Role of Computer Assisted Nuclear Morphometric Analysis in Grading of Infiltrating Ductal Carcinoma of Breast (Nos Type) and Correlation with Established Prognostic Markers

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ABSTRACT

Background: *Grading of Infiltrating Duct Carcinoma (IDC) of the breast forms one of the very important prognostic indicators of breast cancer. The grading system that is well established is the Nottingham modification of Bloom Richardson's grading system which takes into account architectural features (mainly tubule formation), the degree of nuclear atypia and evaluation of mitotic activity. In this system, only the mitotic activity is evaluated in a quantitative manner, while the evaluation of nuclear pleomorphism and tubule formation is still dependent on the pathologist's subjective factors. To improve the clinical value of malignancy grading, it has been suggested to quantify nuclear pleomorphism by measuring nuclear features such as area, perimeter and diameters. Various attempts quantitating these changes (particularly the nuclear aberration) have been made by computer analysis. Computer assisted nuclear morphometry helps in objective grading of breast cancer and reduces the interobserver variability.*

Objectives: *The aim of the study was to quantitate the nuclear pleomorphism in IDC (NOS) of breastby computer assisted nuclear morphometry and to correlate values obtained with other established prognostic indicators.*

Materials and methods: *Sixty cases of mastectomy specimens with proved histological diagnosis of IDC (NOS) were subjected to the study. Computerised Nuclear morphometry was done by using Olympus BX-41 research microscope with jenoptix (Germany) progress CCD camera with progress capture pro imaging software. Fifty nuclei from each case were outlined using the sketch command under 0.00001 increments by the computer mouse.*

Results: *As the grade of tumour increased, the morphometric values increased. A statistically significant correlation was obtained between the morphometric size parameters and histological prognosticators. No correlation was found between morphometric shape parameters and prognostic factors.*

Conclusion: *Computer assisted morphometry can be used in objective grading and standardizing grading performance between different laboratories.*

Key words: *Computer Assisted Morphometry, Breast Cancer, Prognostic Factors.*

INTRODUCTION

Grading of IDC forms one of the very important prognostic indicators of breast cancer. To improve the clinical value of malignancy grading of breast, it has been suggested to quantify nuclear pleomorphism by measuring nuclear features such as area, perimeter and diameters.[1,2] The morpho-metrical grading system is a method that applies quantitative morphometrical measurements and numerical assessment criteria for determining the degree of malignancy in IDC.[3] It is a scientific tool to evaluate cellular changes and enhance the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form.[4] True measurements, statistically assessed, can be expected to be more reproducible than the subjective methods.[5] Nuclear morphometry in combination with other objective prognostic criteria, can improve the evaluation of the patient's prognosis, and possibly predict response to therapy.[6] Hence nuclear morphometrical analysis of these features will bring a factor of objectivity and help in quantification of the nuclear atypia. Thus, limiting the subjective variability in grading breast cancers.

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MATERIALS and METHODS

The two year study was conducted between the July 2012 - July 2014.Sixty mastectomy specimens of carcinoma breast with proved histological diagnosis of IDC (NOS) were subjected to the study. Haematoxylin and Eosin sections of all

the cases were studied and graded according to Bloom Richardson's (BR) scoring system. The sections were screened for areas showing dispersed cells and nuclear atypia. All the measurements were performed in the area showing maximum nuclear atypia preferably at the invasive border of the most cellular part of the tumor, rejecting areas showing necrosis and inflammation. These areas were focused under oil immersion and the nuclear morphometry was done.

Computerised Nuclear morphometry was done by using Olympus BX-41 research microscope with jenoptix (Germany) progress CCD camera with progress capture pro imaging software. The digital images were captured with 1X C mount CCD adapter. Fifty nuclei from each case were outlined using the sketch command under 0.00001 increments by the computer mouse (Fig 1). The system automatically displayed four parameters – Area, Perimeter, Minimum Nuclear Diameter and Maximum Nuclear Diameter (ref table 1). These parameters were saved in the excel sheet and later were used to calculate the other four parameters – Axis ratio, Compactness, Shape factor and Nuclear size.

Measured Parameters (µm)

- Mean Minimal Nuclear Diameter (Mmnd)
- Mean Maximum Nuclear Diameter (MMND)
- Mean Nuclear Perimeter (MNP)
- Mean Nuclear Area (MNA)

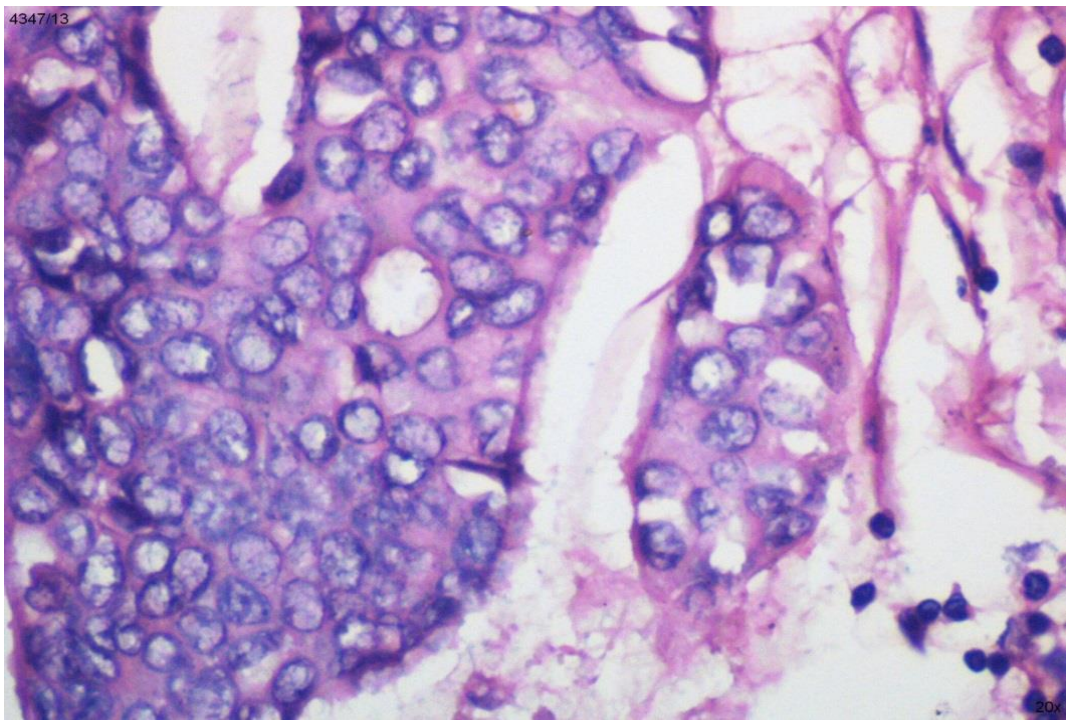
Calculated Parameters

- Mean Axis ratio (MAR) = Mmnd/MMND.
- Mean nuclear Compactness (MNC) =MNP^2 / MNA
- Mean Shape factor (MSHF) = 4 x π x MNA/ MNP^2
- Mean Nuclear size (MNS) = 2 x $(MNA/\pi)^{0.5}$

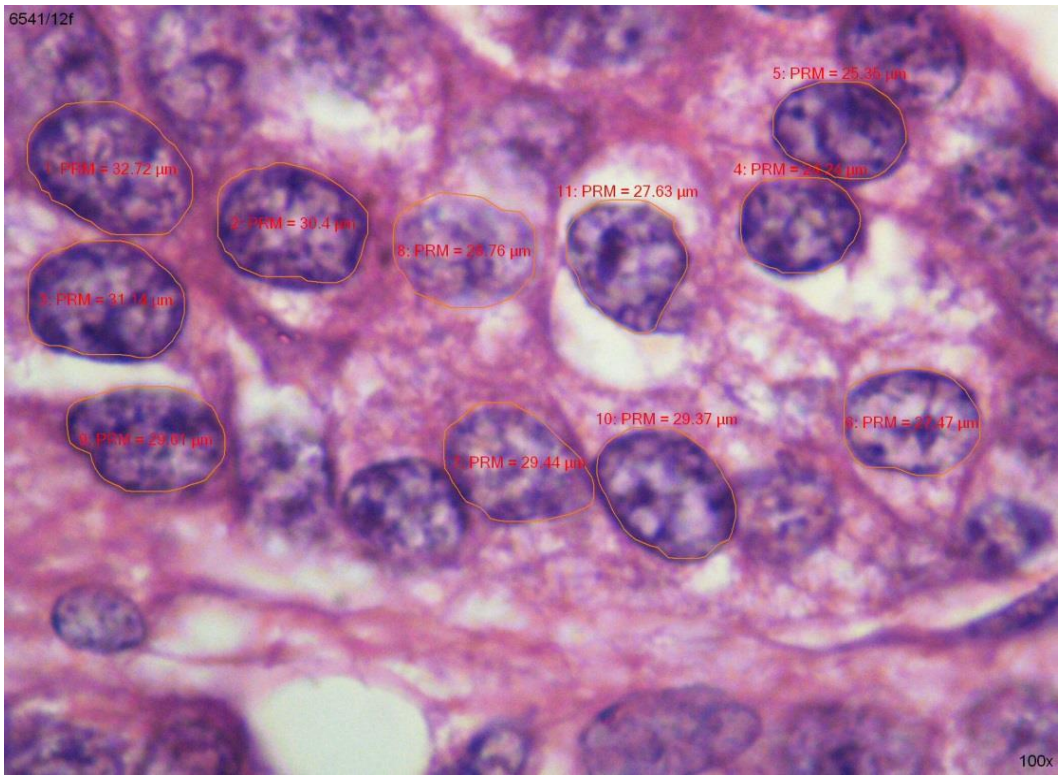
The Statistical software namely SPSS (version 16.0) and Minitab (version 11.0) were used for the analysis of the data. p value <0.05 was considered as statistically significant.

Table 1: The measured morphometric values as displayed by the system

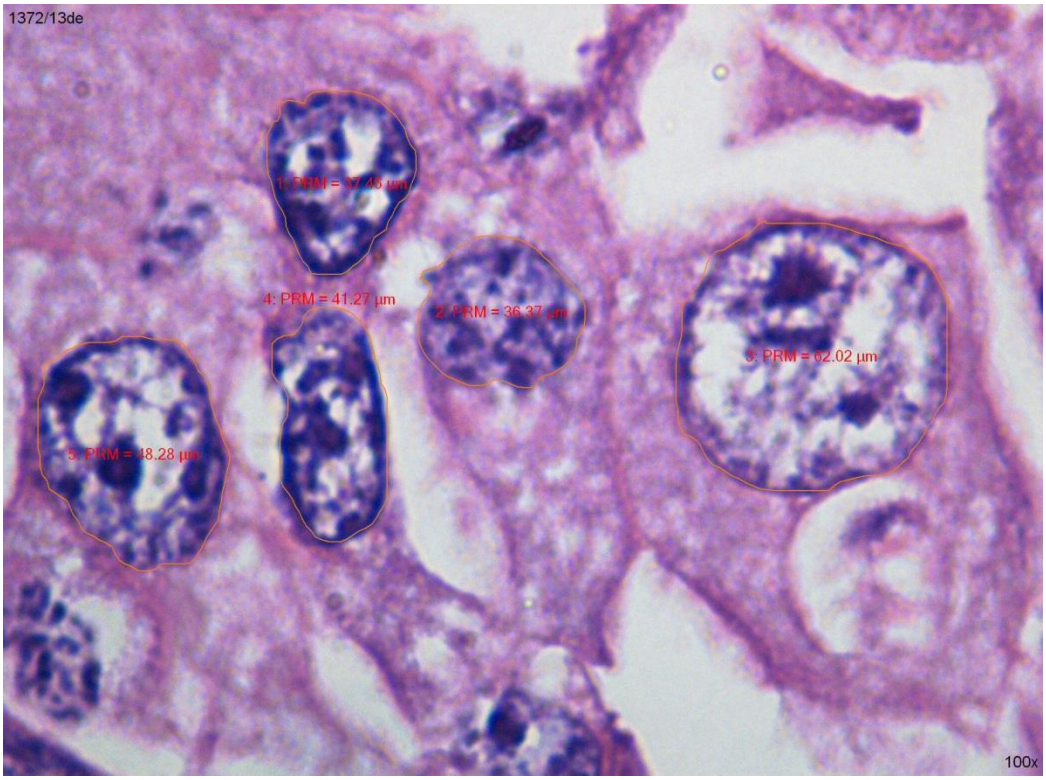
ID	Type	Param.1	Value 1	Param.2	Value 2	Param.3	Value 3	Param.4	Value 4
1	Free form	**WD**	7.14655	**HI**	6.9384	**PRM**	22.8511	**AR**	37.4588
2	Free form	**WD**	7.84039	**HI**	9.36684	**PRM**	26.7325	**AR**	44.7209
3	Free form	**WD**	8.39546	**HI**	7.56285	**PRM**	25.8658	**AR**	43.7846
4	Free form	**WD**	9.64437	**HI**	6.38333	**PRM**	25.8954	**AR**	48.5409
5	Free form	**WD**	8.25669	**HI**	9.85252	**PRM**	28.9514	**AR**	62.7041
6	Free form	**WD**	6.9384	**HI**	4.44057	**PRM**	18.7534	**AR**	24.7423



Microphotograph 1: Tumor cells in nests with Bloom Richardson's Nuclear grading 2. (H & E, X400)



Microphotograph 2: Nucleus of each cell being outlined using free form Bloom Richardson's Nuclear grading 2. (H &E, oil immersion)



Microphotograph 3: Bloom Richardson's Nuclear grading 3. (H & E, oil immersion)

RESULTS

The histological grading system of IDC of the breast is subjective and leaves a large group of patients with unclear prognosis. The combination of morphometry and conventional prognosticators gives us a significant improvement of the prognosis prediction. The clinicopathological data of the 60 cases are depicted in table 2.

Table 2: The clinicopathological data of 60 patients

Clinicopathological data	No of cases (%)
Age:	
<45	21 (35)
≥45	39 (65)
Histological grade	
1	3 (5)
2	28 (47)
3	29 (48)
B R Nuclear Grade	
1	0 (0)
2	21 (35)
3	39 (65)
Lymph node status	
Positive	33 (55)
Negative	27 (45)
Lymph node stage	
1 (negative)	27 (45)
2 (1-3)	19 (32)
3 (>3)	14 (23)
NPI	
1 (<3.41)	2 (3)
2 (3.41-5.41)	32 (53)
3 (>5.41)	26 (44)
Tumor size (cm)	
1 (<2)	5 (8)
2 (2-5)	38 (64)
3 (>5)	17 (28)
Tumor stage (TNM Stage)	
I	2 (3)
II	33 (55)
III	25 (42)

In the present study, majority of the cases were in histological grade 2 and 3, 65% of the cases had BR Nuclear grade 3, and 57% of cases had Nottingham Prognostic Index (NPI) 2.The mean values and the Standard Deviation (S.D) of all the morphometric parameters are shown in table 3.

Table 3: Mean and standard deviation of the morphometric parameters.

Morphometric parameters	Mean values	S.D
MmND (Mean Minimal nuclear diameter)	8.6430 µm	1.48339
MMND (Mean Maximal nuclear diameter)	9.9150 µm	1.50782
MNP(Mean Nuclear Perimeter)	29.7881 µm	4.56544
MNA (Mean Nuclear Area)	67.1399 μm^2	21.81675
MAR (Mean Axis Ratio)	0.8730	0.08685
MNC (Mean Nuclear Compactness)	13.5632	0.35043
MSHF (Mean Nuclear Shape Factor)	0.9275	0.02337
MNS (Mean Nuclear Size)	9.1326 μm^2	1.44246

The correlation between morphometric parameters and histological parameters are shown in table 4 and 5.

Table 4: Correlation between morphometric parameters and histological parameters

Histological parameters	Mmnd+/-SD	p value	MMND+/-SD	p value	MNP+/-SD	p value	MNA+/-SD	p value
Histological Grade								
1	7.4421+/- 1.33309	0.045	8.5474+/- 1.25230	0.024	25.8619+/- 3.89873	0.026	50.3179+/- 15.92028	0.034
2	8.3057+/- 1.20755		9.5493+/- 1.26013		28.6575+/- 3.70284		61.6313+/- 16.50857	
3	9.0929+/- 1.62039		10.4096+/- 1.59998		31.2859+/- 4.93551		74.1988+/- 24.69338	
B R Nuclear Grade								
2	7.8940+/- 1.23535	0.003	9.0747+/- 1.19198	0.001	27.2872+/- 3.64441	0.001	55.8536+/- 15.60719	0.003
3	9.0462+/- 1.46164		10.3675+/- 1.47708		31.1347+/- 4.47817		73.2172+/- 22.41661	
NPI								
I	7.4944+/- 1.88091	0.06	8.5056+/- 1.76806	0.07	25.9270+/- 5.51134	0.05	50.6702+/- 22.49813	0.04
II	8.3201+/- 1.28805		9.6341+/- 1.16056		28.8074+/- 3.63844		62.0868+/- 16.51277	
III	9.1287+?- 1.58429		10.3693+/- 1.75948		31.2921+/- 5.17528		74.6260+/- 25.53948	
Lymph Node Stage								
I	8.8335+/- 1.60855	0.22	10.0947+/- 1.44613	0.05	30.3666+/- 4.67239	0.105	69.7316+/- 23.31124	0.109
II	8.1554+/- 1.30003		9.2551+/- 1.30398		28.0041+/- 3.83067		58.7315+/- 16.21351	
III	8.9373+/- 1.39837		10.4642+/- 1.65925		31.0936+/- 4.84086		73.5531+/- 23.40482	
Tumor Stage								
I	7.6539+/- 0S.45457	0.2	9.0601+/- 0.87331	0.16	26.9291+/- 1.98826	0.17	53.5614+/- 7.28834	0.13
II	8.4215+/- 1.37704		9.6505+/- 1.30509		29.0219+/- 3.93286		63.1423+/- 17.80007	
III	9.0145+/- 1.60616		10.3326+/- 1.71681		31.0282+/- 5.23082		73.5030+/- 25.80525	
Tumor Size								
I	7.8641+/- 1.16562	0.1	9.2324+/- 1.40813	0.13	27.5514+/- 3.85758	0.1	56.3792+/- 15.24888	0.09
II	8.4806+/- 1.39914		9.7458+/- 1.44629		29.2523+/- 4.26611		64.5491+/- 20.22572	
II	9.2350+/- 1.61954		10.4940+/- 1.57511		31.6437+/- 5.01485		76.0960+/- 24.77218	

Table 5: Correlation between morphometric parameters and histological parameters

Histological parameters	MAR+/-SD	p value	MNC+/-SD	p value	MSHF+/-SD	p value	MNS+/-SD	p value
Histological Grade								
1	0.8681+/- 0.03155	0.988	13.5556+/- 0.35136	0.999	0.9278+/- 0.02370	0.999	7.9343+/- 1.27693	0.032
2	0.8718+/- 0.08489		13.5644+/- 0.32415		0.9273+/- 0.02186		8.7826+/- 1.16346	
3	0.8747+/- 0.09417		13.5629+/- 0.38551		0.9276+/- 0.02549		9.5945+/- 1.57012	
B R Nuclear Grade								
2	0.8701+/- 0.07855	0.85	13.5902+/- 0.35791	0.665	0.9256+/- 0.02343	0.657	8.3561+/- 1.15088	0.002
3	0.8746+/- 0.09196		13.5487+/- 0.35018		0.9285+/- 0.02358		9.5507+/- 1.42156	
NPI								
I	0.8771+/- 0.03882	0.68	13.6605+/- 0.42531	0.56	0.9207+/- 0.02867	0.53	7.9284+/- 1.80579	0.05
II	0.8638+/- 0.08430		13.6015+/- 0.33590		0.9248+/- 0.02241		8.8171+/- 1.14839	
III	0.8840+/- 0.09324		13.5086+/- 0.37004		0.9313+/- 0.02460		9.6135+/- 1.63119	
Lymph Node Stage								
I	0.8748+/- 0.09600	0.65	13.5709+/- 0.36012	0.551	0.9270+/- 0.02398	0.538	9.3081+/- 1.47979	0.101
II	0.8836+/- 0.10013		13.6143+/- 0.35937		0.9240+/- 0.02347		8.5669+/- 1.19685	
III	0.8551+/- 0.03754		13.4792+/- 0.32801		0.9332+/- 0.02262		9.5619+/- 1.53301	
Tumor Stage								
I	0.8463+/- 0.03140	0.9	13.5657+/- 0.15334	0.31	0.9268+/- 0.01048	0.32	8.2469+/- 0.56240	0.15
II	0.8743+/- 0.10034		13.6246+/- 0.40113		0.9235+/- 0.02641		8.8788+/- 1.25594	
III	0.8734+/- 0.07082		13.4821+/- 0.27263		0.9328+/- 0.01886		9.5385+/- 1.63498	
Tumor Size								
I	0.8526+/- 0.03175	0.83	13.6886+/- 0.19022	0.5	0.9185+/- 0.01272	0.5	8.4036+/- 1.19070	0.09
II	0.8728+/- 0.09655		13.5781+/- 0.40526		0.9266+/- 0.02687		8.9643+/- 1.35764	
II	0.8795+/- 0.07638		13.4931+/- 0.23094		0.9319+/- 0.01598		9.7233+/- 1.56581	

1. Correlation between Histological Grade and Morphometric parameters (table 4& 5)

In the present study, Mmnd, MMND, MNP, MNA and MNS were higher in tumors with histological grade III than those with grade I and II. The difference was statistically significant. The shape factors- MAR, MNC and MSHF didn't show any correlation with the histological grade of the tumor.

2. Correlation between Bloom Richardson Nuclear Grade and Morphometric parameters (table 4 & 5)

In the present study, all cases had B R Nuclear Grade of II and III. No cases with grade I were seen. Mmnd, MMND, MNP, MNA and MNS were higher in tumors with B R Nuclear Grade III than in Grade II and the difference was statistically significant.

3. Correlation between Nottingham Prognostic Index and Morphometric parameters(table 4 & 5)

MNP, MNA and MNS were higher in tumors with NPI III than those with NPI I and II. The difference was statistically significant. No correlation was observed between other morphometric parameters.

4. Correlation between Lymph Node Stage and Morphometric parameters(table 4 & 5)

MNND was greater in tumors with lymph node stage III than with II and I. The difference was found to be statistically significant. MNA was higher in tumors with lymph node stage III than with II and I but the difference was not found to be statistically significant.

5. Correlation between Tumor Stage and Morphometric Parameter(table 4 & 5)

There was no statistically significant association between tumor stage and morphometric parameters but the mean nuclear area was higher in tumors with TNM Stage III.

6. Correlation between Tumor Size and Morphometric Parameters (table 4 & 5)

There was no statistically significant association between tumor size and morphometric parameters but the mean nuclear area was higher in tumors with size >5 cms.

7. Correlation between Lymph Node Status, Age and Morphometric Parameters

There was no correlation between the lymph node status, age and morphometric parameters.

DISCUSSION

The most common histologic type of invasive breast cancer by far is invasive (infiltrating) ductal carcinoma.[7,8,9] The grading of infiltrating carcinoma is an important form of classification from the point of view of prognosis. Prognostic factors provide information useful in assessing the outcome at the time of diagnosis.[10]

The histological grading system is associated with high prognostic potential[11,12,13] but is still subjective, and leaves a large group of patients with unclear prognosis[14]. Subjective grading has been successfully used for breast cancer prognostication[15,16,17] but, by applying quantitative methodology, standardization and accuracy of grading can still be promoted.[18] Baak et al and Tosi et al introduced nuclear morphometry for prognostication of breast cancer.[1,19]

In the present study the morphometric parameters related to size – Mean Minimal Nuclear Diameter, Mean Maximal Nuclear Diameter, Mean Nuclear Perimeter, Mean Nuclear Area and Mean Nuclear Size correlated in terms of numerical values with tumor size, histological grade, lymph node stage, tumor stage, NPI and B R Nuclear grade and statistical significance was observed with all these prognostic factors except with that of tumor size and stage. MNA was found to be the strongest prognostic parameter among all. Axis ratio, nuclear compactness and shape factor were not found to be statistically significant.

The Mmnd and MMND in the present study were 8.6430 µm and 9.9150 µm respectively which were comparable with the study by Radwan MM et al with Mmnd of 6.60 µm and MMND of 10.26 µm.[20] The Mmnd, MMND and MNP was greater in tumors with histological grade 3 than grade 2 and grade1 and was statistically significant. These values were in correlation with the studyby Radwan MM et al.[20] These parameters were greater in tumors with nuclear grade 3 than 2 and were statistically significant. The MMND was greater in tumors with lymph node stage 3 than in stage 1/2 and was found to be statistically significant. This was in contrast with a study in which no statistically significant association was found. [20]In the present study, the MNA ranged between 34.76µm^2 and 142.01 µm^2 with a S.D of 21.81675 µm^2 and mean of 67.1399 µm^2. In a study done by Kronqvist et al, range of morphometrically determined nuclear area was between 32µm^2 and 47µm^2 and S.D of 15 µm^2 and mean of 38.6 µm^2.[18] In various other studies, the range of

morphometrically determined nuclear area were between 24.4 μm^2 and 67.8 μm^2 and S.D of nuclear area between 12.8 μm^2 and 18.35 μm^2.[21,22,23,24,25] Most differences in the observed nuclear size and size variation among different publications may be due to factors related to patient material and application of the morphometric method.[18] The MNA was greater in tumors with histological grade 3 and was statistically significant. The similar results were observed in these studies.[6,20,26] MNA was greater in tumors with nuclear grade 3 than 2 and was statistically significant as was also see in a study conducted by Abdalla F in tumors with higher nuclear grade.[27]

The Nottingham Prognostic Index is the most widely accepted and uses three prognostic factors - lymph node stage, tumor size and histologic grade. The index formula is:[28, 29]

NPI = [Size (cm) x 0.2] + [lymph node stage (1-3)] + [grade (1-3)]

To calculate NPI, the traditional prognostic factors like size, lymph node stage and histological grade are considered. This may lead to subjective variation. Nuclear morphometry can be applied for the objective grading. The MNA was greater in tumors with NPI 3 and were found to be statistically significant. The study conducted by Radwan MM et al found no statistically significant association between NPI and the morphometric parameters.[20]

The MNA was higher in tumors with lymph node stage 3 but was not found to be statistically significant as was seen by Radwan MM et al.[20]The mean nuclear size increased with the increasing grade of the tumor and was found to be statistically significant as was observed by other studies. [20,27] MNS was greater in tumors with nuclear grade 3 than 2 and were statistically significant. It was also high in tumors with NPI 3.The morphometric parameters related to size, i.e, Mmnd, MMND, MNP, MNA and MNS increased as the tumor size and stage increased, more with MNA. But there was no statistically significant difference as was also observed in other study.[27] Radwan MM et al found MNA to be statistically significant with the tumor stage.[15] Various other studies found tumor size to be statisticaly significant with these morphometric parameters.[20,26,27]

We didn't find any correlation between lymph node status and the morphometric parameters as was seen by Pienta et al and B.Arora et al.[23,30] In contrast, other studies found significant correlation between morphometric parameters and lymph node status.[20,27] Thus, nuclear morphometric parameters can be used as a prognostic tool in infiltrating duct carcinoma breast (NOS type).Size parameters (Mmnd, MMND, MNP, MNA and MNS) inparticular showed association with aggressive tumour nature and correlated well with prognostic indicator like histological grade, lymph node stage, Bloom Richardsons Nuclear grading and NPI. The MNA showed the strongest correlation with histological grade, Bloom Richardson Nuclear Grade and NPI. Shape indices like mean axis ratio, mean nuclear compactness and mean nuclear shape factor showed no significant association. A positive correlation between the nuclear morphometric parameters and clinicopathological features were observed. The morphometric grading system provides breast cancer grading new, more exact and reproducible principles, methods and criteria. The present study focused on the quantitative criteria for nuclear grading in breast cancer and by this means to improve the consistency and the accuracy of the grading. Quantitation of nuclear parameters is a powerful tool that may be an adjunct to other cytologic and molecular indicators of cancer diagnosis and prognosis. The procedure is a bit laborious and time consuming compared to subjective grading. Computerised morphometry can be used to standardize the grading performance between different laboratories and for a better clinical quality control.

REFERENCES:

1. Baak J.P., Van Dop H., Kurver P.H.J. and Hernans J.O.: The value of morphometry to classic prognosticators in breast cancer. Cancer 1985; 56: 374-82.
2. Kronqvist P., Kuopio T. and Collan Y.: Breast cancer prognostication: Morphometric thresholds for nuclear grading. Br. J. Cancer 1998; 78: 800-5.
3. P Kronqvist, T Kuopio et al. Morphometrical malignancy grading is a valuable prognostic factor in invasive ductal breast cancer. Br J Cancer Nov 18, 2002; 87(11): 1275–80.
4. Sheng-Lan Wang, Ming-Tsang Wu, Sheau-Fang Yang, Hon-Man Chan et al. Computerized nuclear morphometry in thyroid follicular neoplasms. Pathology International 2005; 55(11):703–06.
5. Frierson H.F., Wolber R.A., Berean K.W., Franquemount D.W., Gaffey M.J., et al, Inter-observer reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating duct carcinoma. Am. J. Clin. Pathol 1995; 103: 195-8.
6. Buhmeida A, Al-Maghrabi J, Merdad A, Al-Thubaity F, Chaudhary A et al. Nuclear Morphometry in Prognostication of Breast Cancer in Saudi Arabian Patients: Comparison with European and African Breast Cancer. Anticancer Research 2010; 30: 2185-92.
7. Fisher ER, Gregorio RM, Fisher B, et al. The pathology of invasive breast cancer. A syllabus derived from findings of the National Surgical Adjuvant Breast Project (protocol No. 4). Cancer 1975;36:1-85.
8. Rosen PP. The pathological classification of human mammary carcinoma: past, present and future. Ann Clin Lab Sci 1979;9:144-156.
9. Page DL, Anderson TJ. Diagnostic histopathology of the breast. Edinburgh: Churchill Livingstone, 1987

10. Carter D, Schnitt SJ, Millis RR. The Breast. In: Millis SE (ed). Sternberg's Diagnostic Surgical Pathology, 5th ed. Lippincott Williams and Wilkins. 2010;286-346.
11. Collan Y, Kuopio T and Alanen K: Scope and concepts of quantitive histopathology. ActaStereol 1992; 11: 3-23.
12. Sotiriou C, Pratyaksha W, Loi S, Harris A, Fox S et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 2006; 98: 1460-2105.
13. Rosai J. Breast. Roasi and Ackerman Surgical Pathology. 10th edition.Missouri; Elsevier;2009;1659-1770.
14. Page DL: Prognosis and breast cancer. Recognition of lethal and favorable prognostic types. Am J SurgPathol 1991; 15: 334-49.
15. Davis BW, Gelber RD, Goldhisch A, Hartman WH, Locher GW et al. Prognostic significance of tumor grade in clinical trials of adjuvant therapy for breast cancer with axillary lymph node metastasis. Cantice-58: 2662-2670.
16. Henson DE, Ries L, Freedm-an LS and Carriaga M (1991). Relationship among outcome, stage of disease, and histologic grade for 22 616 cases of breast cancer: the basis for a prognostic index. Cancer 68: 2142-2149.
17. Dalton LW, Page DL and Dupont WD (1994) Histologic grading of breast carcinoma. A reproducibility study. C&incer 73: 2765-2770.
18. P Kronqvist, T Kuopio and Y Collan. Morphometric grading of invasive ductal breast cancer. 1. Thresholds for nuclear grade. Br J Cancer 1998; 78(6):800-05.
19. Tosi P, Luzi P, Sforza V, Santopietro R, Bindi M et al. Correlation between morphometrical parameters and disease-free survival in ductal breast cancer treated by surgery. ApplPathol 1986; 4:33-42.
20. Radwan MM, Amer KA, Mokhtar NM, Kandil MA, EL-Barbary AM et al. Nuclear Morphometry in Ductal Breast Carcinoma with Correlation to Cell Proliferative Activity and Prognosis. Journal of the Egyptian Nat. Cancer Inst 2003; 15(3): 169-82.
21. Baak JPA, Kurver PHJ, De Snoo-Niewlaat AJE, De Graaf S, Makkink B and Boon M (1982) Prognostic indicators in breast cancer – morphometric methods. Histopathology 6: 327-339.
22. Aaltomaa S, Lipponen P, Eskelinen M, Alhava E and Syrjinen K (1991). Nuclear morphometry and mitotic indexes as prognostic factors in breast cancer. Eur J Surg 1991; 157: 319-24.
23. Pienta KJ, Coffey DS. Correlation of nuclear morphometry with progression of breast cancer. Cancer 1991; 68: 2012-6.
24. Ladekarl M and Sorensen FB (1993). Quantitative histopathological variables in in-situ and invasive ductal and lobular carcinomas of the breast. APMIS 1993; 101: 895-903.
25. Kronqvist P, Collan Y, Kuopio T and Kujari H (1995) Nuclear morphometry in breast cancer: the influence of sampling rules and freezing of samples. Mod Path 1995; 8: 187-92.
26. Ikpatt OF, Kuopio T et al. Nuclear Morphometry In African Breast Cancer. Image Anal Stereol 2002; 21:145-50.
27. Abdalla F, Boder J, Markus R, Hashmi H, Buhmeida A et al. Correlation of Nuclear Morphometry of Breast Cancer in Histological Sections with Clinicopathological Features and Prognosis. Anticancer Research 2009; 29: 1771-76.
28. Ellis IO, Pinder SE, Lee AHS. Tumors of the breast. In: Fletcher CDM. Diagnostic Histopathology of Tumors. 3rd ed(vol.1). Philadelphia: Churchill Livingstone; 2007: 903-70.
29. Fletcher
30. B. Arora, Renu, A. C. Kakade& B. Rekhi : Value Of Computerized Morphometric Analysis In Diagnosis Of Breast Lesions . The Internet Journal of Pathology 2007; 6(2).