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Original Research Article

SIGNIFICANCE OF NUCLEAR MORPHOMETRY IN CYTOLOGY OF BREAST CARCINOMA IN CORRELATION WITH HISTOPATHOLOGY AT A TERTIARY CARE CENTRE

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Abstract

Background: Fine Needle Aspiration of the breast is effective for diagnosis of the breast lesions, eventhough the procedure and reporting is largely subjective. Due to morphological overlap, a smaller percentage of lesions cannot be undoubtedly interpreted as benign or malignant lesions. Morphometry in combination with FNAC improves the diagnostic accuracy of Fine Needle Aspiration. The objective is to examine the nuclear morphology with respect to nuclear radius and nuclear area on all Fine Needle breast aspirates performed at Department of Pathology, for a period of two years. Statistical analysis was performed to determine the significance of nuclear parameters in benign and malignant lesions of the breast. **Materials and Methods:** In this study, Fine needle aspiration was performed on 100 patients with breast lumps who were referred to department of pathology. Period of study: 2 years. Nuclear morphometric parameters were evaluated on Hematoxylin and Eosin stained breast aspirates using Scopeimage 9.0 version software. The nuclear parameters evaluated were nuclear radius and nuclear area. Student t-test and one way ANOVA were employed for stastistical analysis. **Result:** Nuclear radius and nuclear area were found to be remarkably higher in malignant lesions of the breast as compared to benign lesions with a P-value of <0.0001. **Conclusion:** Nuclear morphometric analysis is an proficient tool to distinguish between benign and malignant lesions of the breast in addition routine light microscopic diagnosis.

INTRODUCTION

Breast cancer is the most prevalent cancer among the women from western countries, accounting for 50% mortality rate in these countries1. However in India, Breast cancers are the second leading cause of cancer mortality which is preceded by carcinoma cervix[2] . Fine needle aspiration is one of the various diagnostic modalities used in diagnosis of carcinoma breast. The diagnostic accuracy reported in the literature ranges from 95.86% to 97.72%[3,4,5]. Fine needle aspiration being one of the components of triple test with the diagnostic accuracy ranging from 98 to 100%[6,7,8] .

. Advantages of Fine needle aspiration cytology are safe, less traumatic, cheap and carried out as an outpatient procedure providing quick results[9].

Results of Fine needle aspiration can be misleading in grey zone areas like atypical ductal hyperplasia, ductal hyperplasia and some cases of fibroadenoma where an erroneous diagnosis can lead to false positivity. The frequence of false positive results in the literature ranges from 6% to 20%[10].

Furthermore, benign lesions such as fibroadenoma and fibroadenosis showing epithelial proliferations with cytological atypicality but prognostically insignificant can be mistaken for preneoplastic lesions. The number of equivocal diagnosis in Fine needle aspiration could be minimised to avoid operational biopsies in benign lesions of the breast[11].

Nuclear morphometry can aid in the differentiation between benign and malignant lesions of the breast. In addition to conventional light microscopy, Nuclear morphometry can help in resolving cases with equivocal diagnosis[12].Various parameters of the cell morphometry are available as commercial packages and the parameters such as Nuclear Area (Area with in the outlined nuclear borders), Perimeter

(Length around the nuclear border), Diameter (Diameter of the nucleus).

The current study focuses on using simple nuclear morphometric parameters on the fine needle aspirates of the histopathologically documented breast lesions in order to assess their significance and in delineating benign from malignant breast lesions. To compare the differences in the measurements of nuclear parameters observed between benign and malignant lesions and to evaluate the significance of nuclear parameters (Nuclear area and Nuclear radius) in distinguishing benign and malignant lesions of breast supported by statistical analysis.

MATERIALS AND METHODS

Materials

All patients(outpatients and inpatients) attending fine needle aspiration clinic of the Pathology department with request for fine needle aspiration of breast were included for the study. All the case sheets of the included patients were reviewed for clinical history and details of the breast lump.

Methods

The study design: A hospital based retrospective analysis was conducted.

Duration of the study: The study period lasted for two years .

Sample size and inclusion criteria: Hundred cases were studied which had both FNAC and histopathological confirmation. Of 100 cases studied, 50 cases were of benign lesions and 50 cases were of malignant breast lesions.

Exclusion criteria: Cases for which proven biopsy report not correlating with fine needle aspiration cytology report was excluded. Also, those cases for which biopsy report was not available were excluded. Well stained Hematoxylin and Eosin FNAC smears with adequate cellularity and good nuclear morphology with a well spread monolayered sheets as well as a isolated single cells were selected for the study.

The breast carcinomas were graded as per the Modified Scarf Bloom Richardson grading system.

FNAC and histopathology correlation: Fine needle aspiration cytology results of the study patients were correlated with their Histopathological diagnosis. Any disparities in cytologic diagnosis were rectified after correlation.

Software used for nuclear morphometry: Scopeimage 9.0 version was used for image processing and analysis in our study. A Mayo – Lewis microscope and an attached camera were used for microscopic photographs. Under 400X, cells in monolayered sheets with better nuclear details were selected for photomicrographs for measurements of cells in clusters and single cells well spread and separated from each other. For Analysis, 100 cells seen in clusters and 100 single cells in malignant lesions of breast were studied. In malignant lesions, dimensions (nuclear radius and nuclear area)were measured on both cell clusters and single cells . In benign lesions, measurements were done on the cells in aggregates due to the absence of single cells. All measurements were taken in micrometre and were exported to an excel file for further evaluation. The mean value of each parameter was calculated for benign and malignant lesions from the measurements obtained for each case.

Statistical Analysis

The statistical analysis for comparison of nuclear parameters of both benign and malignant lesions was done by applying Student T test in SPSS17 version.

RESULTS

Of the total 100 cases selected for the nuclear morphometric analysis, 50 cases (50%)were categorized as benign (fibroadenoma and fibrocystic changes), while other 50 cases (50%) were classified as malignant populations (only ductal carcinoma was included for our study while others were excluded). The graphical representation of sample under study is elaborated

Total female population selected for the study was categorized according to the age groups that included both benign and malignant cases. About 33% of the population were in the age group of 15-30 years of age, 23% of the population were in the age group of 41-50 years of age, 14% of the population were in the age group of 51-60 years of age, 12% of the population were in the age group of 61-70yearsofage, 2%of the population were in the age group of more than 70 years of age [Table 1].

The age distribution between those patients who presented with benign breast lumps was 15-50 years of age and malignant breast lumps were in the age groups of 30-70 years of age. The classification of patients based on the age of occurrence and fine needle aspiration diagnosis is as follows, about 33% of benign breast lumps were in the age group of 15-30 years, about 15% of benign and 8% malignant breast lumps were in the age group of 31-40years, about 2% of benign and 14% of malignant lumps were in the age group of 41-50 years, 14% malignant breast lumps in the age group of 51-60years,12% of malignant breast lumps were in the age group of 61-70 years and 2% malignant breast lumps were in the age group more than 70 years.

Pearson chi – square test was used for analysis of the patient age groups and Fine Needle aspiration diagnosis of breast lumps and a value of 72.130 was attained as T test value with a probability value of 0.0001 that was statistically significant. The results collected by the computerized cytomorphometry were compared between the two groups (benign and malignant cases). Data were analyzed to evaluate the differentiating features between benign and malignant groups. [Table 1]. Of all the nuclear parameters, only nuclear area and nuclear radius were found to be statistically significant. The statistical analyses of the collected data were evaluated by analysis of variance (ANOVA)

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The mean nuclear area and nuclear radius calculated from 50 cases presented with benign breast lumps to the fine needle aspiration clinic were found to be 45.982 ± 10.819 (24.665- 69.733) µm2and 3.871 ± 0.467 (2.802-4.883)µm. The mean nuclear area and nuclear radius calculated from 50 cases presented with Malignant breast lumps to the Fine Needle Aspiration Clinic were found to be 118.673 ± 26.331 (78.456–198.004) µm2 and 6.428± 0.922(5.001–8.450) µm [Table 2 & Figure 1,2]

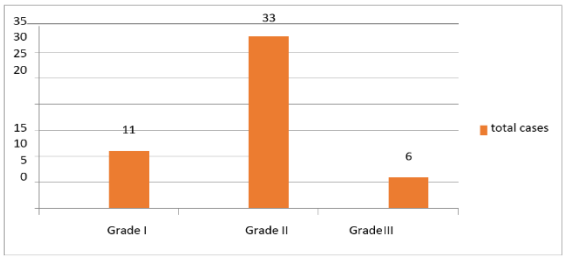
Out of total 50 cases studied from patients with malignant breast lumps, it was found that 22% (11 cases) were found to be of histological grade I, 66% (33 cases) were found to be histological grade II, 12% (6 cases) were of histological grade III [Graph 1].

Table 1: Pearson chisquare-test between Age and FNAC Diagnosis.

Age group comparison cross tabulation					
			Group		Total
			Fibroadenoma	Malignancy	
Age Group	Upto20Years	Count	14	0	14
		%withingroup	28.0%	0.0%	14.0%
	21-30Years	Count	19	0	19
		%withingroup	38.0%	0.0%	19.0%
	31-40Years	Count	15	8	23
		%withingroup	30.0%	16.0%	23.0%
	41-50Years	Count	2	14	16
		%withingroup	4.0%	28.0%	16.0%
	51-60Years	Count	0	16	16
		%withingroup	0.0%	32.0%	16.0%
	Above60Years	Count	0	12	12
		%withingroup	0.0%	24.0%	12.0%
Total		Count	50	50	100

Table 2: Nuclear morphometric analysis between the groups

Nuclear Features	Fibro Adenoma(N=50) Mean±SD(range)	Ductal Carcinoma(N=50) Mean±SD(range)	ANOVA	p-value
Nuclear Area(µm2)	45.982± 10.819 (24.665-69.733)	118.673± 26.331 (78.456-198.004)	326.005	.005**
Nuclear Radius(µm)	3.871±0.467 (2.802-4.883)	6.428±0.922 (5.001-8.450)	305.711	.002**



Graph 1: total malignant cases against histological grades.

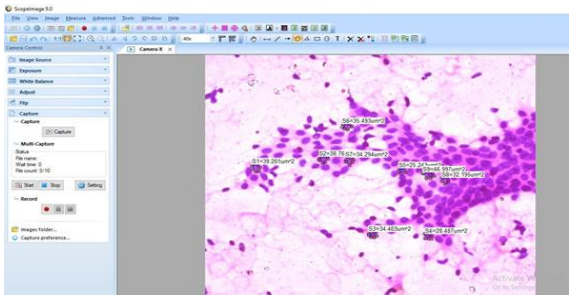


Figure 1: Nuclear morphometric assessement of benign breast aspirate (400X, H&E)

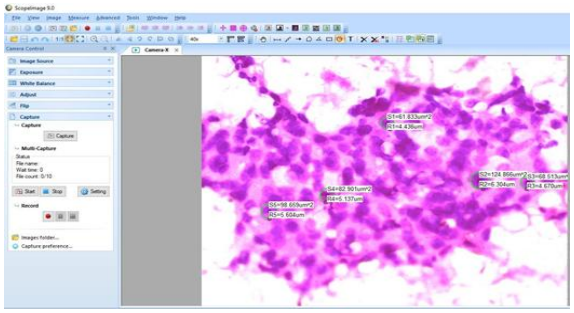


Figure 2: Nuclear morphometric assessement of malignant breast aspirates (400X,H&E)

DISCUSSION

Breast lesions are a heterogenous group of disorders compassed of usual ductal hyperplasia, atypical ductal hyperplasia, fibrocystic disease of breast as well as other benign and malignant neoplasms.

For several years, fine needle aspiration cytology is used as the primary modality in accessing breast lumps at the outpatient department. Although fine needle aspiration cytology is simple and cost-effective procedure, it is based on the visual subjective assessment of cytologic features like cellularity, cell morphology and type of chromatin seen on the fine needle aspirates.

The reporting cytologist are often in the state of diagnostic dilemma while assessing the grey zone lesions of usual hyperplasia, atypical hyperplasia and ductal carcinoma, well differentiated grade1

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carcinomas and benign hyperplasia.[10-12] The grey zone in cytology is estimated around 8.9% as reported by Kaisi et al.[12] Various factors attributable to the grey zone are technical limitations, inexperienced cytopathologist and overlapping features of benign versus malignant lesions in cytology.

Fine needle aspiration cytology is a simple, easier and cost effective method. However, it is based on the subjective visual evaluation of cytologic features like cellularity, morphology and chromatin pattern which is attributable to interobserver and intraobserver variation. The various spectrum of breast lesions in normal breast ranges from ductal fibroadenoma to atypical ductal hyperplasia to ductal and lobular carcinoma in situ to invasive carcinomas which has to be identified as they represent sequence from precancerous to cancerous lesions.

Measurement of morphometric parameters would provide vital points of reference. Nuclear morphometry is the quantitative analysis of morphologic nuclear changes by means of nuclear size, parameters (nuclear enlargement) and nuclear changes by means of nuclear shape pleomorphism, nuclear membrane irregularity and nuclear density measuring nuclear chromasia. Alterations in the nuclear features are the morphologic hallmarks of malignancy diagnosis. Though the histopathologic diagnosis is being considered the gold standard, the time, energy and cost involved at the correct diagnosis, gives the optimal space for this procedure for preliminary diagnosis.

In the current era, several literature studies have emphasized the importance of nuclear morphometry as a highly objective and supplementary tool to the subjective visual interpretation in the differentiation of benign and malignant lesions of the breast and thereby improves the sensitivity and specificity of a particular diagnosis.[13-18] A variety of morphometric parameters have been studied but nuclear morphometry relating to size, area, perimeter, axis, diameter have found to be significant both in histology and cytology for distinguishing benign and malignant lesions. Of all the parameters, mean nuclear area is the most consistent parameter.

Our study was aimed at validation of the role of nuclear morphometry on the cytologic aspirates obtained by fine needle aspiration in differentiating benign from malignant lesions. 100 cases of patients with breast lumps presented to FNAC clinic were randomly selected for our study and were further classified as benign and malignant cases based on fine needle aspiration diagnosis which was further ratified by histopathology. A total of 50 cases of benign and 50 cases of malignant breast lumps were included in our study. Only hematoxylin and Eosin stained samples were considered for our study.

In our study, out of 50 benign breast lumps, FNAC diagnosis of fibroadenoma was made in 46 patients followed by a diagnosis of fibrocystic cystic change in 4 cases. These breast lumps were later followed with excisional biopsy that resulted in 42/50 cases as fibroadenoma, fibrocystic changes in 5/50 cases, 2/50 cases as fibroadenoma with fibroadenosis and lastly one case was reported as blunt duct adenosis.

Out of 50 malignant breast lumps, FNAC diagnosis of ductal carcinoma was made in 41/50 cases followed by proliferative breast disease with atypia in 8/50 cases and one case was reported as proliferative breast disease without atypia. These malignant breast lumps after confirmation with incisional biopsy were later followed with modified radical mastectomy resection that resulted in invasive breast carcinoma(NOS), ductal type (NST) with histological grade I in 11/50 cases, Grade II in 33/50cases and Grade III in 6/50cases.

A study done by Rezanko et al,[14] obtained average nuclear radius of 4.5µm in benign cases and average of 5.4µm in malignant cases. Their observations were made on hematoxylin and eosin stained aspirates. These findings were in concurrence with our study where mean nuclear radius of 3.871±0.467µm in benign breast lesions and mean nuclear radius of 6.428±0.922µm in malignant breast lesions. A variation in nuclear radius arises due to extent of fixation of cells resulting in cell shrinkage. Furthermore, inter-observer and intra-observer variation on nuclear morphometric studies have been previously documented in literature that summed upto 28.9%. A study done by Kronquist et al,[19] that analyzed nuclear radius on 170 cytological samples of malignant breast lesions (only ductal carcinoma was included) to find out objective threshold for nuclear grading. Nuclear radius of about 5.74 – 7.66µm was found as cut off to differentiate between various grades of ductal carcinomas of breast.

A study by Anamika Kashayap et al,[20] studied nuclear morphometric parameters on various breast aspirates of 122 patients, of which 58 cases were with benign breast lumps and 64 patients with malignant breast lumps. At the end of the study, it was concluded that mean nuclear radius of 5.66±0.44µm as a cut-off for benign lumps and a value of 7.86±1.50µm as a cut-off for malignant lesions. In our study, measurement values of mean nuclear radius obtained in both benign and malignant lesions were statistically significant with T value of 17.486 and P value of <0.001 by ANOVA statistical analytic tool.

In a study by Shivani Harman et al,[18] on 82 cases of breast lumps with 53 cases being benign and 29 cases being malignant. They found out the mean nuclear area in benign breast lumps were 28.46±7.72µm2 with a range of 16.9-40.59 µm2 and mean nuclear area in malignant lesions were 94.19±19.49µm2 with a range of 57.36-137.98µm2.

Our current study was extended to correlate the mean nuclear area of tumor cells obtained on fine needle aspiration cytology with the histological grading on resection specimen. It was found that the histological grade I breast carcinoma had a mean nuclear area of 124.2813±28.50099µm2 with a range of 85.353-163.509µm2 on aspiration cytology, histological grade II breast carcinoma had a nuclear area of

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128.0411±29.28µm2 with a range of 78.456-198.004µm2, histological grade III breast carcinoma had a mean nuclear area of 131.52±34.556µm2 with a range of 91.623-198.830µm2.

In a study conducted by Anamika Kashayap et al,[20] it was found mean nuclear area in grade I breast carcinoma were 102.62±28.60µm2 with a range of 60.83-159.28µm2, grade II breast carcinoma were 108.13±28.30µm2 with a range of 60.89-213.69µm2, grade III breast carcinoma were 136.41±35.14µm2 with a range of 67.79-208.56µm2.

The findings observed in our current study were consistent with findings of the study conducted by Anamika Kashayap et al,[20] Hence, it was concluded that distribution of nuclear size related parameters (Nuclear area) of malignant samples were distinctly different from benign samples. However, nuclear size related parameter was not applicable for grading of malignant samples as the ranges obtained in the three grades show significant overlapping of values.

Summary

The age distribution of cases for benign breast lesions were commonly seen in the age groups less than 40 years whereas malignant breast lesions were seen more commonly seen in the age groups more than 40 years. The mean nuclear radius calculated from 50 benign lesions was 3.871±0.467µm with a range of 2.802-4.883µm. The mean nuclear radius calculated from 50 malignant lesions was 6.428±0.922µm with a range of 5.001-8.450µm.

The mean nuclear area calculated from 50 benign breast lesions was 45.982±10.819µm2 with a range of 24.665-69.733µm2. The mean nuclear area calculated from 50 malignant breast lesions was 118.673±26.331µm2 with a range of 78.456-198.004 µm2. Of 50 malignant breast lesions studied, 11 were of grade I, 33 were of grade II and 6 were of grade III. The mean nuclear area obtained in cytology with regards to histopathological grade I breast carcinoma was 124.2813±28.50099 µm2, grade II breast carcinoma was 128.0411±29.286262 µm2, grade III breast carcinoma was 131.52±34.556µm2.

CONCLUSION

Nuclear morphometry is an efficient tool in distinction of benign from malignant breast lesions and has been an objective tool particularly in grey zone areas of diagnosis. Morphometry in terms of overestimating the size of the nuclear profile as a result of over-riding the nuclear cytoplasmic boundary during tracings, magnifications and speed of conducting the analysis can be reduced due to an internal calibration and standardization by an expert observer. However, nuclear morphometry can supplement in distinction of benign and malignant breast lesions along with light microscopy evaluation by a cytologist. The drawback of nuclear morphometry is that it does not aid in the grading of malignant samples due to significantly overlapping values of mean nuclear area.

REFERENCES

1. Marciniak A, Obuchiwicz A, Monzack R. Cytomorphometry of fine needle biopsy material from the breast cancer.Advances in soft computing 2005:30:603-09.
2. Raina V, Tyagi BB, Manoharan N. Cancer incidence and mortality inDelhi UT suburban.2002 and 2003:Delhi cancer registry 2007-14.
3. Singh K, Sharma S, Dubey VK. Role of Fine needle aspiration cytology in diagnosis of breast lumps.JK science2001july-september:3:3.
4. Gupta SK, Ghosh AK, Chowdry T. Aspiration cytology in the diagnosis of breast cancer. Ind Jcan 1979:16:1-8.
5. Bansal RL, Sankaran V, Vallah AJ. Role of aspiration cytology in the diagnosis of breast lumps and its histopathologic correlation. J cytol J1985:2:62-66.
6. Ghimire B, Khan MI, Bihbusal T. Accuracy of triple test score in the diagnosis of palpable breast lumps. JNMA Jnep Med Assoc 2008 oct-dec:47(172):189-92.
7. Morris A, Pommier RF, Schmidt WA. Accurate evaluation of palpable breast masses by triple test score. Arch surg1998:133(9):330-4.
8. Dabb DJ. Prognostic information from fine needle aspiration biopsy of breast cancers. Amj ClinPathol 2000:113(1):76-83.
9. Tiwari M. Role of fine needle aspiration cytology in the diagnosis of breast lumps. KUMJ2007 apr-jun:5(2):215-17.
10. Al- kaisi N. The spectrum of grey zone in breast cytology. A review of 186 cases of atypical and suspicious cytology. ActaCyto1994 nov-dec :38(6):898-908.
11. Stanley MW, Sidaway M.current issues in breast cytopathology .AmJClinPathol2000:113(1):49-75.
12. Wolberg WH, Street N, Mangasarian L.Machine learning techniques to diagnose breast cancer from image processed nuclear features of fine needle aspirates.Cancerletter1994: 77(2):163-171.
13. Rajesh T, Dey P,Joshi K.Automated image morphometry of lobular breast carcinoma.Anal Quant CytolHistol2002 Dec:22 (6):483-85.
14. RezankoT, Pehlivan F, Evcim G. Can nuclear morphometric analysis aid for definitive diagnosis in cases with equivocal cytology. Turk Patoloji Dergisi 2008:24(2):93-99.
15. Elzagheid A, CollanY. Fine needle aspiration biopsy of breast . Value of nuclear morphometry after different sampling methods. Anal Quant Cytol Histol 2003Apr:25(2):73–80.
16. Kaushik N , Sardana S, Das DK. Quantitative Analysis of nuclear area variation in benign and malignant breast fine needle aspirates. Ind J can1991 Dec:29(4):202-7.
17. Sohn JH, Choi YH, ParkYE. Nuclear morphometry of fibroadenoma and carcinoma of breast: comparison between fine needle aspiration cytology and biopsy. Kor J Cytopathol 1998 Dec:9(2):61-68.
18. Mapstone NP, Zakhour HD. Morphometric analysis of fine needle aspirates from the breast aspirates. Cytopathology1990:1(6):349-55.
19. Kronqvist P, Kuopio T, CollanY. Morphometric grading of invasive ductal carcinoma of breast. Thresholds for nuclear grade. BJ of cancer 1998:78(6):800-805.
20. Anamika kashyap et al . Role of Nuclear Morphometry in Breast Cancer and its Correlation with Cytomorphological Grading of Breast Cancer: A Study of 64 Cases .J Cytol 2018 Jan-Mar;35(1):41-45.

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