Comparative analysis of six cytological grading systems in breast carcinoma

Shirish S. Chandanwale, Neha Mishra, Supreet Kaur, Sourabh Paranjape, Aditi Pandey, Megha Jha

Department of Pathology, Dr. D. Y. Patil Medical College, Pune, Maharashtra, India

ABSTRACT

Background: Fine-needle aspiration cytology (FNAC) gives the necessary information of various breast lesions for surgical management of patients and can be extremely useful for establishing the best neoadjuvant therapy in patients where surgical removal of tumor is not the best option. The National Cancer Institute, Bethesda sponsored conference recommended that the tumor grading on FNAC should be incorporated in reports for prognostication. **Objective:** The purpose of this study is to determine the correlation between cytomorphologic and histomorphological features and to evaluate the utility of different cytological grading systems and to determine which cytology grade corresponds best to the histology grade. **Materials and Methods:** The present prospective study included fifty cases of breast carcinoma diagnosed on FNAC and confirmed on histopathology. Detailed cytomorphological features were studied. Cytology grading was done using six grading systems. Detailed histological features were studied, and carcinomas were graded as per modified Scarff-Bloom-Richardson's histopathological grading system. Cytology grading was correlated with histology grading. **Results:** Out of fifty cases of breast carcinomas, the 29 (58%) cases of each Robinson's cytological grading and Fisher's modification of Black grading correlated with histological grading. The cytological features, namely, nuclear chromatin, nuclear pleomorphism, cell size, nuclear margins, and naked tumor nuclei used in grading correlated well with histological grade. **Conclusion:** Cytology grading adds to objectivity, reproducibility, and authenticity to the report. The cytology features such as nuclear chromatin, nuclear pleomorphism, cell size, nuclear margins, and naked tumor nuclei had influential role in predicting the final cytology grade. Among the six cytology grading systems, Robinson's system is simple to interpret, more objective, takes less time, and is reproducible and correlates precisely with histological grade as evident from our study. It should be used for routine evaluation of aspirates of breast carcinoma.

Key words: Aspiration, breast, cytology, fine needle, grade

INTRODUCTION

Breast carcinoma is the most common malignant tumor, and the leading cause of death in women, with more than 1,000,000 cases occurring worldwide annually.[1-3] The increasing trend of its incidence in urban population of developing world is because of changing to Western lifestyles.[4-8] Fine-needle aspiration cytology (FNAC), radiological imaging (mammography and ultrasonography), and clinical examination (triple assessment), have become the standard approach, to the investigation of palpable breast lumps. FNAC gives the necessary information of various breast lesions for surgical management of patients.[9,10]

The information provided by FNAC can be extremely useful while establishing the best neoadjuvant therapy for patients in cases where surgical removal of tumor is not the best option.[11-13] The National Cancer Institute, Bethesda sponsored conference has also recommended that the cytology tumor grading should be incorporated in reports for prognostication.[14,15]

The present study was conducted over a period of 2.5 years prospectively using six cytology grading systems, namely, Robinson's cytological grading, Khan's grading, Fisher's

Address for correspondence: Dr. Shirish S. Chandanwale, Department of Pathology, Dr. D. Y. Patil Medical College, Pimpri, Pune - 411 018, Maharashtra, India. E-mail: shirishchandanwale@gmail.com

modification of Black's nuclear grading, Hunt's nuclear grading, Mouriquand's grading, and Taniguchi grading. The cytology grade was compared with histology grading by modified Scarff-Bloom-Richardson's (SBR's). The purpose of this study was to determine the correlation between cytomorphologic and histomorphological features and to evaluate the utility of different cytological grading systems and to determine which cytology grade corresponds best to the histology grade.

MATERIALS AND METHODS

The present study included fifty cases of breast carcinoma. Inclusion criteria were all breast carcinomas diagnosed on FNAC and confirmed on histology in mastectomy specimens. Exclusion criteria were patients having a history of preoperative chemotherapy or radiotherapy for breast carcinoma.

Fine-needle aspiration was done with aseptic precautions and using FNAC gun. The contents of aspiration were smeared on the glass slides. Smears were made, and air-dried smears were stained with Leishman's stain and alcohol-fixed smears were stained with hematoxylin and eosin stain. Detailed cytomorphological features were studied, and grading was done using six cytology grading systems.

Adequate representative tissue sections from the mastectomy specimens were formalin fixed and paraffin processed. 3–5 thick micron sections were cut and stained with hematoxylin and eosin. Detailed histological features were studied, and breast carcinomas were graded as per modified SBR's grading system.

Concordance value among different cytological features, absolute concordance rate, and the linear-weighted kappa value were calculated using the software OpenEpi, CDC, Atlanta, Georgia, USA.

RESULTS

Out of fifty cases, the twenty cases were between 51 and 60 years, followed by ten cases each in age group 41–50 and 61–70 years, eight cases belonged to age group 31–40, and two cases belonged to the age group 21–30 years. The 32 patients had carcinoma in the left breast and 18 patients had in the right breast.

Table 1 shows cytology grading by five systems and SBR histology grading of fifty cases. Hunt *et al.* used only nuclear features for grading breast carcinoma into high-grade and low-grade [Table 2]. Out of 50 cases, 29 (58%) cases of each Robinson's cytological grading and Fisher's modification of black grading correlated with histological grading. Table 3 shows correlation of various cytologic and histological grading systems.

Robinson's cytology grading system showed concordance value of 14.2% for Grade I tumors, 75% each for Grade II and Grade III tumors. The absolute concordance rate was 58.0%. The linear-weighted kappa value was 0.28.

Khan *et al.* grading system showed concordance value of 0% for Grade I tumors, 71.4% for Grade II, and 30.2% for Grade III tumors. The absolute concordance rate was 36.0%. The linear weighted kappa value was 0.01.

Mouriquand's grading system showed the concordance value 0% for Grade I tumors, 59.0% for Grade II tumors and 0% for Grade III tumors. The absolute concordance rate was 52.0%. The linear weighted kappa value was −0.18.

Taniguchi grading system showed the concordance value of 0% for Grade I tumors, 40% for Grade II tumors, and 28.8% for Grade III tumors. The absolute concordance rate was 30.0%. The linear-weighted kappa value was −0.03.

Fisher's grading system showed the concordance value of 0% for Grade I tumors, 70% for Grade II tumors, and 43.3% for Grade III tumors. The absolute concordance rate was 58.0%. The linear-weighted kappa value was 0.23.

Hunt *et al.* nuclear grading system showed the concordance rate of 66.6% for low-grade tumors and 0.42% for high-grade tumors. The absolute concordance rate was 0.80%.

Out of 50 cases, 29 (58%) showed abundant cellularity, 17 (34%) cases showed moderate cellularity, and only 4 (8%) cases showed scanty cellularity. The 16 cases showed correlation between cytology score of cellularity and histology grade.

Table 1: Cytology and histology grading of fifty breast carcinomas according to different grading systems

| Grade | Histology grade | Cytology grade | | | | |
	SBR	Robinson's	Fisher's	Mouriquand's	Khan	Taniguchi
I	3 (6.0)	14 (28)	0	0	0	0
II	32 (64)	28 (56)	27 (54.0)	44 (88.0)	7 (14.0)	5 (10.0)
III	15 (30)	8 (16)	23 (36.0)	6 (12.0)	43 (86.0)	45 (90.0)
Total	50 (100.0)	50 (100.0)	50 (100.0)	50 (100.0)	50 (100.0)	50 (100.0)

SBR: Scarff-Bloom-Richardson's

Table 2: Hunt *et al.*: Grading of fifty breast carcinomas

Nuclear grade	Number of cases (%)
Low-grade	3 (6)
High grade	47 (94)
Total	50 (100)

Table 3: Correlation of various cytology grading systems with histology grade

Cytology grading system	Number of cases correlating with the histology grade (%)
Robinson's grading system	29 (58)
Fisher's modification of black grading system	29 (58)
Mouriquand's grading system	26 (52)
Khan's grading system	18 (36)
Taniguchi grading system	15 (30)
Hunt nuclear grading system	4 (8)

The 25 (50%) cases showed cells predominantly in clusters, 22 (44%) showed mixture of cells and clusters, and only 3 (6%) showed dispersed single cells [Figure 1a-c].

The 28 (56%) cases showed cell size more than five times the size of red blood cell (RBC), and 22 (44%) showed cell size three to four times the size of RBC [Figure 2]. None of the cases showed cell size 1–2 times the size of RBC.

The 31 (62%) cases showed nuclear size three to five times the size of RBC, 18 (36%) cases showed nuclear size more than five times the size of RBC, and only one (2%) case showed nuclear size of less than three times the size of RBC [Figure 2]. The 22 out of 50 cases showed correlation of cytological score of nuclear size with histological grade.

The 37 cases showed a nuclear-cytoplasmic (N/C) ratio of 50:80, 10 cases showed an N/C ratio of >80%, and only three showed an N/C ratio of <50. The 29 cases showed correlation of cytological score of N/C ratio with histological grade.

The 28 (56%) cases showed moderate degree of nuclear pleomorphism, 16 (32%) showed marked nuclear pleomorphism, and 6 (12%) showed mild degree of nuclear pleomorphism [Figure 3]. None of the cases showed uniform nuclei. Total 28 cases showed correlation of cytological score of nuclear pleomorphism with histological grade.

The 17 (34%) showed regular/smooth nuclear margin, 31 (62%) cases showed irregular nuclear margins. The 2 (4%) cases showed buds in the nuclear margin [Figure 4a-c]. The 23 cases showed correlation between cytological score of nuclear margin and histological grade.

The 25 (50%) cases showed noticeable nucleoli and 22 (44%) showed prominent nucleoli. The 3 (6%) cases showed

indistinct nucleoli in our study, 20 cases showed correlation between cytological score of nucleoli and histological grade.

The 45 (90%) cases showed granular chromatin [Figure 5], 4 (8%) showed clumped coarse chromatin, and only 1 (2%) showed vesicular chromatin; the 30 cases showed correlation between cytological score of nuclear chromatin and histological grade.

The 37 (74%) cases showed moderate nuclear hyperchromatism and 13 (26%) cases showed marked hyperchromatic nuclei [Figure 5]. The 22 cases showed correlation between cytological score and histological grade.

In our study, 35 (70%) cases showed naked tumor nuclei of three to five times the size of RBC [Figure 2]. The 22 cases showed correlation of cytological feature of naked tumor nuclei with histological grade.

The 12 (24%) cases showed the presence of necrosis. The 40 (80%) out of 50 cases showed no or mild lymphocytic response and 10 (20%) cases showed moderate lymphocytic response [Figure 6].

DISCUSSION

Neoadjuvant therapy is becoming increasingly popular as a primary medical treatment for breast cancer, the idea of cytology grading is to assess the tumor *in situ* so that most suitable treatment could be selected immediately and the morbidity associated with overtreatment on low-grade tumors could be avoided. In neoadjuvant therapy, Tamoxifen is administered to high-grade tumors which act mainly on proliferating cells thereby reducing the size of tumor. Low-grade tumors are not benefitted by this therapy and results in unnecessary morbidity.[12] Therefore, it is essential not only to diagnose breast carcinoma but also to grade them.

Our study showed a steady increase in the incidence of breast carcinoma with age. Left breast was commonly involved than the right breast. We found predominance of Grade II tumors on FNAC, which is in accordance with many studies.[16-21]

In our study, Robinson's cytology grading and Fisher's modification of black grading systems correlated most well with histology grade as compared to other cytology grading systems [Table 3].

A double-blind study was conducted by Das *et al.*[20] and they concluded that Robinson's cytology grading was a better choice due to its simplicity, specificity, and better reproducibility. Similar observations were made by Frias *et al.*[17] We made same observations in our study.
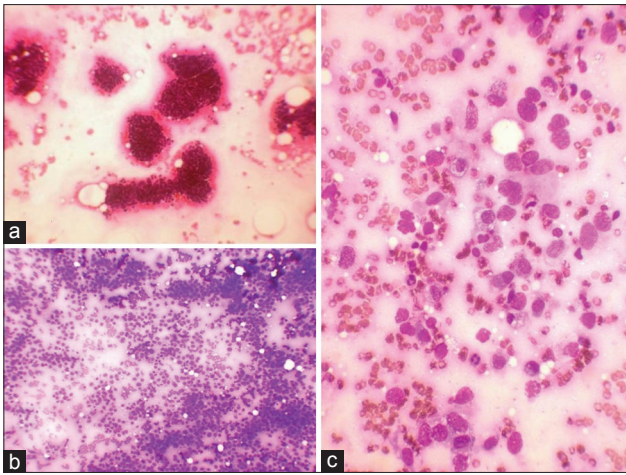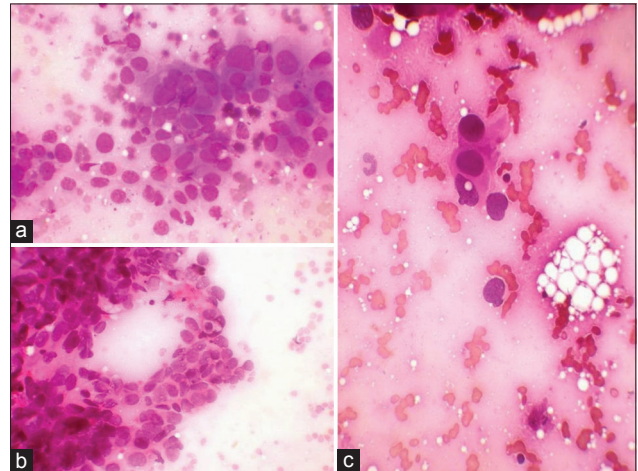
Figure 1: Cytology smears. (a) Cells in clusters (H and E, ×100). (b) Cells dispersed and in clusters (H and E, ×100). (c) Dispersed cells (H and E, ×400)
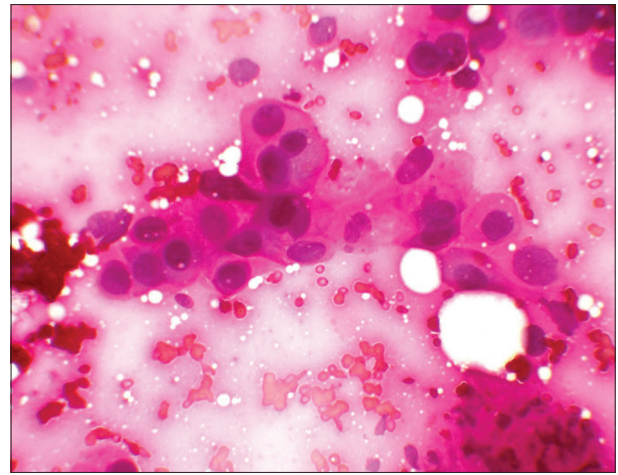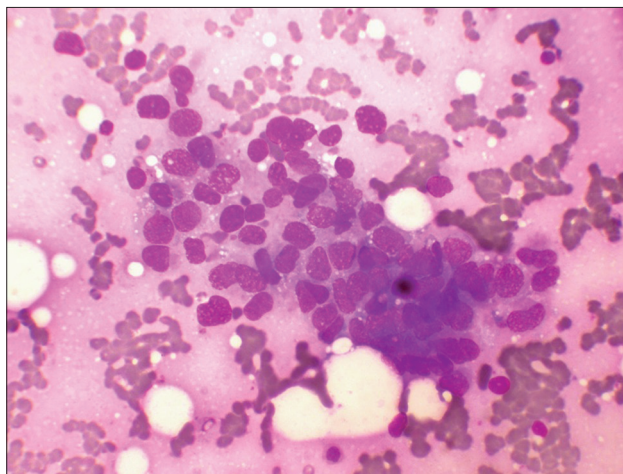

Figure 2: Cytology smears showing size of cells, nuclei, and naked tumor nuclei in comparison with red blood cells (H and E, ×400)
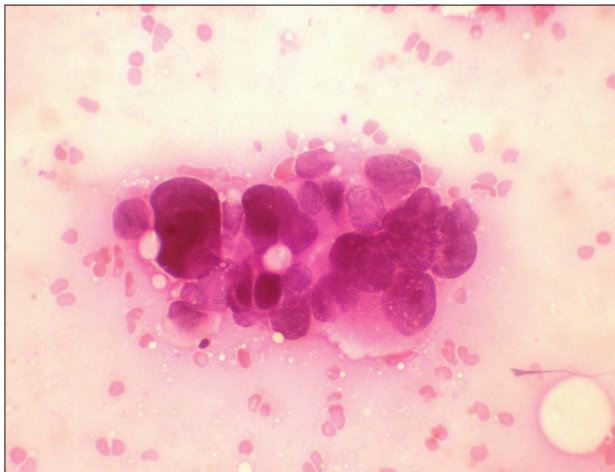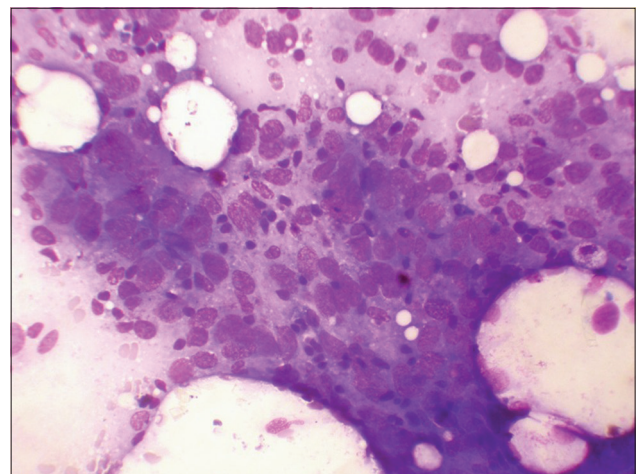

Figure 3: Smears showing nuclear pleomorphism (H and E, ×400)

Figure 4: Smears showing (a) regular nuclear membrane, (b) irregular nuclear membrane, (c) nuclear bud (H and E, ×400)

Figure 5: Smears showing cells with hyperchromatic nuclei (H and E, ×400)

Figure 6: Smears showing loose clusters of tumor cells with lymphocytes on the background (H and E, ×400)

Different agreements were observed in different studies comparing Robinson's cytology grading with histology grading by the Nottingham modification of SBR's system.

The agreement was 57% by Robinson *et al.*,[16] 71.2% by Das *et al.*,[20] 65% by Chhabra *et al.*,[19] 83% by Meena *et al.*,[22]

88.89% by Bhargava *et al.*,[5] 81% by Sinha *et al.*,[23] 88% by Khan *et al.*,[24] and 64% by Lingegowda *et al.*[25] In our study, it was 58%.

The absolute concordance rate for Hunt's nuclear grading in our study was 0.80%. We feel that only nuclear features alone were not adequate on FNAC for grading the breast carcinoma.

Khan *et al.*[24] used cellularity in their grading system, and they observed that the degree of smear cellularity showed no significant correlation with the histological grade. Similar observations were made in our study. The cellularity of the smear depends on the skill of the aspirator and nature of the lesion.

Wallgren *et al.*[26] first evaluated cell dissociation on FNAC and found it to be useful for cytology grading. Subsequently, it was used in Robinson's and Khan. The degree of cell dissociation indicates cell cohesion status and the degree of expression of the E-cadherin/catenin complex. Several studies showed that neoplasms with greater cell dissociation shows a higher incidence of regional lymph node metastasis.[16,27,28] Chhabra *et al.*[19] noticed cell dissociation and nucleoli as the most influential predictive factors of cytology grading. In contrast in our study, 25 (50%) of the cases showed clusters only. In our study, only 19 cases correlated with the final histological grade. We observed cell cohesion was not a very significant factor in cytological grading.

Nuclear size can be assessed by comparing the size of the tumor nuclei to nearby erythrocytes or lymphocytes that have been subjected to similar conditions during smear preparations. Nuclear size has long been established as a significant prognostic parameter for grading of breast carcinoma on FNAC.[29-33] Similar observations were made in our study.

N/C ratio was used by Taniguchi *et al.*[12] and Khan *et al.*[20] in their cytology grading systems. A high N/C ratio is an established criterion of malignancy-associated phenotype. With increasing nuclear volume, there is a reciprocal decrease in the volume of cytoplasm. Ultimately, nuclei are extruded out of fragile cytoplasm even after minor trauma experienced during the procedure.[24] Taniguchi *et al.* found N/C ratio had a nonsignificant correlation with the final grade.[12] In contrast, we found N/C ratio is a useful feature in grading breast carcinoma.

Nuclear pleomorphism is a subjective morphological variation of nuclear size and shape that plays a discriminatory role in FNAC grading of breast carcinoma. The assessment of variation in size and shape of cells in smears is a relatively straightforward criterion that can be easily judged by comparing the size and shape of the adjacent cells with intact cytoplasm for the degree of variability. Cellular pleomorphism was found to be a useful parameter in the previous studies.[29,34-36]

The nuclear margin was used as a cytology feature in Robinson's, Khan, and Fishers grading systems. Normal cells have smooth and rounded nuclei while malignant cells show irregularities of nuclear margins, the degree of which depends on the type and the stage of tumor differentiation. Irregular nuclear margins have been selected as a malignancy-associated phenotype.[24] Nuclear margins investigated in the study by Khan *et al.*,[24] assessed either independently or in combination with other parameters, showed significant correlation with the tumor grade and also showed significant concordance with the histology grades.[16] Similar observations were made in our study.

All the grading systems used presence of nucleoli as a cytology feature in grading of breast carcinoma. The presence of multiple nucleoli was essential for histology grading of breast carcinoma.[36] van Diest *et al.* showed that the a total number of nucleoli was the best single prognostic variable exceeding the value of other nuclear criteria.[37] The presence of nucleoli in histology sections has also been shown to be of prognostic significance.[38] The presence of nucleoli has been found useful in cytological grading both independently and in combination with the other cytological parameters.[16] However, in another study by Thomas *et al.*[34] found no significant concordance of nucleoli with histological grading. In our study, twenty out of fifty cases showed correlation of features of nucleoli on cytological score with histological grade.

In the study, conducted by Robinson's cytological grading system, nuclear chromatin was important in deciding the cytological grade.[16] In our study, 45 cases showed granular chromatin and correlated well with histology grade.

Taniguchi *et al.*[12] used the density of chromatin as a cytological feature in grading breast carcinoma. The 37 (74%) cases showed moderate hyperchromatism, 13 (26%) cases showed marked hyperchromatism. The 22 cases showed a correlation of cytological score of density of chromatin with histological grade.

Naked tumor nuclei size was used as a cytology feature for grading of breast carcinoma by Khan *et al.*[24] In our study, 22 cases showed correlation with histological grade which was significant.

In the study conducted by Khan *et al.*,[24] no significant correlation was found between the cytological parameter

of lymphocytic response and corresponding histological grade. Similar observations were made in our study.

Necrosis was used as a cytology feature to grade breast carcinoma by the grading systems proposed by Taniguchi, Khan, and Mouriquand's. Necrosis in aspirate may be seen in association with *in situ* or invasive carcinoma. The presence of necrosis, therefore, does have a significant practical implication, when interpreted within the proper context.[24] In our study, only 12 cases showed necrosis.

Mitotic activity is an integral part of histology grading systems, and its significance has been documented in the previous studies.[39,40] Significant correlation was found in the study conducted by Khan *et al*.[24] between cytological scores for mitoses and histology grade. The previous studies showed no significant correlations between number of mitoses found on FNAC and in histological sections. This discrepancy can be explained in terms of sampling problem. Similar observations were made in our study.

Cytology grading used by Robinson had the highest concordance rate and the kappa value of agreement (κ = 0.28; fair agreement) followed by Fisher's nuclear grading (κ = 0.23, fair agreement). Khan's grading system showed slight agreement (κ = 0.01). Mouriquand's grading system (κ = −0.18) and Taniguchi's grading system (κ = −0.03) showed less than chance agreement.

In the study conducted by Saha *et al*.,[41] substantial agreement (κ = 0.61–0.80) was observed in histology grading and all cytology grading, except in Taniguchi's grading where the kappa value was in the moderate agreement range (0.41–0.60). The variations of kappa value indicate subjectivity in the assessment of cytology parameters. Robinson's system revealed the best kappa value of agreement (κ = 0.62).

CONCLUSION

Cytology grading mentioned in a cytology report adds to objectivity, reproducibility, and authenticity of the particular report. Cytology grading system in various breast aspirates enables the surgeon to plan definitive treatment for the patient and also avoids unnecessary patient anxiety and unwanted surgery. The cytological features such as nuclear chromatin, nuclear pleomorphism, cell size, nuclear margins, and naked tumor nuclei had an influential role in predicting the final cytological grade. The cytological features correlating with the final histological grade in decreasing order were nuclear chromatin, N/C ratio, nuclear pleomorphism, cell size, nuclear margins, naked

tumor nuclei, density of chromatin, nucleoli, cellularity, cell cohesion, lymphocyte response, mitoses, and necrosis. Among the six cytological grading systems, Robinson's system is simple to interpret, more objective, takes less time and effort, is reproducible and correlates precisely with histological grade as evident from our study. This is because of the multifactorial nature of the system. We conclude that it should be used for routine evaluation of aspirates of breast carcinoma.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-6.
2. Bhatt JV, Shah JM, Shah FS. Pathophysiology of breast lesions: Vision beyond the clinical eye. J Appl Basic Med Sci 2002;4:81-4.
3. Nandakumar A. National Cancer Registry Programme Consolidated Report of the Population Based Cancer Registries 1990-96. ICMR; 2001. Available from: http://www.icmr.nic.in/ncrp/ncrp_p/cancer_reg.pdf. [Last cited on 2016 Jun 01].
4. Wingo PA, Cardinez CJ, Landis SH, Greenlee RT, Ries LA, Anderson RN, *et al.* Long-term trends in cancer mortality in the United States, 1930-1998. Cancer 2003;97 12 Suppl: 3133-275.
5. Bhargava V, Jain M, Agarwal K, Thomas S, Singh S. Critical appraisal of cytological nuclear grading in carcinoma of the breast and its correlation with ER/PR expression. J Cytol 2008;25:58-61.
6. Joshi A, Maimoon S. Limitations of fine needle aspiration cytology in subtyping breast malignancies – A report of three cases. J Cytol 2007;24:203-6.
7. Kaufman Z, Shpitz B, Shapiro M, Rona R, Lew S, Dinbar A. Triple approach in the diagnosis of dominant breast masses: Combined physical examination, mammography, and fine-needle aspiration. J Surg Oncol 1994;56:254-7.
8. Pisani P, Parkin DM, Bray FI, Ferlay J. Estimates of the worldwide mortality from twenty-five major cancers in 1990. Implications for prevention, and projections of future burden. Int J Cancer 1999;83:18-29.
9. Zagorianakou P, Fiaccavento S, Zagorianakou N, Makrydimas G, Stefanou D, Agnantis NJ. FNAC: Its role, limitations and perspective in the preoperative diagnosis of breast cancer. Eur J Gynaecol Oncol 2005;26:143-9.
10. Khan N, Afroz N, Rana F, Khan M. Role of cytologic grading in prognostication of invasive breast carcinoma. J Cytol 2009;26:65-8.
11. Robinson I, McKee G. Cytologic grading of breast carcinoma. Acta Cytol 1995;39:1257.
12. Taniguchi E, Yang Q, Tang W, Nakamura Y, Shan L, Nakamura M, *et al.* Cytologic grading of invasive breast carcinoma. Correlation with clinicopathologic variables and predictive value of nodal metastasis. Acta Cytol 2000;44:587-91.
13. Richards MA, Smith IE, Dixon JM. ABC of breast diseases. Role of systemic treatment for primary operable breast cancer. BMJ 1994;309:1363-6.
14. The National Cancer Institute. Bathesda Conference. The uniform approach to breast fine needle aspiration biopsy. A synopsis. Acta Cytol 1996;40:1120-6.

15. Hunt CM, Ellis IO, Elston CW, Locker A, Pearson D, Blamey RW. Cytological grading of breast carcinoma – A feasible proposition? Cytopathology 1990;1:287-95.

16. Robinson IA, McKee G, Nicholson A, D'Arcy J, Jackson PA, Cook MG, *et al.* Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. Lancet 1994;343:947-9.

17. Robles-Frías A, González-Cámpora R, Martínez-Parra D, Robles-Frías MJ, Vázquez-Cerezuela T, Otal-Salaverri C, *et al.* Robinson cytologic grading of invasive ductal breast carcinoma: Correlation with histologic grading and regional lymph node metastasis. Acta Cytol 2005;49:149-53.

18. Muddegowda PH, Lingegowda JB, Kurpad R, Konapur P, Shivarudrappa A, Subramaniam P. The value of systematic pattern analysis in FNAC of breast lesions: 225 cases with cytohistological correlation. J Cytol 2011;28:13-9.

19. Chhabra S, Singh PK, Agarwal A, Singh SN, Bhagoliwal A. Cytological grading of breast carcinoma-a multivariate regression analysis. J Cytol 2005;22:62-5.

20. Das AK, Kapila K, Dinda AK, Verma K. Comparative evaluation of grading of breast carcinomas in fine needle aspirates by two methods. Indian J Med Res 2003;118:247-50.

21. Masood S, Vass L, Ibarra JA Jr., Ljung BM, Stalsberg H, Eniu A, *et al.* Breast pathology guideline implementation in low- and middle-income countries. Cancer 2008;113 8 Suppl: 2297-304.

22. Meena SP, Hemrajani DK, Joshi N. A comparative and evaluative study of cytological and histological grading system profile in malignant neoplasm of breast – An important prognostic factor. Indian J Pathol Microbiol 2006;49:199-202.

23. Sinha S, Sinha N, Bandyopadhyay R, Mondal SK. Robinson's cytological grading on aspirates of breast carcinoma: Correlation with Bloom Richardson's histological grading. J Cytol 2009;26:140-3.

24. Khan MZ, Haleem A, Al Hassani H, Kfoury H. Cytopathological grading, as a predictor of histopathological grade, in ductal carcinoma (NOS) of breast, on air-dried Diff-Quik smears. Diagn Cytopathol 2003;29:185-93.

25. Lingegowda JB, MuddeGowda PH, Ramakantha CK, Chandrasekar HR. Cytohistological correlation of grading in breast carcinoma. Diagn Cytopathol 2011;39:251-7.

26. Wallgren A, Silfverswärd C, Zajicek J. Evaluation of needle aspirates and tissue sections as prognostic factors in mammary carcinoma. Acta Cytol 1976;20:313-8.

27. Zoppi JA, Pellicer EM, Sundblad AS. Cytohistologic correlation of nuclear grade in breast carcinoma. Acta Cytol 1997;41:701-4.

28. Abati A, Abele J, Bacus SS, Bedrossian C, Beerline D, Bibbo M, *et al*. The uniform approach to breast fine-needle aspiration biopsy. Diagn Cytopathol 1997;81:16-21.

29. Franzén S, Zajicek J. Aspiration biopsy in diagnosis of palpable lesions of the breast. Critical review of 3479 consecutive biopsies. Acta Radiol Ther Phys Biol 1968;7:241-62.

30. Zajdela A, De LaRiva LS, Ghossein NA. The relation of prognosis to the nuclear diameter of breast cancer cells obtained by cytologic aspiration. Acta Cytol 1979;23:75-80.

31. Mouriquand J, Gozlan-Fior M, Villemain D, Bouchet Y, Sage JC, Mermet MA, *et al*. Value of cytoprognostic classification in breast carcinomas. J Clin Pathol 1986;39:489-96.

32. Nguansangiam S, Jesdapatarakul S, Tangjitgamol S. Accuracy of fine needle aspiration cytology from breast masses in Thailand. Asian Pac J Cancer Prev 2009;10:623-6.

33. Mossler JA, McCarty KS Jr., Woodard BH, Mitchener LM, Johnston WW. Correlation of mean nuclear area with estrogen receptor content in aspiration cytology of breast carcinoma. Acta Cytol 1982;26:417-21.

34. Thomas JS, Mallon EA, George WD. Semiquantitative analyses of fine needle aspirates from benign and malignant breast lesions. J Clin Pathol 1989;42:28-34.

35. Kuenen-Boumeester V, Hop WC, Blonk DI, Boon ME. Prognostic scoring using cytomorphometry and lymph node status of patients with breast carcinoma. Eur J Cancer Clin Oncol 1984;20:337-45.

36. Pienta KJ, Coffey DS. Correlation of nuclear morphometry with progression of breast cancer. Cancer 1991;68:2012-6.

37. van Diest PJ, Mouriquand J, Schipper NW, Baak JP. Prognostic value of nucleolar morphometric variables in cytological breast cancer specimens. J Clin Pathol 1990;43:157-9.

38. Black MM, Speer FD. Nuclear structure in cancer tissues. Surg Gynecol Obstet 1957;105:97-102.

39. Kalhan S, Dubey S, Sharma S, Dudani S, Preeti, Dixit M. Significance of nuclear morphometry in cytological aspirates of breast masses. J Cytol 2010;27:16-21.

40. Schulte E, Wittekind C. The influence of the wet-fixed Papanicolaou and the air-dried Giemsa techniques on nuclear parameters in breast cancer cytology: A cytomorphometric study. Diagn Cytopathol 1987;3:256-61.

41. Saha K, Raychaudhuri G, Chattopadhyay BK, Das I. Comparative evaluation of six cytological grading systems in breast carcinoma. J Cytol 2013;30:87-93.