CYTOLOGY OF BREAST LESIONS – A MORPHOMETRIC APPROACH

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ABSTRACT

Aim & Objective;

1. To evaluate the morphometric variation in cytology of breast lesions.

2. To correlate cytologic grade with nuclear morphometry in breast carcinomas.

Methods and Materials :- A retrospective study of thirty seven patients of breast lesions diagnosed clinically, were sampled for FNAC in the Department of Pathology, Vinayaka Mission's Krubananda variyar Medical College, Salem, from Jan 2008 to Dec 2008. Cases were subclassified as benign and malignant, and those which were malignant cytological Grading and histologic grading were done by Robinson's criteria and Elston's modification of the Bloom- Richardson system respectively. Grading was intern compared with nuclear morphometric studies.

Results: Our study showed, 9 cases of benign and 15 cases of malignant breast lesions. The mean nuclear diameter were significantly increased from benign to all grades of malignant lesions. However, a good correlation was identified between mean nuclear diameter and pathological tumor grading.

Conclusion : This study suggests that Mean nuclear diameter is an efficient and successful tool distinguishing between benign and malignant lesions.

It can be used as a diagnostic tool in "Gray Zone" areas of breast lesions.

Key words: *Fine needle aspiration cytology, Cytological Grading, Histologic grading and Nuclear morphometry.*

INTRODUCTION

The breast presents a plethora of benign pathological conditions, benign breast conditions are 4 – 5 times more common than breast cancer. The benign conditions are also associated with morbidity and are great concern to the patient, hence extensive research investigations and treatment of benign conditions are required.

Fibroadenomas are the most common benign tumor, found in all groups with incidence of 4.2% . The next common condition is fibroadenosis or fibrocystic disease of breast, the incidence being 3.4% The other rare lesions are chronic mastitis, Cystosarcoma phylloides, lipoma of the breast, duct papilloma, galactocele and sebaceous cyst of the breast.

Clinically, the breast disease present as Breast lump, Nipple discharge. Nipple discharge is a common problem of the breast that has been reported in 10%-15% of women with benign breast disease and in 2.5%-3% of women with breast cancer. Nipple discharge should be of concern when a woman reports it as unilateral and spontaneous[1]. the risk for breast cancer increases with the increase in age.

Breast cancer comprises 10.4% of all cancer incidence worldwide, among women and the fifth most common cause of cancer death[2]. Incidence of breast cancer exceeds all female cancers with high mortality rates worldwide[3,4]. In India, cancer of breast is second most common cancer in women after the cervical cancer with annual incidence exceeding 80,000.

The malignant lesions were more common among fourth and fifth decade of female life. Overall survival and mortality due to this disease are influenced strongly by the stage of the disease at diagnosis. About 54% of the women are diagnosed in stage II, while only 16% are diagnosed in stage I[5].

Fine needle aspiration cytology continues to gain clinical acceptance as a diagnostic technique for collecting cells from palpable and non-palpable breast lesions.

Lump in the breast is the most common clinical presentation, which has to be categories, as the treatment and prognosis varies with mass lesions.

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[6]. The National Cancer Institute (NCI), Bethesda sponsored conference on the uniform approach to breast fine needle aspiration biopsy had also recommended that

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tumor grading on FNA material should be incorporated in FNA reports for prognostication[7].

Automated image cytometry is relatively newer technique, which can rapidly and accurately measure the various morphometric parameters on cytology smears which can be used to differentiate benign lesions from malignant lesions of the breast. The nuclear morphometry reinforced by image cytometry may separate breast carcinoma into low and high nuclear grades.

METHODS AND MATERIALS

This was a retrospective study which included all the patients presenting with breast lumps for the period of 1 year from Jan2008 to Dec 2008. In all cases, the preoperative diagnosis on FNA was confirmed histologically. The FNAC material was fixed with 95% alcohol and stained using routine Haematoxylin and Eosin. These cases were graded cytologically and histologically by using Robinson's criteria and Elston's modification of the Bloom- Richardson system respectively and finally subjected to morphometric analysis using the most common geometric parameter the nuclear diameter.

In the malignant cases, the smears were evaluated based on the grading system described by Robinson's 5 (Table1) , which takes into account six parameters. Scores for each of the six cytologic features were added together to give a total score for each case. In each case the final score ranged between 6 and 18. Grade1, score 6-11; Grade 2, 12-14; Grade 3, score 15-18.

Table 1: Cytologic grading with the method of Robinsonet al.

Criterion	Score 1	Score 2	Score 3
Cell dissociation	Mostly clusters	Single cells, clusters	Mostly single cells
Nuclear size	1-2 times size of RBC's	3-4 times size of RBC's	= 5 times size of RBC's
Cell uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct/small	Noticeable	Abnormal
Nuclear margin	Smooth	Slightly irregular/folds	Buds, clefts
Chromatin pattern	Vesicular	Granular	Clumping/ Clearing

Histopathological samples were evaluated based on the histologic grade according to Elston's modification of the Bloom- Richardson system. Three parameters were taken into account, degree of tubule formation, nuclear pleomorphism and number of mitoses. Each parameter was scored between one and three. Thus the overall score for all cases ranged between three and nine.

Table 2: Histological grading with Elston's modification of the Bloom-Richardson system.

Histologic features	Score 1	Score 2	Score 3
Tubule formation	Tubule formation in > 75% of the tumor	Tubule formation 10 to 75%	Tubule formation < 10%
Nuclear pleomorphism	Mild Pleomorphism	Moderate Pleomorphism	Marked Pleomorphism
Mitosis/HFP	0-5 mitosis	6 - 10 mitosis	> 11 mitosis

The same H&E stained FNAC smears were studied for computerised morphometric s analysis of nuclear diameter. At least 100 cells from each case were measured with an image analyzer and the mean nuclear diameter were calculated and compared with different clinicopathological features.

RESULTS

In total of 37 breast cases, 15 cases were malignant breast lesions, 9 were benign breast lesions, 13 inflammatory lesions and others.

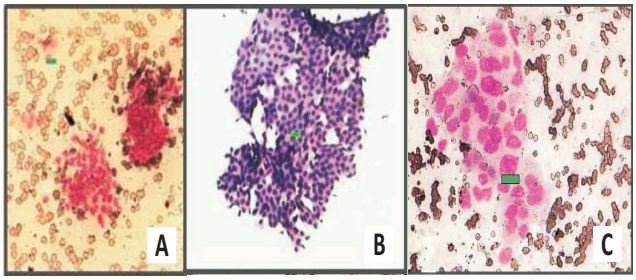

Fig.A - Benign lesion **Fig.B - ADH & DCIS** **Fig.C-Malignant lesion**

Out of 15 cases ten cases were received for histopathological examination as trucut biopsy or as mastectomy specimens. All the 10 cases were also evaluated for pathological TNM staging (pTNM).

On FNAC, cases were cytologically graded according to Robinson's criterias into grade I- 9 cases, grade II – 4cases and grade III -2 cases.

Out of 15 cases , 10 cases were received as Trucut biopsy or Mastectomy for histopathological examination. All the histopathological samples were diagnosed and graded according to Elston's modification of the Bloom-Richardson criteria. According to which Grade 1- 5, Grade 2 - 3 and Grade 3 – 2.

Cytology grading correlated with histological grading .

In the present study, total of 35 breast lesions, 15 could be diagnosed as cytologically malignant lesion. The cytological grading of breast carcinomas correlated well with that of histological grade. (Table:3) shows the comparison of cytological grading with that of cytological grading along with strength of association for each grade.

Table 3: cytological with histological correlation (Concordance rate = approximate sensitivity)

Robinson's cytologic grade (CG)	No of Cases (Cytology)	Histological grade (HG) Gr .I	Histological grade (HG) Gr .II	Histological grade (HG) Gr .III	Concordance rate %
I	4	4	0	0	100
II	4	1	3	0	75
III	2	0	0	2	100
Total	10	5	3	2	91.6

The Mean nuclear diameter was significantly increased from benign to malignant grade I and grade I versus grade II and III. In benign breast lesions, the mean nuclear diameter varies from 9.1 to 12.5µ and in malignant breast lesions varies from 15.0 to 32.0 µ. (Table:4)

Further in the study, also compared the tumor size, tumor stage and lymph node status. The tumor size in malignant breast lesions ranges from 2cm to 10cm. Size is more than 3cm was prone to develop lymph node metastasis. Out of 15 cases, 4 cases were of stage I, 10 cases were of stage II and 1 case was of stage IV.

Out of 15 cytologically malignant cases, 13 cases showed lymph node deposits and other 2 cases showed reactive changes.

Pitfalls:- In two cases, Atypical ductal hyperplasia (ADH) and in DCIS with Sclerosing adenosis the mean nuclear diameter was 12.5 to 15 µ.

Table 4: Morphometry: Benign vs . malignant lesions.

Morphometeric parameter	Benign	ADH & DCIS	Malignant
Mean nuclear diameter (microns)	9.1 ± 3.5	12.5 ± 2.5	15.0 ± 17.0

DISCUSSION

Breast carcinoma is the common emerging cancer after the cervical carcinoma globally[3]. It is the leading cause of cancer mortality in Indian women, with 80,000 new cases of breast cancer diagnosed annually in India[4]. FNAC is the first diagnostic modality in diagnosing breast masses. Cytologically benign lesions show cohesive clusters and Bimodel population of cells and the malignant lesions show noncohesive and pleomorphic nuclei.

Nuclear grading in cytology was first introduced by Black et al[8]. Further modified by various workers and finally composite cytonuclear grading system was introduced by Robinsonet al[9]. According to this system, the cells are graded with the help of six parameters and the scores ranges from 6 to 18 .

Histological grading was done according to Elston's modification of the Bloom- Richardson system. According to this system, the histopathological biopsies were graded with the help of three parameters and the scores ranges from 3 to 9. Histopathological grading is helpful in Gray zone areas where cytology remains as a diagnostic dilemma.

This gray Zone in cytology is estimated to constitute 8.9% of cases[10].

To give strength to this system the cytological grading compared and correlated with Elston's modification of the Bloom- Richardson Histopatholgical grading .

Our study, showed 100% concordance,75 and 100% in grade I,II and III. The overall concordance rate of 91.6%. Cytohistological correlation has been found to vary from 57.1 to 95% in the literature, and our study lies at the higher end of the spectrum.

To confirm the cytological grading, computerised nuclear morphometry was done in breast lesions on cytology. A large number of parameters have been studied by morphometry but in our study, the most important, mean nuclear diameter alone was analysed. Mean nuclear area is the most studied parameter in nuclear morphometry in the literature[11]. Alteration in nuclear structure are the morphological hallmarks of cancer diagnosis[12]. The progression from normal breast to ductal hyperplasia and ADH going into DCIS and invasive carcinoma[13] reveal sequential increase in nuclear diameter. In our study, Mean nuclear diameter ranges from 15.0 to 32.0 µ, in malignant lesions and 9.1 to 12.5µ in benign lesions which is well in agreement with

the other studies. It can be used as a quality control procedure in the Fine needle aspiration cytology.

The cytological grading, histopathological grading and morphometric analysis go hand in hand with clinicopathological features of malignant tumors as tumor size, lymph node status and tumor staging. Tumor size[14], node positivity[14] and mitotic count[15] showed an increase with increasing cytologic grades and morphometeric parameters in our study.

CONCLUSION

The study suggests that computerized nuclear morphometry is an efficient and successful tool distinguishing between benign and malignant lesions.

It can be used as a quality control procedure in the Fine needle aspiration cytology. In future, it is a useful method to diagnose the "Gray Zone" areas in breast cytology.

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