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Original Research Article

Nuclear Morphometric Analysis in Fine Needle Aspirates of Breast Neoplasms

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Abstract

Background: Fine needle aspiration cytology (FNAC) is applied as the primary tool for the diagnosis in breast masses because of its ease and rapidity. However, it is a largely subjective tool. The morphological overlap among the sequential lesions from the precancerous group to frank carcinoma further cause "gray zone" in cytology, estimated to constitute 8.9%. Morphometry is the measurement of cell parameters microscopically by image analysis. The present study has been undertaken to evaluate role of nuclear morphometry in benign and malignant breast neoplasms. *Aim:* To classify breast lumps according to guidelines given by UK National Health Service Breast Screening Program (NHSBSP) and to compare the nuclear morphometry among those categories. *Setting and design:* A retrospective-prospective study. *Material and Methods:* Cases with palpable breast lumps were evaluated by FNAC and were cytologically classified according to UKNHSBSP guidelines. Nuclear Morphometric parameters: nuclear area, perimeter, long and short axis and diameter were studied and compared within the various categories. *Statistical Analysis used:* Student's t test was used as the test of significance. *Result:* Mean age was 37.7 years. 47.3% cases belonged to Category C2 (benign), 6.8% cases to Category C3 (atypia probably benign), 17.6% cases Category C4 (suspicious of malignancy) and 27% belonged to Category C5 (malignant). The nuclear morphometric parameters including nuclear area, perimeter, short axis, long axis and diameter were found to be statistically significant ($p<0.05$) in differentiating between benign and malignant categories. Conclusion: Nuclear morphometry serves as a valuable method in distinguishing benign and malignant breast aspirates and also correlates well with the cytological categories.

Keywords: FNAC; UK NHSBSP Guidelines; Nuclear Morphometry.

Introduction

Breast lesions account for the largest group of conditions necessitating pathological, radiological and surgical intervention. Breast presents with a plethora of benign and malignant pathological

conditions, benign being four to five times more common than malignant [1]. Fine needle aspiration cytology(FNAC) is applied as the primary tool for the diagnosis in breast lumps because of the ease and rapidity of the technique and is minimally invasive and inexpensive procedure. However, it is a subjective tool [2]. The morphological overlap among the sequential lesions from the precancerous group to frank carcinoma cause "gray zone" in cytology, estimated to constitute 8.9% [3].

Morphometry is the measurement of various cell parameters microscopically by image analysis [4]. The progression pattern of nuclear morphometric parameters have shown gradually increased values from benign to atypical, ductal carcinoma in situ(DCIS) and further to Invasive Carcinoma [5]. Morphometry acts as an objective tool to supplement FNAC in the crucial differentiation of benign from malignant lesions [5].

Material and Methods

This was a retrospective-prospective study conducted over a period of five years from June 2012 to May 2017 on patients presenting with breast lumps. A total of 74 cases were studied. Ethical clearance was procured from the Ethical committee of the institute after taking informed and written consent from the patients before the procedure. Patients presenting with palpable breast lumps or radiologically detected lumps were included. Detailed clinical data was recorded including history, physical examination, findings regarding size of breast lump and associated features.

Exclusion criteria included all non-neoplastic lesions of the breast.

Under all aseptic conditions, FNAC was performed using a 21-23 gauge needle by aspiration using the syringe fitted holder or non aspiration (direct needle puncture) technique.

Both dry and wet slides were prepared. Air dried smears were stained with Leishman Giemsa stain and wet fixed (Ethyl alcohol) smears were stained with Papanicolau and Hematoxylin and Eosin (H&E) stains. Smears obtained from radiologically guided aspirations were also processed similarly and examined under light microscope.

Cytomorphological features were recorded according to the guidelines given by UK National Health Service Breast Screening Program(NHSBSP) and smears were classified into five categories: [6]

1. Category C1: inadequate
2. Category C2: benign
3. Category C3: atypia probably benign
4. Category C4: suspicious of malignancy
5. Category C5: malignant

Nuclear morphometric analysis was done on wet fixed stained cytological smears with the help of image analysis software Motic Image Plus 2.0. Images were taken by a camera coupled with Motic BA310 microscope at magnification of X400 and were stored on the host computer.

The area on the slide to be imaged was selected. Random clusters with minimum overlapping of nuclei were assessed excluding any degenerated cell cluster. Measurements were made with 40X objective magnification which when added to the 10X result in an image magnification of X400 on the monitor screen. Around 25 nuclei/ case were analysed.

Following parameters were noted:

1. Nuclear area: area within the outlined nuclear perimeter.
2. Nuclear perimeter: length around the nuclear border.
3. Diameter: diameter of the circle with the same area.
4. Long axis: longest axis of the best fitted ellipse.
5. Short axis: measured perpendicular to the long axis.

The mean and standard deviation were calculated for all parameters and the results obtained by the computerized cytomorphometry were compared between the five cytological categories.

Histo-cytological correlation was done in cases where histopathology reports were available.

Statistical analysis

Student's t test was used as the test of significance using SPSS computer software. A p-value of less than 0.05 was considered as statistically significant.

Results

The age of the patients ranged from 13 years to 67 years with the mean age of 37.7 years. Out of a total of 74 patients, majority of the patients were in 4^{th} decade of their life 25.7% (19/74) followed by 21.6% (16/74) in the 5^{th} decade. 95.9% (71/74) cases were females and 4.1% (3/74) cases were males.

Hundred percent (74/74) patients presented with the chief complaint of breast lump. Ninety eight point seven percent patients presented with palpable breast lumps and in 1.3% cases Ultrasound (USG)

showed presence of a lump for which guided FNA was done. Nipple discharge was seen in 2.7% (2/74) cases. 54.1% (40/74) presented with left side breast lump followed by right side involvement in 40.5% (30/74). Bilateral involvement was seen only in 5.4% (4/74) cases. A total of 78 lumps (70 unilateral+4 bilateral) were analysed. Majority of the patients presented with lumps in upper inner quadrant 27/78 (34.6%) cases, followed by 20 /78 (25.6%) cases in upper outer quadrant. Out of the 20 cases presenting with lumps in the upper outer quadrant, eight were malignant as compared to lumps presenting in the upper inner quadrant where only 6/27 cases were reported as malignant (40% vs 22.2%).

The cytological smears were divided into various categories according to the guidelines given by UK National Health Service Breast Screening Program (NHSBSP) [6]. Category C1 (inadequate) consisted of 1/74(1.3%) cases, Category C2 (benign) 35/74 (47.3%) cases, Category C3 (atypia probably benign) 5/74 (6.8%) cases, Category C4 (suspicious of malignancy) 13/74 (17.6%) cases and Category C5 (malignant) consisted of 20/74 (27%) cases. (Table 1)

Nuclear morphometry was carried out using the Motic Image Plus 2.0 software (Figure 1-3). Five nuclear parameters viz, nuclear area, perimeter, short axis, long axis and diameter were analysed. Mean and standard deviation (SD) were calculated for each category (Table 2). All the parameters were found to be statistically significant (*p value<0.05*) on comparing Category C2 with Category C5 as well as on comparing C2 and C4 while only nuclear area, long axis and diameter were found to statistically significant when Category C2 and C3 were compared. However, results were statistically non significant on comparing C4 and C5 (Table 3).

Histopathological diagnosis was available in 62/74 (84.9%) cases. Out of which 57/62(91.9%) cases showed Cyto-histopathological correlation while 5/62 (8.1%) cases showed non-concensus. In the analysis of the study, a sensitivity of 89.2%, specificity of 94.1%, positive predictive value of 92.6% and a negative predictive value of 91.4% was observed.

Table 1: Cytological Categorization of cases According to U.K. Guidelines

Category (UK Guidelines)	Number of Cases
Category C1: Inadequate	01(1.3%)
Category C2: Benign	35(47.3%)
Category C3: Atypia Probably Benign	05(6.8%)
Category C4: Suspicious of Malignancy	13(17.6%)
Category C5: Malignant	20(27%)
Total	74(100%)

Table 2: Nuclear Morphometric Parameters in Various Categories

Category	Nuclear Area (Mean ± Sd)	Nuclear Perimeter (Mean ± Sd)	Short Axis (Mean ± Sd)	Long Axis (Mean ± Sd)	Diameter (Mean ± Sd)
C2 (N=35 cases)	50.4±11.6µm	30.3±3.6µm	7.0±0.8µm	8.4±1.1µm	8.0±0.9µm
C3 (N=5 cases)	78.3±55.1µm	36.0±11.4µm	8.2±2.8µm	10.3±3.4µm	9.6±3.2µm
C4 (N=13 cases)	135.9±42.4µm	48.8±7.1µm	11.4±1.9µm	14.0±2.0µm	13.0±2.1µm
C5 (N=20 cases)	134±28.9µm	48.5±4.9µm	11.5±1.4µm	13.8±1.4µm	13±1.4µm

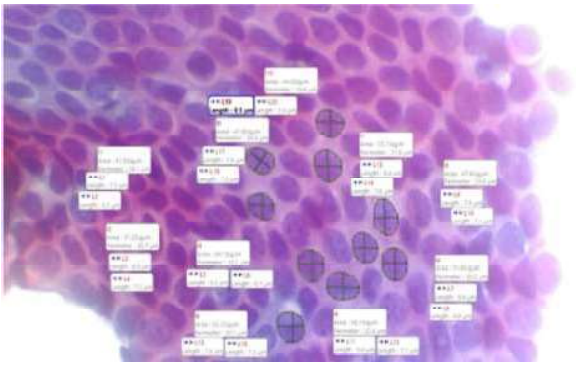

Fig 1: Nuclear Morphometric Analysis-C2 (Benign) category (H&E, 400 x)

Table 3: Comparison of Morphometric Parameters in Various Categories

	Nuclear Area	Perimeter	Short Axis	Long Axis	Diameter
Category C2/ Category C3	S*	NS†	NS	S	S
Category C2/ Category C4	S	S	S	S	S
Category C2/ Category C5	S	S	S	S	S
Category C3/ Category C4	S	S	S	S	S
Category C3/ Category C5	S	S	S	S	S
Category C4/ Category C5	NS	NS	NS	NS	NS

*S: Statistically Significant ($p<0.05$)
†NS: Statistically Non-Significant ($p>0.05$)

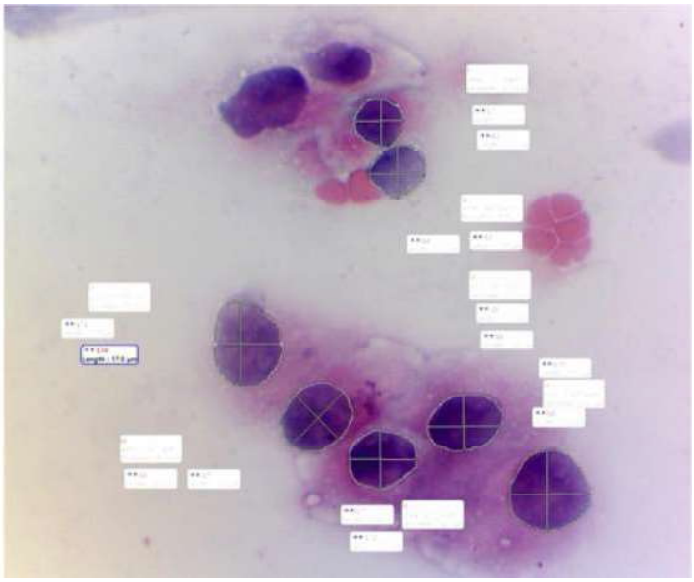

Fig 2: Nuclear Morphometric Analysis- C4 (suspicious of malignancy) category (H&E stain, 400 x)

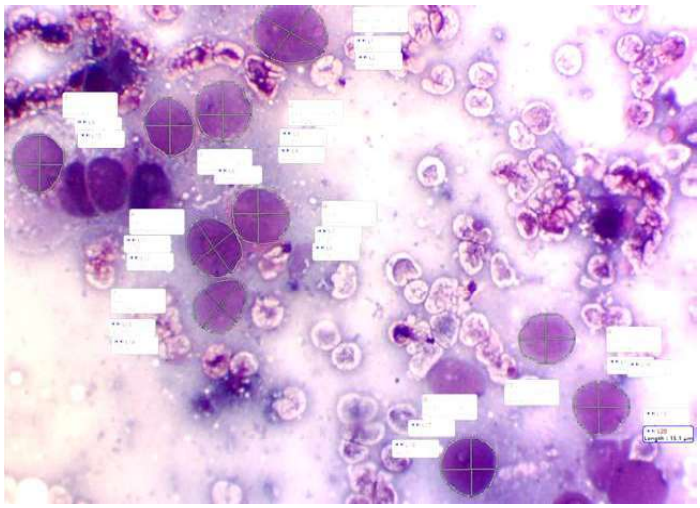

Fig 3: Nuclear Morphometric Analysis-C5 (malignant) category (H&E stain, 400 x)

Discussion

Fibroadenomas are the most common benign tumor found in all age groups followed by Fibroadenosis and Fibrocystic disease. The less common lesions include Cystosarcoma phyllodes, Lipoma of breast, Ductal papilloma and Gynecomastia [7]. Breast Cancer is the most common malignancy now replacing cervical cancer as the leading cause of cancer in females [4].

A palpable mass is the most common symptom of underlying breast pathology and preoperative evaluation of breast lumps is an essential part of the management of breast lesions [8]. FNAC is applied as the primary tool for the diagnosis in breast lumps however there is some morphological overlap among the sequential lesions in few cases where an unequivocal diagnosis cannot be made [3].

Morphometry is the measurement of various cell parameters microscopically by image analysis. Various parameters have been studied but nuclear parameters such as nuclear area(NA), nuclear perimeter (NP), diameter or axes have been found to be consistent [5],[9]. The progression pattern of these parameters have shown gradually increased values from benign to atypical, ductal carcinoma in situ (DCIS) and further to Invasive Carcinoma [5].

In the present study, the mean age of the patients was 37.7 years. Majority of the patients were in the 4^{th} decade followed by the 5^{th} decade. Similarly, in the studies conducted by Prasanth et al and Rathi et al the age incidence ranged from 16 to 74 years and 16 to 64 years respectively with majority of patients presenting in the 4^{th} decade [10,11].

54.1% cases presented with lump in the left breast, followed by right breast involvement in 40.5% cases and bilateral involvement in 5.4% cases. This is comparable to the results obtained by other studies [10,11].

Majority of the lumps were found to be present in the upper inner quadrant (34.6%) followed by upper outer quadrant (25.6%) while Yalavarthi et al. [12] and Rathi et al. [11] observed that most of the lumps were seen in the upper outer quadrant. However, in the present study, malignant lumps were more commonly seen involving the upper outer quadrant (40%) as compared to the upper inner quadrant where only 22.2% cases were reported as malignant. This is in concensus to the study conducted by Aljarrah et al. [13] where the majority of malignant tumors affected the upper outer quadrant.

The FNA smears were divided into various categories according to the guidelines by UK National Health Service Breast Screening Program (NHSBSP). We observed that benign (Category C2) lesions were most frequent, accounting for 47.3% of the cases, followed by malignant (Category C5) cases being 27% of the total. Similar findings were described by other authors [8,14].

This study aimed to explore the possible role of nuclear morphometry to differentiate benign and malignant lesions. Nuclear area (NA) is the most studied parameter in published literature. In present study, the mean nuclear area of 50.4±11.6µm was observed in the C2 (benign) cases, 78.3±55.1µm was seen in C3 (atyia probably benign) cases while 135.9±42.4µm and 134±28.9µm in the C4(suspicious of malignancy) and C5 (malignant) cases respectively. In literature other studies also revealed increased nuclear area in C4 and C5 categories [15].

All the parameters were found to be statistically significant on comparing C2 (benign) with C4 (suspicious of malignancy) and C5 (malignant) categories and C3 (atypia probably benign) with C4 (suspicious of malignancy) and C5 (malignant) categories similar to findings observed by Nnodu et al. [15]. Other studies comparing the benign and malignant categories also showed corresponding results [2,3,16,17].

Conclusion

In conclusion, for the interpretation of FNAC smears from breast lumps, Nuclear Morphometry can act as an adjunct in differentiating between benign and malignant breast lesions and also can act as a helpful tool in the "gray zone" areas. Use of morphometry could overcome the limitations of inter-observer variation and not only improve the diagnostic assessment of patients with breast lumps, it can also be helpful in deciding patient management in cases with a diagnostic dilemma.

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