The Journal of Medical Research

Clinical Research

JMR 2015; 1(3): 72-75
May- June
ISSN: 2395-7565

www.medicinearticle.com

Significance of nuclear morphometry on fine needle aspirates of breast lesions

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Abstract

Background: 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. **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. Statistical analysis was done to find out the significance of the five nuclear parameters in the benign and malignant categories. **Methods:** A total of 60 patients with a history of breast mass referred for FNAC to the Department of Pathology, MVJ MC &RH were taken for the study. Period of study: 2 years - August 2010 and 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 100 cells spread evenly on the slide surface. 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. **Results:** In this study, all the nuclear parameters were found to be significantly higher in the malignant lesions when compared to benign lesions ($p<0.0001$). **Conclusion:** Interactive computerized nuclear morphometry is an efficient and successful tool in distinguishing benign and malignant lesions. When faced with an inconclusive diagnosis of aspirates of breast masses, image analysis can help in the further classification of such lesions providing a more appropriate triage for surgical biopsy.

Keywords: Morphometry, Benign, Malignant, Haematoxylin & Eosin.

Introduction

Breast carcinoma is the most common cancer among women in the western world and accounts for 50% of the mortality rate in these countries [1]. In India it stands second preceded by the carcinoma of the cervix in the data of cancer registries [2].

Fine needle aspiration cytology (FNAC) of the breast is one of the modalities of detecting carcinoma of the breast. The accuracy rate in literature is reported to range from 95.8% to 97.87% [3-5]. FNAC is also one of the prongs of the triple test, the accuracy in such instances ranging from 98% to 100% [6,7]. Where facilities for imaging are not available and in the diagnosis of a breast lump being benign or malignant, FNAC interpretation is invaluable towards an accurate diagnosis. Since biopsy specimens from palpable abnormalities are benign, FNAC can have a substantial role in separating patients in need of a surgical biopsy from patients who can be followed up clinically [8]. Also, it is safe, less traumatic, easy to perform, quick, cheap and can be performed as an office procedure in obtaining quick results [9].

Results in FNAC can be inaccurate particularly in the gray zone areas such as atypical ductal hyperplasia, ductal hyperplasia and even in some cases of fibroadenoma where overdiagnosis may lead to false positive results. In such gray zone areas an unequivocal diagnosis cannot be made. The incidence of these reports varies in literature from 6.9% to 20% [10].

Since lesions as distinct as fibroadenoma and carcinoma sometimes can be confused in FNA material, it is not surprising that instances of false-negative diagnosis (carcinoma mistaken for DH or ADH) and false-positive diagnosis (DH or ADH considered to represent carcinoma) have been described.

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The accuracy of visually diagnosed breast FNAC is over 90%. The overall accuracy was 94.3% in a 37-series study reported by Frable and 25 more recent series; with a total of 23,741 satisfactory breast FNA's. Individually, the mean sensitivity for these series was 0.91 +/- 0.07 and the mean specificity was 0.87 +/- 0.18. The relatively high standard deviations indicate that the accuracy achieved in individual series varies considerably and reflects the subjectivity of visual diagnosis [11].

Nuclear morphometry can improve the distinction between benign and malignant lesions and in combination with a visual impression can help resolve several equivocal cases [12-17].

The present study aims at using simple morphometric cell characteristics on aspirates of histologically confirmed breast lesions in order to assess their values as parameters on diagnosis and delineating benign from malignant lesions.

Materials & Methods

The proposed study was conducted on 60 cases of surgical breast specimens received in the Department of Pathology, MVJ Medical College between 2010 to 2012. Sixty cases (30 benign breast masses & 30 Malignant breast Lesions) were studied which had both FNAC and histopathology correlation. All aspirates after processing were stained with Haematoxylin and Eosin to categorized as- Fibrocystic Disease and breast adenomas. The carcinomas were categorized into specific and NOS types. Grading of carcinomas was done by Modified Scarf-Bloom-Richardson Grading System.

The quantitative study was done by an image J analysis system. The digital images generated by Olympus camera linked to Olympus microscope at a total magnification 400 were used to create photomicrograph that was processed by Image J Software.

A total of hundred cells were randomly selected and measured in each case. In malignant lesions, measurements on both cell clusters and single cells were calculated for all the five parameters mentioned, except NC ratio on single cells (due to presence of cytoplasmic vacuoles and artefacts on spreading). In benign lesions, only cells in clusters were taken due to lack of adequate single cells. With the help of an internal calibration, various parameters were average nuclear diameter (AVD), mean nuclear area (MNA), coefficient of variation of nuclear area (NACV), NC ratio, and average largest to smallest nuclear diameter ratio (LS ratio) After obtaining all the parameters for each case, finally the average value of each parameter was calculated for both the benign and malignant conditions.

Statistical analysis

Applying SPSS 17 version, Student t-Test was used to compare benign and malignant lesions and one way ANOVA test was used for comparing the histological grade. 2σ limits was also calculated.

Results

A total of 60 consecutive cases with corresponding histology to include 30 benign and 30 malignant lesions were assessed. The distribution of various types of lesions both the category are fibro adenoma (23), fibrocystic disease (06), tubular adenoma (01), ductal carcinoma, NOS (29), and metaplastic carcinoma (01). Majority (86.7%) of benign cases belongs to most reproductive 21-40 yrs of age group while 79% of malignant cases were represented by 30-60 yrs of sample population. After histological grading of malignant lesions, Grade II constitutes 14 cases followed by Grade I (11) and Grade III (05).

Morphometric study of the breast lesions

Using Image J software, the nuclear parameters were studied in 100 cell clusters in both the benign & malignant categories.

Table 1: Measurements comparing all benign and all malignant breast lesions in cell clusters

Nuclear Parameters	Benign		Malignant	
	Range	Mean±SD	Range	Mean±SD
Nuclear Diameter (μ)	4.02 – 5.83	4.78±0.42	4.93 – 8.84	6.91±0.93
Nuclear area (μ^2)	12.92 – 27	18.59±3.36	19.35– 3.73	39.59±10.73
NC ratio	0.42 - 0.51	0.47±0.02	0.62 - 0.75	0.68±0.03
LS ratio	1.3 – 1.63	1.46±0.08	1.54 – 2.38	1.78±0.16
NACV (%)	17.14 – 34.56	23.93±3.98	25.5– 53.68	38.94±6.85

The table indicates that all the nuclear parameters measured were higher in malignant breast lesions in comparison to benign breast 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.

Mean nuclear area showed the highest difference with benign lesions measuring 18.59μ^2 and malignant lesions measuring 39.59μ^2 with a difference of 21μ^2 between them. A marked difference was also observed in the mean NACV in which the benign lesions showed the percentage to be 23.93% and malignant lesions 38.94% (difference in the mean value was 15.01%). A difference of 0.21 was observed in the mean NC ratio and a difference of 0.32 was observed in the LS ratio.

Discussion

Breast lesions are a complex group of disorders which encompass hyperplasias, atypical hyperplasias, fibrocystic disease as well as benign and malignant neoplasms. A surgical intervention is not a necessity in some of the lesions outlined above. Therefore, the selection of cases for such a surgical intervention needs caution and precision in order to avoid unnecessary surgery. For several decades, fine needle aspiration cytology is used as the first modality in accessing breast masses in the outpatient clinic. Although FNAC is a simple and cost effective method, it is based on the visual subjective evaluation of cytologic features like cellularity, cell morphology and type of chromatin seen on the smears, the interpretation of which can be associated with interobserver variability and sometimes even intraobserver variations. The reporting pathologist is often in a state of dilemma when it comes to the "gray zone" areas such as differences between hyperplasias and atypical hyperplasias; atypical hyperplasias and ductal carcinomas; well differentiated Grade I carcinomas and benign hyperplasias [10] [18,19].

Studies in literature have shown nuclear diameters to be measured in combination with nuclear areas. These have been performed on aspirates[20] as well as paraffin embedded tissue [21].

Rezanko *et al*[20] obtained an average nuclear diameter of 9.9μ in benign category and an average of 10μ in the malignant category. Their observations were based on Haematoxylin and Eosin stained samples. The present study shows an average of almost 5μ in the benign category and approximately 7μ in the malignant category. Variations in diameters maybe due to the degree of fixation of cells resulting in cell shrinkage.

Kronqvist *et al*[22] analysed nuclear diameters on 170 histological samples of invasive ductal carcinoma to find objective and quantitative

thresholds for nuclear grade. Their mean thresholds of nuclear diameter were 6.4µ and 7.4µ to differentiate Grade I, Grade II and Grade III carcinomas. Their measurements of nuclear diameters are closer to those obtained in the present study.

In the present study, there was statistically significant difference between the nuclear diameters of benign and malignant lesions ($p<0.0001$). This has been supported by several studies in literature which suggest that objective measurements on cell spreads and even cells in reasonably thin paraffin embedded sections go along with in giving additive information to a diagnostic cytologic impression [21], [23,24].

The parameter of nuclear area too has been particularly useful in the differentiation of benign from malignant breast lesions. Workers have found a gradual increase in the mean nuclear area from baseline value of normal epithelium through benign diseases to invasive cancers [16], [24]. Arora *et al*[25] found that the mean nuclear area for benign lesions was significantly low ($24.33 \pm 0.77\mu^2$) as compared to atypical ductal hyperplasia ($42.21 \pm 1.84\ \mu^2$) ($p<0.05$) and mean nuclear area was found to be significantly higher in cases of infiltrating ductal carcinoma as compared to benign breast disease. Their study showed that the mean nuclear area and NC ratio was found to be statistically significant.

Kalhan *et al*[26] found their geometric parameters of high significance ($p<0.0001$) in differentiating benign from malignant lesions. They found no overlap between benign and malignant aspirates when these parameters were applied and could even differentiate atypical ductal hyperplasia from ductal carcinoma in situ.

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 $25.31\mu^2$, 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 $61.05\mu^2$, there being 95% chance for the lesion to be malignant in this range.

In a study by Pienta et al(27), it was found that MNA increased with a more malignant histologic finding- The step up for an increase being the following: normal controls, intraductal carcinoma, node-negative infiltrating carcinoma and node-positive infiltrating carcinoma. The present study however did not project parameters of so many variables due to the small number of cases studied.

Abdalla *et al*[17] have even suggested that MNA is the most powerful feature in distinguishing benign from malignant lesions. However, Wittekind and Schulte[28] are of the opinion that the perimeter and not the MNA was the most powerful feature for differentiation between benign and malignant lesion.

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. Arora *et al*[25] also found in their study that there was 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 ($p<0.05$). Abdalla *et al*[17] disagreed that such a design should be avoided because outlining of cellular margins is difficult making the measurement less reproducible and more subjective.

In the present study, LS ratio is the ratio of the five largest diameters to the five smallest diameters. The mean LS ratio for benign lesions was 1.46 ± 0.08 and the LS ratio for malignant lesions was higher being 1.78 ± 0.16 which was statistically significant ($p<0.0001$) in the present study. Nagashima *et al*[29] however found no significant difference in LS ratio in the study conducted by them.

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*[30] found that patients with high NACV > 35% had lower rates of disease free survival than those with low NACV <35%. Tajima *et al*[31] and Nagashima *et al*[29] 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*[32] 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.

Conclusion

All the nuclear parameters were higher in the malignant lesions when compared to benign lesions and were statistically significant ($p<0.0001$). Mean nuclear area was the most important parameter to differentiate between benign and malignant lesions. Therefore, 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 managemen.

Authors' Contribution: Dr. Shameem Shariff has been my guide and teacher who supervised me throughout the study.

Conflict of interest – Nil

Funding - Nil

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