | <0.05 | 0.31 (Fair) |
| Chromatin pattern 2 | 30 (59%) | 50 (83%) | 25 (64%) | | |
| Chromatin pattern 3 | 00 (0%) | 03 (05%) | 14 (36%) | | |
assigned to the tumors by the two systems. Pleomorphism is a subjective intrasample morphologic variation of nuclear size and shape. On cytology, four components of cell pleomorphism were assessed separately—cell uniformity (similarity of cell appearance), nuclear size, nuclear outline and chromatin pattern, each adding strength to the one feature of the histological grading system which can be reliably assessed on cytological smears.

The significant correlation between the cytological and the histological grades as observed in our study is supported by the studies of Robinson et al., Frias et al., and Pradhan et al. who had used the same grading system as us. Moroz et al. and Khan et al., using their respective cytological grading systems, have demonstrated similar results.

In addition, our study showed a significant correlation ($P < 0.05$) between the score of each cytological feature and the corresponding histological grade. Robinson et al. and Khan et al., in their studies, have found similar results. This would be expected because each of the cytological features reflects two of the three parameters evaluated in the histological grade.

Of the six cytological features evaluated in our study, cell uniformity (Kappa coefficient = 0.50) and large, abnormal nucleoli (Kappa coefficient = 0.52) were found to have a better correlation with the histological grade. Cell uniformity, as reflected by nuclear pleomorphism, has been found to be a significant feature in Moroz’s and Robles-Frias’ study. This feature is straightforward to assess and correlates with nuclear pleomorphism on histology, which is one of the components of the Bloom Richardson system. Cell uniformity is of paramount interest in cytological examination because tubule formation and mitotic index are difficult to evaluate cytologically.

The appearance of nucleoli is the other important feature that we found to correlate well with the histological grade. The appearance of nucleoli has also been noted as important by Moroz et al. and Khan et al. in predicting the histological grade.

To conclude, our study has shown that cytological grading of breast carcinoma by Robinson’s system reliably correlates with histological grading by Elston–Ellis modification of Scarff-Bloom-Richardson grading system, and therefore, it is possible to accurately grade invasive ductal carcinoma of breast on FNAC. This study has identified the most reliable cytological features of Robinson’s system in grading of FNAC smears of invasive duct carcinomas, i.e., cell uniformity and nucleolar prominence. This allows accurate prognostic evaluation, in addition to diagnosis on simple FNAC material, without additional morbidity or expense to the patient. This is particularly valuable in resource-constrained healthcare facilities where patients are exposed to neoadjuvant therapy prior to definitive surgery based on preoperative FNAC diagnosis.

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**Conflicts of interest**
There are no conflicts of interest.

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