Significance of nuclear morphometry in benign and malignant breast aspirates

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Abstract

Background: Breast carcinoma is one of the most common cancers occurring in the female population world-wide. Normal cells gradually transform to form the cancer cells through several stages. Nuclear changes occurring during these transformational steps need to be assessed objectively. Hence nuclear morphometry can be used as a diagnostic tool. **Aim:** To compare the nuclear morphometric parameters of benign and malignant breast aspirates. **Study Design:** Cytology was used to categorize aspirates from the breast lumps in to malignant (30 cases), and benign (30 cases). Nuclear parameters were calculated using the Image J 1.44C morphometric software. Several nuclear size parameters were analyzed. **Results:** The nuclear area, perimeter, diameter, compactness, and concave points were found to be statistically significant ($P < 0.05$) parameters in differentiating benign, and malignant aspirates. **Conclusion:** Nuclear morphometry was thus, a useful objective tool in the differentiating benign, and malignant breast lesions.

Key words: Breast lesions, fine needle aspiration cytology, nuclear morphometry

Introduction

Breast carcinoma is one among the most common cancers occurring globally. In India, breast cancer is one among the top three cancers and the incidence of breast cancer in Kolar district is around 6.4%.[1]

Variations in nuclear structure are the morphologic hallmark of cancer diagnosis. There is a gradual shift in the nuclear parameters as the disease progresses from benign to malignant.

Nuclear size, shape, chromatin pattern, and nucleoli size and a number have all been reported to change in breast cancer.[2]

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These nuclear morphometric features have been shown to predict the prognosis of the breast cancer patients.[3]

Fine needle aspiration cytology (FNAC) has been routinely employed as a screening test for the breast cancer along with mammography and the clinical examination. However, cytological diagnosis is based on the subjective evaluation of nuclear features and thus, may raise a difficulty in establishing the precise diagnosis pre-operatively.[4]

There have been studies on computerized nuclear morphometric analysis of benign and malignant breast aspirates, and these may be supportive in diagnostic decisions.[4]

Studies on nuclear morphometric analysis of breast aspirates in South- Indian population are limited. Hence we have undertaken this study with an aim to compare the nuclear morphometric parameters of benign and the malignant breast aspirates.

Materials and Methods

This was a retrospective study. We collected sixty fine needle aspiration samples from the archives of our department. Cytology was used to categorize

aspirates from the breast lumps into 4 groups. Group I-fibroadenomas (10 cases), Group II-fibrocystic disease (10 cases), Group III-hyperplasia (10 cases), and Group IV-carcinoma (30 cases). Only those cases which had confirmed histopathological correlation, were included in the study. We used a microscope with an $\times 2.5$ ocular and an $\times 40$ objective to visually select a field for analysis. A 640×400 pixel digital image of the field was produced by a camera on the microscope and frame grabber card in a PC. Around 50 nuclei/case were analyzed using the Image J 1.44C morphometric software for image processing, and analysis (JAVA) developed by the National Institute of Health, USA.

The following nuclear features were analyzed:
*   Radius computed by averaging the length of radial line segments from the center of the nuclear mass to each of the points of the nuclear border.
*   Nuclear area was the area within the outlined nuclear perimeter.
*   Perimeter was measured as the distance around the nuclear border.
*   Diameter was the diameter of the circle with the same area as the outlined nucleus.
*   Compactness of the cell nuclei calculated using the formula: Perimeter^2/area.
*   Concave points counted the number of points on the nuclear border that lie on an indentation.[5]

The computer calculated the mean, standard deviation, and range for all the nuclear features.
*   Inclusion criteria: Only ductal carcinomas were considered for the study.
*   Exclusion criteria: Lobular, medullary, and metaplastic carcinomas were excluded.
*   Ethical clearance was obtained by the Institutional Ethics Committee.

Statistical analysis
The results obtained by the computerized cytomorphometry were compared between the four groups. Data were analyzed to evaluate the most distinctive morphometric features of all the features available. The nuclear parameters between all the 4 groups were compared using Analysis of variance (ANOVA) and between the groups using a *post hoc* test i.e., Bonferroni Multiple Comparisons Test. Statistical analysis was performed using the statistical software Graph Pad Instat.

A $P < 0.05$ was considered as statistically significant.

Results

Nuclear morphometric analysis
Our sample size was 60, which was categorized in to 4 groups: Group I-fibroadenomas (10 cases), Group II-fibrocystic disease (10 cases), Group III-hyperplasia (10 cases), and Group IV-carcinoma (30 cases).

Cytological features
Fibroadenoma
Benign appearing ductal epithelial cells in sheets, antler horn pattern or honeycomb pattern. Background shows bare nuclei [Figure 1].

Fibrocystic disease
Benign appearing ductal epithelial cells in sheets with cyst macrophages in the background [Figure 2].

Hyperplasia
Hypercellular smear showing ductal epithelial cells arranged in sheets showing mild variation in size and shape. Few cells may show nuclear atypia [Figure 3].

Carcinoma
Loosely arranged clusters of ductal epithelial cells showing nuclear pleomorphism, increased nuclear cytoplasmic ratio [Figure 4], nuclear indentations [Figure 5], and hyperchromatic nucleus. Mitotic activity may be seen. No bare nuclei.

The age distribution of the cases is shown in Table 1. Benign lesions were in the age group ranging from 21 to 40; hyperplasia was seen between 31 and 60 and malignancy between 40 and 70.
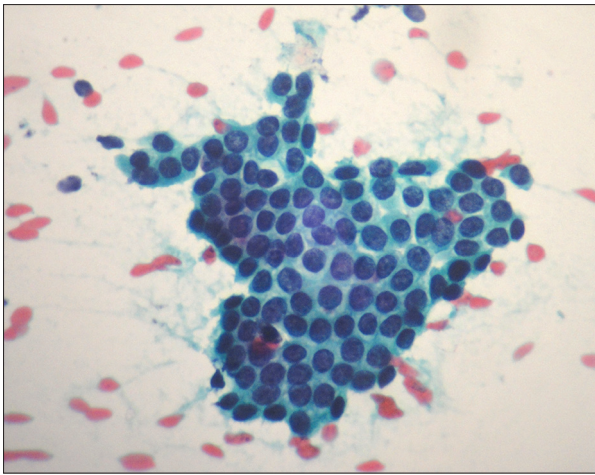
Nuclear morphometric analysis was carried out using the Image J 1.44C morphometric software for image processing and analysis [Figure 6]. The basic results of our study are shown in Table 2.

Using one-way ANOVA, the nuclear area, perimeter, diameter, compactness, and concave points were found to be statistically significant ($P < 0.05$).

For comparisons between the individual groups we employed *post hoc* test i.e., Bonferroni Multiple Comparisons Test. There was a significant difference in the nuclear area and diameter between fibroadenoma, fibrocystic disease, and carcinoma with a *P* value of (0.0009) and (0.0007), which is considered to be extremely significant. There was a significant difference in

| Age groups	Group I fibroadenoma	Group II fibrocystic disease	Group III hyperplasia	Group IV malignancy
21-30	7	8		
31-40	3	2	4	2
41-50			3	14
51-60			3	12
61-70				2
Total	10	10	10	30

Table 1: Age distribution of cases

Figure 1: Microphotograph of fibroadenoma showing ductal epithelial cells arranged in sheets with bare nuclei (Pap, x400)
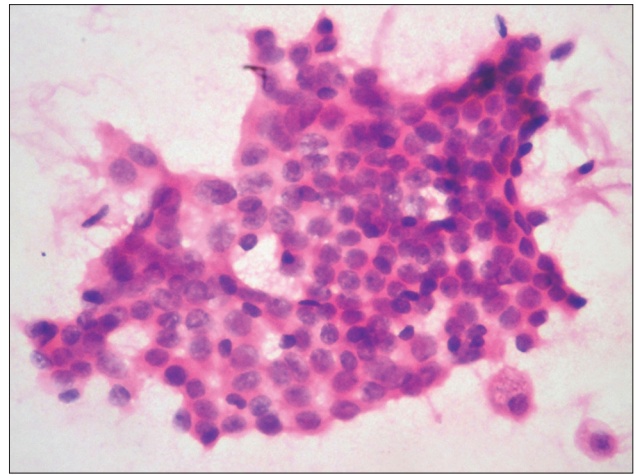

Figure 2: Microphotograph of fibrocystic disease showing benign appearing ductal epithelial cells and cyst macrophages (H and E, x400)
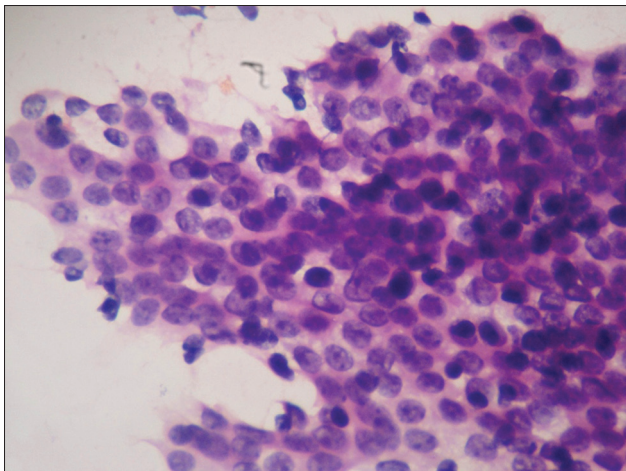

Figure 3: Microphotograph of ductal hyperplasia showing ductal epithelial cells in sheets show mild atypia (H and E, x400)
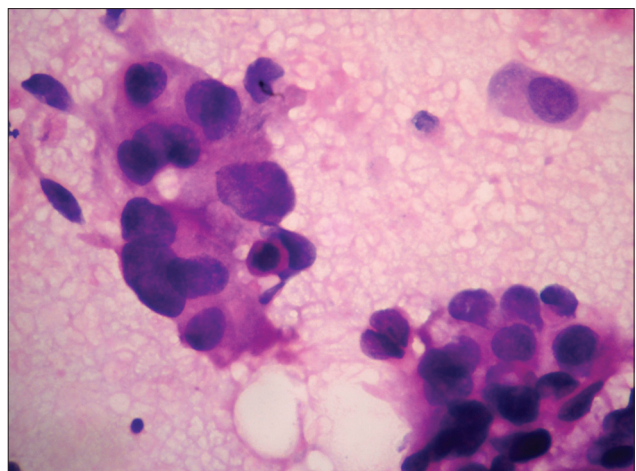
Figure 4: Microphotograph of carcinoma breast showing pleomorphic cells with nuclear indentations (H and E, x400)
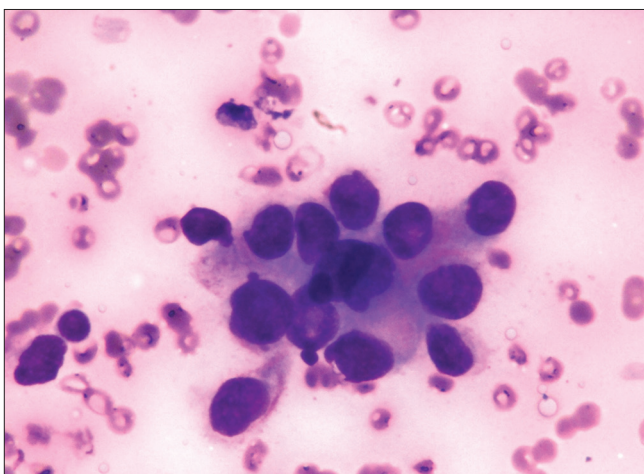
Figure 5: Microphotograph of carcinoma breast showing pleomorphic nuclei and nuclear indentations (concave points) (Leishman stain, x400)
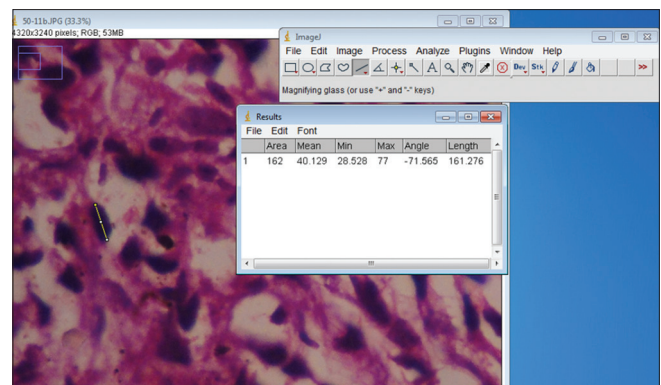
Figure 6: Image of the software used for morphometric analysis

perimeter and compactness between fibroadenoma, fibrocystic disease, hyperplasia, and carcinoma with a $P < 0.0001$, which is considered to be statistically significant.

Unpaired "t" test was used to find the significance of concave points between hyperplasia and carcinoma, which was highly significant ($P < 0.0001$).

The mean nuclear area and perimeter were useful in differentiating benign and malignant breast aspirates. The ductal carcinoma cells showed higher values for nuclear area, perimeter, diameter, compactness, and concave points when compared to fibroadenomas, fibrocystic disease, and hyperplasia.

Discussion

The leading cause of cancer mortality in Indian women is breast cancer with an annual diagnosis of 80,000 new cases every year.[6] Hence, adequate screening of the breast lumps is essential to safeguard the health of women. The progress of normal breast to carcinoma follows a sequence of events. There are several diagnostic modalities starting from the clinical examination, mammography, FNAC, biopsy etc., However, how precise are each one of them in giving accurate diagnosis. Though cytology is able to categorize benign and the malignant breast diseases, there are gray zones is cytology where an inconsistent diagnosis may be offered.

The gray zones in cytology are around 8.9% as reported by al-Kaisi.[7] These included technical limitations (4.5%), inexperience of the cytopathologist (2.4%), and overlap of cytological features of benign vs. malignant (2%).[7]

Our study aimed to explore the possible role of nuclear morphometric analysis to differentiate benign from the malignant lesions. Morphometric analysis of nuclear parameters has been studied by several authors.[2,4,8-11]

In the present study, the size related parameters (area, perimeter, diameter, concave points and compactness) of the nucleus were appropriate parameters to differentiate between benign lesions and infiltrative ductal carcinoma of the breast. These parameters showed significant differences between the benign breast lesions and carcinoma ($P < 0.05$). Some studies have also measured long axis and short axis

as nuclear morphometric parameters.[3,4,8] However, among the nuclear parameters nuclear area and perimeter are important.

In our study, there was a gradual increase in the nuclear area and perimeter in carcinomas when compared to benign lesions. Our results were in concordance with that of Fathi *et al*.[4] with the mean nuclear area being 64-82 um^2 for benign cases and 72-163 um^2 for malignant cases. Abdalla *et al*.[4] also showed that clearly reduced cohesiveness was associated with larger nuclear size. Wittekind and Schulte in their study showed that perimeter was the most powerful feature to differentiate between benign and malignant breast lesions.[12]

In our study, nuclear perimeter and compactness was highly significant in differentiating hyperplasia from carcinoma ($P < 0.0001$). Concave points represent the number of indentations present on the nuclear border. This parameter was found to be statistically significant ($P < 0.0001$) in differentiating hyperplasia from carcinoma.

Shape is one of the factors to assess nuclear atypicality. Shape factors have been shown to have prognostic value in breast cancer as proved by Yan *et al*.[3] He reported that the shape factor that includes short nuclear axis and the longest nuclear axis is of value to predict subsequent development of breast cancer among women with benign breast disease.[3] Nuclear form factor, a measure of the regularity of the nuclear perimeter was shown to have predictive value for discriminating benign and malignant conditions as proved by Mapstone and Zakhour.[9]

However, studies by Abdalla *et al*.[4] and Kalhan *et al*.[8] showed that shape factors were not significant in differentiating benign from the malignant lesions. Hence we did not analyse shape factors in our study.

Many studies have shown that there is a progressive

Table 2: Nuclear morphometric analysis between the groups

Nuclear features	Fibroadenoma (*n*=10) Mean±SD (range)	Fibrocystic disease (*n*=10) Mean±SD (range)	Hyperplasia (*n*=10) Mean±SD (range)	Carcinoma (*n*=30) Mean±SD (range)	ANOVA *P* value
Nuclear area	71.6±9.29* (64-82)	70.21±11.68* (57.27-80)	96±39.5 (93-137)	117.33±45.50 (72-163)	0.0009
Perimeter	29.95±1.91# (28.28-32.05)	29.51±5.93# (26.77-31.42)	34.27±7.26# (26.96-41.48)	40.87±3.80 (30.10-45.25)	0.0001
Diameter	9.53±0.61$ (8-10.2)	9.42±0.80$ (8.52-10.08)	10.90±2.31 (8.58-13.2)	12.05±2.41 (9.58-14.4)	0.0007
Radius	4.7±0.30 (4.5 − 5.1)	4.71±0.40 (4.26-5.04)	5.45±1.15 (4.29-6.6)	6.02±1.20 (4.79-7.2)	0.841
Compactness	12.55±0.07^ (12.49-12.70)	12.47±0.11^ (12.34-12.56)	12.59±0.09^ (12.53-12.70)	12.70±0.11 (12.58-12.85)	0.0001
Concave points	Nil	Nil	1±0.5 (0-2)	3.5±1.5 (2-5)	Unpaired "*t*' test 0.0001

*significant between fibroadenoma, fibrocystic disease as compared to carcinoma; #significant between fibroadenoma, fibrocystic disease and hyperplasia as compared to carcinoma; $significant between fibroadenoma, fibrocystic disease as compared to carcinoma; ^significance between fibroadenoma, fibrocystic disease and hyperplasia as compared to carcinoma; ANOVA: Analysis of variance

pattern of nuclear morphometric parameters with gradually increasing values from benign to atypical, to ductal carcinoma in-situ (DCIS), further to invasive carcinoma and carcinoma with the lymph node involvement.[8]

Keunen-Boumeester *et al.* in a prognostic study of breast carcinoma aspirates concluded that the standard deviation of nuclear area along with the presence of axillary metastases was the most important predictor of prognosis.[13] Similarly, Pienta and Coffey[2] showed that there was a sharp increase in the nuclear area in patients with the node positive disease when compared to node negative disease.

Boon *et al.*[14] used nuclear/cytoplasmic ratio for characterizing cells of different tumors; however, Abdalla *et al.*[4] opined that such parameter to be avoid as outlining of cellular margins is difficult due to indistinct cytoplasmic outline than nuclear outline, thus making the analysis less reproducible and more subjective.

Conclusions

Nuclear morphometry is thus, a useful objective tool in the differentiating benign and the malignant breast lesions. It can be of immense help when diagnostic dilemmas are encountered especially in gray zones.[8] It can be combined with other ancillary methods such as mammography, DNA cytometry chromatin texture analysis, flow cytometry, and cDNA array analysis for selecting the patients for adjunct therapy.[4]

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