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Role of Nuclear Morphometry in distinguishing gray areas of breast lesions

Sabina Laishram[1], Shameen Shariff[2]

1 *Assistant Professor, Department of Pathology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India*
2 *professor & Head of Department, MVJ Medical college and Research Hospital, Hoskote, Bangalore, India*

Abstract

FNAC of the breast, although effective for the diagnosis of breast lesions is largely subjective and a minority of cases cannot be classified as benign or malignant due to the morphological overlap. This hinders a definite diagnosis which may sometimes lead to unnecessary surgical biopsy. Morphometry in combination with FNAC is one such method of improving the diagnosis. **Aims & Objective:** To study the nuclear morphology with regard to nuclear diameter; nuclear area; coefficient of variation of nuclear area; nuclear/cytoplasmic ratio and the ratio of largest to smallest nuclear diameter (L:S ratio) on all breast aspirates (after histopathology correlation) performed at the Department of Pathology, MVJ MC and RH in a two year period for distinguishing benign lesions from Grade I Carcinoma. **Methods:** Sixty consecutive FNAC breast aspirates ratified by histology were studied from patients referred for a breast lump evaluation to the Department of Pathology, MVJ Medical College & Research Hospital between Aug 2010 to July 2012. Morphometric analysis was done on Haematoxylin & Eosin stained aspirates using the Image J Morphometric Software for image processing and analysis developed by National Institutes of Health, USA. **Results:** Nuclear morphometry calculated showed all the nuclear parameters were higher in the Grade I (well differentiated) carcinoma as compared to benign lesions, the highest difference being the mean nuclear area. **Conclusion:** Nuclear morphometry does helps in distinguishing the benign from Grade I carcinoma which is responsible for equivocal diagnosis in cytology.

Keywords: FNAC, Breast, Morphometry.

***Corresponding author:**
Dr. Sabina Laishram
Assistant Professor, Department of Pathology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India
Email:
sabina_laishram[at]yahoo.com

INTRODUCTION

Fine needle aspiration cytology (FNAC) has become a critical component in the investigation of palpable breast masses and has become popular as a valuable tool in preoperative assessment of breast masses. It has gained popularity due to its fast and easy approach, being inexpensive, and can be performed with little complications.

FNAC plays a major role in the diagnosis of benign disease in symptomatic palpable lumps as part of triple assessment, staging of breast carcinoma, in particular preoperative axillary lymph node FNAC and helps to diagnose metastatic disease at distant sites following treatment for carcinoma [1]. Reports in literature shows an efficient role of FNAC in the evaluation of breast masses with a high accuracy rate (95.8 % to 97.87%) sensitivity rate (95% to 98.4%) and specificity rate (60% to 93%) [2-6]. Inspite of these, there are instances where the smear is reported as inadequate which ranges from 0.7% to 25.3%, and this is influenced by the nature of the lesion, the available technology, and the experience of the operator. It has been reported that the nature of the lesion (schirrous lesions) was the most common cause of inadequacy of FNAC, accounting for 68% of the inadequate aspirates, followed by the experience of the aspirator that accounted for 32% of the inadequacy rate [7].

In order to overcome such circumstances, most centres have now adopted a triple assessment approach, i.e. clinical, imaging and FNAC as the first-line of investigations in both screening and symptomatic populations. This increases the accuracy rate to 98% to 100% [8,9]. Triple assessment is a cost effective, easy to perform and time saving approach [1].

However, a "gray zone" exists between benign and malignant lesions in FNAC of breast where an unequivocal diagnosis cannot be given [10]. Nuclear morphometry in combination with FNAC can improve the distinction between benign and malignant lesions and in combination with visual impression can help resolve several equivocal cases. The present study was undertaken to validate the role of the nuclear

morphometry on specimens obtained by fine needle aspirates in differentiating benign from grade I carcinoma.

MATERIALS AND METHODS

Sixty consecutive FNAC breast aspirates ratified by histology were studied from patients referred for a breast lump evaluation to the Department of Pathology, MVJ Medical College & Research Hospital between Aug 2010 to July 2012. Morphometric analysis was done on Haematoxylin & Eosin stained aspirates using the Image J Morphometric Software for image processing and analysis developed by National Institutes of Health, USA. The five parameters were measured on 200 cells spread evenly on the slide surface. 100 cells from cell clusters in both benign and malignant lesions and 100 cells from single cells in malignant lesions were selected for the measurements. NC ratio was avoided in single cells due to cytoplasmic vacuoles and artefacts. Correlation of results with histopathology was done using it as the gold standard. Any discrepancy in preformed cytological diagnosis was rectified after correlation. Statistical analysis was done using Student t-Test and one way ANOVA wherever applicable.

RESULTS

A total of 30 benign and 30 malignant breast lesions with corresponding histopathological correlation were included for nuclear morphometric analysis. Fibroadenoma comprises 23 cases of benign breast lesion followed by fibrocystic disease (6) and tubular adenoma (1). Malignant category was represented by ductal carcinoma, NOS (29) followed by one case of metastatic carcinoma. All the nuclear parameters analysed were higher in malignant lesions in comparison to benign lesions. The distribution of the mean nuclear diameters of cells obtained from the benign breast lesions is 4.78 while the mean nuclear diameters of malignant breast lesions is 6.9 which shows a difference of 2.13µ. After histological grading of malignant lesions, Grade II constitutes 14 cases followed by Grade I (11) and Grade III (05). All nuclear parameters obtained were in proportion with the histological grade, being the highest in grade III (poorly differentiated) carcinoma and lowest in grade I (well differentiated) carcinoma. The nuclear parameters obtained for cell clusters of benign lesions and Grade I (well differentiated) carcinoma on comparison showed significant difference.

Table 1: Nuclear parameters of cell clusters in benign & Grade I carcinoma

Nuclear Parameters	Benign		Grade I	
	Range	Mean±SD	Range	Mean±SD
Nuclear Diameter(µ)	4.02 – 5.83	4.78±0.42	4.93 – 6.49	5.94±0.54
Nuclear area(μ^2)	12.92 – 27	18.59±3.36	19.35 – 34.34	28.66±5.09
N:C ratio	0.42 - 0.51	0.47±0.02	0.62 – 0.68	0.65±0.02
L:S ratio	1.3 – 1.63	1.46±0.08	1.54 – 1.69	1.64±0.06
NACV (%)	17.14 – 34.56	23.93±3.98	25.5 – 38.21	32.41±3.57

Table 2: Statistical correlation between benign and Grade I Carcinoma

Nuclear parameters	Benign	Grade I	P value
MND(µ)	4.78±0.42	5.94±0.54	<0.0001(Significant)
MNA (μ^2)	18.59±3.36	28.66±5.09	<0.0001(Significant)
Mean NC ratio	0.47±0.02	0.65±0.02	<0.0001(Significant)
Mean LS ratio	1.46±0.08	1.64±0.06	<0.0001(Significant)
Mean NACV (%)	23.93±3.98	32.41±3.57	<0.0001(Significant)

Table 3: 2σ limits for benign and Grade I Carcinoma

Nuclear parameters	Benign	Malignant
Nuclear diameter(µ)	3.94 – 5.62	4.86 – 7.02
Nuclear area (µ2)	11.87 – 25.31	18.48 – 38.84
NC ratio	0.43 – 0.51	0.61 – 0.69
LS ratio	1.3 – 1.62	1.52 – 1.76
NACV (%)	15.97 – 31.89	25.27 – 39.55

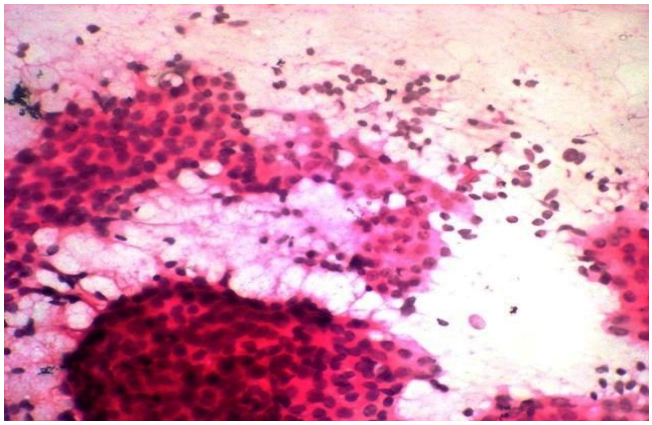

Figure 1: Cellular spread of a fibroadenoma with single cell layer in the cluster and a shower of bare nuclei in the background. (Haematoxylin & Eosin X 400).

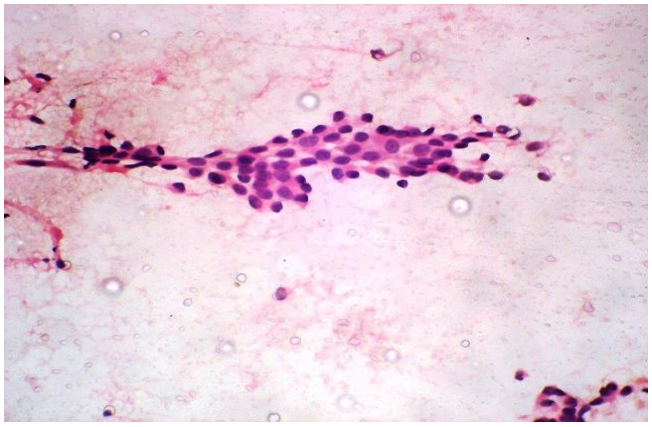

Figure 2: Cytologic smear of Grade I carcinoma: cell clusters with small, uniform cells. (Haematoxylin & Eosin X 400.

DISCUSSION

The "grey zone lesions" are represented both by benign and malignant lesions including fibroadenoma, fibrocystic disease of the breast, papilloma and other papillary lesions of the breast, proliferative breast disease with or without atypia including radial scar and sclerosing adenosis, fat necrosis, phyllodes tumor, lactating breast, gynaecomastia, lobular carcinoma, tubular carcinoma, mucinous carcinoma, low-grade *in situ* carcinoma, or ductal carcinoma [11]. In the UK, a survey programme for breast carcinoma was established recommending FNAC results to be reported from C1 through to C5 categories where C1 was considered inconclusive due to lack of material, C2 definitely benign, C3 probably benign with atypia, C4 probably malignant and C5 was definitely malignant.(7) Lesions in "gray zone" are categorized as "probably benign with atypia" (C3) and "probably malignant" (C4) [10]. Some cases of low grade ductal carcinoma and fibroadenoma, may present with overlapping features causing erroneous diagnoses. The root causes contributing to this misdiagnoses were large branching sheets of carcinoma mimicking folded sheets of fibroadenoma; fibroblasts mimicking myoepithelial cells; apocrine cells mimicking carcinoma cells; and not recognizing the loose myxoid matrix presenting as soap bubbles in fibroadenoma [11]. Since these lesions can be easily confused one for the other, much care must be given while giving their diagnosis as overdiagnosis may lead to unnecessary biopsy for a benign lesion and underdiagnosis may lead the patient to further complications and advancements of malignancy. In an attempt to evaluate the role of nuclear morphometry in distinguishing benign from low grade (Grade I) carcinoma, a comparison was done between these categories and all the nuclear parameters (MND, MNA, NACV, NC ratio and LS ratio) measured in the present study was found to be statistically significant (P<0.0001). Dey *et al.* [13] found significant difference in the MND, MNA and SD of MNA between benign lesions and Grade I carcinomas. Nijhawan *et al.* [14] noted significant difference between fibroadenoma and fibroadenoma with atypia categories and also between fibroadenoma and Grade I ductal carcinoma. They also observed that there was no significant difference between fibroadenoma with atypia and Grade I ductal carcinoma.

The mean nuclear diameter shows an average of almost 5µ in the benign category and approximately 7µ in the malignant category in our study. There was statistically significant difference between the nuclear diameters of benign and malignant lesions (p<0.0001). The present study shows that the mean nuclear area was the most significant parameter of all other parameters studied in differentiating benign from malignant lesions. It illustrates a reasonable 2σ limits for the mean nuclear area: For benign lesions, the range was 11.87 to 25.31μ^2, if the value falls within this limit, there is a 95% chance for the lesion to be benign. For malignant lesions, 2σ limits for the lesion was between 18.13 to 61.05μ^2, there being 95% chance for the lesion to be malignant in this range. The MND and MNA observed in the present study in the various grades were comparatively lower than that observed in the other studies. These variations in the results could be due to technical problems or even a genetic basis as suggested by Buhmeida *et al*. [15]. These authors also suggest that the MNA was higher in lymph node positive patients as compared to lymph node negative patients and in advanced stages as compared to early cancer. MNA was significantly higher in higher grades, in cases of tumor invasion, in recurrent cases than in non recurrent cases. The MND and MNA were higher in the single cells when compared to cell clusters in the various histological grades and it is suggested that in malignancy a cell morphometry yielded objective finding on single cells as compared to cell clusters. The present study ratifies these findings in literature.

In the present study, the mean NC ratio for benign lesions measured 0.47 ± 0.02 and the mean NC ratio for malignant conditions measured 0.68 ± 0.03 which was statistically significant (p<0.0001) in cell clusters. There was also statistically significant correlation in the various histological grades (p<0.0001) being directly proportional to the grade of the carcinoma. Arora *et al.* [16] also found statistically significant difference in the NC ratio which contributed in distinguishing various benign lesions like fibroadenoma, fibroadenosis as well as ADH from IDC without lymph node metastasis and IDC with lymph node metastasis (p<0.05). The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. It denotes the extent of variability in relation to mean of the population. The present study shows a statistically significant difference between the benign and malignant lesions (p<0.0001) with regard to NACV. Suzuki *et al.* [17] found that patients with high NACV > 35% had lower rates of disease free survival than those with low NACV <35%. They also observed that NACV was significantly associated with hormone receptor status of a tumor, ploidy status as well as histological grade (p<0.05) but did not correlate with tumor size and lymph node status. Tajima *et al.* [18] and Nagashima *et al.* [19] have mentioned in their studies that NACV together with the MNA is a good indicator for identifying DCIS from lesions like benign intraductal hyperplasia, papilloma and fibrocystic disease. However, Cornelisse *et al*. [20] mentioned that NACV had considerably less discriminatory power and also showed the lowest correlation with the MNA. The present study showed that NACV correlated with MNA. Therefore it is observed that nuclear morphometry supplements FNAC interpretation particularly in cases of well differentiated small-cell duct carcinoma (Grade I) where FNAC diagnosis can be problematic as the cytology features are hypercellular with cohesive and rarely discohesive cells with no demonstrable nuclear atypia. This is often confused with benign conditions like fibrocystic disease, papillary neoplasia and fibroadenoma and also with malignant lesions like lobular carcinoma [21].

CONCLUSION

Morphometry is efficient in distinguishing benign from malignant lesions and has been proved to be useful objective tool especially in the "gray zone" areas. Inspite of obtaining an objective results with the help of morphometric analysis, errors occur due to technical problems and application of "Stepwise" algorithms can reduce the technical problems in Computerized Interactive Morphometry in terms of overestimation of the size of the profile as a result of overriding the cytoplasmic/ nuclear contours during tracings, magnifications used, speed of conducting the analysis and the shape and size of object being traced. Internal calibration and standardization by an expert observer performing correct tracings can also reduce the errors.

Caution with regard to these factors and careful assessment can make FNAC a valuable tool in the differentiation of benign and malignant lesions, which is the most crucial factor in deciding patient management.

Conflict of Interest

The authors have no conflict of interest.

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