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Nuclear morphometric study of malignant breast lesions with histopathological correlation in South Indian population

Tony Joe Peechatt[1], Rufus K Sam Vargis[2], Shabana Salim[3], Samreen Panjakash[4]

[1]Assistant Professor, Department of Pathology, Al Azhar Medical College and Super Speciality Hospital Ezhalloor, Kumaramangalam, Thodupuzha- 685605, India.
[2]Associate Professor, Department of Pathology Al Azhar Medical College and Super Speciality Hospital Ezhalloor, Kumaramangalam, Thodupuzha- 685605, India.
[3]Associate Professor, Department of Physiology, Al Azhar Medical College and Super Speciality Hospital Ezhalloor, Kumaramangalam, Thodupuzha- 685605, India.
[4]Professor, Department of Anatomy, Al Azhar Medical College and Super Speciality Hospital Ezhalloor, Kumaramangalam, Thodupuzha- 685605, India.

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Abstract:
Background: nuclear morphometric measurements like major axis, minor axis, nuclear area and nuclear perimeter vary according to malignant grades of breast carcinoma. **Materials and methods:** Diagnosis of malignant cases was done in 31 patients by correlation of clinical history, radiological findings, adequacy of smear, predominant patterns and individual cell cytomorphology. Morphometric values like major axis of nucleus, minor axis of nucleus, nuclear area and nuclear perimeter were measured using ProgresR capture pro 2.9.0.1 software. **Result:** 16 cases (51.61%) were diagnosed as grade 1, eight (25.80%) cases were grade 2, seven (22.58%) cases were grade 3 using Robinson's cytological grading. One-way ANOVA test showed that these cytodiagnostic categories are significantly different from each other for the nuclear parameters. (p value < 0.01). **Conclusion:** as there is grey area in diagnosing breast lesions from FNAC, nuclear morphometry can be pivotal in prompt diagnosis and also improvement in mortality of patients with malignant breast carcinoma.

[*]**Corresponding Author:** Dr. Samreen Panjakash, Professor, Department of Anatomy, Al Azhar Medical College and Super Speciality Hospital Ezhalloor, Kumaramangalam, Thodupuzha-685605, India.
Postal address: Al Azhar Medical College and Superspecialty Hospital, Ezhalloor road, Thodupuzha, Idukki district, Kerala 685605, India.
Email: samrin.pjk@gmail.com

Introduction

Breast cancer is the second most common cancer among women in India and accounts for 7% of global burden of breast cancer and one-fifth of all cancers among women in India.[1]
A definitive cancer diagnosis is given in approximately two-thirds of screen detected cancers by fine needle aspiration cytology as a part of triple diagnosis. The other one- third requires further investigation to give the go-ahead for more extensive definitive surgery. The reason may be due to discordance with radiological findings, doubts about invasion, or a relatively bland cytology as in low-grade cancers, mainly lobular carcinoma of classic type and tubular carcinoma.[2]

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Uncertainties in FNAC can hinder a definite diagnosis in breast lesions 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. Study of morphometric parameters of breast lesions have reported from 20th century. Nuclear and histologic grade, lymph node status, tumor size, mitotic activity index, cellularity index, and mean and SD of nuclear area have been reported to be the most important single predictors of prognosis in breast carcinoma.[3]

The aim of the present study is to quantify nuclear morphometric changes on malignant breast aspirates and to correlate these parameters with clinicopathologic features such as cytologic grade, tumor size, lymph node status, mitotic index, and histopathologic grade.

Materials And Methods

Among the patients with palpable and non- palpable breast lumps referred to the department of Pathology Adichunchanagiri Institute of Medical sciences, B.G Nagara during November 2015 to April 2017, who were willing to participate, were included in the present study. Written consent was also taken.

Diagnosis of malignant cases was done in 31 patients by correlation of clinical history, radiological findings, adequacy of smear, predominant patterns and individual cell cytomorphology. In 18 cases where surgical excision was performed, the specimen was fixed in 10% formalin and processed routinely for H&E staining. Histopathological correlation with morphometry was performed in these malignant cases.

Measurements of parameters

A minimum of 100 cells in Papanicolaou stained cytology breast smears were were observed under projection microscope Olympus CX31.

All the cases malignant were graded as per Robinson's grading system.

Morphometric values like major axis of nucleus (MAJX), minor axis of nucleus(MINX), nuclear area(NA) and nuclear perimeter(NP) were measured using ProgresR capture pro 2.9.0.1 software. Values were measured in micrometer which is calibrated to each objective. In this study 40x objective was used for measurement of individual parameters.

The data obtained by morphometry was expressed as mean values and percentage Comparison between groups were done by unpaired t test and one way ANOVA test.

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 Progres R capture pro 2.9.0.1 software. Morphometric parameters measured were major axis of nucleus, minor axis of nucleus, nuclear area and nuclear perimeter. 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 objectives. 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.

Results

16 cases (51.61%) were diagnosed as grade 1, eight (25.80%) cases were grade 2, seven (22.58%) cases were grade 3 using Robinson's cytological grading.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

Table 5: Mean values of nuclear parameters and age with SD and range for malignant lesions

Values	Age (Years)	Major Axis(µm)	Minor Axis(µm)	Nuclear Area(µm²)	Nuclear Perimeter(µm)
Mean	48.03	15.60	13.04	161.26	46.37
Std. Deviation	11.41	2.96	3.14	68.69	8.80
Range	0	12.22	13.43	320.42	40.30
Minimum	25	11.70	8.60	79.58	33.15
Maximum	65	23.92	22.03	400.00	73.45
N=31					

Table 9: Mean values of nuclear parameters and age with SD and range for malignant grades

		Mean	SD	Range	Min	Max
Malignant (Grade 1)	**Age(in year)**	46.81	10.38	40	25	65
	major axis	13.63	1.29	4.8	11.7	16.5
	minor axis	10.78	1.21	3.8	8.6	12.4
	nuclear area	114.83	15.23	54.46	79.58	134.04
	nuclear perimeter	39.92	2.88	10.55	33.15	43.7
Malignant (Grade 2)	**Age(in year)**	43.25	12.51	31	25	56
	major axis	15.49	1.74	5.26	13.94	19.2
	minor axis	13.58	2.04	5.95	10.4	16.35
	nuclear area	158.36	16.86	54.36	132.4	186.76
	nuclear perimeter	47.01	2.81	8.83	42.7	51.53
Malignant (Grade 3)	**Age(in year)**	56.29	9.21	21	44	65
	major axis	17.63	1.74	5.33	15.5	20.83
	minor axis	16.26	1.06	2.56	15.1	17.66
	nuclear area	221.22	32.22	99.2	176.2	275.4
	nuclear perimeter	54.5	3.57	11.4	49.1	60.5

Table 14: The mean values of nuclear parameters with SD for the three malignant grades with p values between them

	Major Axis(µm)	Minor Axis(µm)	Nuclear Area(µm²)	Nuclear Perimeter(µm
Malignant Grade 1 Mean±SD N=16	13.63±1.29	10.78±1.21	114.83±15.23	39.92±2.88
Malignant Grade 2 Mean±SD N=8	15.49±1.74	13.58±2.04	158.36±16.86	47.01±2.81
Malignant Grade3 Mean±SD	17.63±1.74	16.26±1.06	221.22±32.22	54.50±3.57

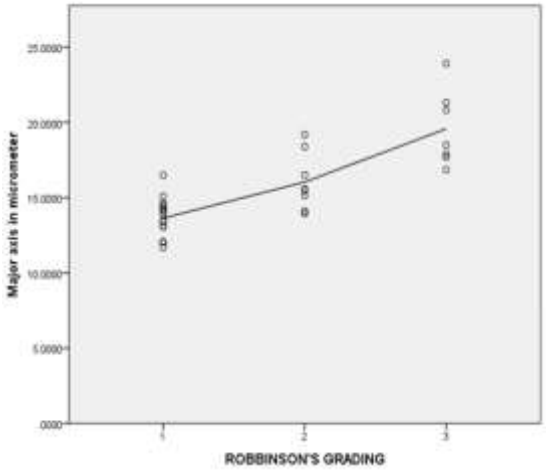
ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

N=7				
P value	< 0.001	< 0.001	< 0.001	< 0.001

One-way ANOVA test showed that these cytodiagnostic categories are significantly different from each other for the nuclear parameters. (p value < 0.01).
Invasive Lobular Carcinoma (ILC) with Invasive Ductal Carcinoma(IDC) one case, IDC grade1-four cases, IDC grade2-five cases, IDC grade3- four cases, papillary carcinoma-one case, medullary carcinoma –one case, malignant phylloides-one case.

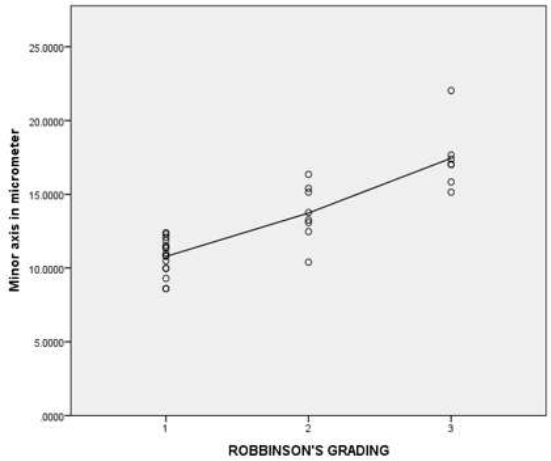
Cytological Grade	**Histopathological Grade**			**Otherhistopathological diagnosis**					**Total**
	Grade 1	**Grade 2**	**Grade 3**	**Fibroadenoma**	**Papillary carcinoma**	**ILC with IDC**	**Medullary carcinoma**	**Malignant Phyllodes**	
Grade 1	3	1	0	1	1	1			7
Grade 2	1	3	1				1	1	7
Grade 3	0	1	3						4
Total	4	5	4	1	1	1	1	1	18

In the case of ILC all the nuclear values were lower than mean values of malignant group but above the cut off values for malignancy. Nuclear morphometric values of medullary carcinoma were higher than grade 1 IDC but lower than grade 2 IDC for all parameters. All the parameters showed lower value than IDC grade 1 in the case of papillary carcinoma but well more than cut off values for malignancy. In the case of malignant phyllodes nuclear parameters were higher than grade 1 IDC but lower than grade 2 IDC. One case of IDC grade 1 diagnosed in cytology was confirmed as fibroadenoma in histopathology. In this case all the nuclear parameters showed less value than malignant lesions.

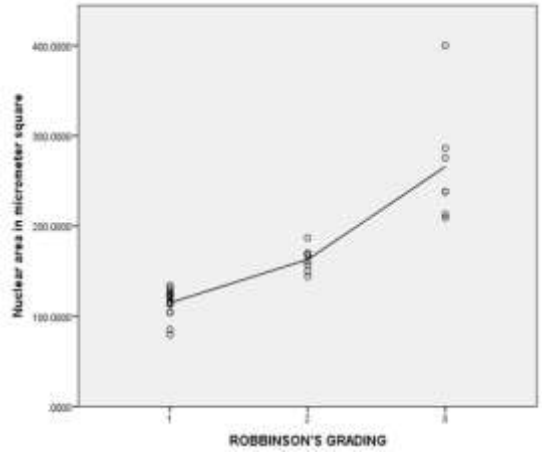


Graph 9: Scatter plot of malignant grades versus MAJX

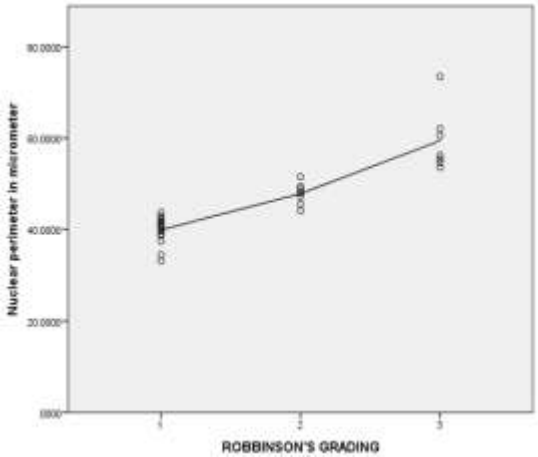
ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023



Graph 10: Scatter plot of malignant grades versus MINX.



Graph 11: Scatter plot of malignant grades versus NA



Graph 12: Scatter plot of malignant grades versus NP

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

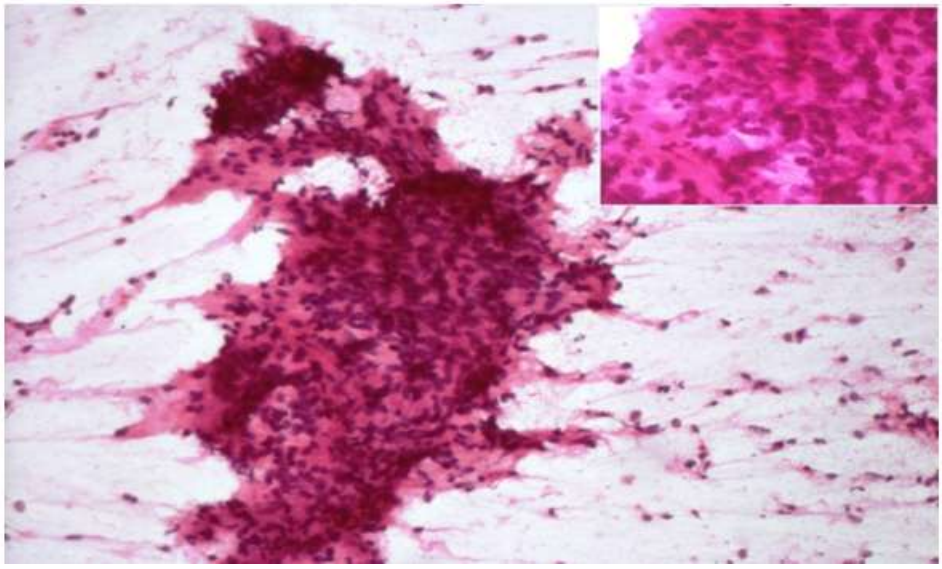


Figure 21: FNAC smears of malignant phyllodes showing stromal fragments with nuclear atypia along with atypical bare nuclei in the background. (H&E, x100). Inset showing high power view of stromal fragments. (H&E, x400).

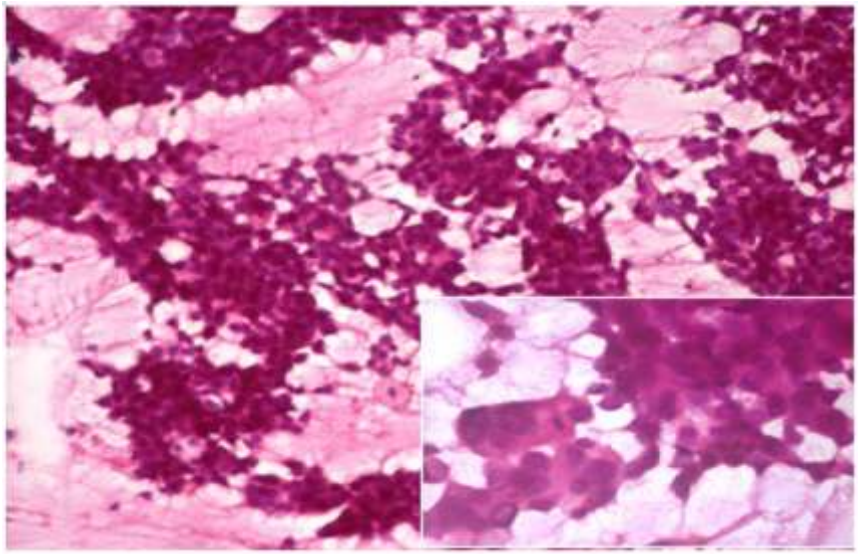


Figure 22: FNAC smears of IDC showing increased cellularity and ductal epithelial cells in loosely cohesive clusters. (H&E, x100).Inset showing loosely cohesive ductal cells with cytological features of malignancy. (H&E,x400).

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

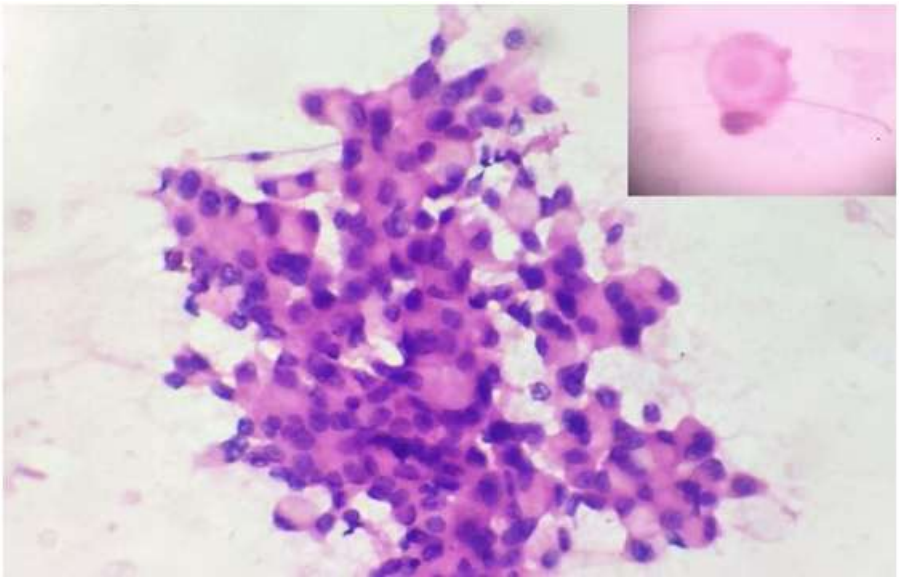


Figure 23: FNAC smears of ILC showing uniform tumor cells with eccentrically placed nucleus and pale eosinophilic to clear cytoplasm(signet ring like cells) (H&E, x400).Inset showing intracytoplasmic neolumina and targetoid cytoplasmic mucin. (H&E, x400).

Discussion

According to **Boruah et al**[4] and **Kalhan et al** [3] three malignant grades showed significantly different values for all nuclear parameters which showed concordance with present study.

Study done by **Mihalache et al**[5] said that mean nuclear area is significantly different among malignant grade 1(168.77± 13.05), grade 2(203.52± 12.40) and grade 3 (243.21± 26.13).

Prvulovic et al[6] also had similar findings. The mean nuclear area among cytology grades were 85.4926±12.49 (grade1), 89.0447±34.24 (grade2) and 116.9542±32.73 (grade3).

Abdalla et al[7] evaluated cut off values of mean nuclear area for diagnostic purposes. They says that for 100% detection of malignant cases: NA>54 µm (specificity 84%), for 100% detection of benign cases: NA<72µm2 (sensitivity 91%).

Study done by **Parmar et al**[8] 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 was 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.

Present study had one case of medullary carcinoma, papillary carcinoma and malignant phyllodes which were confirmed by histopathology. Nuclear morphometric values of medullary carcinoma were higher than grade 1 IDC but lower than grade 2 IDC for all parameters. All the parameters showed lower value than IDC grade 1 in the case of papillary carcinoma but well more than cut off values for malignancy. In the case of malignant phyllodes nuclear parameters were higher than grade 1 IDC but lower than grade 2 IDC. In the present study one case of IDC grade 1 diagnosed in cytology which was confirmed as fibroadenoma in histopathology. In this case all the nuclear parameters showed less value than malignant group which strongly suggest that morphometric analysis is very helpful in differentiating benign and malignant cases.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

Table 18: Correlation of nuclear parameters among malignant grades with other studies

Morphometic parameters Mean±SD	Boruah et al[4]			Kalhan et al[5]			Present study		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
MAJX(µm)	12.87 ±1.82	12.93 ±1.76	14.67 ±1.90	11.68 ±1.07	13.66 ±1.24	14.75 ±1.06	13.63 ±1.29	15.49 ±1.74	17.63 ±1.74
MINX(µm)	9.95 ± 1.37	10.44 ±1.18	11.51 ±1.72	8.10 ±0.81	9.23 ±1.15	10.2± 0.85	10.78 ±1.21	13.58 ±2.04	16.26 ±1.06
NA(μm^2)	102.62 ±28.60	108.13±28.30	136.41±35.14	75.46 ±12.92	92.38 ±13.73	114.09 ±17.59	114.83 ±15.23	158.36 ±16.86	221.22 ±32.22
NP(µm)	35.82 ±4.94	36.69 ±4.56	41.11 ±5.66	31.27 ±3.91	36.67 ±3.84	39.28 ±4.94	39.92 ±2.88	47.01 ±2.81	54.50 ±3.57

Conclusion

Nuclear morphometry has a promising role in not just diagnosis of malignant breast lesions but also in its histological grading. This could be pivotal in the prognostic value in such patients. Morphometric studies being an objective parameter have an added advantage of being free from interobserver and intra observer variability. Thus it can be helpful in diagnosing grey zone lesions of the breast.

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ISSN: 0975-3583,0976-2833 VOL14, ISSUE 10, 2023

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