Image Analysis-Assisted Nuclear Morphometric Study of Benign and Malignant Breast Aspirates

Dayal Johan Nirnanjan Pandian, Anita Ramdas, M. Moses Ambroise

Department of Pathology, Pondicherry Institute of Medical Sciences, Kalapet, Puducherry, India

Abstract

Background: Fine needle aspiration cytology of the breast is well accepted and accurate for the diagnosis of benign and malignant lesions, however, it remains a subjective evaluation. **Aims and Objectives:** The present study was carried out to assess the utility of nuclear morphometry in differentiating benign and malignant breast aspirates. Importantly, we wanted to evaluate the utility of nuclear density parameters using Image J software. **Materials and Methods:** Nuclear morphometry was carried out using image analysis software Image J 1.44 on 20 selected benign and malignant breast aspirates. Assessment was carried out on a total of 1000 cells in benign and 1000 cells in malignant aspirates counting 50 intact nuclei in nonoverlapping cells for each case. Six parameters including three size parameters, namely nuclear area, nuclear diameter, and nuclear perimeter; one shape parameter, i.e., axis ratio; and two nuclear chromasia parameters, namely density (integrated and raw), were measured. **Results:** There were significant differences between nuclear area, perimeter, diameter, integrated density, and raw integrated density of benign and malignant lesions. No significant difference was found for axis ratio. Receiver operating characteristic curve analysis revealed that nuclear area, perimeter, diameter, integrated density, and raw integrated density are helpful in discriminating benign and malignant aspirates. **Conclusions:** Thus, Image J is helpful in the evaluation of nuclear size as well as chromasia. We conclude that nuclear size and density parameters can be used to derive cutoff values of various parameters to differentiate between benign and malignant cells in breast cytology.

Keywords: Breast, fine-needle aspiration cytology, morphometry, nuclear

INTRODUCTION

An accurate preoperative diagnosis of carcinoma breast is essential, which is witnessing an alarming rise of this entity in women.^[1]

Fine-needle aspiration cytology (FNAC) is a first-line preoperative investigation in women presenting with breast lumps for rapid diagnosis of benign or malignant lesions.^[2]

FNAC diagnosis of breast lumps has a reported high accuracy rate varying from 95.8% to 97.87%, with a sensitivity rate of 95%–98.4% and a specificity rate of 60%–93%.^[2] FNAC is routinely employed as part of a “triple test” (including mammography and trucut biopsy for histopathological examination) for screening breast malignancy.

In spite of these advantages, there are many pitfalls of breast FNAC as cytological diagnosis is based on subjective evaluation of cellular details including nuclear features for differentiating benign and malignant lesions and may raise a difficulty in establishing diagnosis, particularly for “gray areas” which account for about 10% of cases.^[3,4]

In order to overcome this subjectivity, studies based on computer-assisted image analysis and morphometric quantification of nuclear features have shown utility in differentiating benign and malignant aspirates.^[5-8]

Morphological changes in nuclear structure are often the diagnostic hallmark of carcinoma. These changes include

Address for correspondence: Dr. Anita Ramdas,
Department of Pathology, Pondicherry Institute of Medical Sciences,
Kalapet - 605 014, Puducherry, India.
E-mail: anitaramdas2002@yahoo.co.in

Received: 01-04-2020
Accepted: 11-07-2020

Revised: 04-07-2020
Published: 22-01-2021

Access this article online

Quick Response Code:

Website:
<http://www.jmau.org/>

DOI:
10.4103/JMAU.JMAU_17_20

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Nirnanjan Pandian DJ, Ramdas A, Ambroise MM. Image analysis-assisted nuclear morphometric study of benign and malignant breast aspirates. *J Microsc Ultrastruct* 2021;9:114-8.

variation in nuclear size, shape, chromatin, and size of nucleoli. These parameters are better quantified in cytology rather than histopathology as nuclear preservation is optimum in alcohol-fixed cytology smears. A large number of parameters have been assessed objectively by morphometry, but nuclear size parameters have been found to be significant in various studies.^[5-9] These nuclear morphometric features have also been shown to be related to carcinoma grade and may predict the prognosis of the carcinoma patients.^[10]

An automated system for breast cancer cytology is not yet available unlike for cervical cancer screening where a computer-assisted neural network system “PAPNET” is used for minimizing false-negative cytology smears.^[11] Morphometric studies using freely available image analysis software may help in creating an effective low-cost automated screening system for breast aspirates.^[7-10]

The present study was undertaken to compare some of the nuclear morphometric parameters of benign and malignant breast aspirates of histologically confirmed lesions. We also wanted to evaluate the utility of nuclear density parameters (nuclear chromasia) in addition to size and shape parameters. Nuclear density calculation is now possible using Image J software, developed by Wayne Rasband at the National Institute of Health at Bethesda, Maryland, USA.

The objectives of this study were to measure nuclear morphometric parameters including nuclear diameter, nuclear area, nuclear perimeter, axis ratio, and density in benign and malignant breast aspirates using Image J software. Image J is a freely available Java-based public-domain image processing and analysis program developed at the National Institutes of Health, USA.^[12]

MATERIALS AND METHODS

The study was carried out on breast FNAC smears retrieved from archival material. Cases were included in the study only if histopathological correlation was available. Women who underwent breast FNAC with subsequent histopathological diagnosis of benign lesions (fibroadenoma or fibrocystic disease) and malignant invasive ductal carcinoma (not otherwise specified [NOS]) were included. The study included 20 aspirates from women with breast lumps cytologically diagnosed as benign lesions and 20 from breast lump FNAC in women with a diagnosis of carcinoma. Only hematoxylin and eosin (H and E)-stained smears of the selected aspirates were used for the study purpose as nuclear preservation is optimum in alcohol-fixed smears.

FNAC slides as per inclusion criteria were selected by the pathologists. The slides were then coded to avoid bias. Images of the FNAC slides were captured using an Olympus microscope CX31 (ocular lens 10 × and objective 40×, high power) equipped with a Canon Digital Camera (Model DS126491 Systems). Each image had a resolution of 5184 × 3456 pixels and was saved in a JPEG format. A digital

picture was obtained from five different fields for each case and stored as JPEG files on a laptop with image analysis software.

Image J 1.44C software for image analysis and processing was used for the morphometric study. Image J has an interactive menu bar which is displayed at the top of the screen when the program is run. Selected microphotograph was converted to grayscale.

Fifty representative nuclei (from five different fields) were evaluated for each case. Nuclei of nonoverlapping well-preserved cells with sharp nuclear boundaries were chosen for analysis. The representative nuclei in each image were outlined and analyzed using Image J. The investigator who was blinded to the diagnosis carried out morphometric measurements of 6 nuclear parameters [Table 1] on 50 nonoverlapping cells with intact nuclei in each of the 40 aspirates selected. Nuclear morphometric parameters analyzed in a total of 2000 cells (1000 each for benign and malignant categories) were included for analysis.

Institutional ethics committee approval was obtained before the start of the study.

Statistical analysis

Nuclear parameters were subsequently compared between benign and malignant groups. The mean, median, and standard deviation (SD) of various parameters were analyzed. Comparisons between the two groups (benign and malignant lesions) were performed using the Mann–Whitney test. The diagnostic ability of various parameters to predict carcinoma was assessed using receiver operating characteristic (ROC) curve analysis. The ROC plots the true-positive rate (sensitivity) against the false-positive rate (1-sensitivity). The area under the curve (AUC) gives the overall diagnostic value. A perfectly accurate test would yield an AUC of 1.0, and an AUC of 0.5 indicates a predictive efficacy no better than chance. $P < 0.05$ was considered statistically significant for all tests.

Table 1: Nuclear morphometric and density parameters used in the study

Nuclear morphometry parameter	Measures
Size parameters	
Area	Area of the nucleus
Perimeter	The length of the nuclear contour (the length of the outside boundary of the selection)
Feret diameter	The longest distance between any two points along the selection boundary, also known as maximum caliper
Nuclear density parameters	
Integrated density	The product of area and mean gray value
Raw integrated density	The sum of the values of the pixels in the selection
Shape parameters	
Axis ratio	Is a shape descriptor (aspect ratio) It is the ratio of length of major and minor axis

Data processing and analysis were performed using SPSS for Windows (version 20.0. Armonk, New York: IBM corporation, USA).

RESULTS

Distribution of cases

Of the 20 selected benign aspirates, 16 were histologically confirmed fibroadenomas and 4 were cases of fibrocystic disease. All 20 malignant aspirates were confirmed invasive ductal carcinomas (NOS).

Clinical features of cases

Side of breast

Majority of the lesions (80%) in both benign (11/20 patients) and malignant (16/20) cases were present in the left breast.

Age distribution of cases

The range was 16–75 years, with a mean age for benign lesions noted as 31 years and 51 years for malignant lesions, respectively. Majority of the benign lesions were in the age group of 21–40 years (40%), whereas majority of the malignant cases were in the age group of 51–60 years (45%).

Presenting symptom

Thirty-seven cases in both benign and malignant groups presented with complaints of a lump in the breast, whereas three cases presented with breast pain (two in malignant category).

Quadrant of breast

The right breast upper inner quadrant showed lumps in 40% of benign cases (8/40). The majority of breast lumps in malignant category were in the left upper inner quadrant (50%).

Family history

only one patient with a diagnosis of carcinoma breast had a positive family history.

Figure 1: Receiver operating characteristic curves of nuclear area, nuclear perimeter, Feret diameter, and integrated and raw nuclear densities for identification of carcinoma

Morphometric study of breast aspirates

Nuclear morphometry was carried out on 50 nonoverlapping nuclei selected in each of the cases, totaling 2000 nuclear measurements in the 40 cases (1000 for benign and 1000 for malignant). The morphometric measurements of mean nuclear area, perimeter, diameter, and density parameters (both integrated and raw nuclear densities) showed a significant difference between the two categories with $P < 0.05$.

Axis ratio did not show a statistically significant difference between benign and malignant nuclei, as shown in Table 2.

The ROC curve analysis shown in Table 3 and Figure 1, reveals that mean nuclear area, perimeter, diameter, and density parameters (both integrated and raw nuclear densities) are helpful in discriminating carcinoma cases from benign cases. The best cutoff point with sensitivity and specificity is shown in Table 4.

DISCUSSION

Breast cancer is the most common cancer in women worldwide and ranks number one among Indian women, with an incidence rate as high as 25.8/100,000 and a mortality of 12.7/100,000. Future projection suggests that numbers will increase. Better awareness and availability of adequate screening programs with early diagnosis will lead to a favorable picture.^[1]

Various diagnostic modalities employed include the “triple test,” but although FNAC is widely accepted as a rapid, inexpensive procedure with a high sensitivity and specificity, there are pitfalls where an inaccurate diagnosis may be rendered. Al-Kaisi^[4] in their study on the spectrum of the “gray zone” in breast cytology reviewed 186 cases of atypical and suspicious cytology and concluded that subjective evaluation of aspirates with overlapping features of hyperplasia and malignancy may result in diagnostic errors. Furthermore, variation in cell morphology, nuclear features, and chromatin pattern also contribute to subjectivity.^[4,13]

Our study aimed to assess the utility of objective nuclear parameters in differentiating benign and malignant breast aspirates. Many authors have studied different nuclear morphometric parameters including size and shape parameters like our study.^[5-10,14-17] In the present study, six nuclear parameters including size parameters (nuclear area, perimeter, and diameter), density (integrated and raw) parameters measuring nuclear chromasia, and axis ratio (shape parameter) were assessed morphometrically. All these parameters except the axis ratio showed statistically significant differences with $P < 0.05$.

Parmar *et al.*^[5] in their study had similar results like ours with axis ratio, with the difference between benign and malignant cells not statistically significant. Similarly, Abdalla *et al.*^[17] did not find any of the shape parameters such as axis ratio to be statistically significant.

Narasimha *et al.*^[15] in their study using similar image analysis software as our study (Image J1.44C) reported that size-related

Table 2: Comparison of nuclear morphometric parameters between benign and malignant cases

Nuclear morphometric parameters	Mean $\pm$ SD, median (minimum-maximum)		P
	Benign (n=1000)	Malignant (n=1000)	
Nuclear area (μm^2)	16.67 $\pm$ 5.41, 16.48 (3.64-39.32)	39.63 $\pm$ 22.41, 34.25 (13.33-211.23)	<0.001
Nuclear perimeter (μm)	15.79 $\pm$ 2.90, 15.80 (6.78-24.91)	23.62 $\pm$ 6.93, 22.01 (13.23-67.80)	<0.001
Diameter (μm)	5.71 $\pm$ 1.04, 5.73 (2.34-9.69)	8.38 $\pm$ 2.15, 7.99 (4.69-19.67)	<0.001
Axis (aspect) ratio	1.375 $\pm$ 0.29, 1.29 (1.000-3.600)	1.376 $\pm$ 0.27, 1.32 (1.023-3.09)	0.153
Integrated density	1501.72 $\pm$ 674.9, 1412.71 (238.08-4288.39)	3837.78 $\pm$ 2349.15, 3269.18 (1007.73-22199.40)	<0.001
Raw integrated density	209,808.52 $\pm$ 94,285.06, 197,373.00 (33,263.00-599,141.00)	536,184.90 $\pm$ 328,205.86, 456,745.50 (140,792.00-3,101,532.00)	<0.001

SD: Standard deviation

Table 3: Receiver operating characteristic curve analysis of nuclear parameters

Parameter	AUC	SE	P	95% CI	
				Lower limit	Upper limit
Nuclear area (μm^2)	0.926	0.005	<0.001	0.916	0.937
Nuclear perimeter (μm)	0.892	0.007	<0.001	0.879	0.906
Feret diameter (μm)	0.893	0.007	<0.001	0.879	0.907
Integrated density	0.900	0.007	<0.001	0.887	0.913
Raw integrated density	0.900	0.007	<0.001	0.887	0.913

CI: Confidence interval, SE: Standard error, AUC: Area under the curve

Table 4: Cutoff values determined from receiver operating characteristic curve analysis

Parameter	Cutoff	Sensitivity (%)	Specificity (%)
Nuclear area (μm^2)	22.0	83.3	85.2
Nuclear perimeter (μm)	19.0	73.5	88.7
Feret diameter (μm)	7.0	72.3	90.2
Integrated density	2417	72.5	89.8
Raw integrated density	337705	72.5	89.8

parameters such as area, perimeter, diameter, concave points, and compactness of the nucleus were appropriate to distinguish between benign and malignant cells in aspirates. They found significant differences in all parameters but determined that nuclear area and perimeter are the most important. The mean nuclear area in their study ranged from 64 to 82 μm^2 for benign cells and between 72 and 163 μm^2 for malignant cells. The mean nuclear diameter in their study was 9.53 ± 0.61 μm for benign cells and 12.05 ± 2.4 μm for malignant cells and nuclear perimeter 29.95 ± 1.91 μm (benign cells) and 40.87 ± 3.80 μm (malignant cells). Although their values for mean nuclear area, perimeter, and diameter were slightly higher than in our study, there was a significant statistical difference. The difference in values may be due to different methodologies.

Other authors like Laishram and Shariff^[6] and Abdalla *et al.*^[17] also found nuclear area, perimeter, and diameter to be useful in distinguishing benign from malignant lesions. Nuclear area is the most common morphometric measurement used by various authors.^[6-9,14-17] The present study also demonstrates that the

use of nuclear size parameters shows high SD for malignant aspirates as compared to that of benign due to high degree of nuclear pleomorphism in malignancy.

Only a few studies have attempted to identify the cutoff value to distinguish benign and malignant aspirates. The cutoff value between benign and malignant lesions derived in the study by Kashyap *et al.*^[9] was 31.93 μm^2 for nuclear area, 21.55 μm for nuclear perimeter, and 7.855 μm for maximum Feret diameter. The cutoff value between benign and malignant lesions in our study was 22 μm^2 for nuclear area, 19 μm for nuclear perimeter, and 7 μ for maximum Feret diameter. Abdalla *et al.*^[17] found the mean nuclear area cutoff to be 60.5 μm . This difference in mean values and best cutoff values could be due to the software, calibration factor, ethnicity, slide preparation, and staining methods. Image morphometry needs to be standardized in each institute.

In contrast to nuclear size parameters, nuclear density parameters such as integrated and raw integrated densities have not been studied extensively. Kashyap *et al.*^[9] studied mean density and sum density (using Nikon Imaging Software-AR with integrated NIS-Elements software) and found the difference between benign and malignant lesions for density parameters to be statistically significant. Our results were similar to those of Kashyap *et al.* Kashyap *et al.* have not evaluated the cutoff for the density parameters. Our data reveal that the density parameters also have good diagnostic value in distinguishing benign and malignant aspirates.

However, morphometric measurements may not be helpful in distinguishing between lobular carcinoma and benign proliferative disease. Lobular carcinoma nuclear parameters are only slightly different from benign cell nuclei.^[14,18] Lobular carcinoma aspirates were not included in our study because of this pitfall. Subsequently, after establishing the cutoff in our study, we did try a few cases of lobular carcinoma, but nuclear morphometric analysis was not helpful in the distinction from borderline and benign proliferative cases. We also agree that this is a pitfall of nuclear morphometry.

CONCLUSION

Computerized image analysis for nuclear morphometry is able to offer an objective assessment of parameters to help differentiate between benign and malignant breast aspirates.

Nuclear morphometry can be used as an adjunct to FNAC breast. In the present study, size parameters such as nuclear area, nuclear perimeter, and nuclear diameter along with nuclear density parameters were found to be useful. Recent advances in image analysis software are helpful in analyzing nuclear size as well as nuclear chromasia. Training, standardization of methodology, and software are, however, essential to increase the efficacy.

Financial support and sponsorship

The authors acknowledge the Indian Council of Medical Research, New Delhi, India, for providing sponsorship through Short-Term Research Studentship (STS 2019-02083) to the first author.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. *Asia Pac J Clin Oncol* 2017;13:289-95.
2. Mane PS, Kulkarni AM, Ramteke RV. Role of fine needle aspiration cytology in diagnosis of breast lumps. *Int J Res Med Sci* 2017;5:3506-10.
3. Mehta R, Tewari K, Goyal N, Basak U, Gupta A. Triple approach for diagnosing breast lesions – Experience at a tertiary care hospital. *J Mar Med Soc* 2017;19:123-7.
4. al-Kaisi N. The spectrum of the “gray zone” in breast cytology. A review of 186 cases of atypical and suspicious cytology. *Acta Cytol* 1994;38:898-908.
5. Parmar D, Sawke N, Sawke GK. Diagnostic application of computerised nuclear morphometric image analysis in fine needle aspirates of breast lesions. *Saudi J Health Sci* 2015;4:51-5.
6. Laishram S, Shariff S. Role of nuclear morphometry in distinguishing gray areas of breast lesions. *JMR* 2017;3:132-35.
7. Chowdhury AR, Talukdar M, Adhikari A. Role of nuclear morphometry as objective parameter to evaluate cytology smears of epithelial breast lesions. *JCDR* 2017;11: EC26-28.
8. 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.
9. Kashyap A, Jain M, Shukla S, Andley M. Study of nuclear morphometry on cytology specimens of benign and malignant breast lesions: A study of 122 cases. *J Cytol* 2017;34:10-5.
10. Kashyap A, Jain M, Shukla S, Andley M. Role of nuclear morphometry in breast cancer and its correlation with cytomorphological grading of breast cancer: A study of 64 cases. *J Cytol* 2018;35:41-5.
11. Koss LG, Lin E, Schreiber K, Elgert P, Mango L. Evaluation of the PAPNET cytologic screening system for quality control of cervical smears. *Am J Clin Pathol* 1994;101:220-9.
12. Rasband WS, Image J, U.S National Institutes of Health, Bethesda, Maryland, USA. Available from: <http://Imagej.nih.gov/ij/>;1997-2018.
13. Saraf S, Khare M, Kalgutkar A. Fine needle aspiration cytology of breast lumps A correlation with histopathology diagnosis. *Indian J Pathol Oncol* 2016;3:103-6.
14. Krishnappa I, Parthiban R, Sharma A, Rani P. Significance of nuclearmorphometry as a diagnostic tool in fine-needle aspirates of breast masses. *Indian J Pathol Oncol* 2018;5:592-7.
15. Narasimha A, Vasavi B, Kumar HM. Significance of nuclear morphometry in benign and malignant breast aspirates. *Int J Appl Basic Med Res* 2013;3:22-6.
16. Yadav H, Gill M, Srivastava D, Gupta V, Gupta S, Sen R. Significance of morphometric parameters in the categorization of breast lesions on cytology. *Turk Patoloji Derg* 2015;31:188-93.
17. Abdalla F, Boder J, Buhmeida A, Hashmi H, Elzagheid A, Collan Y. Nuclear morphometry in FNABs of breast disease in Libyans. *Anticancer Res* 2008;28:3985-9.
18. Rajesh L, Dey P, Joshi K. Automated image morphometry of lobular breast carcinoma. *Anal Quant Cytol Histol* 2002;24:81-4.