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Role of Nuclear Morphometry in The Diagnosis of Various Breast Lesions By Fine Needle Aspiration Cytology- A Study of Diagnostic Accuracy

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Abstract

Introduction

The Nuclear Morphometric Study can improve. During the past several years, it has been well-established that several clinical, cytological and histopathological variables are helpful in predicting the clinical outcome of cancer patients.

Objectives

1. To study the Nuclear Morphometric parameters of the benign and malignant breast FNA aspirates
2. To determine suitable cutoff values differentiating benign and malignant in case of nuclear parameters with significant difference.
3. To study correlation of nuclear parameters with cytological grades

Materials and Methods

Nuclear Morphometric parameters (MAJX-Major Axis ,MINX-Minor Axis, NAR-Nuclear Aspect Ratio, NA-Nuclear Area, NP- Nuclear Perimeter) study on 50 histopathologicaly proven cases of both benign and malignant breast lesions each. Nuclear Morphometric parameters were evaluated on FNAC slides using Digital Image analyzing software. Appropriate statistical tests where applied wherever necessary and P value of less than 0.05 was considered for significant difference. Receiver Operating Characteristics (ROC) curve between sensitivity (true positive) and 1-specificity (false negative) was plotted to determine the optimal cut-off of various nuclear parameters

Results -The nuclear parameters showed a significant difference. All nuclear parameters showed positive correlation with their variability. Cut-off values with sensitivity=1 for differentiation between the benign and malignant cases were MAJX>5.6µmicron(specificity =1), MINX>4.6µmicron(specificity=0.99),NA>88.98µmicron^2(specificity=1), NP>33.67µmicron(specificity =1)

Conclusion Nuclear Morphometric analysis along with routine FNAC proves very helpful in further grading and appropriate management of the patient. Nuclear Morphometry approach overcomes interobserver variability &can be gainfully exploited in the diagnosis

of breast carcinoma Thus, Computerized nuclear morphometry is an efficient objective Tool to supplement the subjective FNAC, improving the diagnostic capabilities of FNAC

Keywords: Breast FNAC, Nuclear Morphometry, Nuclear parameters, Morphometric Analysis

Introduction

Breast lesions is a complex group of disorders which includes hyperplasias, atypical hyperplasias, fibrocystic disease as well as benign and malignant neoplasms. Benign breast diseases include a heterogeneous group of lesions arising from mammary epithelium or in other mammary tissues and they may also be linked to vascular, inflammatory or traumatic pathologies[1].

Some lesions are palpable masses, which may be nodular, sometimes with specific or unspecific characteristics, but mostly (particularly in lesions of greater prognostic significance such as atypical hyperplasia) there are no specific clinical signs, and detection is difficult also at diagnostic imaging examinations[1]. The incidence of benign breast lesions begins to rise throughout the second decade of life and peaks within the fourth and fifth decades, as opposed to malignant diseases and the incidence continues to increase after menopause, but at a less rapid pace.[2]

Breast cancer is the most frequent cancer among women, impacting 2.1 million ladies each year, and also causes the greatest number of cancer-related deaths among women. In 2018, it was estimated that 627,000 women died from breast cancer – which is approximately 15% of all cancer deaths among women. While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally.[3]

Fine Needle Aspiration Cytology (FNAC) has gained popularity for the preoperative assessment in breast lesions. It is a good diagnostic technique because of the high sensitivity and specificity which are 68-99% and 99% respectively. FNAC is also a part of the 'triple test' due to its good accuracy and reduced need for excisional biopsy[4].The nuclear morphometric study is one way to understand about the tumors and grades. During the past several years, it has been well established that several clinical, cytological and histopathological variables are helpful in predicting the clinical outcome of cancer patients.

The cytological grading, histopathological grading and morphometric analysis go hand in hand with clinico-pathological features in malignant tumours (like tumour size, lymph node status &tumour staging)[5].

The present study was carried out with the need to role of nuclear morphometric study in the diagnosis of various breast lesions by fine needle aspiration cytology as there are very few studies on such evaluation.

Material and Methods

The present study was a hospital based cross sectional study to evaluate the role of nuclear morphometric study in the diagnosis of various breast lesions by fine needle aspiration cytology. This study was carried out for 2 years from August 2017 to December 2019. -The study was carried out in the department of Pathology at a Tertiary care hospital.

The study population included 50 benign and50 invasive ductal FNA cytology cases which were histopathologically proven cases. In-patient /out-patient cases with request for FNAC of breast lump/ lumps of either sex, presented for Fine Needle Aspiration cytology section of pathology department were included for the study. Sampling technique used was convenient sampling.

Inclusion criteria included histopathologically proved benign (fibroadenoma) and malignant (invasive ductal

carcinoma) cases. Cases with insufficient FNA material and invasive lobular carcinoma, metastatic carcinoma and special types of invasive ductal carcinoma were excluded.

Ethical Consideration

The study was started after the approval from institutional ethics committee. Informed consent was taken from the cases before enrolling them in the study.

Methods of Data Collection

All necessary data were obtained from patient's file, cytology request form /case record form which included demographic details an informed written consent was taken prior to the FNAC procedure.

FNAC Procedure

Under aseptic precaution, Breast FNAC was done using standard procedure with 20 ml syringe, syringe holder and 22-23 gauze needles. Multiple direction technique was performed maintaining suction throughout the procedure. Aspirated material was expressed onto the glass slide. Smears were made and immediately fixed in 95% ethanol and stained with H&E/ PAP stain Slides were examined by the reporting pathologist and graded according to ROBINSON cytological grading and reported in various categories. Well stained smear with adequate cellularity showing properly spread monolayered sheets, clusters and single cells without overlapping were selected in the nuclear morphometric study

Staining Procedure

Histopathology:

Biopsies / modified radical mastectomy were routinely processed using the paraffin wax method and 5µ sections were cut and stained with Haematoxylin and Eosin stain. Carcinoma cases were categorized into specific and NOS types. Modified Bloom Richardson Grading System was used to grade the carcinomas. IDC – NOS Cases with available FNAC Smear were included.

Cyto-histological correlation

Cytology results were correlated with histopathology considering it to be Gold standard. If any discrepancy cases were present, those were removed.

Software for nuclear morphometric analysis

Motic image plus 2.0 software was used for nuclear morphometric analysis. This digital microscopy software was complimentary with the Motic light microscope also provided with calibrated slide for caliberation at various power.

Features

Image analysis

To calculate the various area related and pixel related measurements. It has auto-calculation and auto - caliberation systems. It has measure table like MS Excel sheet format .Calculation can be also done on digital photos taken with camera

Image control – To capture the microscopic view, edit, analyze, save images. It provides with advance processing function like color adjustment, White balance, Scale tools, Region zoom.

Nuclear morphometric analysis /Methods of measurement – with the help of image analyzing system, measurement of various nuclear parameters for 50 nuclei of D:\MET\project\sdm\sdm project final 29 9 18\51 poster11 11 18each sample (50 BENIGN AND 50 MALIGNANT) was obtained. Nuclear morphometric parameters assessed were MAJX, MINX, NA, and NP AND NAR. NAR is shape related and all rest is size related nuclear parameters. It is ratio of long axis of nucleus to short axis of nucleus and an estimate of nuclear pleomorphism.

Mean and Standard deviation was calculated or... each (50 BENIGN AND 50 MALIGNANT) sample (i.e

MEAN- MAJX, MEAN-MINX, MEAN-NA, MEAN-PERIMETER,MEAN NAR AND SD- MAJX, SD MINX , SD-NA , SD-NP, SD- NAR)

Statistical analysis

Student t-test and p value was obtained between the Mean of age and nuclear morphometric parameters of benign and malignant cases .Age and nuclear parameters were correlated with their variability and p value was obtained. p-value of less than 0.05 was considered to be of stastically significant difference. Cut offs were determined in those parameters with significant difference Nuclear parameters of cytological grades(I,II,III) were compared with ANOVA test to obtain Pearson correlation coefficient ('r') and p-value & regression line will be drawn.

Results

Table 1: Mean values of all 5 nuclear parameters and AGE with their SD, range for benign and malignant group

Sr no	Parameters(UNIT)	Benign(n=50)	Malignant (n=50)	P value
1	AREA (μm^2)	46.2 +/- 10.4 (73.2-28.6)	180.7 +/- 42.9 (279.3-104.7)	<0.0001*
2	PERIMETER (µm)	24.0 +/- 2.6 (30.2-18.9)	47.6 +/- 5.57 (58.2-37.1)	<0.0001*
3	MAJX (µm)	3.98 +/- 0.43 (4.8-3.0)	8.05 +/- 1.02 (10.9-6.5)	<0.0001*
4	MINOR (µm)	3.62 +/- 0.44 (4.774-2.92)	6.99 +/- 1.04 (9.26-4.65)	<0.0001*
5	NAR	1.11 +/- 0.08 (1.38-1.00)	1.19 +/- 0.21 (2.41-1.00)	0.1
	AGE	30.46 +/- 4.67 (39-19)	54.02 +/- 6.98 (68-39)	<0.0001*

Table 2: Mean values of variability (standard deviation) of five nuclear morphometric parameters

SR NO	PARAMETERS (UNIT)	BENIGN(n=50)	MALIGNANT(n=50)	P value
1	SD-NA(μm^2)	8.15 (5.07-13.1)	51.2 (24.86-108.8)	<0.0001* 1.07608E-25
2	SD-NP(µm)	2.15 (1.55-3.37)	6.61 (3.64-10.7)	<0.0001* 4.9385E-05
3	SD- MAJX(µm)	0.45 (0.31-0.75)	1.26 (0.78-2.04)	<0.0001* 8.22649E-24
4	SD-MINX(µm)	0.41 (0.25-0.68)	1.17 (0.74-2.00)	<0.0001* 6.4428E-23
5	SD-NAR	0.18 (0.031-0.46)	0.23 (0.035-0.49)	0.006

Table 3: parameters of cut off values and specificity at sensitivity 1(100%) for the differentiation of malignant from benign.

Parameters	SENSITIVITY	CUT -OFF VALUES
MAJX	1	>5.6 µmicron (Specificity=1)
MINX	1	> 4.6 µmicron (Specificity=0.99)
NA	1	> 88.98 $\mu micron^2$ (Specificity=1)
NP	1	> 33.67 µmicron (Specificity=1)

The above table shows that all size-related parameters showed the significant difference and thus the cut off were derived of each nuclear parameter at with sensitivity of 1.

Table 4: Correlation between Cytological and Histological Grading

HISTOLOGICAL GRADE	CYTOLOGICAL GRADE						TOTAL	
	1		2		3			
	NO	%	NO	%	NO	%	NO	%
I	17	89.4736842	2	15.3846154	0	0	17	34
II	2	10.5263158	11	84.6153846	0	0	14	28
III	0	0	0	0	18	100	19	38
TOTAL	19	100	13	100	18	100	50	100

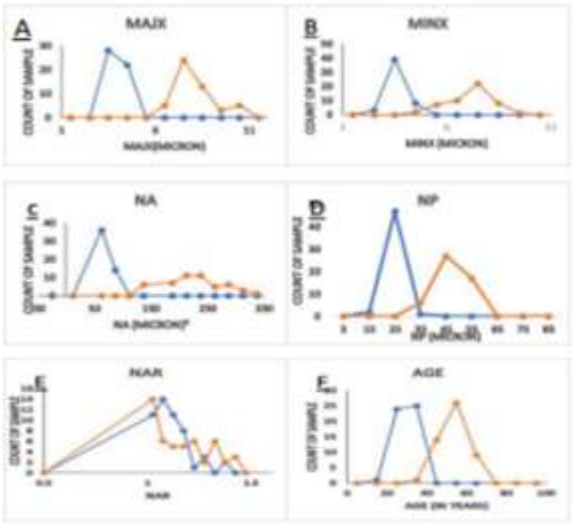
The concordance rate for cytological grade I, II and III were 89.4%, 84.6% and 100% respectively.

Table 5: Mean Values of Nuclear Morphometric Parameter With SD and Range for Three Cytological Grades With _R'- Value And P-Values (Using One-Way ANOVAs).

SR.NO	PARAMETER	Grade I (n=19)	Grade II (n=13)	Grade III (n=18)	'r' value	p value
		Mean ± std dev	Mean ± std dev	Mean ± std dev		
1	MAJX (µm)	7.22±1.24 (7.836-6.504)	8.01±1.06 (10.962-7.374)	8.94±1.44 (10.21-7.868)	0.730483	<0.0001*
2	MINX (µm)	6.00±1.08 (7.357143-4.984)	7.14±0.965 (7.654-4.658)	7.95±1.41 (9.268-7.274)	0.821051	<0.0001*
3	AREA (μm^2)	137.89±36.61 (158.186-104.718)	180.59±41.56 (194.208-160.826)	227.10±71.96 (279.304-191.416)	0.90083	<0.0001*
4	PERIMETER (µm)	41.92±5.99 (45.248-37.144)	47.98±5.50 (53.006-45.692)	53.36±8.05 (58.284-48.612)	0.89188	<0.0001*
5	NAR	1.24±0.34 (1.441302-1.056996)	1.17±0.20 (2.410898-1.008251)	1.15±0.19 (1.442577-1.005626)	-0.21025	0.394

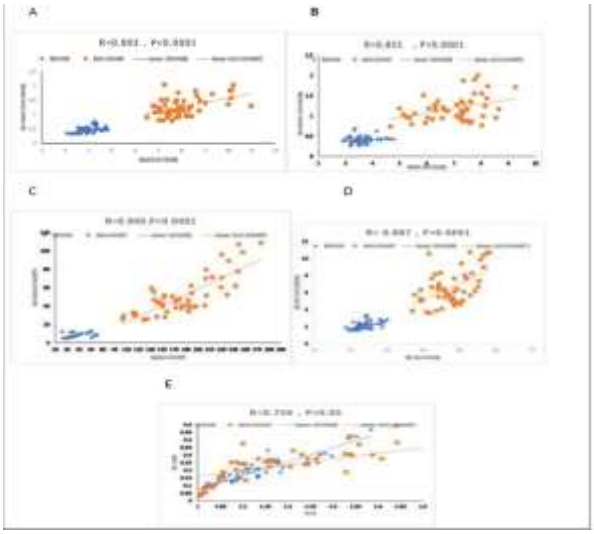
Values are expressed as: mean +/- standard deviation (maximum minimum value), n- number of sample, *- difference is significant

Figure 1: Distribution Of Studied Samples Of Benign And Malignant Groups With A) MAJX, B) MINX, C) NA, D) NP, E) NAR AND F) AGE



Malignant samples of all studied parameters and Age were shifted towards larger values than benign samples, except NAR.

Figure 2: (A-E). Scatterplot of mean: a) majx b) minx c) na d) npE) nar with their sd (variablity) for all benign and malignant Samples along with their _r' value and p values. Linear Regression of these parameters with their SD is shown by Solid lines in their respective plots.



Two different clusters of benign and malignant samples were seen in all analyzed morphometric parameters, except NAR.

Discussion

The present study included 100 cases out of which 50 cases each were included in benign and malignant categories. Benign cases included all fibroadenomas and malignant cases included invasive ductal carcinoma. All study subjects were females. All 50 cases of benign lesions were of fibroadenoma as it is the most common benign lesion as per literature which mentions that fibroadenomas account for 68% of all breast masses and 44%–94% of biopsied breast lesions.

Also in malignant category, all 50 cases were of invasive ductal carcinoma as it is the most common type in malignant lesions of breast. Male breast carcinoma is a rare condition, accounting for less than 1% of all breast carcinomas. Voinea C et al[6] in their review of literature on male breast lesions concluded that only 0.1% of mortality among male oncologic conditions is attributed to this pathologic entity. Hence, only female cases were included in present study.

Another study by Badge et al[7] observed that benign tumors contributed 73.64% cases and malignant tumors to 26.36%. Among benign tumors, fibroadenoma was the most common tumor found by them which constituted 55% of total cases. Infiltrating duct carcinoma was the most common malignant breast tumor found by them and it comprised of 24.09% cases. The current study evaluates the role of nuclear morphometry as an adjuvant to FNAC in accurate distinction between benign and malignant lesions preoperatively. Most of the studies conducted on nuclear morphometry have been done on histo- pathological sections; however we are of the opinion that cytological specimens are better for morphometric analysis when compared to histopathology

sections as the cells are better preserved in FNAC smears.

The present study found MAJX for benign lesions as 3.98± 0.43 µm ranging from 3.0-4.8 µm and 8.05±1.02 µm ranging from 6.5 -10.9 µm for malignant lesions. This difference is statistically significant (p value<0.0001) Parmar et al[8] found in their study MAJX to be 8.34±0.38 µm for benign lesions and 13.14±0.99 µm malignant lesions which is higher for both benign and malignant lesions . Kalhan et al[9] in the similar study as the present study found mean MAJX as 6.87 ± 0.87 µm ranging from 5.72–8.12 µm for benign lesions and 13.52 ± 1.56 µm ranging from 10.04–15.96 µm. These findings were also higher than present study findings. Boruah et al[10] in their study found MAJX for benign lesions as 12.87 ±1.82 µm ranging from 10.08-15.97 µm and 14.67 ± 1.90 µm ranging from 0.72-16.79 µm for malignant lesions which is again higher than present study.

The present study shows minor axis (MINX) as 3.62±0.44 µm ranging from 2.92-4.772 µm in benign lesions and 6.99±1.04 µm ranging from 4.65-9.26 µm for malignant lesions. This difference is statistically significant (p value<0.0001) Parmar et al[8] in their study on nuclear morphometry for breast lesions found MINX value for benign lesions as 6.02±0.33 µm and 9.83±1.00 µm for malignant lesions. These findings were higher than the present study. Boruah et al[10] in their similar study on nuclear morphometry found that MINX for benign lesions was 6.93± 0.51 µm ranging from 5.68-8.03 µm and 10.58 ± 1.42 µm ranging from 7.66-14.15 µm for malignant lesions. These findings are higher for benign lesions when compared to the present study but nearly similar for malignant lesions.

The mean nuclear area (MNA) in current study was 46.2±10.4 µm2 in benign lesions with range of (28.6-73.2) and 180.7±42.9 µm2 for malignant lesions and (104.7- 279.3) as range. It was clearly more in malignant lesions as compared to benign ones.(p<0.0001).Abdalla et al[11] found similar results for benign lesions as the MNA by cell groups was 47±6 µm2 in fibroadenoma but slightly lower for malignant i.e. 98±23 µm2 in ductal carcinoma. The MNA in cell groups of carcinomas ranged between 59.2 and 137.8 µm2 and in benign cases between 27.8 and 61.8 µm2.Chowdhury et al[12] found MNA in benign lesions as 47.32±3.53 µm2 and 140.75±22.94 µm2 in malignant lesions. These findings were nearly similar for benign lesions for present study. But the MNA for malignant lesions was lower than the present study. Kalhan et al[9] in their study concluded MNA as 28.46 ± 7.72 µm2 ranging from (16.9–40.59) for benign lesions and 94.19 ± 19.49 µm2 (57.36–137.98) for malignant lesions. These results were lower than the present study findings.

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present study findings. Wittekind et al[13] mentioned nuclear perimeter to be their best indicator of malignancy, but again do not mention standard deviation of nuclear area. The present study had consistent results. Narasimha et al[14] found nuclear perimeter and compactness as highly significant in differentiating hyperplasia from carcinoma (P < 0.0001). Concave points represent the number of indentations present on the nuclear border.

The present study found following results on cyto-histological grading correlation:

Table 6:

Studies	Cytological Grade	Histological Grade			Accuracy (%)
		I	II	III	
Chandanwale et al[10]	I	08	0	0	100
	II	0	39	5	88.63
	III	0	4	13	76.47
	Total	08	43	18	
Kalhan et al[9]	I	05	01	0	100
	II	01	24	01	88.63
	III	0	02	08	76.47
	Total	06	27	09	
Meena et al[16]	I	19	03	00	82.6
	II	04	32	04	88.89
	III	00	01	08	66.67
	Total	23	36	12	
Present study	I	07	02	00	89.47
	II	02	11	00	84.61
	III	00	00	18	100
	Total	09	13	18	

Out of 19 cases in grade 1 cytology, 17 were in grade 1 of histology and 2 were in grade 2 of histology. Out of 13 in cytological grade 2, 11 were in grade 2 of histology but 2 were in grade 1histology. In grade III, all cases were in concordance in both cyto and histological grading.

Conclusion

We concluded in our study that nuclear morphometry study is also helpful further grading of malignant lesions for appropriate management of patients. Nuclear Morphometry approach overcomes interobserver variability &can be gainfully exploited in the diagnosis of breast carcinoma Thus, Computerized nuclear morphometry is an efficient objective Tool to supplement the subjective FNAC, improving the diagnostic capabilities of FNAC.

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