Diagnostic Application Of Mean Nuclear Area (MNA) Measured By Computerized Interactive Morphometry In Breast Cancer

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Citation

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Abstract

Breast carcinoma cells develop significant nuclear and cytoplasmic alterations during the development of this cancer through its various stages. These alterations are the cornerstone for typing and grading various breast lesions, including carcinomas. The present study was undertaken to evaluate the role of computerized interactive morphometry (CIM) for an objective analysis of cellular details by measuring mean nuclear area (MNA), mean cytoplasmic area (MCA) and nuclear/cytoplasmic (N/C ratio) in 75 breast specimens with 15 benign cases, in form of lumpectomies (93.3% cases), including 9 cases of fibroadenoma, 5 of fibrocystic disease harboring foci of ductal hyperplasia of usual type (DUT) and 1 case of duct ectasia; another 10 for cases of atypical ductal hyperplasia (ADH), mostly wide-excision specimens (70% cases) and 50 mastectomies with a diagnosis of infiltrating ductal carcinoma, not otherwise specified (IDC NOS) . MNA for benign lesions ($24.33\pm0.77\mu m^2$) was found to be significantly lower than in cases of ADH ($42.21\pm1.84\mu m^2$) ($p<0.05$). MNA was found to be significantly higher in cases of IDC, as compared to benign and atypical cases ($p<0.05$). Moreover, it significantly correlated directly with the histological grade of IDCs. While the values for MCA were found to be significantly higher in atypical vs. benign cases, the same were not found to be significant between atypical and malignant cases. Significant differences were also obtained with N/C ratio amongst the various categories. CIM analysis can be included as a useful tool for objective assessment of various breast lesions. Among the parameters studied, MNA and N/C ratio are the most significant.
Breast carcinoma cells develop significant nuclear and cytoplasmic alterations

INTRODUCTION

Breast lesions are among the commonest biopsy specimens in surgical pathology[1, 2]. Carcinoma breast ranks among the most frequent female malignancies in metropolitan cities of India like Delhi, Mumbai etc.[3]. Several breast carcinoma cases in our country present at a locally advanced stage[4]. Breast cancer development reveals sequential events from precancerous to frank carcinoma: Normal breast to ductal hyperplasia, usual type, to atypical ductal hyperplasia (ADH), to Ductal carcinoma-In-situ (DCIS) to invasive carcinoma to nodal and distant metastasis[5]. Presence of certain breast lesions such as usual ductal hyperplasia in an otherwise benign biopsy indicates a risk of 1.5-1.9 of subsequent invasive cancer development. Atypical ductal hyperplasia has been reported with even higher risk i.e. up to 3% [6, 7]. Hence, identification of these lesions assumes importance.

However, while dealing with breast lesions on biopsy, frequently there is a morphological overlap, even though definite criteria have been established. Application of ancillary techniques like immunohistochemistry and flow cytometry then becomes imperative. Computerized interactive morphometry (CIM) forms another useful technique in providing an objective and a reproducible estimate of the various lesions[8, 9]. Apart from enabling an appraisal of several parameters, CIM can also be used for automated analysis for several specimens.

The current study was aimed at analyzing the value of morphometric parameters like MNA, MCA, N/C ratio in various breast specimens including benign, atypical and malignant cases, with and without lymph node metastasis.

MATERIAL AND METHODS

The proposed study was conducted on 75 cases of surgical breast specimens received in the Department of Pathology, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, India.

Out of these, 15 cases (20%) were of benign breast lesions and 10 cases (13.3%) were of atypical ductal hyperplasia. Remaining 25 cases (33.3%), each, were of infiltrating duct carcinoma, with and without regional nodal metastasis, respectively.

While the surgical specimens for benign breast lesions were in form of lumpectomies and rarely wide-excisions (1 case of duct ectasia), 70% cases of ADH were of wide-excisions. Remaining 30% cases were incidentally diagnosed on lumpectomies

Among 15 benign cases, 9 cases (60%) were of fibroadenoma, 5 (33.3%) were of fibrocystic disease, including foci of ductal hyperplasia of usual type (DUT) and the remaining 1 case was of duct ectasia. Out of the 25 cases of infiltrating ductal carcinoma (IDC) without lymph node metastasis, 11 cases (44%) were of Grade-1, 10 cases (40%) were of Grade-2 while 4 cases (16%) were of Grade-3. Out of the 25 cases included in the category of IDC with lymph node metastasis, 5 cases (20%) were of Grade-1, 10 cases (40%) were of Grade-2 and remaining 10 cases (40%) were of Grade-3.

All malignant cases, as retrieved from the case files, were operable breast cancers (OBC), who had undergone modified radical mastectomy (MRM) with infiltrating duct carcinoma (NOS) as the histological type. (Table 1).

Figure 1

Table 1: Clinicopathological features of various cases along with various morphometric categories

No.	Age	Type of surgery	Pathological Diagnosis	Grade	'M' Category
1	30	Lumpectomy	Fibroadenoma	NA	B
2	15	Lumpectomy	Fibroadenoma	NA	B
3	17	Lumpectomy	Fibroadenoma	NA	B
4	45	Lumpectomy	Fibroadenoma	NA	B
5	32	Lumpectomy	Fibroadenoma	NA	B
6	17	Lumpectomy	Fibroadenoma	NA	B
7	18	Lumpectomy	Fibroadenoma	NA	B
8	30	Lumpectomy	Fibroadenoma	NA	B
9	32	Lumpectomy	Fibrocystic disease	NA	B
10	45	Lumpectomy	Fibrocystic disease	NA	B
11	32	Lumpectomy	Fibrocystic disease	NA	B
12	24	Lumpectomy	Fibrocystic disease	NA	B
13	47	Lumpectomy	Fibrocystic disease	NA	B
14	20	Wide-excision	Duct ectasia	NA	B
15	45	Lumpectomy	Fibroadenoma	NA	B
16	45	Lumpectomy	Atypical Ductal Hyperplasia	NA	A
17	40	Wide-excision	Atypical Ductal Hyperplasia	NA	A
18	25	Wide-excision	Atypical Ductal Hyperplasia	NA	A
19	28	Wide-excision	Atypical Ductal Hyperplasia	NA	A
20	21	Wide-excision	Atypical Ductal Hyperplasia	NA	A
21	35	Lumpectomy	Atypical Ductal Hyperplasia	NA	A
22	20	Wide-excision	Atypical Ductal Hyperplasia	NA	A
23	27	Wide-excision	Atypical Ductal Hyperplasia	NA	A
24	50	Lumpectomy	Atypical Ductal Hyperplasia	NA	A
25	60	Wide-excision	Atypical Ductal Hyperplasia	NA	A
26	38	MRM	Infiltrating duct carcinoma	2	M
27	46	MRM	Infiltrating duct carcinoma	2	M
28	75	MRM	Infiltrating duct carcinoma	2	M
29	55	MRM	Infiltrating duct carcinoma	3	M
30	38	MRM	Infiltrating duct	1	M

Figure 2

			carcinoma		
		MRM	Infiltrating duct		
31	40		carcinoma	2	M
		MRM	Infiltrating duct		
32	50		carcinoma	3	M
		MRM	Infiltrating duct		
33	60		carcinoma	2	M
		MRM	Infiltrating duct		
34	66		carcinoma	1	M
		MRM	Infiltrating duct		
35	45		carcinoma	3	M
		MRM	Infiltrating duct		
36	52		carcinoma	1	M
		MRM	Infiltrating duct		
37	55		carcinoma	2	M
		MRM	Infiltrating duct		
38	35		carcinoma	1	M
		MRM	Infiltrating duct		
39	45		carcinoma	2	M
		MRM	Infiltrating duct		
40	78		carcinoma	1	M
		MRM	Infiltrating duct		
41	65		carcinoma	1	M
		MRM	Infiltrating duct		
42	40		carcinoma	1	M
		MRM	Infiltrating duct		
43	67		carcinoma	1	M
		MRM	Infiltrating duct		
44	65		carcinoma	2	M
		MRM	Infiltrating duct		
45	36		carcinoma	2	M
		MRM	Infiltrating duct		
46	48		carcinoma	1	M
		MRM	Infiltrating duct		
47	45		carcinoma	2	M
		MRM	Infiltrating duct		
48	32		carcinoma	1	M
		MRM	Infiltrating duct		
49	60		carcinoma	1	M
		MRM	Infiltrating duct		
50	60		carcinoma	3	M
No.	**Age**	**Type of surgery**	**Pathological Diagnosis**	**Grade**	**'M' Category**
51	60	MRM	IDC + LN	1	M
52	48	MRM	IDC + LN	3	M
53	45	MRM	IDC + LN	2	M

Figure 3

54	49	MRM	IDC + LN	2	M
55	40	MRM	IDC + LN	1	M
56	45	MRM	IDC + LN	3	M
57	25	MRM	IDC + LN	1	M
58	40	MRM	IDC + LN	2	M
59	35	MRM	IDC + LN	1	M
60	80	MRM	IDC + LN	3	M
61	40	MRM	IDC + LN	3	M
62	52	MRM	IDC + LN	2	M
63	50	MRM	IDC + LN	2	M
64	45	MRM	IDC + LN	2	M
65	65	MRM	IDC + LN	3	M
66	60	MRM	IDC + LN	2	M
67	30	MRM	IDC + LN	3	M
68	38	MRM	IDC + LN	2	M
69	43	MRM	IDC + LN	2	M
70	30	MRM	IDC + LN	1	M
71	60	MRM	IDC + LN	3	M
72	45	MRM	IDC + LN	3	M
73	60	MRM	IDC + LN	3	M
74	50	MRM	IDC + LN	3	M
75	45	MRM	IDC + LN	2	M

Key: MRM: Modified radical mastectomy; IDC: Infiltrating duct carcinoma; LN: Lymph node; NA: Not Applicable 'M'Category: Morphometric. B: Benign; A: Atypical; M: Malignant.

The routine diagnosis was made on Hematoxylin and Eosin (H&E) stained sections. Grading of IDC cases was done by Nottingham's modification of Richardson and Bloom's grading system[10].

One representative section from every case was subjected to morphometric analysis by an independent observer to remove subjective bias. Frozen sections were excluded.

The quantitative study was done by an image analysis system. The digital images generated by a charge coupling device (CCD) video camera (Sony) linked to a Olympus microscope at a total magnification of 400X were stored on a host computer through a digital frame grabber. The processing was done by image analysis software viz. Image pro-express version 4.5 by Cyber Natics Inc. USA. This was integrated into the host computer.

A total of hundred cells were randomly selected and measured in each case. The cells of interest were identified on the screen and the contours of their nuclear and cytoplasmic profiles were traced. Inside each tracing, a semiautomatic procedure consisting of threshold based boundary detection was implemented to determine the nuclear and cytoplasmic areas.

With the help of an internal calibration, various parameters were studied like Mean nuclear area (MNA), mean cytoplasmic area (MCA) and mean N/C ratio.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS system (version 14). The mean values ± standard deviations (SD) were calculated for all the three variables including mean nuclear area (MNA), cytoplasmic area (MCA) and nuclear-cytoplasmic (N/C) ratio. Values for MNA and MCA followed normality of data, which was tested by graphical as well as statistical tests. One-way ANOVA (analysis of variance) was used to compare MNA and MCA among 4 categories. Posthoc comparison was done by using Dunnett C test. P value <0.05 was considered as statistically significant.

N/C ratio did not follow normality of data despite log transformation. Thus, a non-parametric test i.e. Kruskal Wallis test was applied. In cases of IDC with/without lymph node positivity (LN), MNA was correlated with the histological grade using One-way ANOVA

RESULTS

The age of the patients selected for present study ranged from 15 to 80 years with a mean of 43.68± 14.96 years. It was observed that maximum numbers of cases (22) were

between 41-50 years of age group, forming 29.3% of the study group.

On morphometric analysis, MNA for benign, atypical and malignant cases, without and with lymph node metastasis was found to be 24.33±0.77, 42.21±1.84, 52.17±14.87, and 60.31±17.50μm^2 respectively. MCA for these various categories was found to be 77.37±2.65, 115.85±5.43, 119.37±33.45, and 138.28±40.74μm^2 respectively. N/C ratio in the categories was found to be 0.31±0.005 in benign cases, 0.36±0.003 in cases of ADH, 0.43±0.01 in cases of IDC without lymph node metastasis and 0.43±0.02 μm in cases of IDC with nodal metastasis. (Table 2), (Figure 1), (Diagrams 1 and 2).

Figure 4

Table 2: Comparison of mean nuclear area (MNA), mean cytoplasmic area (MCA) and mean N/C ratio in the various categories (Total cases N=100).

Category	No. of cases	MNA±SD (μm^2)	MCA±SD (μm^2)	N/C ratio ±SD
Benign Hyperplasia	15	24.33±0.77	77.37±2.65	0.31±0.005
Atypical ductal Hyperplasia	10	42.21±1.84	115.85±5.43	0.36±0.003
IDC without lymph node metastasis	25	52.17±14.87	119.37±33.45	0.43±0.010
IDC with lymph node metastasis	25	60.31±17.50	138.28±40.74	0.43±0.024

IDC: Infiltrating duct carcinoma

Figure 5

Figure 1: A. Benign ductal hyperplasia of usual type. Photomicrograph showing nuclear and cytoplasmic contours of benign cells. (H & E x 400). B. Atypical ductal hyperplasia (cribriform pattern). Arrows show arrangement of cells around the lumina. Nuclear and cytoplasmic contours of individual atypical cells seen. (H & E x 400). C. Ductal carcinoma-in-situ (High nuclear grade) in a case of infiltrating ductal carcinoma. Nuclear and cytoplasmic contours of malignant cells seen. (H & E x 400) D. Infiltrating ductal carcinoma (High grade). Nuclear and cytoplasmic contours of malignant cells seen. (H & E x 400).

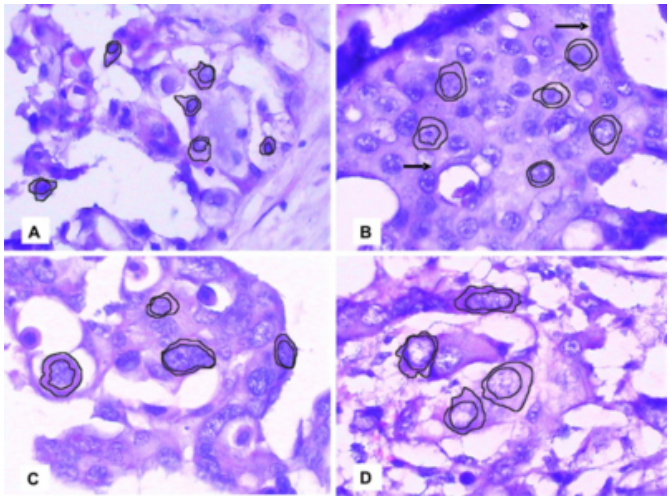


Figure 6

Diagram 1: Representation of the mean nuclear area (MNA) in the various categories.

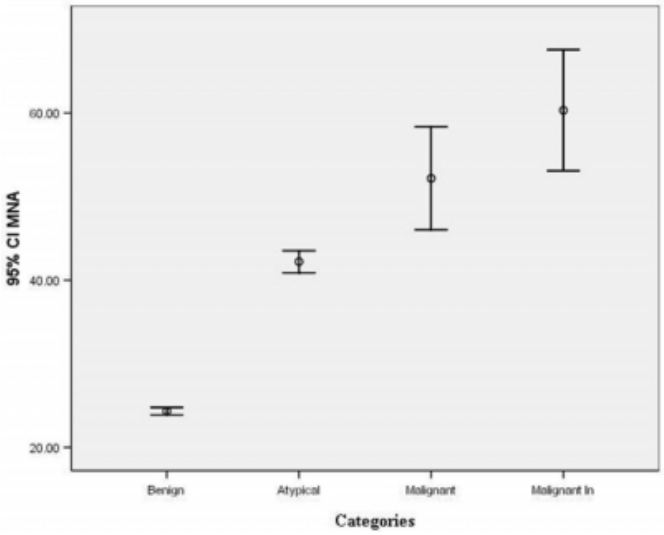
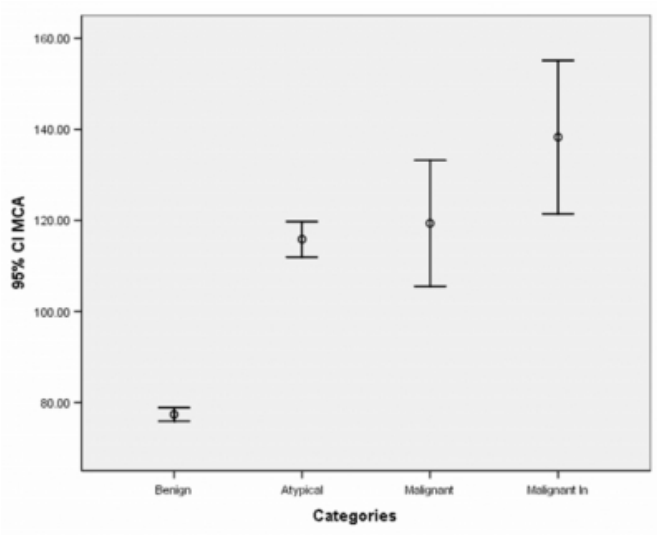


Figure 7

Diagram 2: Representation of the mean cytoplasmic area (MCA) in various categories.



Significant differences were observed with MNA in the various categories with lowest values in benign cases, intermediate in ADH cases, higher in IDC cases without nodal metastasis and highest in IDC cases with nodal metastasis. While significantly higher values for MCA were noted in atypical vs. benign cases, the same were not found to be significant between atypical and malignant cases. Significant differences were also obtained with N/C ratio amongst the various categories ($p<0.05$).

Correlation of MNA values with varying histological grades of IDC with/without metastatic lymph nodes showed significant differences in the 3 histological grades with the lowest MNA values i.e. 40.00±6.3 observed in grade 1 IDCs; intermediate values i.e. 55.58±10.51 in grade 2 cases and highest i.e. 75.75±9.76 in cases of grade 3 IDC.

DISCUSSION

While dealing with breast lesions in histopathology, difficulties exist as a result of a morphologic overlap. There is considerable subjective variability in distinguishing between lesions like atypical ductal hyperplasia vs. a well-differentiated low-grade $DCIS_8$. Apart from this, predictability of certain tumor cases for metastatic potential is difficult. Nearly 30% of breast cancer cases with node negativity succumb to the $disease_{11, 12}$. Morphological criteria supplemented with immunohistochemistry are available but at times their utility is limited as a result of subjective variability. Analysis of cellular measurements by CIM can be a useful adjunct in providing an objective and a more reproducible diagnosis for these lesions. A range of parameters can be evaluated like mean nuclear and cytoplasmic diameters and perimeters, MNA, MCA, N/C ratio, feret ratio, as studied by different authors in various $cancers_{13, 14, 15}$.

In the present study various parameters like MNA, MCA and N/C ratio were analyzed for differentiating benign vs. atypical vs. malignant breast cases with and without nodal metastasis.

Among the various parameters studied in the current study, MNA was found to be significantly different in various categories with lowest values in benign cases ($24.33\pm0.77\mu m^2$) and highest values in malignant cases having lymph node metastasis ($60.31\pm17.50\mu m^2$). While the observed values for malignant cases without lymph node metastasis (52.17±14.87) were comparable to the findings of Pienta $et\ al_{11}$ ($MNA=55.30\mu m^2$), the same were higher in cases of IDC with node metastasis. The values for atypical lesions ($42.21\pm1.84\mu m_2$) were found to be similar as observed by a manual morphometric analysis on breast lesions by Bhattacharjee $et\ al_{15}$ ($42.50\pm7.32\mu m^2$). In accordance with our study, these studies have observed MNA as a useful parameter for differentiating atypical lesions vs. carcinoma cases. In their study, Skjorten $et\ al_{16}$ have highlighted MNA as a significant parameter in differentiating normal breast vs. proliferating vs. malignant breast lesions. This highlights importance of nuclear alterations as an important hallmark in development of breast cancer. In terms of malignant lesions, they found that there were no significant differences in MNA of in-situ and infiltrating carcinomas. This aspect is reflected in histopathological grading when the nuclear grade of in-situ carcinomas is observed to be similar to the adjacent foci of infiltrating ductal carcinomas. However, within the 3 grades of DCIS, MNA has been found to be increasing from grade I to grade III. In our study, the IDC cases were with or without DCIS and the values were calculated from the cells in the IDC areas. A correlation of MNA with the varying histological grades of IDCs showed a significant increase in the values in grade 1 vs. grade 2 vs. grade3 IDCs. Similar observation has been made by $others_{17}$. MNA has also been found to be significantly different in lobular vs. ductal $carcinomas_{16}$. The present study was however restricted to IDC as the included cancer subtype. Even in the spectrum of benign and proliferating lesions, MNA has been found to be useful as an objective parameter in differentiating

fibroadenomas with and without atypia[18].

In the current study, apart from MNA, N/C ratio was also found to be significant in differentiating benign vs. atypical vs. malignant cases with the lowest values in the former 0.31±0.005, intermediate in the atypical group i.e. 0.36±0.003 and highest in the malignant category i.e. 0.43±0.02.

Earlier studies[8, 11, 13, 16,17,18,19] have indicated nuclear area as the most relevant parameter in distinguishing benign from malignant cases. However, cytoplasmic parameters like MCA help further to refine the predictive power of discriminating function. MCA was found to be useful in differentiating benign vs. atypical lesions but was not found to be statistically significant in differentiating atypical vs. malignant lesions.

Another observation in our study included an increase in MNA in IDC cases with lymph node metastasis vs. node negative cases. There has always been a limitation to predict which IDC cases have metastatic potential. Certain invasion tumor suppressor genes have been implicated. Even though in a similar study, Pienta et al[11] did not observe significant differences of MNA values between node negative vs. node positive cases like in our study, it can be suggested that there might be another mutation in primary lesion that could be implicated in both increase in nuclear area as well as metastatic potential.

In this way, evaluation of morphometric parameters including nuclear variables like MNA can form a useful adjunct in a more objective assessment of various breast lesions. In spite of obtaining an objective result with the help of morphometric analysis, however, errors occur due to individual and technical problems. Application of "Step-size" algorithms can reduce technical problems in CIM in terms of overestimation of the size of the profile as a result of overriding the cytoplasmic/ nuclear contours during tracings, magnifications used, speed of conducting the analysis and the shape and size of object being traced[20, 21]. Internal calibration and standardization by observer carefully performing correct tracings, as in the present study, can also reduce the errors. However, we acknowledge the limitations in our study, including wide standard deviation for values of MNA and MCA, especially in cases of carcinoma. While one of the reasons is an increased variation in nuclear and cytoplasmic characteristics (pleomorphism) that is seen with carcinoma cells, the other relates to inclusion of relatively limited number of cases. Inclusion of more number of cases would enhance the value of this technique. Nevertheless, morphometric values like MNA can be used for an objective assessment of certain breast lesions. With the help of correct applications, CIM could form a part of an automatic screening process. A metanalysis of the various earlier studies would be useful in identifying exact values and relevance of this technique in the current pathology practice.

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