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Original Research Article

Evaluation of AgNOR Scores in Fine Needle Aspiration Cytology Smears of Breast Lesions

Anisha Tirumala S[1], GK Parvathi Devi[2]

[1]Assistant Professor, Department of Pathology, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka 560074, India.
[2]Professor, Department of Pathology, Sri Siddartha Medical College, Tumakuru, Karnataka 572107, India.

Corresponding Author:
Anisha Tirumala S, Assistant Professor, Department of Pathology, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka 560074, India.

E-mail: anishats@gmail.com

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Abstract

Aim: The study evaluates the AgNOR (Argyrophilic Nucleolar Organizer Region) scores in the aspiration cytology smears of breast lesions to differentiate benign from malignant. *Material and methods*: A total of 100 cases who presented with palpable breast lump were subjected to FNAC (Fine Needle Aspiration Cytology). AgNOR staining was done on all the smears to differentiate benign from malignant lesions. Of the 100 cases, 72 cases were benign, 4 were borderline cases and 24 were malignant cases on FNAC. AgNOR stain was done on all the smears and the mean AgNOR count was calculated. *Satistical Analysis*: The collected data was analyzed by student '*t*' test. The *p* values less than 0.05 were considered significant. *Results:* On FNAC, the distribution of AgNORs in benign lesions were small, uniform, compact, 1–4 dots, central and peripherally placed and in malignant lesions they were small, medium and large, coarse, clumped and 1–8 dots. The mean AgNOR count in FNAC smears of benign lesions was 2.67 and of malignant lesions was 5.71, thus the mean AgNOR counts in malignant lesions were higher when compared to benign lesions on FNAC. The mean AgNOR count in HPE (Histopathological Examination) sections of benign lesion was 1.42 and of malignant lesions was 3.77. The observations revealed statistically significant results between benign and malignant lesions. Thus, AgNOR technique is simple, inexpensive and useful for evaluation of proliferative activity in breast lesions and can be used as an adjunct to FNAC to differentiate benign from malignant lesions of breast.

Keywords: AgNOR; Breast lesions; Aspiration cytology.

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Introduction

The nucleolus plays a vital role in control of cell proliferation and protein synthesis. Rapidly dividing cells and cells with high metabolic activity have prominent nucleoli. Nucleolar Organizer Regions (NORs) are segments of DNA closely associated with nucleoli containing coding genes for ribosomal RNA and they contribute to regulation of cellular protein synthesis.[1]

They are located in the short arm of chromosomes 13, 14, 15, 21 and 22. Associated with these regions are certain acidic, argyrophilic and non-histonic proteins called NOR-associated proteins (NORAPs). NORs can be rapidly identified in light microscopy by a simple, one-step colloidal silver technique by staining their associated proteins with colloidal silver and these silver stained reaction products represent the Argyrophilic Nucleolar Organizer Regions (AgNORs) and appear as black dots within the nucleus.[2,3]

The study of AgNOR has enjoyed a vogue in diagnostic tumor pathology as increased number of AgNORs correlate with increased cellular proliferation.[4]

Counting the number of AgNORs have been of use in distinguishing benign from malignant cells than in normal, reactive or benign cells.[5] Quantification of AgNOR's is valuable parameter in tumor pathology. An increased number of AgNORs are associated with increased tumor aggressiveness as the mean number of AgNORs (mAgNOR) per nucleus is higher in malignant than in benign, higher in high grade malignancies and in tumors with a poor prognosis compared to those with good prognosis.[6]

AgNORs also seem to correlate with other markers of proliferative activity such as mitotic counts, immunostaining with monoclonal antibody (Ki67), Proliferating Cell Nuclear Antigen (PCNA) as well as Cell fraction of cell division as assessed by DNA flow cytometry.[7]

FNAC (Fine Needle Aspiration Cytology) is an indispensible investigation tool for any palpable lump, clinically benign or malignant as a guide to clinical management and also to obtain tumor cells for special analysis and research. The sensitivity of FNAC in the diagnosis of breast cancer is 90–95% in most series.[8]

Hence, the present study uses the AgNOR technique in the aspiration cytology smears of breast tumors to differentiate benign and malignant tumors and histopathological correlation of the same in the specimens available.

The AgNOR method: The principal advantages of the AgNOR technique are the relative simplicity of the staining method and the ease of application to archival tissues. Disadvantages include, time consuming and tedious counting of little dots, often tightly associated with the usual vagaries of observer error.[9]

In brief, one-step method consists of mixing silver nitrate and formic acid with gelatin acting a colloid stabilizer. Paraffin or frozen sections or cytological smears are incubated in this mixture for variable periods of time and then washed and mounted.

Ultra structural and light microscopic studies have indicated that this method is remarkably specific as a means of detecting interphase and metaphase NOR's by the virtue of their associated proteins. Sequential staining with radiolabelled rDNA (Ribosomal Deoxyribonucleic Acid) and rRNA (Ribosomal Ribonucleic Acid) has shown correspondence between the binding sites and silver stained NOR's on chromosomes and in interphase nucleoli.[9]

The silver reaction product is seen as discrete black dots at the light microscopic level and these can be enumerated using an oil immersion lens. Counts in 100–150 cells are usually made and the results expressed as mean number per nucleus.

The internal control such as lymphocytes are usually employed. With minor modifications, the technique can be used in success with semiautomatic and automatic image analysis hardware. Here, the total amount of AgNOR material per nucleus is measured rather than the number of sites counted.[10]

AgNOR count: Mean AgNOR/ Nucleus (mAgNOR): It was hypothesized that the mAgNOR represents the mean DNA content of the cells (ploidy).

AgNOR score: Each AgNOR dot was classified as small, medium and large according to its size. A small dot was defined as just visible but distinct one, under the oil immersion objective. Dots about three times the size of a small one were classified as medium and those about five times or more the size of a small dot were classified as large.

The AgNOR score was calculated by multiplying the number of small dots by a factor of one, number of medium dots by a factor of three and number of large dots by a factor of five and adding up the three.[11]

Materials and Methods

The present study was carried out on the FNAC of breast lesions and their respective histopathology specimens of breast, of patients attending the Out Patient Department (OPD) in our institute for a period of 18 months.

A detailed history was taken and examination of breast with lymph nodes was carried out in all cases. After obtaining consent, fine needle aspiration of breast using a 22- or 23- guage needle was

performed from the breast lump. Multiple smears were made simultaneously, but in all cases at least a minimum of three smears were made of which one was air dried and the other two were methanol fixed. The air-dried smear was stained routinely with MGG (May Grunwald Giemsa) or Leishman and one of the methanol fixed smears was stained with haematoxylin and eosin and the other was subjected to AgNOR staining. The AgNOR staining was performed using the technique described by Crocker and Nar.[12] AgNORs were seen as brownish black dots in the nucleus; i.e. both within the nucleus and scattered and within the nucleoplasm against a light yellow background.

Under oil immersion lens (magnification of 100X), number of AgNORs within the nuclei of randomly selected epithelial cells were counted and mean number of AgNORs per nucleus for each case was evaluated.

Histopathological correlation was done in specimens which were available. For histopathology, two paraffin sections were cut from each paraffin block. One was stained with Haematoxylin and Eosin (H & E) and the other was subjected to AgNOR. Under oil immersion lens, number of AgNORs within the nuclei of 200 breast epithelial cells were counted and mean number of AgNORs per nucleus for each was evaluated.

The collected data was analysed by Students '*t*' test, The '*p* values' less than 0.05 were considered significant.

Women with lactating breast, patients with breast abscess and cases with infections and inflammatory conditions were excluded from the study.

The AgNOR staining technique in aspiration cytology followed was that the FNA smears were fixed in alcohol. Smears were hydrated through descending grades of alcohol to three changes of deionised water. Freshly prepared silver colloidal solution containing one part by volume of 2 gm gelatin dissolved in 100 ml of 1% aqueous formic acid solution and two parts by volume of 50% aqueous silver nitrate solution was made and mixed in above proportions just before use and the solution was poured over the smears and kept for 45 minutes at room temperature in a dark place. Smears were washed off the silver colloidal solution with three changes of deionised water and treated with 5% sodium thiosulphate for 5 minutes and then washed with deionised water and dehydrated through increasing grades of alcohol, cleared in xylene and mounted in DPX (Dibutyl phthalate polystyrene xylene).

For tissue sections. 4 micron thick sections were made for each case and the sections were dewaxedin two changes of xylene, hydrated through descending grades of alcohol to three changes of deionised water. Freshly prepared silver colloidal solution as mentioned above was made just before use and poured over the tissue sections and kept for 45 minutes at room temperature in dark place. The solution was washed off with three changes of deionised water, then treated with 5% sodium thiosulphate for 5 minutes, washed with deionised water and the sections were finally dehydrated through increasing grades of alcohol, clear in xylene and mount in DPX.

AgNORs were identified as brownish black dots within nucleus and the background was pale yellow.

Results

The present study included evaluation of AgNOR staining on a total of 100 cases of patients with palpable breast lumps sent for FNAC to the Department of Pathology in our institute. Of the 100 cases on FNAC, majority of the cases (29%) were between the ages of 21 and 30 years, were female patients (97%). Of the 100 cases, seventy two cases (72%) were benign lesions, four cases (4%) were borderline and rest twenty four cases (24%) were malignant lesions. The benign cases were seen in younger age group, whereas the borderline and the malignant lesions were seen in the fourth, fifth, sixth and seventh decades of life (Table 1). Majority of the benign lesions were fibroadenoma, borderline lesions were

Table 1: Distribution of cases of benign, borderline and malignant lesions according to different age in years

Age in years	Benign Lesions	Borderline Lesions	Malignant Lesions	Total	*p* value
10–19	05 (6.94%)	00 (0%)	00 (0%)	05 (5%)	0.453
20–29	27 (37.5%)	00 (0%)	00 (0%)	27 (27%)	<0.001**
30–39	16 (22.22%)	01 (25%)	07 (25.92%)	24 (24%)	0.820
40–49	18 (25%)	00 (0%)	04 (16.66%)	22 (22%)	0.587
50–59	02 (2.7%)	01 (25%)	06 (25%)	09 (9%)	0.004**
60–69	02 (2.7%)	02 (50%)	05 (20.80%)	09 (9%)	0.001**
70–79	02 (2.7%)	00 (0%)	02 (8.3%)	04 (4%)	0.373
Total	72 (100%)	04 (100%)	24 (100%)	100 (100%)	—

** Strongly significant (*p* - value: $p \leq 0.01$)

Atypical Ductal Hyperplasia (ADH) and majority of malignant lesions were IDC Nos (Infiltrating Ductal Carcinoma, Not otherwise Specified). A total of 27 out of the 100 cases were available for histopathology correlation.

All the 100 FNAC smears of the breast lesions done during the 18 months study period were stained for AgNOR and they were correlated with FNAC diagnosis. Amongst the benign lesions, majority of the cases; i.e. 45 were fibroadenoma and showed AgNOR range between 1–3.0 with a mean AgNOR of 2.14 (Table 2), out of 4 borderline lesions, majority of cases; i.e. 3 were Atypical Ductal Hyperplasia with AgNOR range between 4.9–5.3 and a mean AgNOR count of 5.08 (Table 3), out of 24 cases of malignant tumors diagnosed by FNAC, majority of the cases i.e 21 cases were IDCNoS and the AgNOR count ranged between 5.04 and 7.3 with a mean AgNOR count of 6.21 (Table 4). AgNOR discriminates the benign and malignant with $p < 0.001$ as the the mean AgNOR count in benign was much lower than the mean AgNOR count in malignant lesions (Table 5). Of the 27 specimens for histhopathological correlation, the mean AgNOR count of benign

Table 2: AgNOR distribution in FNAC of benign lesions

Benign lesions	Number of patients	AgNOR count		
		AgNOR range	Mean AgNOR	SD
Fibroadenoma	45	1–3.0	2.14	0.69
Fibrocystic change	21	1.4–3.2	1.98	0.52
Benign phyllodes tumor	03	2.04–3.9	2.2	0.63
Gynecomastia	03	2.2–2.60	2.6	0.20

Table 3: AgNOR distribution in FNAC of borderline lesions

Borderline lesions	Number of patients	AgNOR count		
		AgNOR range	Mean AgNOR	SD
Atypical ductal hyperplasia	3	4.90–5.30	5.17	0.23
Borderline phyllodes tumor	1	4.00–4.00	4.00	–

Table 4: AgNOR Distribution In FNAC of Malignant Lesions

Malignant lesions	Number of patients	AgNOR count		
		AgNOR range	Mean AgNOR	SD
Medullary carcinoma	1	5.60–5.60	5.60	–
IDC-Nos	21	5.04–7.30	6.21	0.74
Inflammatory carcinoma	1	6.04–6.04	6.04	–
Apocrine carcinoma	1	5.01–5.01	5.01	–

Table 5: Mean AgNOR in FNAC in discriminating benign from malignant

AgNOR	FNAC		*p* value
	Benign	Malignant	
Min-Max	1.07–3.06	5.01–7.30	$T = 22.910$
Mean ± SD	2.67 ± 0.72	5.71 ± 0.74	$p < 0.001$**
95% CI	2.04–2.37	5.81–6.44	

** Strongly significant (p - value: $p \leq 0.01$)

Table 6: AgNOR distribution on histopathology section

Cases studied	No. of cases	AgNOR range	Mean AgNOR count	SD
A. Benign				
Fibroadenoma	11	1.1–1.9	1.43	0.24
Fibrocystic change	2	1.1–1.68	1.28	0.33
Gynaecomastia	–	–	–	–
Benign phyllodes tumor	2	1.1–2.0	1.55	0.64
B. Borderline				
Atypical ductal hyperplasia	3	2.9–3.4	3.2	0.39
C. Malignant				
IDC-Nos	6	3.1–4.1	3.87	0.39
Lobular carcinoma, solid variant	2	3.3–3.86	3.58	0.54
Medullary carcinoma	1	4.01–4.01	4.01	–
Mucinous carcinoma	1	3.4–3.4	3.4	–
Tubular carcinoma	1	4.03–4.03	4.03	–

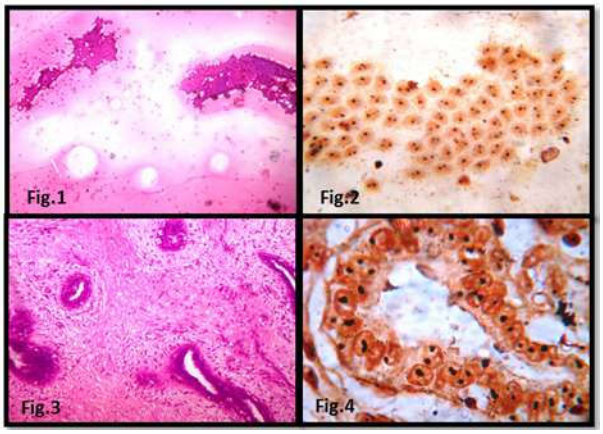

Fig. 1: FNA smear from fibroadenoma [H & E, 100X]. **Fig. 2:** AgNOR stain of FNA smear from fibroadenoma. Distribution of AgNOR dots are 1–3, discrete, central. [AgNOR stain, 1000X], **Fig. 3:** HPE of fibroadenoma [H & E, 400X], **Fig. 4:** AgNOR stain of HPE of fibroadenoma. AgNOR dots are 1–2, uniform and compact. [AgNOR stain, 1000X]

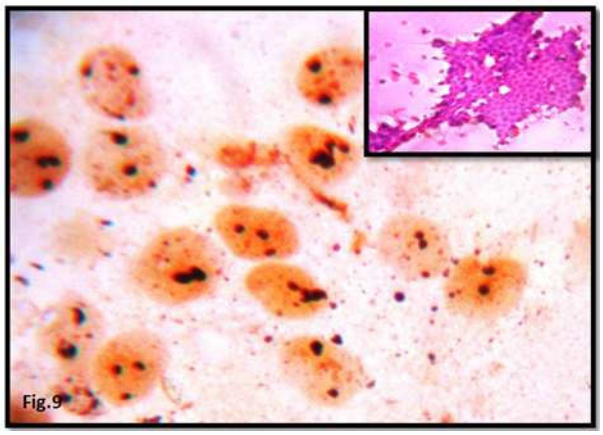

Fig. 9: AgNOR stain of FNA smear of gynecomastia. The dots are 1–3, central and peripheral and compact. [AgNOR stain, 1000X], inset picture shows FNA smear from Gynecomastia, The cells are showing epithelial hyperplasia [H & E, 400X]

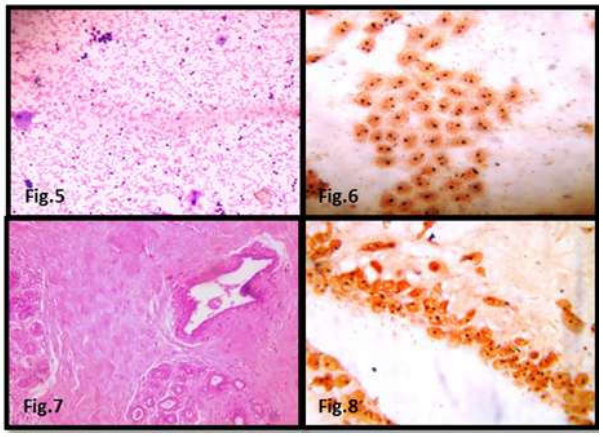

Fig. 5: FNA smear from fibrocystic disease [MGG, 100X] **Fig. 6:** AgNOR stain of FNA of fibrocystic disease. The AgNOR dots are small 1–3, central, discrete. [AgNOR stain, 1000X], **Fig. 7:** HPE of Fibrocystic change. [H & E, 100X], **Fig. 8:** AgNOR stain of HPE of fibrocytic change with uniform 1–2, compact and discrete AgNOR dots. [AgNOR stain, 400X]

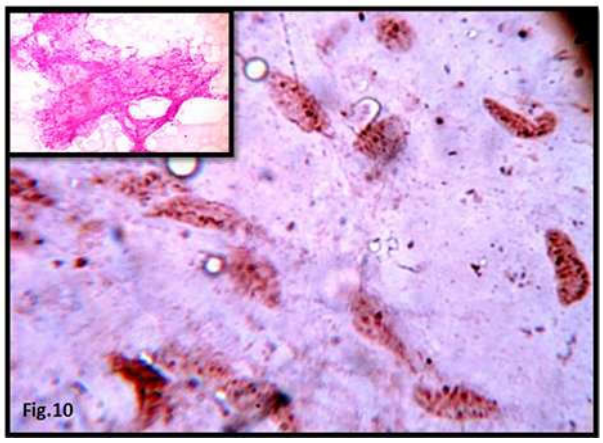

Fig. 10: AgNOR stain of FNA smear from phyllodes tumor. The AgNOR dots are tiny, 1–2, central and discrete. [AgNOR stain, 1000X], inset picture shows FNA smear from phyllodes tumor with predominantly spindle shaped stromal cells in fibrous stroma [H & E, 400X].

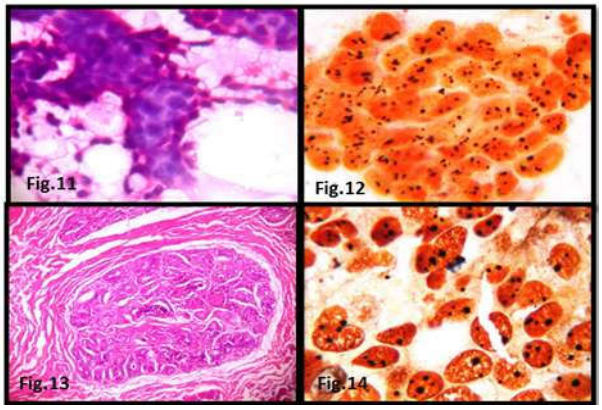

Fig. 11: FNA smear of atypical ductal hyperplasia [H & E, 400X], **Fig. 12:** AgNOR stain of FNA smear of atypical ductal hyperplasia. The dots are 1–6, scattered throughout the nucleus and coarse. [AgNOR stain, 1000X], **Fig. 13:** HPE of atypical ductal hyperplasia [H&E, 400X], **Fig. 14:** AgNOR stain of HPE of atypical ductal hyperplasia. AgNOR dots are 1-4, small and medium sized, distributed throughout the nucleus [AgNOR stain, 1000 X].

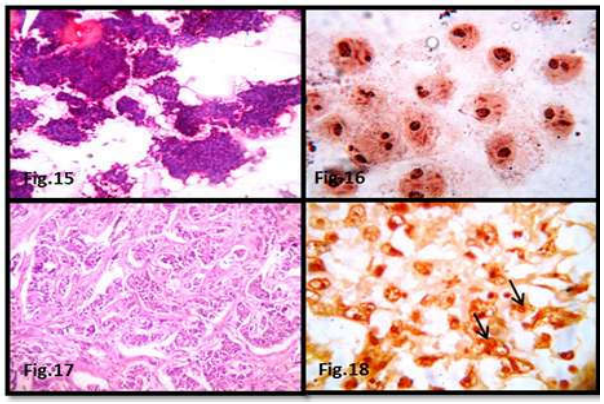

Fig. 15: FNA smear of IDC-Nos [H&E, 100X], **Fig. 16:** AgNOR stain of FNA smear from IDC. The dots are medium to large, coarse and clumped. [AgNOR stain, 1000X]. **Fig. 17:** HPE of IDC-Nos [H & E, 100X], **Fig. 18:** AgNOR stain of HPE from IDC-Nos. The AgNOR dots are large 1–2, clumped, peripheral and central (arrows) [AgNOR stain, 400X].

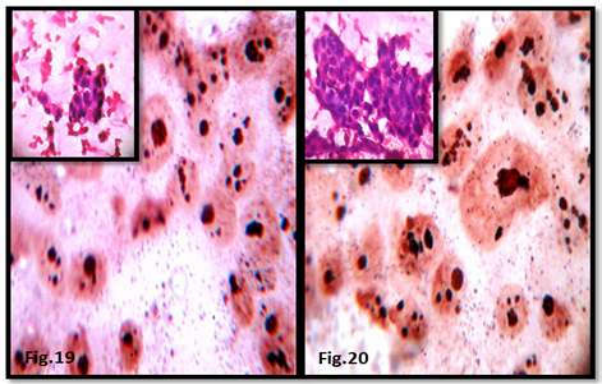

Fig. 19: AgNORstain of FNA smear from apocrine carcinoma. The AgNOR dots are large, irregular,coarse, clumped. [AgNOR stain, 1000X] inset picture shows FNA smear of apocrine carcinoma [H & E, 400X], **Fig. 20:** AgNOR stain of FNA smear from medullary carcinoma. The AgNOR dots are small to large, irregular, coarse, clumped and scattered throughout the nucleus.[AgNOR stain, 1000X] inset picture shows FNA smear of medullary carcinoma [H & E, 400X].

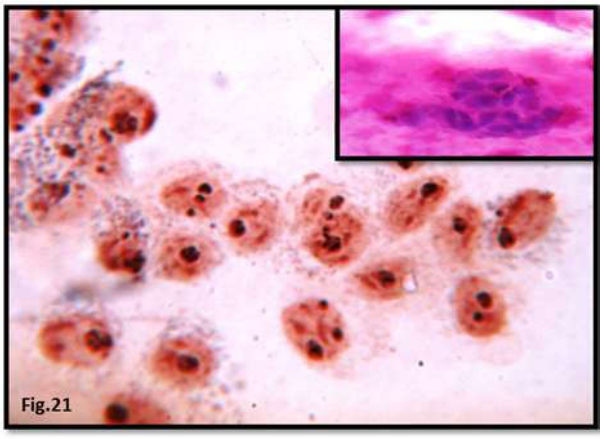

Fig. 21: AgNOR stain of FNA smear from inflammatory carcinoma. The dots are small to large, central and peripheral, coarse, clumped. [AgNOR stain, 1000X] inset picture shows FNA smear of inflammatory carcinoma [H&E, 400X]

lesions in histopathology was 1.62 and 3.78 for maligant lesions (Table 6).

Discussion

AgNORs have found widespread application in tumor pathology, in assessing the growth potential and malignant potential of tumors, in distinguishing between benign from malignant neoplasms, to assess the prognosis and also to evaluate the risk of recurrence.[2]

AgNOR staining has already been applied and shown to be of great value in differentiating benign from malignant in lesions of breast, cervix, oral cavity, skin, soft tissue tumors, lymphomas and melanomas.

So, the present study was undertaken to evaluate the AgNOR scores in aspiration cytology smears of breast lesions to differentiate benign lesions from malignant lesions.

A total of 100 patients who presented with breast lump were subjected to fine needle aspiration and subsequent AgNOR staining. The Age of the patients varied from 16 to 72 years with mean age of presentation being in the range of 20–29 years. Out of 100 patients, 97 patients were females and 3 patients were males. Out of 100 cases, 72 cases were diagnosed as benign lesions, 4 cases were borderline lesions and 21 cases were malignant lesions (Table 7) on H&E stain of fine needle aspiration smears.

The AgNOR stain showed that the AgNORs were clearly visible in aspiration smears as brown / black intranuclear dots upon 100X magnification.

Benign breast tumors generally showed a fine, round and singly dispersed dots in the nucleus and the mean AgNOR count was lesser, our study was in concordance with the study conducted by K Rajeevan et al.[11] and Alpana Basu et al.[15] In our study we had 4 cases of borderline lesions, 3 cases of atypical ductal hyperplasia and 1 borderline phyllodes tumor. The cases diagnosed as atypical ductal hyperplasia on H & E stain had mean AgNOR score in the malignant range of more than 5 and the case diagnosed as borderline phyllodes tumor on H & E stain had mean AgNOR score of 4.0 which was the higher limit for benign lesions. These lesions were not compared in any of the studies in our references published. The present study showed that the mean AgNOR count on FNA of malignant lesions of breast was 5.71. The reason for lower or higher counts can be due to overlapping of cells, higher counts because malignant cells contained extremely large AgNORs whose number is got by multiplying by a factor 5, and also due to interobserver variations.

Morphology of AgNOR dots in malignancy was at variance with benign lesions. Dots were coarse, large, often deeply stained and had variable size and distribution. The present study showed that the mean AgNOR count in benign was 2.67, borderline lesions were 4.59 and in malignant lesions were 5.71. The present study shows concordance with other studies (Table 8) i.e. the mean AgNOR count in malignant was more than the mean AgNOR count in benign.

Although the present study shows a good correlation between NOR's of benign and malignant, it does not help to solve the most important diagnostic problem in the field namely the differential diagnosis between atypical ductal hyperplasia and invasive carcinoma as the mean AgNOR of borderline lesions in 4.6. However, seen in conjunction with morphological characteristics, AgNOR's can provide additional and helpful information.

In the present study, the mAgNOR in histopathology of benign lesions was 1.8 ± 0.3 and in malignant lesions was 3.74 ± 0.4. Thus, the present study is in concordance with the study conducted by Hasnan J and Gita Jayaram[14] and with the study conducted by Hena Ansari et al.[13] The reason for varying NOR counts include different section thickness, different staining procedures and different counting methods. Prolonged fixation appears to cause AgNOR's to coalesce, thus resulting in low count, especially in small biopsy specimens fixed in an excess of formalin overnight. These problems can be overcome by the use of aspiration smears, wherein staining time is better controlled and the counting of dots becomes easier due to cellular dispersal.[13] Thus, it is not possible to directly compare counting results from different institutions.[7] Though the scores cannot be standardized and fixed for a particular lesion as there being laboratory variations, each laboratory has to establish its cut off scores for various lesions.[5]

Table 7: Comparative study of distribution of cases for FNAC

Sl. No.	Study	Total	Benign	Borderline	Malignant
1	K. Rajeevan et al.[11]	163	102	7	43
2	Hena A Ansari et al.[13]	27	17	—	10
3	Hasnan J and Gita Jayaram.[14]	56	31	—	25
4	Present Study	100	72	4	24

Table 8: Comparison of overall AgNOR distribution in FNAC smears of breast lesions in various studies.

FNAC Diagnosis	Mean AgNOR Count		
	Rajeevan K et al.[11] (163 cases)	Hasnan J and GitaJayram.[14] (56 cases)	Present study (100 cases)
Benign lesions	2.8	3.82	2.67
Borderline lesions	—	—	4.59
Malignant lesions	5.42	9.42	5.71

Conclusion

The present study included the evaluation of the AgNOR scores in the aspiration cytology smears of breast lesions to differentiate benign from malignant.

This study suggests that AgNOR scores are reliable markers to differentiate benign breast lesions from malignant lesions.

Therefore the AgNOR technique is found to be a simple, one step, inexpensive staining procedure which can be effectively utilized to differentiate benign lesions from malignant lesions.

The findings in the present study indicate that the AgNOR technique can be used as a useful adjunct in FNAC and histopathology diagnosis of breast lesions and that in fine needle aspiration cytology smears of breast lesions, an AgNOR score of less than 5.0 strongly suggests a benign lesion and a score of 5.0 or more is in favor of a malignant lesion.

References

1. Dervan PA, Gilmartin LG, Loftus BM, Carney DN. Breast Carcinoma Kinetics. Argyrophilic Nucleolar Organizer Region counts correlate with Ki-67 Scores. Am J Clin Pathol 1989 Oct;92(4):401–7.
2. Elangovan T, Mani NJ, Malathi P. Argyrophilic nucleolar Organizer regions in inflammatory, pre malignant and malignant oral lesions: A quantitative and qualitative assessment. Indian J Dent Resp 2008;19(2):141–6.
3. Rosai J. Special techniques in surgical pathology, In. Rosai and Ackerman's surgical pathology. 9th ed, vol 1. St. Louis: Elsevier 2004.pp.37–92.
4. Grizzle WE, Bancroft JD. The dispersed neuroendocrine system, cytoplasmic granules, and other organelles. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 6th ed. Philadelphia: Elsevier 2008. pp.283–307.
5. Kaushik R, Sharma S, Mahajan V, Gulati A, Sharma BB. AgNORs in endometrial lesions. Indian J Pathol Microbiol 1999;42(4):451–4.
6. Terlikowski S, Lenczewski A, Sulkowski S, Kulikowski M. Nucleolar Organizer regions in differentiated preneoplastic and neoplastic endometrial lesions. Gynecol Obstet Invest 1999; 47:205–9.
7. Brustmann H, Riss P, Naude S. Nucleolar Organizer Regions as markers of endometrial proliferation: A study of normal, hyperplastic and neoplastic tissue. Human Pathology 1995; 26(6):664–7.
8. Karin Lindholm. Breast. Orell RS, Sterrett FG, Sterrett GF, Whitaker D. Fine Needle Aspiration Cytology. 4th ed. Churchill Living Stone: Elsevier 2005;165.
9. Egan MJ, Crocker J. Nucleolar Organizer Regions in Pathology. Br J Cancer. 1992 Jan;65(1): 1–7.
10. Ruschoff J, Plate KH, Contractor H, Kern S, Zimmermann R, Thomas C. Evaluation of Nucleolar Organizer Regions by automatic image analysis: A contribution to standardization. J Pathol 1990;161(2):113–8.
11. Rajeevan K, Aravindan KP, Kumari BC. Value of AgNOR's in Fine Needle Aspiration Cytology of Breast Lesions. Indian J Pathol Microbiol 1995; 38(1):17–24.
12. Crocker J, Nar P. Nucleolar Organizer regions in lymphomas. J Pathol 1987;151:111–8.
13. Ansari HA, Mehdi G, Maheshwari V and Diddiqui SA. Evaluation of AgNOR scores in aspiration cytology smears of breast tumors.Jcytol 2008;25(3):100–4.
14. Hasnan J, Jayaram G. Nucleolar Organizer Regions distribution in Fine Needle Aspiration of Cytological smears from breast lesions. Malaysian J Pathol 1996;18(1):35–41.
15. Basu A et al. A comparative study of Silver Binding Nucleolar Organizer Regions (AgNOR's) of breast lesions in histological sections and Fine Needle Aspiration Smears. J Indian Med Assoc 1997;95(8):443–7.