International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2012 Vol. 2 (2) May-August, pp.189-195/Das et al.
Research Article

BREAST CARCINOMA ASPIRATES: A STUDY ON CYTOLOGICAL GRADING

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ABSTRACT

Cytological nuclear grading is one of the most important prognostic factors that should be considered in the study of breast cancer. The aim was to study the importance of the nuclear grading in breast carcinoma in imprint smears along with its histological correlation. The fine needle aspiration smears were stained with Papanicolaou stain and graded using the Robinson's cytological grading system. Subsequently histological grading was performed on their Hematoxylin &Eosin stained tissue sections by Nottingham modification of Scarff-Bloom-Richardson method. The study shows nuclear features of breast carcinoma can play important prognostic factor with significant diagnostic and therapeutic value.

Key Words*: Cytology, Grading, Breast Carcinoma*

INTRODUCTION

Fine needle aspiration cytology (FNAC) is being employed for pre-operative diagnosis of breast lumps (Massod *et al.,* 1995). It is recommended that grading and other prognostic markers should also be included in all FNAC reports of breast carcinoma (Issac *et al.,* 2001)**.** As systemic adjuvant therapy becomes popular as the primary medical treatment for breast cancer in the pre-surgical period, attention is being drawn towards a tumor on FNAC. Such grading would allow assessment of tumor ' in-situ", so that most suitable treatment could be selected immediately on diagnosis and the morbidity associated with over –treatment of low-grade tumors could be avoided (Chhabra *et al.,* 2005).

MATERIAL AND METHODS

The present study was conducted on cytologically proven cases of primary breast carcinoma, diagnosed in the department of pathology of a teaching hospital from 2006-2007. The inclusion criteria was a pre-operative cytological diagnosis of breast cancer, which included mastectomy or partial breast resection with the dissection of axillary lymph nodes.

Wet fixed FNAC smears were stained with Papanicolaou stain and used for reporting. This staining permits assessment of nucleoli, chromatin pattern and nuclear membrane (Scultze *et al.,* 1987). Cell size is measured by comparison with associated red blood cells (Hunt *et al.,* 1901). Cytological grading was done on the smears according to the grading system of Robinson *et al.,* (1994)**.** [Table-1] Grading is done at the time of cytological diagnosis and forms part of the cytology report. Two observers independently reviewed both the FNAC smears and biopsies of all cases. The scores for each of the cytological features were summed to arrive at a total score for a given case which includes (a) Score 6- 11- Grade I (b) Score 12- 14-Grade II (c) Score 15-18- Grade III. Subsequently , the excised specimens were processed and graded according to the Nottingham modification of Scraff-Bloom-Richardson method (SBR) (Bloom, *et al.,* 1957). For histological grading which includes (a) Score 3-5 – Grade –1 (Well differentiated) (b) Score 6-7 – Grade – II (Moderately differentiated) (c) Score 8-9 – Grade III (Poorly differentiated).

The total cytological scores were compared with the final histological grade (Table 1).

Statistical analysis was done. The non-parametric Pearson's correlation test was used to examine the degree of correlation between cytological and histological grades. Multiple regression analysis was used to assess the significance of each cytological feature (Table2).

Table 1: Comparison of histological grade with cytological grade

Cytological grade	Histological grade (I)	Histological grade (II)	Histological grade (III)	Total.
I	14	2	4	20.
II	5	35	10	50
III	5	5	20	30
Total	24	42	34	100

Table 2: Correlation of cytological features with cytological grading.

Cytological Features.	Cytological grade. (r value)	
Dissociation	0.86	
Cell size	0.97	
Cell uniformity	0.95	
Nucleoli.	0.65	
Nuclear margin	0.84	P= 0.000
Chromatin.	0.87	

All the cytological features are showing a strong correlation with cytological grade(p= .000 in each case).

RESULTS

All the hundred patients were females in the age-group of 30-78 years. The mean age at presentation was 43 years. All the cases included were infiltrating duct carcinoma (IDC) of non-specific type (NOS). The size of the tumor varied from 4-15 cms. Maximum number of cases were in the range of 7-9 cms. Most of the cases were in stage T4.None of the cases were in stage T1. Around 37 cases had ipsilateral lymphnode involvement. 7 cases had contralateral lymphnode involvement whereas 4 cases had supra-clavicular lymphnode involvement. None of the cases had distant metastasis. The results were tabulated as shown in Tables 1-4.

DISCUSSION

The evaluation of malignant breast aspirates, as pointed out in an editorial by Katz should provide not only the diagnosis of malignancy and type of tumor, but also information on the cytological grade of duct carcinoma (Katz 1994). The purpose of cyto-prognostic grading is to identify fast growing tumors (Grade-3) (Chhabra, *et al.*, 2005). Fast growing tumors are more likely to respond to chemotherapy than the low grade, slow growing tumors, which are better suited to pre-treatment with Tamoxifen. Assessment of biological aggressiveness by cytological grading without removing the tumor would, therefore, be valuable (Chhabra *et al.*, 2005).

Table 3: Correlation of histological grading with cytological grading

Pearson's correlation	Cytological grade	Histological grade	Cytological score	Histological score
Cytological grade	1.000	0.438	0.987	0.689
Histological grade	0.438	1.000	0.645	0.859
Cytological score.	0.987	0.645	1.000	0.673
Histological score	0.689	0.859	0.673	1.000

The cytological grade showed a positive correlation with the histological grade (r=0.438)

Table 4: Multiple regression analysis of cytological features

Model	Regression coefficients		Significance
	B	**t**	**p**
Constant	0.568	3.113	0.003
Dissociation	0.804	7.064	0.000
Cell size	-5.42	-1.302	0.254
Cell uniformity	1.156	3.789	0.001
Nucleoli	0.708	8.653	0.000
Nuclear margin	0.695	-5.764	0.000
Chromatin	0.593	-1.5432	0.086

This table highlights that cell dissociation, cell uniformity and nucleoli are the most influential features.

The National Cancer Institute (NCI), Bethesda, sponsored conference had also recommended that tumor grading on FNA material should be incorporated in FNA reports for prognostication (Chhabra S, *et al.*, ., 2005). It was also emphasized that the cytologic grading system on FNA specimens should correspond to the grading system used in the histologic material.

Of the various cytological grading methods for breast cancer, the method proposed by Robinson *et al.*, has been found to be better (Mc Divitt, *et al.*, 1996) (Zoppi, *et al.*, 1997). In our study 20 (20%) cases were in cytological grade 1 (Fig1) whereas 50(50%) and 30 (30%) cases were in grade 2 and grade 3 respectively (Fig2) and (Fig3). On histological grading 24 (24%) cases were in grade 1 whereas 42 (42%) and 34(34%) cases were in grade 2 and 3 respectively. There was agreement between cytological and histological grading in 69% of the cases. Using Fisher's modification of Black's nuclear grading , few authors have found 70.73% agreement between cytology and histology (McDivitt *et al.*, 1996, Zoppi, *et al.*, 1997).

Certain cytologist found 95 % agreement (Dabbs *et al.*, ., 1993) whereas others had an agreement of 65% of (Chhabra *et al.*, 2005) (Moroz , *et al.*, 1997). The lack of coordination in 31% of the cases were due to tumor heterogenecity and observer subjectivity when assigning a cytological nuclear grade.

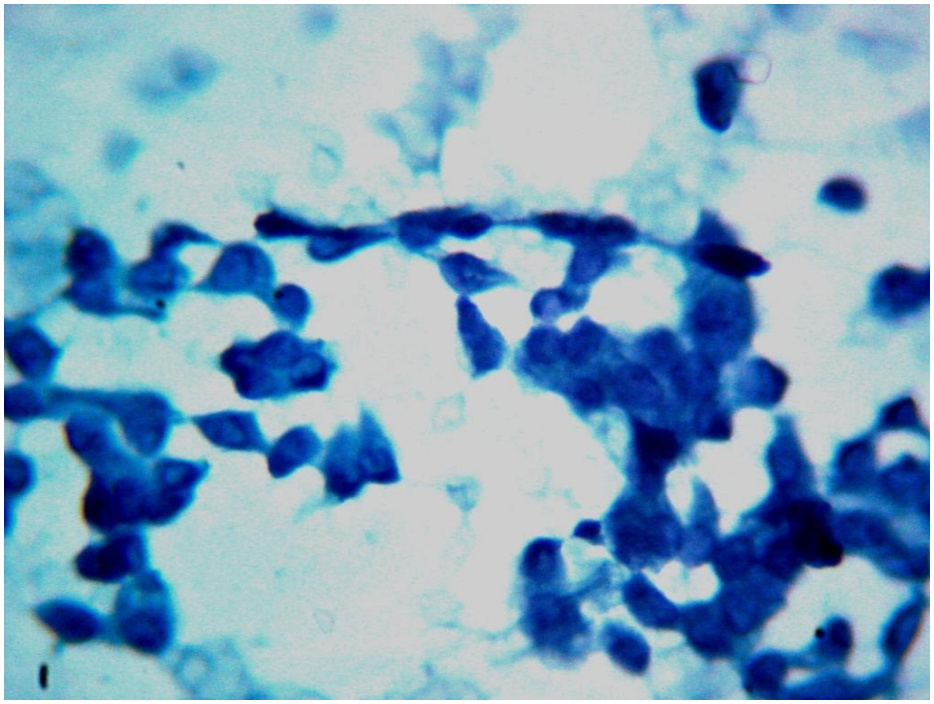

Figure 1: Photomicrograph of FNAC smear: Cytological Grade I showing cells which are monomorphic , having indistinct nucleoli and are lying in clusters(Papanicolaou x 400).

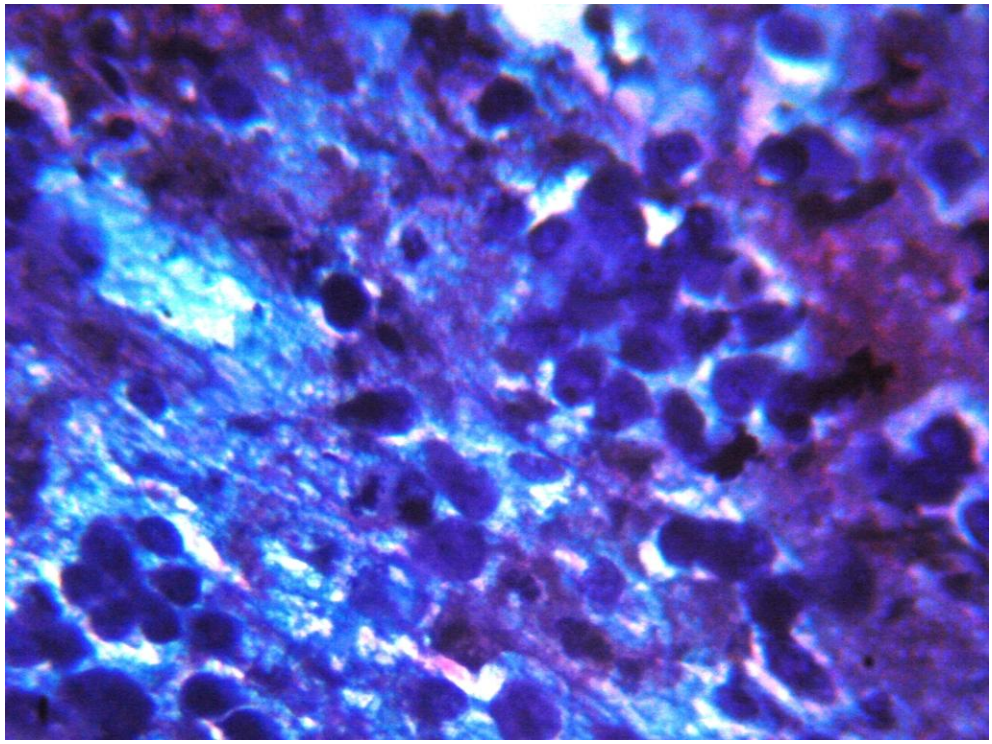

Figure 2: Photomicrograph of FNAC smear: Cytological Grade II showing cells arranged singly with moderate pleomorphism (Papanicolaou x400).

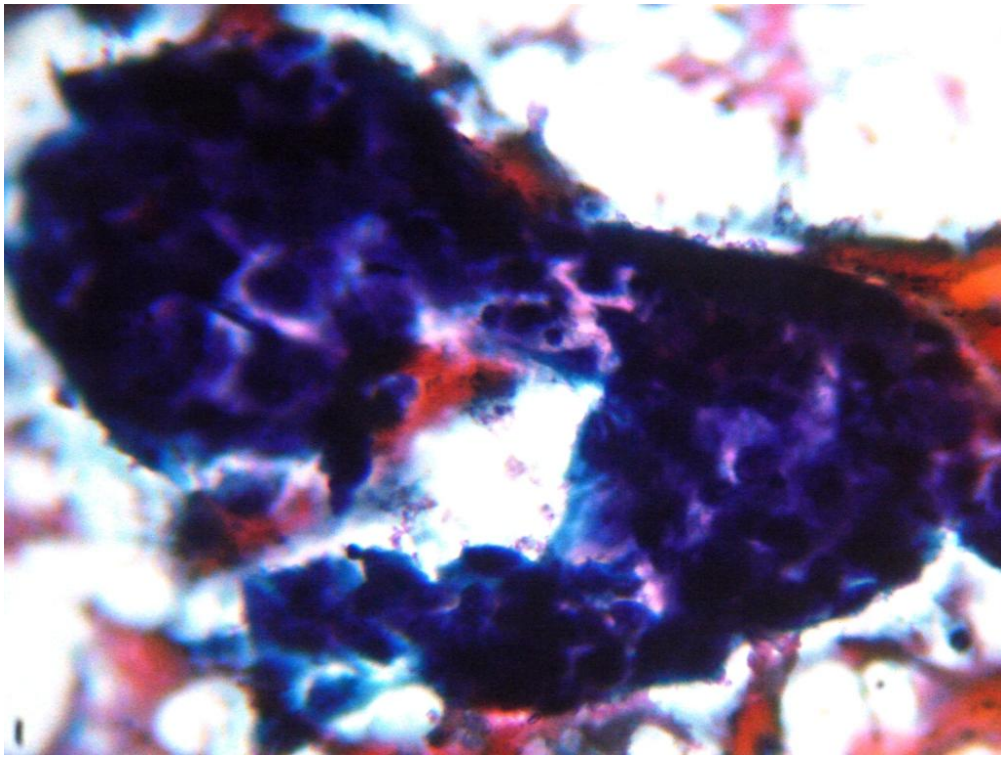

Figure 3: Photomicrograph of FNAC smear: Cytological Grade III showing cells with marked pleomorphism, prominent nucleoli and granular chromatin (Papanicolaoux400)

In the present study, histological grade correlated positively with the cytological grade (r = 0.438). The high value of coefficient of correlation showed a significant and marked association (p <0.001) between the grade assigned to cytological and histological specimens. The relationship was true for the scores to even a larger extent (r =0.673, p =0.000) (Table 3). FNA samples evaluated by Moroz. (Moroz *et al.,* 1997) noted a strong correlation (r =0. 8059, p< 0.0001) between cytological nuclear grade and the modified histological grade, similarly Taniguchi (Taniguchi *et al.,* 2000) observed a significant correlation (r = 0.337, p = 0.0015) between histological and cytological grade.

Multiple regression analysis of cytological features was used to asses the significance of each cytological feature. The coefficients of regression for the following parameters i.e. dissociation, cell uniformity, nucleoli and nuclear margin were 0.804, 1.156, 0.708 and 0.695 respectively and were statistically significant (Table 4). For these 4 parameters the p values were 0.000, 0.001, 0.000 and 0.000 respectively. We have excluded cell size and chromatin from the statistical model as their statistical significance could not be established (p value for cell size was 0.254 and for chromatin was 0.086).The model used for assessing the histological grade is based on following parameters such as Histological grade = 0.568 + 0.804 (dissociation) +1.156 (cell uniformity) + 0.708 (nucleoli) + 0.695 (nuclear margin). This model could predict the histological grade with a great degree of accuracy, as the coefficient of determination (R.Sq.) is 87.3%. Similarly Chhabra *et al.,* (2005) noted a value of R.Sq as 84.1%. However, in contrast to the earlier study, we have included nuclear margin characteristics on Papanicolaou stained cytological smears with a relatively larger sample size.

The overview of all breast cancer adjuvant therapy trials in 1992 showed a long term survival benefit for receiving adjuvant therapy. The Nottingham Prognostic Index is a useful and sensitive guide for selecting adjuvant systemic therapy and there is no reason why such an index should not be developed for neoadjuvant therapy as suggested by Montgomery (Montgomery *et al.,* 2006). Such an index might

imitate the Nottingham Prognostic Index since tumor diameter can be estimated by ultrasound, tumor grade by cytology and lymph node status by a staging lymph-adenectomy while the primary tumor is left in –situ as suggested by Ohri (Ohri, *et al.,* 2006**).**

Mouriquand *et al.,* in their cytological classification of breast carcinoma aspirates, demonstrated that the cyto-prognostication based on three cytological grades stratified the patients into prognostic groups that were distinct (grade1, 95% seven year disease free survival versus 45% for grade 3 (Moriquand, *et al.,* 1986). The results of this study are also in agreement with the study conducted by Sneige (Sneige *et al.,* 1992).

Critics of nuclear grading cite its subjective nature and prefer the data generated by flow cytometry. However, flow cytometry is costly and has not yet established a decisively clear clinical niche for the ploidy and S–phase data it provides as noted by Fechner (Fechner *et al.,* 1991).

Tumor grading, DNA Ploidy and Ki-67 index as prognostic markers in breast carcinoma aspirates was done by Das (Das, *et al.,* 2005). Recent studies have also tried to include other parameters for cytological grading which include apoptosis and bcl-2 as noted by Hemachandran (Hemachandran, *et al.,* 2002). In addition, a immunocytochemical study of ER/ PR receptor (estrogen/progesterone) expression on breast carcinoma aspirates was done by Bhargava *et al.,* (2008).

CONCLUSION

Our study shows that it is possible to grade invasive breast cancer on cytological aspirates and that the cytological grade corresponds well within the histological grade. Moreover, the system used is simple, less tedious and the clinical information obtained is of prognostic value especially when combined with mammographic findings. More studies are necessary to further evaluate whether the discordant cases could be improved by the addition of new features such as the study of the proliferative activity and DNA ploidy pattern of the tumor in the decision making approach of the breast carcinoma aspirates.

REFERENCE

Massod S *et al.*, (1995). Prognostic factors in breast cancer : use of cytologic preparations. *Diagnostic Cytopathology* **13**(1) 388-395.

Issac C, Stearns V and Hayes D (2001). New prognostic factors for breast cancer recurrence. *Seminal Oncology* **28** (2) 53-67.

Chhabra S, Singh PK, Agarwal A, Bhagoliwal A, Singh SN (2005). Cytological Grading of Breast Carcinoma- A Multivariate Regression Analysis. *Journal of Cytology* **22**(3) 62-65.

Scultze E, Wittekind C (1987). The influence of wet fixed Papanicolaou and air dried Giemsa technique on nuclear parameters in breast cancer cytology: a cytomorphometric analysis. Diagnostic Cytopathology **3**(6) 256-261.

Hunt CM, Ellis IO, Elston CW, Locker A, Pearson D, Blamey RW (1901). Cytological grading of breast carcinoma- a feasible proposition ? Cytopathology **1**(9) 287-295.

Robinson IA, McKee G, Nicholson A *et al.*, (1994). Prognostic value of cytological grading of fine needle aspirates from breast carcinoma. *Lancet* **343**(7) 947-949.

Bloom HJG and Richardson WW (1957). Histological grading and prognosis in breast cancer. A study of 1409 cases of which 359 have been followed for 15 years . British Journal of Cancer **11**(9): 359-377.

Katz RL(1994). A turning point in breast cancer cytology reporting: Moving from callowness to maturity. *Acta Cytologica* **38**(12) 881-883.

Pandit AA and Parekh HJ (2000). Cytological grading of breast carcinoma:comparison of four grading systems. *Journal of Cytology* 17 (7) 39-44.

McDivitt RW, *et al.*, (1996). Tumours of the breast. Atlas of tumour pathology, Fascicle 2, 2nd Series. Washington DC: Armed Forces Institute of Pathology.

Zoppi JA, *et al.*, (1997). Cytohistologic correlation of nuclear grade in breast carcinoma. *Acta Cytologica* **4**(6)1701-704.

Dabbs DJ *et al.*, (1993). Role of nuclear grading of breast carcinoma in fine needle specimens. Acta Cytolgica **37**(11) 361-366.
Moroz K *et al.*, (1997). Cytologic nuclear grade of malignant breast aspirates as a predictor of histologic grade. *Acta Cytolgica* **41**(7) 1107-1141.
Taniguchi E *et al.*, (2000). Cytologic grading of invasive breast carcinoma: correlation with clinicopathologic variables and predictive value of nodal metastasis. *Acta Cytolgica* 44 (2) 587-591.
Montgomery E (2006). Soft tissue tumours. In Silverberg's Principles and Practice of Surgical Pathology and Cytopathology. In: Silverberg SG, editor. 4th ed, Elsevier: Churchcill Livingstone.
Ohri A *et al.*, (2006). Cytological grading of breast neoplasia and its correlation with histological grading. *Indian Journal of Pathology and Microbiology* 49(2) 208-213.
Moriquand J *et al.*, (1986). Value of cytoprognostic classification in breast carcinoma. *Journal of Clinical Pathology* **39**(6) 489-496.
Sneige N *et al.*, (1992). Nuclear grading in fine needle aspirates of breast. Cytopathology **3**(9):161-171.
Fechner RE *et al.*, (1991). Anatomic Pathology in the 1980s: A decade of new information and new challenges. *American Journal of Clinical Pathology* **96**(12)10-14.
Das AK *et al.*, (2005). Tumor Grading, DNA Ploidy and Ki-67 Index as Prognostic Markers in Fine Needle Aspirates of Breast Carcinoma. *Journal of Cytology* **22**(7).57-61.
Hemachandran M, Nijhawan R, Joshi K (2002). Cytological grading ,apoptosis and Bcl-2 protein expression in breast cancer. *Diagnostic Cytopathology* **26**(6) 356-359.
Bhargava V, Jain M, Aggarwal K, Thomas S, Singh S (2008). Critical appraisal of nuclear grading in carcinoma of the breast and its correlation with ER/ PR expression. *Journal of Cytology* **25**(11) 58-61.