Original Research Article

Fine Needle Aspiration Cytology of Breast Carcinoma: A Comparative Study between Cytological and Histopathological Grading System with Lymph Node Status Assessment

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International Archives of Integrated Medicine, Vol. 3, Issue 10, October, 2016.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 01-09-2016 **Accepted on:** 22-09-2016
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: Agarwal AA, Tambekar MY, Dhar R. Fine Needle Aspiration Cytology of Breast Carcinoma: A Comparative Study between Cytological and Histopathological Grading System with Lymph Node Status Assessment. IAIM, 2016; 3(10): 27-35.

Abstract

Introduction: Breast carcinoma is the most common malignancy and the leading cause of death in women. Fine needle aspiration cytology (FNAC) plays a critical role in early diagnosis of any palpable breast lump.

Aim and objectives: The aim of the study was to find out the utility of grading malignant breast tumours using Robinson's cytology grading on FNAC and correlating it with Modified Bloom Richardson grading along with lymph node status assessment post-operatively by histopathological examination.

Materials and methods: A retrospective study was conducted; cytological smears of 30 cases of breast carcinomas on FNAC were graded according to the Robinson's cytology grading and correlated with Modified Bloom Richardson histopathological grading system over a period of 1 year.

Results: There were a total of 28 (93.33%) cases of invasive ductal carcinoma, 1 case of mucinous carcinoma and 1 case of Metaplastic carcinoma. All patients were females with a mean age of 52.8 years. The correlation showed 85.71% concordance between the two grading systems with a 93.33% agreement and an observed weighted Kappa value of 0.916 implying a substantial strength of agreement. Chi-square statistical test performed for lymph node metastasis with grade of the tumor was statistically significant with a *p*-value of 0.0189 ($p<0.05$).

Conclusion: Cytological grading of malignant breast carcinoma aspirates will help in the management and planning future treatment including preoperative neoadjuvant chemotherapy. We recommend it to be followed by all cytopathologists, in order to bring uniformity in the reporting of breast FNAs for grading the malignant lesions.

Key words

Biopsy, Fine needle; Breast neoplasms, Lymph node.

Introduction

Breast carcinoma is the most common malignancy and the leading cause of death in women. More than 1,000,000 cases occur worldwide annually. Triple diagnosis refers to the concurrent use of clinical examination, mammography and FNAC for diagnosing palpable breast lumps. Among these, Fine needle aspiration cytology (FNAC) plays a critical role in early diagnosis of any palpable breast lump. FNAC has become popular as a valuable tool in preoperative assessment of breast masses and it shows high accuracy, sensitivity and specificity. The procedure is safe, non-invasive, inexpensive and easy to perform, requires no advanced preparations and can be easily carried out in OPD with minimal complications.

Many factors other than clinical stage like tumor type, histological grading, hormone receptor status, DNA ploidy, cell proliferation markers and expression of different oncogenes determine prognosis in a given patient [1].

There are various cytological grading system such as Fisher's modification of Black's nuclear grading [2-4], Robinson's grading [5, 6], Howell's modification of SBR's system [7], Mouriquand's [8, 9] however, almost all the cytological parameters correlated with Robinson's system in predicting the final cytological grade [10].

Histological grading of breast carcinoma using Nottingham method described by Elston and Ellis is a widely accepted tumor grading system and has been found to have good prognostic correlations [11].

Materials and Methods

A retrospective study of all malignant breast lumps with both cytology and histopathology evaluated at our Department of Pathology, MGM Medical College, Navi Mumbai over a period of 1 year (January 2015 to December 2015) was conducted. The aim of the study was to evaluate the role of scoring system on aspirates of breast carcinoma and to compare cytological grading system with histopathological grading system of breast carcinoma. The inclusion criteria for the study were patients of all age groups and both sexes and cases that were cytologically diagnosed as having breast carcinomas and confirmed on histopathology. The exclusion criteria included non-malignant lesions of the breast and cytologically proven breast carcinomas without histopathological confirmation. The clinical details were obtained from the medical records department and from the cytology requisition forms, histopathology requisition forms accompanying the specimens.

Papanicolau (PAP) stained slides for cytologic evaluation and H&E stained slides for histopathologic evaluation from archives were assessed and scored. Evaluation was done with the use of Robinson's cytological grading system (RCGS, **Table - 1**) for breast aspirates and Modified Bloom Richardson grading system (BRS, **Table - 2**) for histopathology.

Ethical committee approval was obtained prior to the commencement of the study.

The data were analyzed using Microscoft Excel for Windows and Epi-info Version 6.0, CDC, Atlanta, GA. The agreement between the methods was assessed by using Kappa Statistics

and Chi-square test was used to evaluate the significance of correlation among two parameters.

Results

The present study included a total of 30 cases of malignant breast lumps, of which 29 were diagnosed as invasive ductal carcinoma (IDC) and 1 case of mucinous carcinoma on FNAC. Out of the 29 cases of IDC, 1 case was reported as metaplastic carcinoma on histopathology. The reason of discordance could have been the heterogeneity of the tumor that might not have been sampled by FNAC. Thus, 28 (93.33%) cases of IDC, 1 case of mucinous carcinoma and 1 case of metaplastic carcinoma were reported on histopathology. All patients enrolled in the study were females. The mean age at the time of diagnosis was 52.8 years.

A 93.33% agreement was observed on Kappa analysis of the cytological grades and histopathological grades with a weighted Kappa value of 0.916 implying a substantial strength of agreement.

Chi-square statistical test performed for lymph node metastasis with grade of the tumor was statistically significant with a *p*-value of 0.0189 ($p<0.05$) however, lymph node metastasis was seen irrespective of cytological tumor grade. There was no correlation between the cytological grade and lymph node status. The chi-square statistic is 2.8689 with a *p*-value is 0.238. The result is *not* significant at $p< .05$.

Table - 1: Cytological grading according to Robinson's Grading System.

Score	1	2	3
Cell dissociation	Cell mostly in clusters	Mixture of single cells and clusters	Mostly single cells
Cell size	1-2 times size of RBC	3-4 time size of RBC	>= 5 times size of RBC
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct	Noticeable	Prominent or pleomorphic
Nuclear margins	Smooth	Slightly irregular/folds and grooves	Buds and clefts
Chromatin	Vesicular	Granular	Clumped and cleared

Table - 2: Histological grading of breast carcinoma - Modified Blood Richardson Grading System.

Score	1	2	3
Tubule formation	Tubular formation in > 75% of the tumor	Tubular formation in 10 to 75% of the tumor	Tubular formation in <10% of the tumor
Nuclear pleomorphism	Nuclei with minimal variation in size and shape	Nuclei with moderate variation in size and shape	Nuclei with marked variation in size and shape
Mitotic count per 10 high power fields	0-5/HPF	6-10/HPF	>11/HPF

Discussion

FNAC is widely used as one of the primary pre-operative diagnostic tool for the evaluation of all palpable breast lumps and along with clinical and radiological evaluation (Triple diagnostic tool) with high sensitivity and specificity. Breast carcinoma is the leading cause of morbidity and mortality in females and also cause grave anxiety, hence early diagnosis and workup in such cases is mandatory and of great significance. In cases of

breast lumps diagnosed as malignant, cytological grading by FNAC can predict tumor behavior, prognosis and also guide the treating surgeon to plan surgery and neo adjuvant chemotherapy.

Evaluation of cytological tumor grade is simple, quick, cost effective and easy to perform and correlates well with the histopathological grading system. Various cytological grading systems are available, however we chose Robinson's Cytological grading system (**Table - 1**) because in the past, studies performed by different authors revealed an absolute concordance when compared with gold standard BRS system and was considered a better method due to its simplicity, specificity and better reproducibility. This system takes into consideration 6 criteria viz cell dissociation, cell size, uniformity, nucleoli, nuclear margin and chromatin. The total of these scores were then graded as Grade I (score 6 to 11), Grade II (score 12-14) and Grade III (scores 15-18).

The histopathological grading was done using Modified Bloom Richardson grading system (**Table - 2**) that took into consideration tubule formation, nuclear pleomorphism and mitotic activity. The total of these scores were then graded as Grade I (score 3-5), Grade II (score 6-7) and Grade III (scores 8-9).

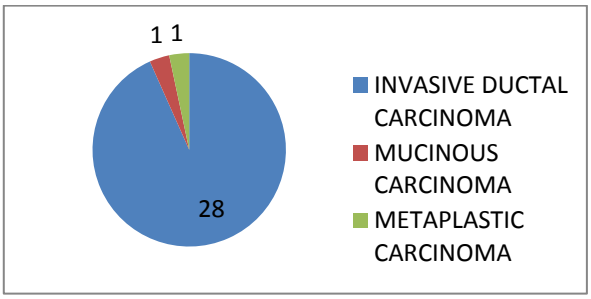
In our study, majority of the patients belonged to the age group of 41-50 years (8 cases, 26.66%) followed by 51-60 years (7 cases, 23.33%) with an overall age range of 29 to 77 years. The mean age of 52.8 years in our study (**Table - 3**) was similar to a study conducted by Ravikumar G, et al. with a mean age of 52.17 years (age ranging from 28-98 years) [18], and also a study conducted by Das S, et al. who had a mean age of 43 years (age ranging from 30-78 years) [19].

On histopathology, 28 (93.33%) cases were of invasive ductal carcinoma, 1 case of metaplastic and 1 case of mucinous carcinoma were diagnosed in this study (**Figure - 1**). Out of the 25 cases enrolled in the study by Sandeepa S, et al., 21 cases (84%) belonged to invasive ductal carcinoma, 1 case each of mixed ductal and lobular carcinoma, tubular carcinoma, metaplastic carcinoma and medullary carcinoma [20]. Saha K, et al. also had predominantly invasive ductal carcinoma (53 out of 57 cases, 93%) followed by 4 cases of invasive lobular carcinoma [10]. A study conducted by Gore CR, et al. reported out of 58 cases, 48 cases (82.76%) of invasive ductal carcinoma, 4 cases of invasive papillary, 2 cases of mucinous, and 1 case of medullary and sarcomatoid carcinoma [17]. In a study conducted by TS Rekha, et al., out of the 50 cases, 40 cases were invasive ductal carcinoma, 5 cases of mucinous carcinoma, 2 cases of invasive lobular carcinoma, 2 cases of tubular mixed cell carcinoma and 1 case of medullary carcinoma [15].

Table - 3: Age Distribution.

Age group (Years)	No. of cases
21-30	2 (6.6%)
31-40	6 (20%)
41-50	8 (26.66%)
51-60	7 (23.33%)
61-70	6 (20%)
71-80	1 (3.3%)
TOTAL:	**30 (100%)**

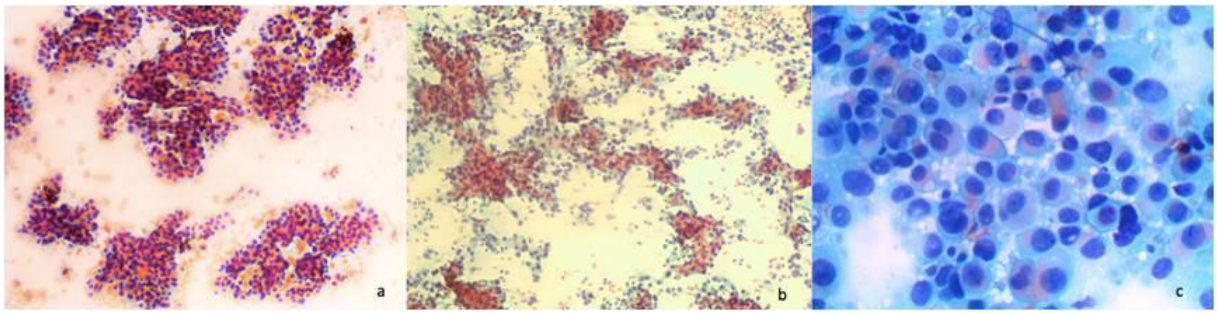
Figure - 1: Types of Breast Carcinomas.



Robinsons cytology grading took into consideration 6 parameters. Grading was done by 1st and 2nd author and a consensus was achieved after reviewing discrepant scoring to reach a unanimous final score and grade for each case (**Table - 3**). In this study for cell dissociation, 22 (73.3%) cases were given score 2 followed by 1 case (3.4%) a score of 1 and 7 cases (23.3%) a score of 3. Cell size was assessed by comparing

the size of tumor cell with adjacent red blood cell present in the aspirates in which 20 cases (66.6%) were scored 2; while 7 cases (23.4%) scored 1 and 3 cases (10%) scored 3. For cell uniformity, 17 and 13 cases were scored 2 and 3 respectively. No case was scored 1 inferring that cellular pleomorphism was a constant feature in our study. In assessing nucleoli, 16 cases (53.4%) were scored 2 while the remaining 5 (16.6%) and 9 (30%) cases were scored 1 and 3 respectively. For nuclear margin, 17 cases (56.6%) were given a score of 2 and 10 (33.4%) were scored 1 and 3 (10%) were scored 3. Chromatin morphology in 24 cases (80%) was scored 2 while 4 cases (13.4%) and 2 cases (6.6%) were scored 1 and 3 respectively. After scoring the aspirates for these parameters, a total score was obtained for each case and graded according to grades I, II or III if the total score was between 6-11, 12-14 and 15-18 respectively (**Figure - 2**). In a study conducted by Gore CR et al maximum cases were scored 2 for cell dissociation, nucleoli, nuclear margin and chromatin pattern, findings that were similar and correlated with our study [17]. However, maximum cases for 2 parameters, i.e. cell size and cell uniformity was scored 3 which did not correlate with our study.

Figure - 2: Cytology of breast carcinomas, PAP stain. a) Shows predominantly cells arranged in clusters, x10. b) Tumor cells arranged in both clusters and singly scattered, x10. c) Tumor cells are dyscohesive with marked nuclear pleomorphism, x40.



In our study, 10 cases (33.4%) were graded as Grade I, 14 (46.6%) as Grade II and 6 (20%) as Grade III on cytology. Gore CR et al had similar observations with maximum cases belonging to Grade II (44, 75.86%), followed by Grade III (8, 13.79%) and Grade I (6, 10.35%) [17]. Wani FA, et al. in their study had 46 cases (41.81%) of Grade II followed by 36 cases (32.72%) of Grade III and 28 cases (25.45%) of Grade I, which showed a substantial higher percentage of high-Grade III tumors when compared with our study as per **Table - 6** [21].

All cases were confirmed and scored histologically by BRS score. This grading system took into consideration 3 parameters (**Table - 4**). The first, percentage of tubule formation, 24 cases (80%) were scored 2 while 6 cases (20%) were scored as 3. No case was scored as 1, inferring that breast carcinomas in our study did not show more than 75% tubule formation. 21 cases (70%) were scored 2, 6 cases (20%) scored 3 and 3 cases (10%) scored 1 while assessing the amount of nuclear pleomorphism. Finally, 14 cases (46.6%) were given a score of 2, 13 cases (43.4%), a score of 1 and remaining 3 cases (1%) a score of 3 for mitotic count per 10 high power fields (**Figure - 3**). Gore CR, et al. had similar findings with parameters of tubule formation and nuclear features with maximum cases reported a score of 2 followed by score 3 and 1 [17]. However, maximum cases for mitotic count were scored 3 which was different from our study.

In our study after BRS scoring on histopathology, the grading system showed 11 cases of Grade I, 12 cases of Grade II and 7 cases of Grade III [15]. In a study of 38 cases conducted by Patil VS, et al. reported a much lower incidence of Grade I (1 case) followed by

Grade III (17 cases) and Grade II (20 cases) cases [22].

Number of cases which on cytology scoring were graded as I and III showed 100 percent concordance on BRS histopathological grade. However, 14 cases graded as II on cytology, 12 had a positive concordance on histopathology, whereas 2 cases showed discordance. Out of two discordant cases, 1 case was over-graded and 1 case was down-graded. The absolute concordance was 85.71%. Robinson's system revealed the best kappa value of agreement (κ = 0.897; substantial agreement), maximum percent of agreement (93.33%; 28 out of 30 cases) with a weighted kappa value of 0.916 (**Table - 5**) which was interpreted according to Landis and Koch classification. Wani FA et al also had similar findings which revealed that most of the discordance was seen in Grade II tumors [21]. Of the 46 (41.81%) cases graded as Grade II cytologically, 39 cases were graded as Grade II whereas two cases were down-graded as Grade I and 5 cases were over-graded as Grade III histologically [21]. Various studies have reported concordance of more than 50% (**Table - 7**). The diagnostic accuracy of the present study is 100% which correlates well with Chiemchanya et al and Rupom TU, et al. [23, 24]. Das S, et al. found Robinson's cytological grading's absolute concordance with histological grading in 37 cases (71.2%) but they considered Robinson's method as a better choice due to its simplicity, specificity and better reproducibility [19]. Saha K, et al. in their study also observed clustering of cases in lower grades in Robinson's system which was comparable with our study [10].

Figure - 3: H&E stain, x40 a) Tubule formation by tumor cells, b) Nuclear pleomorphism in grade 3 tumor with inset showing numerous mitoses.

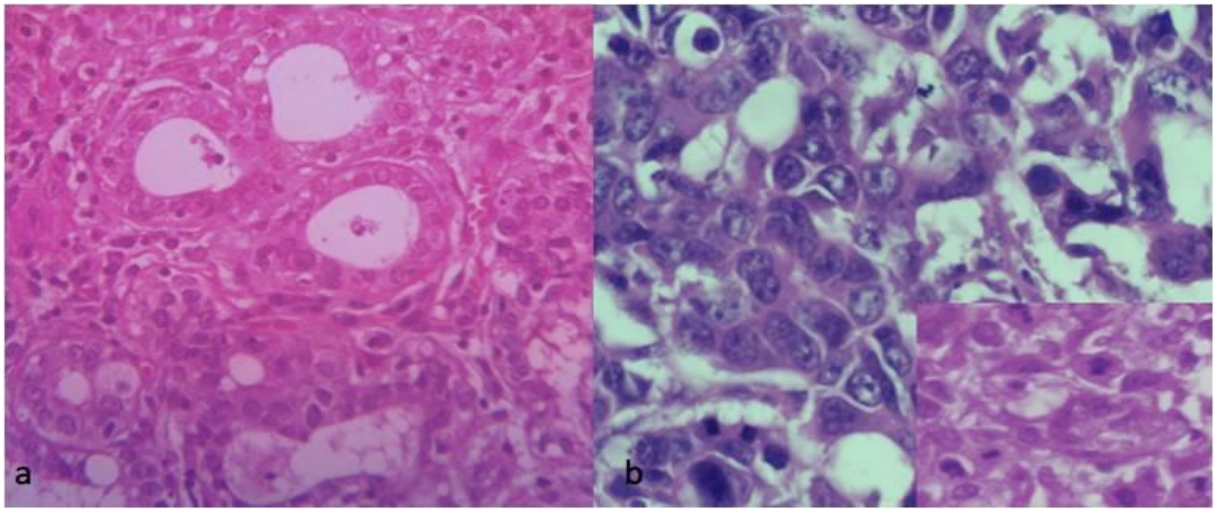


Table - 4: Cytology Scoring Distribution according to Robinson's Grading System.

Scores / Parameters	1	2	3
Cell dissociation	1(3.4%)	22(73.3%)	7(23.3%)
Cell size	7(23.4%)	20(66.6%)	3(10%)
Cell uniformity	0	17(56.6%)	13(43.4%)
Nucleoli	5(16.6%)	16(53.4%)	9(30%)
Nuclear margins	10(33.4%)	17(56.6%)	3(10%)
Chromatin	4(13.4%)	24(80%)	2(6.6%)

The cause for discrepancy may have resulted due to the fact that in cytological grading, nuclear features were predominantly considered for grading whereas the degree of tubule formation and mitotic count were not assessed on cytology which was a similar cause in studies conducted by different authors [18]. Observer and inter-observer variability and subjectivity can also add to the discordance in assigning the scores. Large tumor size or tumor heterogeneity is known in cases of breast carcinomas which is a pitfall of FNAC where entire tumor cannot be assessed at a given time.

Table - 5: Histological Scoring System - Modified Blood Richardson Grading System.

Scores / Parameters	1	2	3
Tubule formation	0	24 (80%)	6 (20%)
Nuclear pleomorphism	3(10%)	21 (70%)	6 (20%)
Mitotic count per 10 high power fields	13 (43.4%)	14 (46.6%)	3 (1%)

Table - 6: Concordance and Comparison between two grading systems.

Cytologic grade	No. of cases in Cytological grading	No. of cases in Histological grading			Concordance rate
		Grade I	Grade II	Grade III	
I	10	10	0	0	100%
II	14	1	12	1	85.71%
III	6	0	0	6	100%
TOTAL	**30**	**Absolute Concordance: 93.33%**			

Table - 7: Comparison of Concordance rates reported in different studies.

Authors	Concordance rate (%)
Robinson, et al. (1994) [6]	56.9
Das A K, et al. (2003) [12]	71.2
Robles-Frias A, et al. (2005) [13]	97.0
Nazoor Khan, et al. (2009) [14]	89.1
TS Rekha, et al. (2011) [15]	82.0
Pandya Amrish N, et al. (2012) [16]	74.5
Chandanwale Shirish, et al. (2013) [17]	82.7
Present Study	85.7

Lymph node status was assessed in all cases, a total of 14 cases showed positive lymph node metastases out of 30 cases. On histopathology, out of the 11 cases Graded I, 2 cases (18.18%) had lymph node metastasis, whereas 12 cases Graded II, 6 cases (50%) had lymph node metastasis and remaining out of 7 cases graded III, 6 cases (85.71%) had lymph node metastasis (**Table - 8**). Chi-square test performed had statistical score of 7.92 with a p-value of 0.01 ($p<0.05$), which was statistically significant. However, cytological grading did not correlate with lymph node metastases and was statistically insignificant (p-value of 0.23), a finding contrasting with the findings of Wani FA, et al. [21]; Patil VS, et al. had similar findings where among 38 cases, 20 cases showed nodal metastasis which accounted for 52.63%. The maximum number of Grade III cases (60%) showed lymph node metastases followed by Grade I and Grade II carcinomas with 50% and 43.75% of cases respectively [22].

Table - 8: Lymph Node Metastasis.

	GRADE I	GRADE II	GRADE III
Total cases	11	12	7
Node positive cases	2	6	6
Node negative cases	9	6	1
% Positive cases	18.18%	50%	85.71%

Conclusion

FNAC is a simple, cost effective and minimally invasive technique, which provides useful preoperative diagnosis. Cytological grading of malignant breast carcinoma aspirates will help in the management and planning future treatment including preoperative neoadjuvant chemotherapy. We recommend it to be followed by all cytopathologists, in order to bring uniformity in the reporting of breast FNAs for grading the malignant lesions. We propose that the sign out reports of all breast FNAs from malignant lesions should include the cytological grading as well.

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