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Original Research Article.

Nuclear Image Morphometry and Cytologic Grade of Breast Carcinoma

Sabina Laishram[1*], Shameen Shariff[2]

[1*]Assistant Professor, Department of Pathology,
Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India.
[2]Professor & HOD, Department of Pathology,
MVJ Medical College & Research Hospital, Bangalore, Karnataka, India.

ABSTRACT

Aims: The present study was undertaken to validate the role of the nuclear morphometry on specimens obtained by fine needle aspirates in differentiating different grades of malignant breast cancer.

Materials and Methods: Sixty needle aspiration samples ratified by histology were studied from patients who were referred for FNAC for a breast lump evaluation to the Department of Pathology, MVJ MC & RH. Thirty benign cases and thirty malignant cases were obtained for the study. Only H & E stained samples were included in the study and Giemsa stained smears were avoided due to the exaggeration of cell sizes and measurements in air dried smears.

Results: Of the 30 benign lesions, 23 (38.3%) were aspirates from fibroadenoma, 6 (10%) were from fibrocystic disease and 1 (1.7%) was of tubular adenoma.

Of the 30 malignant lesions, 29 (48.3%) were diagnosed as infiltrating ductal carcinoma, NOS variety and 1 (1.7%) as metaplastic carcinoma, squamoid type. Categorization of the histological grade on paraffin sections showed 11 (36.7%) aspirates fell under category Grade I, 14 (46.7%) cases as Grade II and 5 (16.6 %) cases as Grade III. All the aspirates were processed using image J software to study nuclear parameters to make comparison between benign and malignant breast lesions.

Conclusion: The study indicates that all the nuclear parameters were much higher in grade III malignant breast cancer as compared to low grade malignant cancer.

Keywords: Nuclear Morphometry, Fine Needle Aspirates, Cytology, Breast Lesion.

***Correspondence to:**

Dr. Sabina Laishram,
Assistant Professor, Department of Pathology,
Sikkim Manipal Institute of Medical Sciences,
Gangtok, Sikkim, India.

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INTRODUCTION

In India, over 100,000 new breast cancers are estimated to be diagnosed annually. In general, breast cancer has been reported to occur decade earlier in Indian patients compared to their western counterparts. More than 80% of Indian patients are younger than 60 years of age.[1] The 5 years survival rate reported in various Indian studies ranges between 40- 62%. Further, 5 years survival rate is most favourable in stage I and other lower grades of cancers which emphasized early and accurate diagnosis in breast lesions.[2-4] The triple test, initially described in 1975, is the evaluation of palpable breast masses by physical examination, mammography, and fine-needle aspiration. Fine needle aspiration cytology (FNAC) of the breast is one of the modalities of detecting carcinoma of the breast. The accuracy rate in literature is reported to range from 95.8% to 97.87%.[5-7] FNAC is also one of the prongs of the triple test, the accuracy in such instances ranging from 98% to 100%.[8,9] Where facilities for imaging are not available and in the diagnosis of a breast lump being benign or malignant, FNAC interpretation is invaluable towards an accurate diagnosis. Since biopsy specimens from palpable abnormalities are benign, FNAC can have a substantial role in separating patients in need of a surgical biopsy from patients who can be followed up clinically.[10] Nuclear morphometry can improve the distinction between benign and malignant lesions and in combination with a visual impression can help resolve several equivocal cases.[11] The present study aims at using simple morphometric cell characteristics on aspirates of histologically confirmed breast lesions in order to assess their values as parameters on diagnosis and delineating benign from malignant lesions and differentiating various histological grades of malignant breast cancer.

MATERIALS & METHODS

The proposed study was conducted on 60 cases of surgical breast specimens received in the Department of Pathology, MVJ Medical College between 2010 to 2012. Sixty cases (30 benign breast masses & 30 Malignant breast Lesions) were studied which had

both FNAC and histopathology correlation. All aspirates after processing were stained with Haematoxylin and Eosin to categorized as- Fibrocystic Disease and breast adenomas. The carcinomas were categorized into specific and NOS types. Grading of carcinomas was done by Modified Scarf-Bloom-Richardson Grading System. The quantitative study was done by an image J analysis system. The digital images generated by Olympus camera linked to Olympus microscope at a total magnification 400 were used to create photomicrograph that was processed by Image J Software. A total of hundred cells were randomly selected and measured in each case. In malignant lesions, measurements on both cell clusters and single cells were calculated for all the five parameters mentioned, except NC ratio on single cells (due to presence of cytoplasmic vacuoles and artefacts on spreading). In benign lesions, only cells in clusters were taken due to lack of adequate single cells. With the help of an internal calibration, various parameters were average nuclear diameter (AVD), mean nuclear area (MNA), coefficient of variation of nuclear area (NACV), NC ratio, and average largest to smallest nuclear diameter ratio (LS ratio) After obtaining all the parameters for each case, finally the average value of each parameter was calculated for both the benign and malignant conditions.

Statistical Analysis

Applying SPSS 17 version, Student t-Test was used to compare benign and malignant lesions and one way ANOVA test was used for comparing the histological grade. 2σ limits was also calculated.

Table 1: Measurements of single cells in comparison to cell clusters:

NUCLEAR PARAMETERS	CELL CLUSTERS		SINGLE CELLS	
	Range	Mean±SD	Range	Mean±SD
Nuclear Diameter(μ)	4.93 – 8.84	6.91±0.93	5.24 – 11.38	7.48±1.18
Nuclear area(μ^2)	19.35 – 63.73	39.59±10.73	22.06 – 106.49	47.1±15.92
N:C ratio	0.62 - 0.75	0.68±0.03	---	---
L:S ratio	1.54 – 2.38	1.78±0.16	1.36 – 2	1.57±0.13
NACV (%)	25.5– 53.68	38.94±6.85	23.32 – 46.18	34.43±5.34

Table 2: Nuclear parameters in the various histological grades of malignant lesions:

Grade			Nuclear diameter (μ)	Nuclear area (μ^2)	NC ratio	LS ratio	NACV (%)
I	Cell clusters	Range	4.93 – 6.49	19.35 – 34.34	0.62 – 0.68	1.54 – 1.69	25.5 – 38.21
		Mean±SD	5.94±0.54	28.66±5.09	0.65±0.02	1.64±0.06	32.41±3.57
	Single cells	Range	5.24 – 7.11	22.06 – 41.82	--	1.36 – 1.65	23.32 – 38.15
		Mean±SD	6.45±0.7	34.52±7.35	--	1.48±0.09	31.08±4.83
II	Cell clusters	Range	6.87 – 7.58	37.42 – 46.49	0.66 – 0.73	1.74 – 1.89	33.53 – 46.75
		Mean±SD	7.19±0.21	42.15±2.54	0.69±0.01	1.8±0.05	41.21±4.18
	Single cells	Range	7.15 – 8.4	43.46 – 56.37	--	1.43 – 1.67	28.49 – 41.03
		Mean±SD	7.66±0.33	47.87±3.92	--	1.56±0.06	35.46±3.74
III	Cell clusters	Range	7.76 – 8.84	48.55 – 63.73	0.7 – 0.75	1.8 – 2.38	38.39 – 53.68
		Mean±SD	8.26±0.52	56.45±6.43	0.74±0.02	2.03±0.09	46.99±5.95
	Single cells	Range	8.2 – 11.38	53.89 – 106.49	--	1.67 - 2	29.41 – 46.18
		Mean±SD	9.26±0.96	72.61±10.07	--	1.77±0.10	38.94±4.95

Table 3: Statistical correlation between the mean of all the nuclear parameters in various histological grades:

		Grade I	Grade II	Grade III	P value
Mean nuclear diameter (μ)	Cluster cells	5.94±0.54	7.19±0.21	8.26±0.52	<0.0001 (significant)
	Single cells	6.45±0.7	7.66±0.33	9.26±1.26	<0.0001 (significant)
Mean nuclear area (μ^2)	Cluster cells	28.66±5.09	42.15±2.54	56.45±6.43	0.0001 (significant)
	Single cells	34.52±7.35	47.87±3.92	72.61±20.07	<0.0001 (significant)
Mean NC ratio	Cluster cells	0.65±0.02	0.69±0.01	0.74±0.02	<0.0001 (significant)
	Single cells	-	-	-	-
Mean LS ratio	Cluster cells	1.64±0.06	1.8±0.05	2.03±0.22	<0.0001 (significant)
	Single cells	1.48±0.09	1.56±0.06	1.77±0.13	<0.0001 (significant)
Mean NACV (%)	Cluster cells	32.41±3.57	41.21±4.18	46.99±5.95	<0.0001 (significant)
	Single cells	31.08±4.83	35.46±3.74	38.94±6.57	0.012 (significant)

Table 4: 2σ limits for the various histologic grades:

		Grade I	Grade II	Grade III
Mean nuclear diameter (μ)	**Cluster cells**	4.86 – 7.02	6.77 – 7.61	7.22 – 9.3
	Single cells	5.05 – 7.85	7 – 8.32	7.34 – 11.18
Mean nuclear area (μ^2)	**Cluster cells**	18.48 – 38.84	37.07 – 47.23	43.59 – 56.45
	Single cells	19.82 – 49.22	40.03 – 55.71	52.47 – 92.75
Mean NC ratio	**Cluster cells**	0.61 – 0.69	0.67 – 0.71	0.70 – 0.78
	Single cells	-	-	-
Mean LS ratio	**Cluster cells**	1.52 – 1.76	1.7 – 1.9	1.85 – 2.21
	Single cells	1.3 – 1.66	1.44 – 1.68	1.57 – 1.97
Mean NACV (%)	**Cluster cells**	25.27 – 39.55	32.85 – 49.57	27.98 – 42.94
	Single cells	21.42 – 40.74	35.09 – 58.89	29.04 – 48.84

RESULTS

A total of 60 consecutive cases with corresponding histology to include 30 benign and 30 malignant lesions were assessed. The distribution of various types of lesions are fibro adenoma (23), fibrocystic disease (06), tubular adenoma (01), ductal carcinoma, NOS(29),and metaplastic carcinoma (01). Majority (86.7%) of benign cases belongs to most reproductive 21-40 yrs of age group while 79% of malignant cases were represented by 30-60 yrs of sample population. After histological grading of malignant lesions, Grade II constitutes 14 cases followed by Grade I (11) and Grade III (05). All the nuclear parameters measured were higher in malignant breast lesions in comparison to benign breast lesions. The distribution of the mean nuclear diameters of cells obtained from the benign breast lesions is 4.78 while the mean nuclear diameters of malignant breast lesions is 6.9 which shows a difference of 2.13μ.

Mean nuclear diameter and mean nuclear area were higher in single cells and mean LS ratio and mean NACV were marginally higher in cell clusters. Using the histological grade as standard, the results obtained on cytology for each grade were evaluated. (NC ratio was not measured for single cells.)

All nuclear parameters obtained were in proportion with the histological grade, being the highest in grade III (poorly differentiated) carcinoma and lowest in grade I (well differentiated) carcinoma. The p value obtained was statistically significant ($p<0.05$) in all the nuclear parameters suggesting nuclear morphometry is significant in categorizing the histological grade of a ductal carcinoma. There is 95% probability for a lesion to be either Grade I carcinoma, Grade II carcinoma or Grade III carcinoma if the nuclear parameters fall within the 2σ limits given above for each parameter in the various grades.

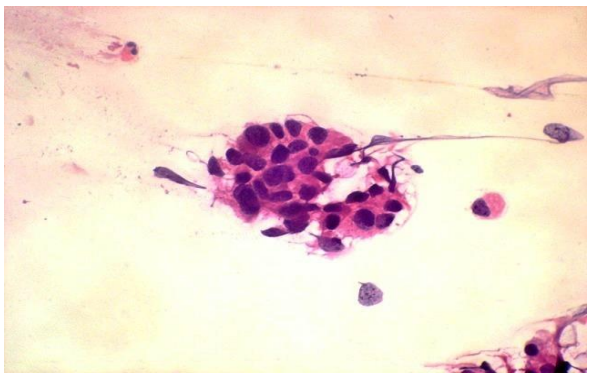

Fig 1: Cytologic smear of Grade III carcinoma showing a sheet of large, highly pleomorphic and hyperchromatic cells with an increased NC ratio. (H& E X 400)

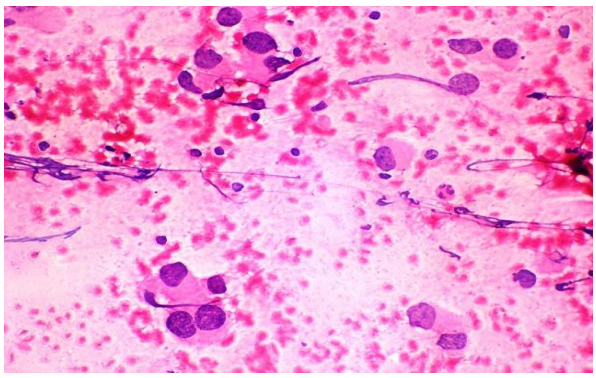

Fig 2: Cytologic smear of Grade III carcinoma showing single cells on which morphometry was done. (Haematoxylin & Eosin X 400)

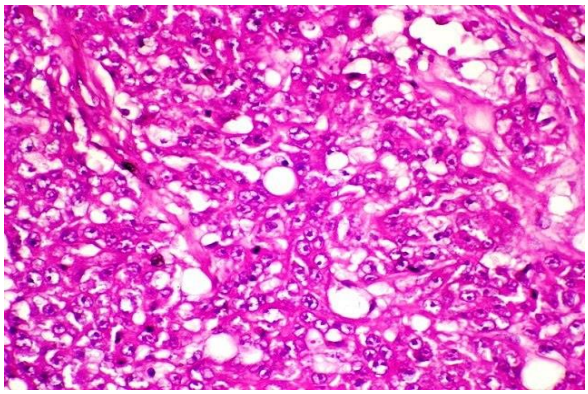

Fig 3: Histopathologic section of corresponding Grade III carcinoma with sheets of highly pleomorphic cells with prominent nucleoli and increased mitotic figures. (H&E X 400)

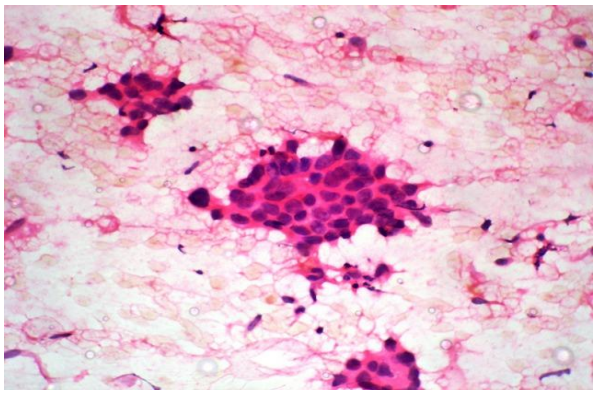

Fig 4: Cytology of Grade II carcinoma: cell clusters (Haematoxylin & Eosin X 400)

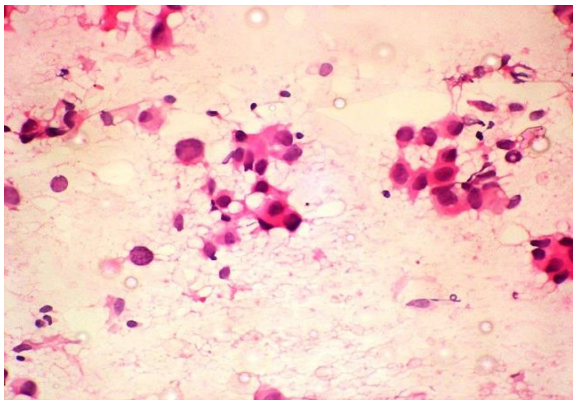

Figure 5: Cytology of Grade II carcinoma: single cells (Haematoxylin & Eosin X 400)

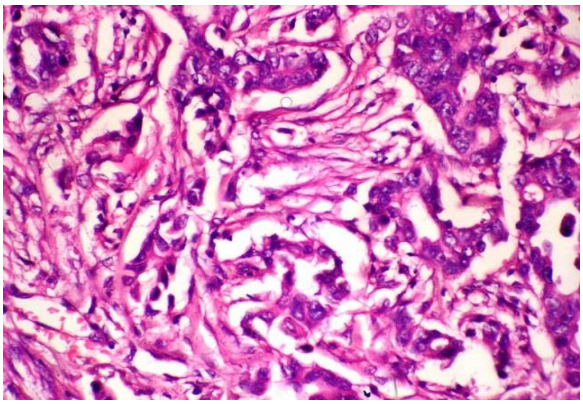

Fig 6: Histopathologic section of Grade II carcinoma corresponding to cytology of figure 9. (H & E 5X 400)

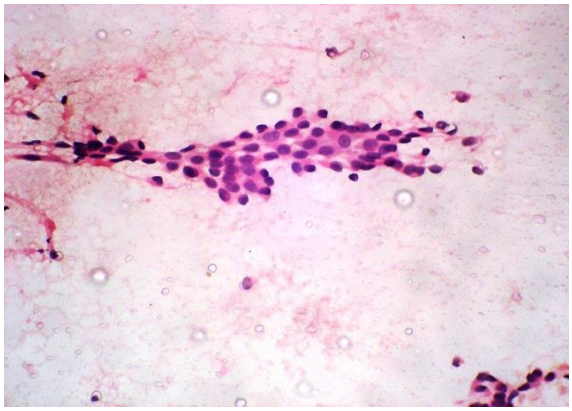

Fig 7: Cytologic smear of Grade I carcinoma: cell clusters with small, uniform cells. (Haematoxylin & Eosin X 400)

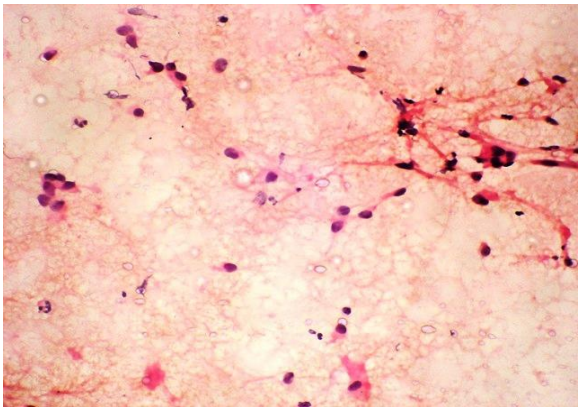

Fig 8: Cytologic smear of Grade I carcinoma: scattered single cells with small, uniform, hyperchromatic nuclei. (H&E X 400)

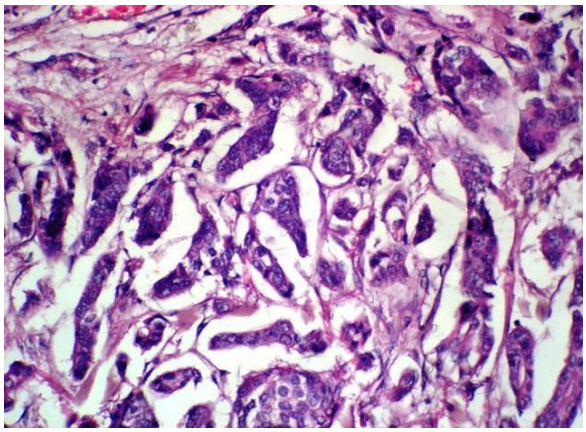

Fig 9: Histopathologic section of Grade I carcinoma corresponding to cytology of figure 12. (Haematoxylin & Eosin X 400)

DISCUSSION

All the nuclear parameters were higher in the malignant lesions when compared to benign lesions and were statistically significant ($p<0.0001$). MNA of malignant lesions measured 39.59 μ^2 compared to 18.59μ^2 in benign lesions.

The MND and MNA observed in the present study in the various grades were comparatively lower than that observed in the other studies. These variations in the results could be due to technical problems or even a genetic basis as suggested by Buhmeida et al.[12] These authors also suggest that the MNA was higher in lymph node positive patients as compared to lymph node negative patients and in advanced stages as compared to early cancer. MNA was significantly higher in higher grades, in cases of tumor invasion, in recurrent cases than in non-recurrent cases. Ikpatt et al[13] found a statistically significant correlation in the MND and MNA between the histological grades. Dey et al[14] in their study also mentioned that there was a significant difference in the MND and MNA between Grade I vs. Grade II and III carcinomas but no significant difference between Grade II and Grade III carcinomas. Suzuki et al[15] however found no significant correlation between the MNA and the histological grade. Kronqvist et al[16] analysed nuclear diameters on 170 histological samples of invasive ductal carcinoma to find objective and quantitative thresholds for nuclear grade. Their mean thresholds of nuclear diameter were 6.4μ and 7.4μ to differentiate Grade I, Grade II and Grade III carcinomas. Their measurements of nuclear diameters are closer to those obtained in the present study.

The MND and MNA were higher in the single cells when compared to cell clusters in the various histological grades and it is suggested that in malignancy a cell morphometry yielded objective finding on single cells as compared to cell clusters. The present study ratifies these findings in literature. In the present study, the mean NC ratio for benign lesions measured 0.47 ± 0.02 and the mean NC ratio for malignant conditions measured 0.68 ± 0.03 which was statistically significant ($p<0.0001$) in cell clusters. There was also statistically significant correlation in the various histological grades ($p<0.0001$) being directly proportional to the grade of the carcinoma. Arora et al[17] also found in their study that there was statistically significant difference in the NC ratio which contributed in distinguishing various benign lesions like fibroadenoma, fibroadenosis as well as ADH from IDC without lymph node metastasis and IDC with lymph node metastasis ($p<0.05$). Abdalla et al[18] disagreed that such a design should be

avoided because outlining of cellular margins is difficult making the measurement less reproducible and more subjective.

In the present study, LS ratio is the ratio of the five largest diameters to the five smallest diameters. The mean LS ratio for benign lesions was 1.46 ± 0.08 and the LS ratio for malignant lesions was higher being 1.78 ± 0.16 which was statistically significant ($p<0.0001$) in the present study. The mean LS ratio for single cells was marginally lower than the mean LS ratio for cell clusters in malignant conditions. There was also statistically significant correlation in the various histological grades ($p<0.0001$) in the LS ratio.

Nagashima et al[19] however found no significant difference in LS ratio in the study conducted by them. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean. It denotes the extent of variability in relation to mean of the population. The present study shows a statistically significant difference between the benign and malignant lesions ($p<0.0001$) with regard to NACV. Suzuki et al[15] found that patients with high NACV > 35% had lower rates of disease free survival than those with low NACV <35%. They also observed that NACV was significantly associated with hormone receptor status of a tumor, ploidy status as well as histological grade ($p<0.05$) but did not correlate with tumor size and lymph node status. Tajima et al[20] and Nagashima et al[19] have mentioned in their studies that NACV together with the MNA is a good indicator for identifying DCIS from lesions like benign intraductal hyperplasia, papilloma and fibrocystic disease. However, Cornelisse et al[21] mentioned that NACV had considerably less discriminatory power and also showed the lowest correlation with the MNA. The present study showed that NACV correlated with MNA. The p value obtained was statistically significant ($p<0.05$) in all the nuclear parameters suggesting nuclear morphometry is significant in categorizing the histological grade of a ductal carcinoma.

CONCLUSION

All the nuclear parameters were higher in the malignant lesions when compared to benign lesions and were statistically significant ($p<0.0001$). Mean nuclear area was the most important parameter to differentiate between benign and malignant lesions. Further all nuclear parameters obtained were in proportion with the histological grade, being the highest in grade III (poorly differentiated) carcinoma and lowest in grade I (well differentiated) carcinoma.

Therefore, morphometry is efficient in distinguishing benign from malignant lesions and has been proved to be useful objective tool especially in the “gray zone” areas. Inspite of obtaining an objective results with the help of morphometric analysis, errors occur due to technical problems and application of “Stepwise” algorithms can reduce the technical problems in Computerized Interactive Morphometry 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. Internal calibration and standardization by an expert observer performing correct tracings can also reduce the errors.

Caution with regard to these factors and careful assessment can make FNAC a valuable tool in the differentiation of benign and malignant lesions, which is the most crucial factor in deciding patient management.

REFERENCES

1. National Cancer Registry Program. Ten year consolidated report of the Hospital Based Cancer Registries, 1984–1993, an assessment of the burden and care of cancer patients. New Delhi: Indian Council of Medical Research; 2001.
Available at: http://ncrpindia.org/Rep1/ten_yrs_hbcr_rpt_.aspx

2. Chopra R. The Indian scene. J Clin Oncol. 2001;19 (18 suppl):106S–111S.

3. Nandakumar A, Anantha N, Venugopal TC, Sankaranarayanan R, Thimmasetty K, Dhar M. Survival in breast cancer: a population-based study in Bangalore, India. Int J Cancer. 1995;60:593–6.

4. Gajalakshmi CK, Shanta V, Swaminathan R, Sankara narayanan R, Black RJ. A population-based survival study on female breast cancer in Madras, India.BrJ Cancer.1997;75:771–5.

5. Singh K, Sharma S, Dubey VK, Sharma PR. Role of FNAC in diagnosis of breast lumps. JKScience 2001 July-Seplember; 3:3.

6. Gupta SK, Ghosh AK, Chowdry T, Dutta TK. Talwar BL, Dutta BN. Aspiration cytology in diagnosis of breast cancer. Ind J Can 1979; 16: 1-8.

7. Bansal RL. Sankaran V, Valiah AJ. Role of fine needle aspiration cytology in diagnosis of breast lumps and its histopathological correlation. J Cyto J 1985; 2: 62-66.

8. Ghimire B, Khan MI, Bibhusal T, Singh Y, Sayami P. Accuracy of triple test score in the diagnosis of palpable breast lump. JNMA J Nep Med Assoc. 2008 Oct-Dec; 47 (172):189-92.

9. Morris A, Pommier RF, Schmidt WA, Shih RL, Alexander PW, Vetto JT. Accurate evaluation of palpable breast masses by the triple test score. Arch Surg 1998; 133(9):930-4.

10. Dabb DJ. Prognostic Information from fine-needle aspiration biopsy of breast carcinoma. Am J Clin Pathol 2000; 113 (1):76-83.

11. Wolberg WH, Street N, Mangasarian L. Machine learning techniques to diagnose breast cancer from image-processed nuclear features of fine needle aspirates. Cancer letters 1994 ;77 (2):163-171.

12. Buhmeida A, Al-Maghrabi J, Merdad A, Al-Thubaity F, Chaudhary A, Gari M, et al. Nuclear morphometry in prognostication of breast cancer in Saudi patients: Comparison with the European and African breast cancer. Anticancer Res 2010; 30 (6), 2185-2191.

13. Radwan MM, Amer KA, Mokhtar NM, Kandil MA, El-Barbary, Aiad H. Nuclear morphometry in ductal breast carcinoma with correlation to cell proliferative activity and prognosis. J Egypt Nat Can Inst Sept 2003; 15(3): 169-82.

14. Dey P, Ghoshal S, Pattari SK. Nuclear image morphometry and cytologic grade of breast carcinoma. Anal Quant Cytol Histol 2000 Dec; 22(6):483-5.

15. Suzuki MM, Oshida M, Nagashima S, Hashimoto H, Yagata H, Suzuki N. Quantitative morphometric analysis of fine needle aspirates of breast carcinoma. Breast Can 2001;8: 138-145.

16. Kronqvist P, Kuopio T, Collan Y. Morphometric grading of invasive ductal breast cancer. Thresholds for nuclear grade. B J of Cancer 1998; 78(6):800-805.

17. Arora B, Renu, Kakade AC, Rekhi B. Diagnostic application of mean nuclear area measured by computerized interactive morphometry in breast cancer. Internet J of Path 2007; 5:2. Assessed on 13 May 2012 05:56:26 -0500 (00002044) — http://www.ispub.com:80/journal/the-internet-journal-of-pathology/volume-5-number-2/diagnostic-application-of-mean-

nuclear-area-mna-measured-by-computerized-interactive-morphometry-in-breast-cancer.html.
18. Abdalla F, Boder J, Markus R, Hashmi H, Buhmeida A, Collan Y. Correlation of nuclear morphometry of breast cancer in histological sections with clinicopathological features and prognosis. Anticancer Research 2009; 29: 1771-76.
19. Nagashima T, Suzuki M, Yagata H, Hashimoto H, Shishikura T, Imanaka N, et al. Cytomorphometric differentiation of intraductal prolifereative breast lesions. BreastCancer2007;7:43-7.
20. Tajima Y, Ishige H, Kondo Y. Morphometric studies for objective diagnosis of intraductal carcinoma of the breast. Acta Pathol Jpn 1991 Aug; 41(8):
604-9.
21. Cornellisse CJ, Koning HR, Arentz PW, Raatgever JW, Van Heerde P. Quantitative analysis of the nuclear area variation in benign and malignant breast cytology specimens. Anal Quant Cytol 1981 Jun; 3(2):128-34.

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