



ROBINSON CYTOLOGICAL GRADING OF BREAST CARCINOMA AND ITS COMPARISON WITH ELSTON'S MODIFICATION OF BLOOM-RICHARDSON HISTOPATHOLOGICAL GRADING

Pathology

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ABSTRACT

Objectives Breast carcinoma is the second most common malignancy in women. It is a malignant with heterogeneous prognosis. Because of the enhanced effects of new chemotherapeutic agents on the survival of patients with breast cancer, there has been an increased interest in the use of prognostic factors like Lymph node status, tumor size, nuclear grade, steroid receptor content, tumor type and cellular proliferation rate. The cytological grading system introduced by Robinson et al. corresponds to the histologic grading method of Elston's modification of Scarff Bloom Richardson. The present study was undertaken to correlate the cyto-histological grading of invasive breast carcinomas and to assess the importance of grading.

Methods The present study was conducted on a sample size of 62 patients for over a span of 26 months. 62 Patients who underwent FNAC and mastectomy for breast carcinoma were cytologically and Histologically Graded. The results obtained by both grading methods were then compared.

Results In the present study, the age of the subjects ranged from less than 40 to above 60 years. All were female. On comparison of the cytological and histological grades, 93% cases were grade III, 91% cases were grade II, 55% were grade I both on cytology and histology. Cytological grading of tumor shows strong agreement with histologic grading of tumors. (weighted kappa = 0.821)

Conclusion The present study proves that, a simple cytological grading helps in evaluating the prognosis of the patient and provides varied treatment options depending on the grade.

KEYWORDS:

Breast cancer, cyto-histological correlation, Scarff Bloom Richardson Histopathological Grading, Robinson Cytological Grading

Introduction

Breast carcinoma is the common malignancy next to the cervical cancer and leading cause of death in women.[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 so that the evaluation of prognostic parameters in breast cancer is of growing interest.[2] Histological 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 tumor grading and has been found to have good prognostic correlation.[4] In the recent year FNAC is increasingly being used for primary diagnosis means preoperative diagnosis of breast cancer. As neoadjuvant chemotherapy is gaining popularity as primary modality of medical treatment of breast cancer, much attention is being focused on grading of breast cancer on FNAC smears.[5] Such grading would allow assessment of the tumor in situ, provides relevant information on the aggressiveness of tumor, its tendency to produce regional metastasis and there by guiding the selection of most suitable treatment before primary surgery and hence avoiding the morbidity associated with overtreatment of low-grade tumors.[6] There is much to be gained by grading a tumor on FNAC of the different cytological grading methods corresponding to Elston and Ellis's histological grading, method described by Robinson et al. was found to be useful in grading breast carcinoma in FNAC.[6]

The purpose of the present study is to find utility of grading breast carcinoma on FNAC as per the criteria proposed by Robinson in 1994 and compare it with histological grading based on method proposed by Nottingham's modification of Bloom and Richardson system by Elston and Ellis.

Material and methods

Sixty two cases of infiltrating ductal carcinoma (IDC) breast, diagnosed on cytology, and undergoing surgery were included in the study. Cytological smears stained with haematoxylin and eosin (H&E), Giemsa, and Papinacolaou's stain were evaluated and tumor was graded based on the grading system described by Robinson et al. (table 1). In the Robinson's grading system, six different cytological parameters, namely cell dissociation, cell size, cell uniformity, nucleolus, nuclear margin, and nuclear chromatin were used to grade the tumors. A score of 1-3 was given to each of these parameters and the tumor was graded by adding up the scores. Tumor that were scored in the range of 6-11 were grade I, those that are scored in the range of 12-14 were grade II and grade III was given for a score ranging from 15 to 18.

Table 1: Robinson Grading System [6,7,8,9]

| sr no. | criteria | score 1 | score 2 | score 3 |
|--------|-------------------|-------------------|--|--------------------------|
| 1 | cell dissociation | mostly in cluster | mixture of single cells and cells in cluster | mostly single cells |
| 2 | cell size | 1-2 | 3-4 | >5 |
| 3 | cell uniformity | monomorphic | mildly pleomorphic | pleomorphic |
| 4 | nucleoli | indistinct | noticeable | prominent or pleomorphic |
| 5 | nuclear margin | smooth | folded | buds/clefts |
| 6 | chromatin | vesicular | granular | clumped and clear |

The surgical specimens stained with H&E were evaluated and histologically graded according to the Elston's modification of Bloom-Richardson system (table 2). Three parameters were taken into consideration: Degree of tubule formation, nuclear pleomorphism, and mitotic figures. Each parameter was given a score of either 1, 2 or 3. The overall score for each case ranged from 3 to 9.

Table 2: Bloom Richardson Grading System [10,11]

| FEATURE | SCORE |
|--|-------|
| Tubule formation | 1 |
| Majority of tumor >75% | 2 |
| Moderate degree 10-75% | 3 |
| Little or none <10% | |
| Nuclear pleomorphism | 1 |
| Small, uniform | 2 |
| Moderate increase in size/variation | 3 |
| Marked variation | |
| Mitotic counts – per 10 hpf (40x fields) | 1 |
| 0-5 | 2 |
| 6-10 | 3 |
| >11 | |

Statistical analysis

Comparison was done between these two grading systems and concordance rates were calculated between two grading systems. Kappa coefficient was calculated to compare the agreement. Sensitivity and specificity of Robinson's cytological grading method were calculated for each cytological grade.

Results

Sixty two cases were cytologically graded on FNAC smears according to Robinson's grading system and subsequently graded on histology using Elston's modification of Bloom-Richardson grading system. The maximum number of cases i.e. 28 (45%) were cytological grade III (Fig 3), 23(37%) cases were cytological grade II (Fig 2) and 11(8%) cases were cytological grade I (Fig 1). On histological grading, 28(45%) cases belonged to histological grade III and grade II, and 6 (10%) cases belonged to histological grade I. out of 11 cases with cytological grade I, 6 cases (55%) were of grade I also on histopathology, while 5 cases were histologically upgraded to grade II. Out of 23 cases with cytological grade II, 21 cases (91%) were of grade II also on histopathology, while 2 cases (9%) were histologically upgraded to grade III. Out of the 28 cases with cytological Grade III, 26 (93%) were of Grade III also on histopathology while 2 cases (7%) were of Grade II on histopathology.

Table 3: Distribution of cases according to cytology and histology grading along with comparison between two grading system

| Cytological grades | Histological grades | | | | | | Total | | Concordance rate(%) |
|----------------------|---------------------|----|-----|----|-----|----|-------|-----|---------------------|
| | 1 | | 2 | | 3 | | No. | % | |
| | No. | % | No. | % | No. | % | | | |
| 1 | 6 | 55 | 5 | 45 | 0 | 0 | 11 | 18 | 54.54% |
| 2 | 0 | 0 | 21 | 91 | 2 | 9 | 23 | 37 | 91.30 |
| 3 | 0 | 0 | 2 | 7 | 26 | 93 | 28 | 45 | 92.85 |
| Total | 6 | 10 | 28 | 45 | 28 | 45 | 62 | 100 | |
| Absolute Concordance | | | | | | | | | 85.48% |

Table 3 shows that concordance rate between grade I tumors in cytology and histology was 54.54% (6 cases), for grade II tumors was 91.30% (21 cases) and for grade III it was 92.85% (26 cases). The absolute concordance rate between all three corresponding grades was 85.48% (53/62). Cytological grading of tumor showed strong agreement with histologic grading of tumor. (weighted kappa - 0.821)

Table 4 : Calculation of sensitivity and specificity of Robinson cytological grading system in each cytological grade considering histological grade as base*

| Cytology Grade | No. of true positive cases | No. of false positive cases | No. of true negative cases | No. of false negative cases | Sensitivity (%) | Specificity (%) |
|----------------|----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------|-----------------|
| I | 6 | 5 | 51 | 0 | 100 | 91.07 |
| II | 21 | 2 | 32 | 7 | 75 | 94.11 |
| III | 26 | 2 | 32 | 2 | 92.8 | 94.11 |

*Total no. of cases =62

Table 4 shows that in cytological grade I tumors, the sensitivity of Robinson's cytological grading system was 100% that's because of 0 number of false negative cases and specificity was 91.07%, in cytological grade II tumors the sensitivity was 75% as the number of false negative cases were 7 and specificity was 94.11%. and in cytological grade III tumors the sensitivity was 92.85% and specificity was 94.11%.

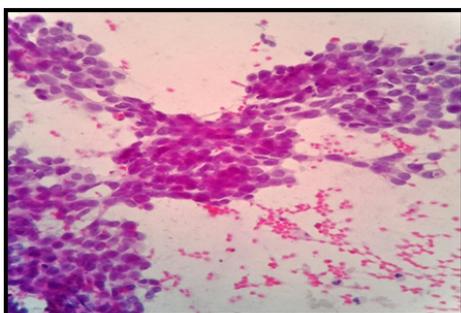


Figure 1: Cytologic grade I showing loosely cohesive cluster of mild pleomorphic ductal cells with smooth nuclear membrane and indistinct nucleoli (H & E 400 x)

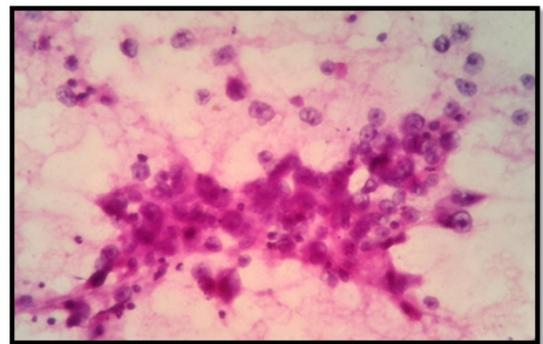


Fig 2: Cytologic grade II showing loosely cohesive cluster as well as single cells with moderate pleomorphism, slightly irregular nuclear membrane and noticeable nucleoli (H & E 400 x)

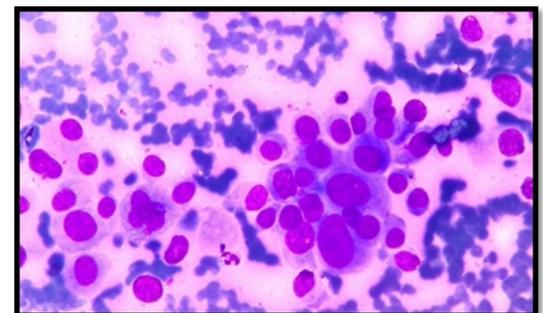


Fig 3: Cytological grade III tumor showing cells with highly pleomorphic nuclei, prominent nucleoli, and budding of nuclear membrane (MGG stain, 400x)

Discussion

FNAC is a routinely used investigation for rapid diagnosis of breast cancer. The ability to predict the accurate grade on cytology smears would add to the diagnostic value of FNAC, without any additional morbidity or expense for the patients.[5] The advantage of FNAC in the diagnosis of breast carcinoma has 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.

Before studying various criteria for grading it was ensured that the FNAC was performed excluding the areas of necrosis and excessively sclerotic areas, material was adequate, smears were cellular, cell morphology was well preserved excluding ruptured cells and H&E, Papanicolaou's and Giemsa stained slides were available. For ensuring accurate results, all the slides were studied and predominant pattern was considered for scoring, while avoiding taking average of scores of two slides.

The degree of cell dissociation is an indicator of cell cohesion status and the degree of expression of e-cadherin/catenin complex. Loss of cell cohesion appears to facilitate vascular infiltration by tumor 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.[12,13] In our study for cell dissociation we got 75% (n=47) cases were given score 2 followed by 15% (n=9) cases of score 3 and 10% (n=6) cases of score 1.

Cell size was assessed by comparing the size of tumor cell with adjacent red cell present in the aspirate. [12,13] In cell size study we got 49% (n=30) of cases with score 3 followed by 45% (n=28) of score 2 and 6% (n=4) cases of score 1.

Pleomorphism is one of the constant features of breast carcinoma. 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. [14]

Pleomorphism was judged independent of cell dissociation and cell size features, as large cells can be large through (cell size) without

varying much among themselves (cell uniformity).[14] In our study we got 59%(n=36) cases with score 3 for cell uniformity followed by 35% (n=22) cases of score 2 and 36%(n=4) cases of score 1.

Robinson et al found the presence and the character of nucleoli valuable in grading independently as well in combination with the cytological feature. [6] An increase in the size and the number of nucleoli is a well-known characteristic of cells engaged in growth and synthesis.[15]In nucleoli 56%(n=35) cases got score 2, 26% (n=16)of cases got score 3 and 18%(n=11) got score 1.

When nuclear margins taken into consideration for scoring 56%(n=35) cases were given score 2, 31%(n=19) cases were given score 3 and 13%(n=8) cases were given score 1.

Chromatin pattern differentiates the nuclei of well and poorly differentiate breast carcinoma but not statistically significant because subjective variability as well as our eye resolution limitation.[16] In present study morphology of chromatin 58% (n=36) cases got score of 2, 32% (n=20) cases got score 3 and 10% (n=6) cases got score 1.

H&E, Pap and Giemsa stained slides were considered for all cases. H&E stained smears were must for chromatin, while Giemsa stained smears were indispensable for judging Nuclear margins.

Table 5 :Distribution of cases according to Robinson's cytologic grade

| TOTAL SCORE | GRADE | NUMBER | PERCENTAGE OF CASES (%) |
|-------------|-------|--------|-------------------------|
| 6-11 | I | 11 | 18 |
| 12-14 | II | 23 | 37 |
| 15-18 | III | 28 | 45 |
| | TOTAL | 62 | 100 |

Table 5 shows that grade III tumors were the commonest comprising of 45% followed by grade II tumors (37%). There were only 11 cases of grade I tumor in our study.

Table 6 : Comparative study of cytological grading of breast carcinoma

| Cytog rade | Robin son et al[6] (1994) | Tanig uchi et al[17] (2000) | Pandi t et al[18] (2000) | Das et al[19] (2003) | Chha bra et al[20] (2003) | Meen a et al[21] (2006) | Nazoo ra khan et al[22] (2009) | Faroo q at el[23] (2010) | Our study |
|------------|---------------------------|-----------------------------|--------------------------|----------------------|---------------------------|-------------------------|--------------------------------|--------------------------|-----------|
| I | 34.07 | 32.23 | 34.66 | 29 | 30 | 31 | 30.23 | 25.45 | 18 |
| II | 43.77 | 40 | 34.66 | 46 | 51.66 | 56.33 | 41.87 | 41.82 | 37 |
| III | 22.06 | 27.77 | 30.68 | 25 | 18.34 | 12.67 | 27.90 | 32.73 | 45 |

Table 6 shows that Robinson et al[6] (1994),Taniguchi et al[17] (2000), Pandit et al[18] (2000), Das et al[19] (2003), Chhabra et al[20](2005), Meena et al[21] (2006), Nazoora Khan et al[22] (2009) and Farooq et al[23] (2010) found grade II tumors the commonest on cytology while in our study grade III tumors were the commonest comprising of 45% followed by grade II tumors 37%.

Table 7: Distribution of cases in which correlation was available according to histopathological grades

| TOTAL SCORE | GRADE | NUMBER OF CASES | PERCENTAGE OF CASES(%) |
|-------------|-------|-----------------|------------------------|
| 3-5 | I | 6 | 10 |
| 6-7 | II | 28 | 45 |
| 8-9 | III | 28 | 45 |
| | TOTAL | 62 | 100 |

Table 7 shows that Among the cases in which both cytology and histology were available, grade III tumors and grade II shows equal incidence (45%) and 6 (10%) cases were of grade I.

Table 8: Comparison of histological grade with other studies

| GRADE | Shet et al[24] | Muddaw a[25] | Nidal m. almasri et al[26] | Dutta et al[27] | OUR STUDY |
|-------|----------------|--------------|----------------------------|-----------------|-----------|
| I | 2 | 14.6 | 3.2 | 10.6 | 10 |

| | | | | | |
|-----|----|------|----|------|----|
| II | 28 | 36.4 | 37 | 76 | 45 |
| III | 70 | 49 | 41 | 13.4 | 45 |

Table 8 shows that Shet et al[24] , Muddawa[25], Nidal M. Almasri et al[26] found grade III tumors the commonest as in our study, while Dutta et al[27] found grade II tumors more common than grade III.

Table 9 : Showing comparison of cyto-histo grading correlation with other studies

| Histo grade Cyto grade | DAS ET AL[19] | | | CHHABRA ET AL[20] | | | MEENA ET AL[22] | | | NAZOOR A KHAN ET AL[24] | | | OUR STUDY | | |
|------------------------|---------------|----|-----|-------------------|----|-----|-----------------|----|-----|-------------------------|----|-----|-----------|----|-----|
| | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III |
| I | 7 | 6 | 2 | 11 | 6 | 1 | 19 | 3 | 0 | 12 | 1 | 0 | 6 | 5 | 0 |
| II | 2 | 20 | 2 | 5 | 21 | 5 | 4 | 32 | 4 | 2 | 15 | 1 | 0 | 21 | 2 |
| III | 0 | 3 | 10 | 1 | 3 | 7 | 0 | 1 | 8 | 0 | 1 | 11 | 0 | 2 | 26 |
| TOTAL | 9 | 29 | 14 | 17 | 30 | 13 | 23 | 36 | 12 | 14 | 17 | 12 | 6 | 28 | 28 |

Table 9 shows that Das et al[19], Chhabra et al[20] , Meena et al[22] and Nazoora khan et al[24] like in our study, found good correlation between Robinson cytologic grade and Elston's modification of Bloom- Richardson system.

Thus besides from having minimal subjective discomfort, insignificant complication, negligible risk of tumor spread, rapidity of diagnosis, utility for multiple lesion, readily repeatability, high accuracy of FNAC; FNAC grading is comparable with histology grading and is useful in assessing the tumor behavior and prognosis and guiding neoadjuvant chemotherapy. Now –a-days, attempts have been made to determine various prognostic parameter on FNA material. The National Cancer Institute Bethesda sponsored conference had also recommended that tumor grading on FNA material should be incorporated in FNA reports for prognostification.[28]

Conclusion

Our study shows that it is possible to grade breast cancer on FNAC aspirates and that the cytological grade corresponds well with the histological grade. The cytological grade also provide relevant prognostic information regarding the aggressiveness of the tumor. Thus, it is suggested that a conscious effort should be made to include the cytological grade in all the FNAC reports of breast cancer so as to guide the surgeon regarding the judicious use of neoadjuvant therapy.

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