Significance of nuclear morphometry in benign and malignant breast aspirates

Aparna Narasimha, Vasavi B, Harendra Kumar ML
Department of Pathology, Sri Devraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India

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

Submission: 26-02-2012  Accepted: 19-10-2012

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;\(^2\)

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 ×2.5 ocular and an ×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: Perimeter²/area.
- Concave points counted the number of points on the nuclear border that lie on an indentation.¹⁵

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 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).

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                          | 4                    | 2                   |
| 31-40      | 3                    | 2                          | 3                    | 14                  |
| 41-50      |                      | 3                          | 12                   |                     |
| 51-60      |                      |                            | 12                   |                     |
| 61-70      |                      |                            | 2                    |                     |
| Total      | 10                   | 10                         | 10                   | 30                  |
Narasimha, et al.: Nuclear morphometry in breast aspirates

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. 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. These included technical limitations (4.5%), inexperience of the cytopathologist (2.4%), and overlap of cytological features of benign vs. malignant (2%).

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. 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. 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. with the mean nuclear area being 64-82 $\mu$m$^2$ for benign cases and 72-163 $\mu$m$^2$ for malignant cases. Abdalla et al. 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.

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. 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. 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.

However, studies by Abdalla et al. and Kalhan et al. 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 increase of nuclear size and perimeter in carcinomas when compared to benign lesions. In our study, the 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.

### 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)            | 70.2±11.6 ($^*$) (57-127)                | 96±39.5 ($^*$) (93-137)            | 117±45.5 ($^*$) (72-163)          | 0.0009       |
| Perimeter        | 29.95±1.91 ($^*$) (28.28-32.05)     | 29.5±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)         | 4.7±0.40 ($^*$) (4.26-5.04)             | 5.42±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±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.[9] 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]

References

1. Kalyani R, Das S, Bindra Singh MS, Kumar H. Cancer profile in the Department of Pathology of Sri Devaraj Urs Medical College, Kolar: A ten years study. Indian J Cancer 2010;47:160-5.
2. Pienta KJ, Coffey DS. Correlation of nuclear morphometry with progression of breast cancer. Cancer 1991;68:2012-6.
3. Cui Y, Koop EA, van Diest PJ, Kandel RA, Rohan TE. Nuclear morphometric features in benign breast tissue and risk of subsequent breast cancer. Breast Cancer Res Treat 2007;104:103-7.
4. 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.
5. Wolberg WH, Street WN, Mangasarian OL. Importance of nuclear morphology in breast cancer prognosis. Clin Cancer Res 1999;5:3542-8.
6. Sinha R, Anderson DE, McDonald SS, Greenwald P. Cancer risk and diet in India. J Postgrad Med 2003;49:222-8.
7. 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.
8. Kallhan 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. Mapstone NP, Zakhour HD. Morphometric analysis of fine needle aspirates from breast lesions. Cytopathology 1990;1:349-55.
10. Bhattacharjee DK, Harris M, Faragher EB. Nuclear morphometry of epitheliosis and intraduct carcinoma of the breast. Histopathology 1985;9:511-6.
11. Tan PH, Goh BB, Chiang G, Bay BH. Correlation of nuclear morphometry with pathologic parameters in ductal carcinoma in situ of the breast. Mod Pathol 2001;14:937-41.
12. Wittekind C, Schulte E. Computerized morphometric image analysis of cytologic nuclear parameters in breast cancer. Anal Quant Cytol Histol 1987;9:480-4.
13. Kuemen-Boumeester V, Hop WC, Blonk DJ, Boon ME. Prognostic scoring using cytomorphometry and lymph node status of patients with breast carcinoma. Eur J Cancer Clin Oncol 1984;20:337-45.
14. Boon ME, Trott PA, van Kaam H, Kerper PJ, Leach A, Baak JP. Morphometry and cytodiagnostics of breast lesions. Virchows Arch A Pathol Anat Histol 1982;396:9-18.

How to cite this article: Narasimha A, Vasavi B, Harendra Kumar ML. Significance of nuclear morphometry in benign and malignant breast aspirates. Int J App Basic Med Res 2013;3:22-6.

Source of Support: Nil. Conflict of Interest: None declared.