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Nuclear morphometric study and its correlation in various breast lesions using FNAC

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Abstract

Background: Early detection through screening, effective investigative pathways and appropriate treatment have the ability to lessen breast cancer mortality rates. Thus the present study was undertaken to assess the role of morphometry on fine needle aspirates in accurate diagnosis of benign and malignant breast lesions. **Materials and methods:** 120 cases of Fine needle aspirates of breast lesions done at the Department of Pathology, Adichunchanagiri Institute of Medical sciences were take for nuclear morphometric study. Nuclear Morphometric parameters were measured using ProgresR capture pro 2.9.0.1 software. Data was expressed as mean values and percentage comparison between groups were done by unpaired t test and one way ANOVA test. **Results:** Nuclear parameters were significantly higher in malignant groups comparing benign ones (P value<.001) also between various cytodiagnostic categories (P value<.001). The Standard Deviation of these parameters is a quantitative measurement of nuclear pleomorphism, which was significantly lower in benign cases. **Conclusion:** Morphometric analysis of nuclear parameters can be used as an adjunct to FNAC for diagnosis of benign and malignant lesions, differentiating various cytodiagnostic categories and malignant grades of breast with precision and accuracy.

Key words: Nuclear parameters, breast lesions, FNAC, nuclear pleomorphism.

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Introduction

Breast carcinoma is one of the most common malignancies in the female population and it also accounts for the major cause of cancer mortality among Indian woman. Early detection through screening, effective investigative pathways and appropriate treatment have the ability to lessen breast cancer mortality rates.

FNAC of the breast is very effective for the diagnosis of breast lesions but it is largely subjective and in a minority of cases an unequivocal diagnosis cannot be achieved due to the existence of grey zone between benign and malignant lesions. Lesions in gray zone are generally categorized as probably benign with atypia and probably malignant.[1]

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All this hinders a definite diagnosis which may sometimes lead to unnecessary CNB or open biopsy. Morphometry is the study of various cell parameters microscopically, which can be used as an objective tool to avoid false positive or false negative diagnosis.
The purpose of this study is to assess the role of morphometry on fine needle aspirates in accurate diagnosis of benign and malignant breast lesions.

Materials And Methods
Source of data: The present study was conducted in the department of Pathology at Adichunchanagiri Institute of Medical Sciences.
Study design: Prospective study
Sample size: This study included 120 cases of Fine needle aspirates of breast lesions done at the Department of Pathology, Adichunchanagiri Institute of Medical sciences, B.G Nagara during November 2015 to April 2017
Sample collection: via FNAC
Inclusion criteria: All the patients with palpable and non- palpable breast lumps referred to the department of Pathology for FNAC during the study period.
Exclusion criteria
1. Inadequate sample with insufficient cellularity.
2. Patient's refusal/ non-willingness to undergo the procedure.
Statistical analysis
Data was expressed as mean values and percentage comparison between groups were done by unpaired t test and one way ANOVA test. Sensitivity, specificity, standard deviation and range were analyzed whenever required. They were compared to similar studies conducted previously and conclusions were drawn which was presented in this study.
Procedure for Morphometric Measurements
Papanicolaou stained cytology breast smears were observed under projection microscope Olympus CX31.A minimum of 100 cells in a smear was measured to obtain mean morphometric values for each parameters using ProgresR capture pro 2.9.0.1 software.
Morphometric parameters measured were Major Axis of Nucleus (MAJX), Minor Axis of Nucleus (MINX), Nuclear Area (NA) and Nuclear Perimeter (NP). Major axis is the longest axis of the nucleus and minor axis is the shortest one. Values were measured in micrometer which is calibrated to each objective. In this study 40x objective was used for measurement of individual parameters. Free form selection tool of the software was used to circle the perimeter of individual nucleus. Major axis, minor axis, nuclear area and nuclear perimeter values were automated by the software.
Morphometric Analysis
ProgresRcapture pro 2.9.0.1 software was used to analyze nuclear morphometric size parameters which were major axis, minor axis, nuclear area and nuclear perimeter. A total of hundred cells are randomly selected and measured in each case for morphometric parameters using Papanicolau stained smears.
Statistical analysis
Data was expressed as mean values and percentage Comparison between groups were done by unpaired t test and one way ANOVA test. Sensitivity, specificity, standard deviation and range were analyzed whenever required. They were compared to similar studies conducted previously and conclusions were drawn which was presented in this study.

Result
Table 1: Distribution of all breast lesions among cytodiagnostic categories with percentage

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Categories	No of cases	Percentage
Benign - Nonspecific	19	15.83%
Benign-Specific	68	56.66%
Atypical	2	1.66%
Suspicious	Nil	Nil
Malignant	31	25.83%
Total	120	100%

Table 2: Mean values of nuclear parameters and age with SD and range for various benign lesions

		Mean	SD	Range	Min	Max
Benign lesions	**age**	32.81	11.61	50	13	63
	major axis	9.83	1.61	7.39	6.11	13.5
	minor axis	8.28	1.43	5.94	5.16	11.1
	nuclear area	63.86	19.35	83.4	26.38	109.78
	nuclear perimeter	29.58	4.65	22.69	18.83	41.52
Malignant	**age**	48.03	11.41	40	25	65
	major axis	15.6	2.96	12.22	11.7	23.92
	minor axis	13.04	3.14	13.43	8.6	22.03
	nuclear area	161.26	68.69	320.42	79.58	400
	nuclear perimeter	46.37	8.8	40.3	33.15	73.45
Benign non specific	**age**	30.47	8.39	32	22	54
	major axis	9.38	1.64	5.17	6.7	11.87
	minor axis	7.88	1.65	5.11	5.9	11.01
	nuclear area	58.59	21.63	62.83	28.07	90.9
	nuclear perimeter	28.56	5.35	16.5	20.1	36.6
Benign specific	**age**	33.01	12.05	50	13	63
	major axis	9.86	1.52	6.97	6.11	13.08
	minor axis	8.35	1.36	5.94	5.16	11.1
	nuclear area	64.57	18.32	83.4	26.38	109.78
	nuclear perimeter	29.65	4.35	22.69	18.83	41.52
Atypical	**age**	48	16.97	24	36	60
	major axis	13.03	0.66	0.94	12.56	13.5
	minor axis	9.85	0.35	0.5	9.6	10.1
	nuclear area	90.07	11.06	15.65	82.25	97.9
	nuclear perimeter	36.63	2.22	3.14	35.06	38.2

Table 3: The mean values of nuclear parameters with SD for different cytodiagnostic categories with p values between them.

Parameters	Major Axis (µm)	Minor Axis (µm)	Nuclear Area (μm^2)	Nuclear Perimeter (µm)
Benign-non Specific	9.38±1.64	7.88±1.65	58.59±21.63	28.56±5.35

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Mean±SD N=19				
Benign-Specific Mean±SD N=68	9.86± 1.52	8.35±1.36	64.57±18.32	29.65±4.35
Atypical Mean±SD N=2	13.03±0.66	9.85±0.35	90.07±11.06	36.63±2.22
Malignant Mean±SD N=31	15.60±2.96	13.048065±3.14	161.26±68.69	46.37±8.80
P value	<0.001	<0.001	<0.001	<0.001

Table 4: The mean values of nuclear parameters with SD for benign and malignant lesions with p values between them

Parametes		**Major Axis (µm)**	**MinorAxis (µm)**	**Nuclear Area (µm²)**	**Nuclear Perimeter (µm)**
Benign N=89	**Mean**	9.83	8.28	63.86	29.58
	SD	1.61	1.43	19.35	4.65
Malignant N=31	**Mean**	15.6	13.04	161.26	46.37
	SD	2.96	3.14	68.69	8.8
P value		< 0.001	< 0.001	< 0.001	< 0.001

ROC curves and cut off values

The nuclear parameters showed remarkable distinction between the benign and malignant lesions. Hence this value can be used to identify malignant lesions. Receiver operating characteristics (ROC) curves between sensitivity and (1- specificity) with cut off values were evaluated for mean MAJX, MINX, NA and NP and presented in graph1, 2, 3 and 4. The cut off values with sensitivity 1(100%) for the differentiation of malignant from benign were: (a) MAJX>11.61 micron (specificity=0.86), (b) MINX> 8.51micron (specificity=0.52), (c) NA>78.36 micron2 (specificity=0.75) and (d) NP> 33.09 micron (specificity=0.75).

Graph 1: ROC curve for MAJX

MAJX		
Cut Off (Micron)	Sensitivity	Specificity
5.11	1	0
11.61	1	0.86
13.72	0 .74	1

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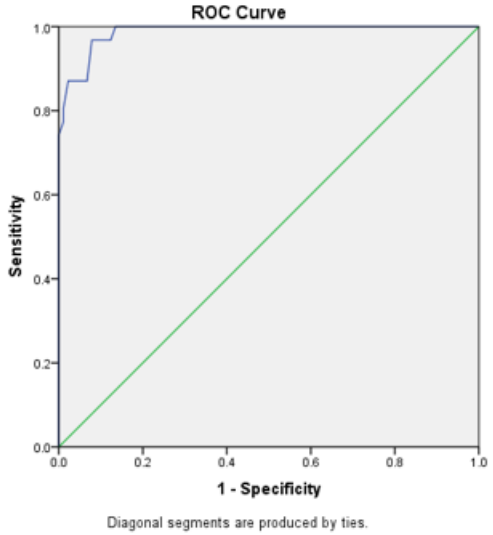
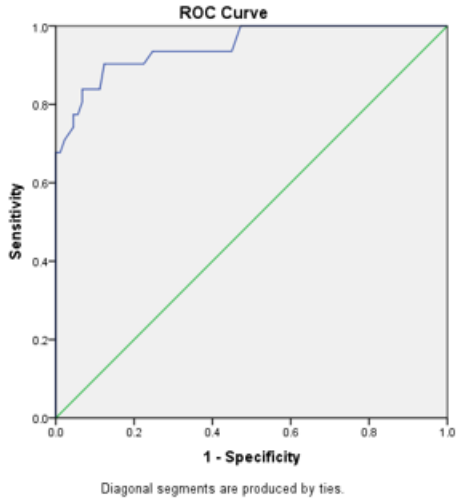


Graph 2: ROC curve for MINX

MINX		
Cut Off (Micron)	Sensitivity	Specificity
4.16	1	0
8.51	1	0.52
11.22	0 .67	1



Graph 3: ROC curve for NA

NA		
Cut Off (Micron^2)	Sensitivity	Specificity
25.38	1	0
78.36	1	0.75
111.54	0 .87	1

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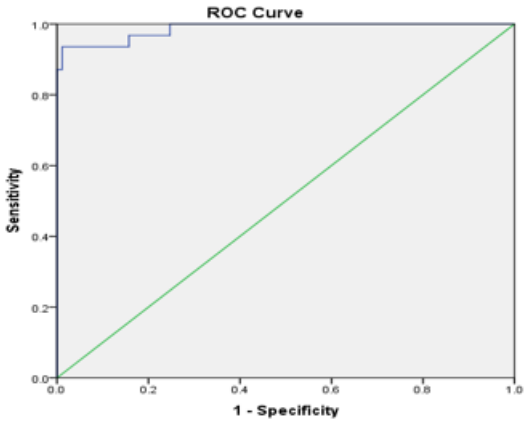
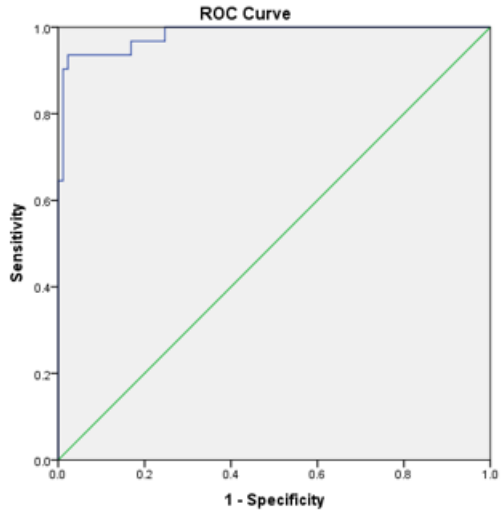


Graph 4: ROC curve for NP

NP		
Cut Off (Micron)	Sensitivity	Specificity
17.83	1	0
33.09	1	0.75
41.56	0.64	1



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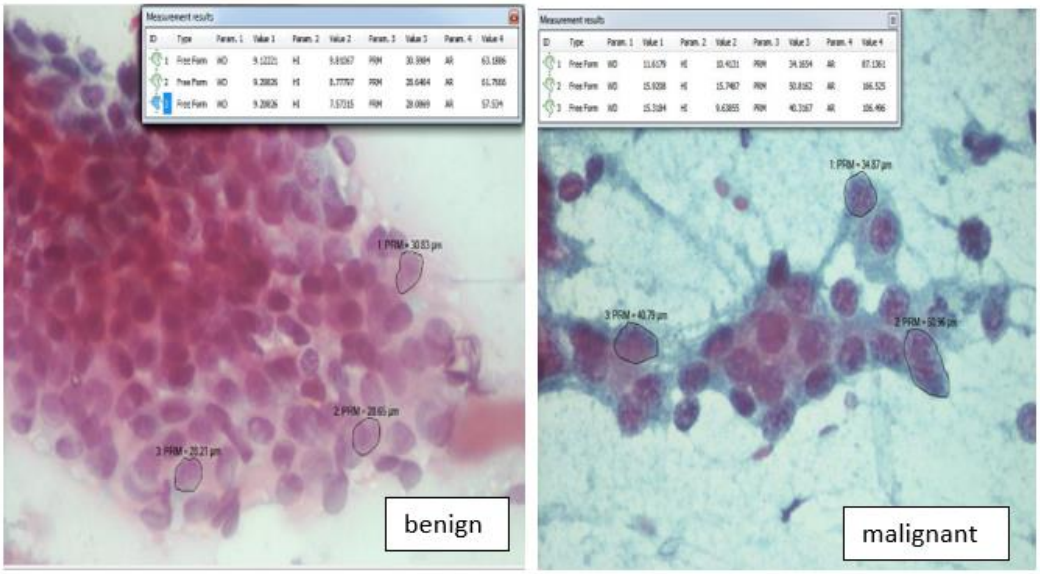

Figure 1: Morphometric measurements of benign lesion; (PAP, x400) and malignant lesion. (PAP, x400)

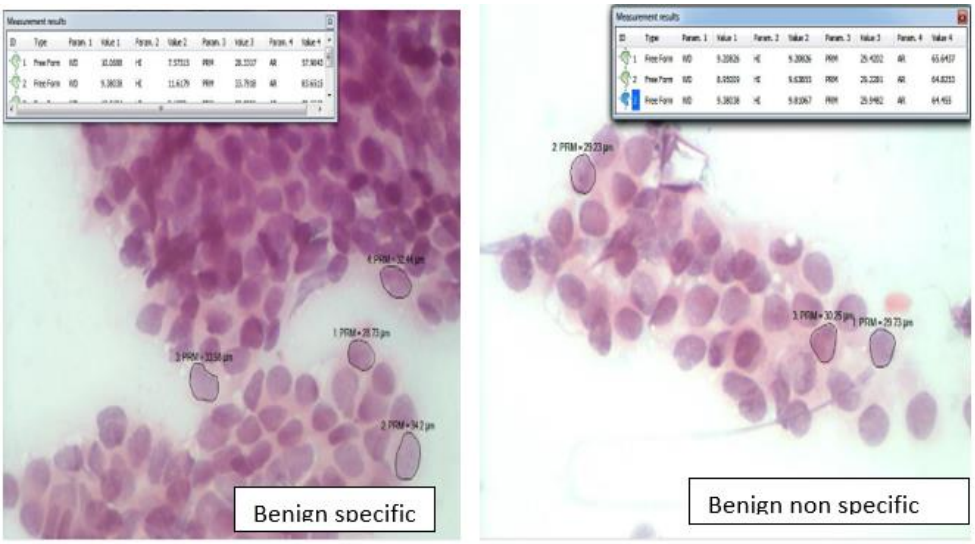

Figure 2: Morphometric measurements of benign category. (PAP, x400); specific category. (PAP, x400) and benign non specific

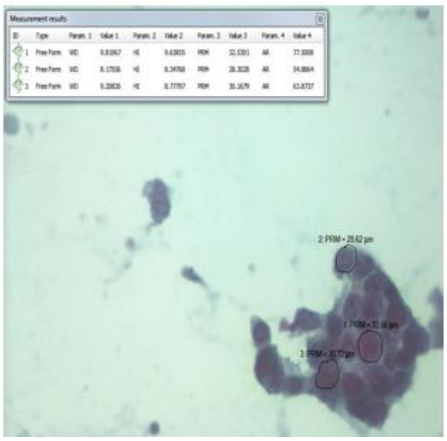

Figure 3: Morphometric measurements of atypical category. (PAP, x400

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Discussion

Breast carcinoma is the leading cause of cancer mortality in Indian women in which 80,000 new cases are diagnosed every year. So, adequate screening of the breast lesions is very essential to protect the health of women.[2]

Morphometric Correlation

In the present study nuclear morphometric parameters in malignant breast lesions were distinctly larger than the benign lesions, which can be utilized in diagnosis for the distinction of the malignant lesions which was also studied by **Abdalla et al** [3] and others.[4,5] The relation of nuclear size which aid in the diagnosis of malignancy was previously studied by many researchers.[5,6]

In the present study the values of mean NA, NP, MAJX and MINX, their standard deviation and variability can be used to make correct diagnosis with high precision. These values were significantly higher in malignant groups comparing benign ones (P value<.001) also between various cytodiagnostic categories (P value<.001). The SD of these parameters is a quantitative measurement of nuclear pleomorphism, which was significantly lower in benign cases.

Boruah et al[5] in their study reported the mean value with SD of MAJX for benign and malignant lesions as 8.98±0.64 and 13.30 ± 1.91.According to **Kashyap et al**[7] it was 6.61±0.57 and 9.26±1.81.**Kalhan et al** [8] documented the values as 6.87 ± 0.87 and 13.52 ± 1.56.In the present study the values of MAJX for benign and malignant lesions were 9.83±1.61 and 15.60±2.96.All these studies showed concordance with present study saying that mean values of MAJX is significantly higher in malignant lesions comparing benign lesions.(P value<.001)

The mean value with SD of MINX for benign and malignant lesions according to **Boruah et al**[5] was 6.93 ± 0.51 and 10.58 ± 1.42. **Kashyap et al**[7] reported it as 5.11±0.41 and 7.09±1.29. **Kalhan et al**[8] documented it as 4.95 ± 0.84 and 9.25 ± 1.24. In the present study the values were 8.28±1.43 and 13.04±3.14 All these studies showed concordance with present study proving that mean values of MINX is significantly higher in malignant lesions comparing benign lesions.(P value<.001)

The mean value with SD of NA for benign and malignant lesions according to **Boruah et al**[5] were 49.34 ± 6.79 and 113.25 ± 31.92. **Kashyap et al**[7] documented it as 25.49±3.88 and 51.43±20.47.

According to **Kalhan et al**[8] the values were 28.46 ± 7.72 and 94.19 ± 19.49.In the present study the values observed were 63.86±19.35 and 161.26±68.69. All these studies showed similar results as present study stating that mean values of NA is significantly higher in malignant lesions comparing benign lesions.(P value<.001)

According to **Boruah et al** the mean value with SD of NP for benign and malignant lesions were 24.98 ± 1.76 and 37.49 ± 5.18. **Kashyap and Kalhan et al** reported it as 18.39±1.49, 25.69±4.99 and 19.77 ± 2.42, 36.19 ± 4.91 respectively. The values of the present study were 29.58±4.65 and 46.37±8.80

All these studies showed that mean values of NP in malignant lesions were significantly higher than benign lesions (P value<.001) which is concordance with our study.

According to this study the cut off values with sensitivity 1(100%) for the differentiation of malignant from benign was: (a) MAJX>11.61 micron (specificity=0.86), (b) MINX> 8.51micron (specificity=0.52), (c) NA>78.36 micron^2 (specificity=0.75) and (d) NP> 33.09 micron (specificity=0.75).

MAJX showed maximum efficiency to differentiate benign and malignant lesions in the present study, followed by NA and NP.

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According to **Kashyap et al** cut-off values for mean nuclear area, maximum feret, minimum feret and perimeter between benign and malignant cases were found to be 31.93 μm^2, 7.855 μm, 5.865 μm and 21.55 μm respectively with specificity (ranging from 98.3 to 100%) and sensitivity (ranging from 79.6 to 81.2%)

These values were not comparable with present study, could be due to the difference in the software used along with difference in other analytical factors such as slide preparation, staining and patient ethnicity.

According to **Boruah et al** the cut off values with sensitivity 1(100%) for the differentiation of malignant lesions from benign were (a) MAJX>10.70 micron (specificity=0.98), (b) MINX>7.53 micron (specificity=0.94), (c) NA>60.61 $micron^2$ (specificity=0.98) and (d) NP> 27.81 micron (specificity=0.96). which is almost similar to present study.

Abdalla et al [3] evaluated cut off values of mean nuclear area for diagnostic purposes. They says that for 100% detection of malignant cases: NA>54 μm^2 (specificity 84%), for 100% detection of benign cases: NA<72μm^2 (sensitivity 91%).

In the present study the mean and SD of MAJX, MINX, NA, NP showed significant difference between various cytodiagnostic categories of breast lesions (P value<.001). The values of MAJX obtained for benign-non specific, benign-specific, atypical and malignant were 9.38±1.64, 9.86 ± 1.52, 13.03±.66 and 15.60±2.96. The values of MINX were 7.88±1.65, 8.35±1.36, 9.85±.35 and 13.04±3.14.The NA values were 58.59±21.63, 64.57±18.32, 90.07±11.06 and 161.26±68.69. The values of NP obtained in the present study were 28.56±5.35, 29.65±4.352, 36.63±2.22 and 46.37±8.80.

Kashyap et al has done a study on BBD, ADH and malignant lesions. The mean nuclear area reported by them for these groups were 24.86±3.59, 29.44±3.51 and 51.43±20.47. The values of mean Maximum feret were 6.51±0.3, 7.26±0.42 and 9.26± 1.81. The mean minimum feret reported by them were 5.07±0.40, 5.39±0.43 and 7.09±1.29. The perimeter were 18.15±1.40, 19.91±1.18 and 25.69±4.99.In this study they concluded that all the four nuclear parameters showed an increasing trend from BBD to ADH to carcinoma with a significance different between ADH and malignant lesions which is concordance with present study.

According to the study done by **Yadav et al**[9] mean values of nuclear area and perimeter were significantly different between benign, borderline and malignant categories. The values of nuclear area were 45.45±3.88, 107.03±6.60 and 115.10±9.01. The perimeter recorded by them were 22.72±1.30, 35.01±2.73 and37.29±3.57.This study also showed similar values as present study Study done by **Parmar et al**[10] reported that mean values of nuclear area for fibroadenoma, atypical hyperplasia, and invasive carcinoma as 36.89 ± 3.53, 64.97 ± 3.12 and 98.9 ± 19.56.The values of perimeter were 26.69 ± 1.45, 32.78 ± 3.1, and 39.86 ± 2.23. The long and short axis were 8.34 ± 0.38 and 6.02 ± 0.33, 10.71 ± 0.45 and 8.10 ± 0.38 and 13.14 ± 0.99 and 9.83 ± 1.0 for three groups respectively. The values of this study are significantly different among these groups and shows concordance with present study.

According to **Narasimha et al**[2] mean values of nuclear area and perimeter were statistically significant between fibroadenoma, fibrocystic disease, hyperplasia and carcinoma which showed similarity with present study.

Study done by **Kalhan et al** also showed significant difference for mean nuclear area, perimeter, long axis, short axis among benign, ADH, DCIS and invasive carcinomas (P value<.0001) which also supports present study.

Arora et al[11] reported significant difference for MNA among benign, atypical and malignant cases which is in concordance with present study.

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Conclusion

Morphometry is the study of various cell parameters microscopically, which can be used as an objective tool for correct diagnosis. Morphometric analysis of nuclear parameters can be used as an adjunct to FNAC for diagnosis of benign and malignant lesions, differentiating various cytodiagnostic categories and malignant grades of breast with precision and accuracy.

FNAC is one of the best diagnostic procedures available now but it has few limitations mainly diagnosing grey zone lesions. We recommend the use of morphometry as an adjunct to FNAC in differentiating malignant lesions and whenever in doubt regarding grey zone lesions.

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