ORIGINAL ARTICLE

SIGNIFICANCE OF SILVER STAINING OF NULCEAR ORGANIZER REGIONS IN FINE NEEDLE ASPIRATION SMEARS OF BREAST LESIONS

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ABSTRACT

Background: Demonstration of Nucleolar Organizer Regions (NORs) by silver impregnation method can differentiate malignant lesions from benign, reactive and inflammatory breast lesions. As the technique is based on mitotic activity, it is also useful in prediction of clinical behavior of diagnosed lesion. Most importantly, it is able to pick up grey zone lesions between benign and malignant.

Objective: To establish AgNOR staining as a diagnostic and prognostic tool in the management of various breast lesions including precancerous lesions.

Method: In the present study, AgNOR silver staining impregnation method was applied on air dried smear of breast FNAC along with routine H & E stain.

Results: Out of total 79 (87.77%) satisfactory smears of breast FNA, 52 (65.8%) benign cases showed mean AgNOR count (mAgNOR) 3.45 + 1.19, proliferation index (pAgNOR) 20.47 + 14.8 and AgNOR score of 9.25± 3.30 and 24 (38.3%) malignant cases showed mAgNOR 9.54 ± 2.53; pAgNOR 65.12 ± 15.12; AgNOR score 26.56±6.98. the difference between AgNOR counts of benign and malignant lesions was statistically highly significant.(p value is <.001).

Conclusions: AgNORs study in fine needle aspiration was found to be simple, feasible and very effective technique. AgNOR counts help to discriminate benign lesions from malignant lesions and other features like size, shape and distribution of the AgNOR dots help to judge aggressiveness of the malignant tumor.

Key Words: Nuclear Organiser Regions, Impregnation, Precancerous

INTRODUCTION

Benign and malignant lesions of breast are quite common in fertile age group. It has been claimed that demonstration of nucleolar organizer regions (AgNORs), by silver impregnation technique, can differentiate malignant cells from normal, reactive or benign neoplastic cells, because of their significantly increased numbers in malignant cells.[1] AgNORs are better demonstrated and easily counted on FNA smears[2,3] as it provides single cell thick preparation. Objectives of the study are to obtain the AgNOR count in various breast lesions and check its utility in differentiation of malignant from benign and borderline cases.

MATERIAL METHODS

A cross sectional descriptive study of breast lesions was conducted in tertiary care hospital in central area of Gujarat. A total 90 cases of breast lumps were aspirated. Conventional fine needle aspiration technique was used in cytology OPD clinic by using 21-22 gauze disposable needles attached to a 10 cc disposable plastic syringe. Multiple smears were prepared from the aspirations.

Out of these, in 79 cases smears were satisfactory and representative and diagnosis was given and remaining 11 smears were unsatisfactory, due to scanty cellularity and poor quality of AgNOR staining. Thus they were not included in the study.

Minimum of two smears were immediately fixed with ether-ethanol mixture, and stained with H & E stain. Other smears were air dried and stained with AgNOR silver staining technique. AgNORs were visible as brown or black intranuclear dots.

The AgNOR count was done by counting nuclei of 100 cells under oil immersion lens using a standard procedure. In each case the mean number of total AgNOR dots per cell (mAgNOR), percentage of cells with more than five AgNOR dots per cell (pAgNOR) and percentage mean AgNOR score (mAgNOR score) were calculated by multiplying the number of small dots

by a factor of one, number of medium dots by a factor of three and the number of large dots by a factor of five and adding up the three.

OBSERVATION

In the present study total 90 cases of breast lumps were aspirated within the age group of 15-70 years. Out of these, 79 cases (87.77%) were satisfactory, representative and diagnosed on H & E stain, 11 cases (12.23%) smears were unsatisfactory.

Out of total 79 cases, 52 cases (65.82%) were benign lesions, 24 cases (30.38%) were malignant lesions and 3 cases (3.8%) were cytologically diagnosed as suspicious of malignancy. 59 cases (49.36%) out of these 79 cases were correlated histologically.

Out of 52 benign breast lesions, 34 (65.38%) cases were cytologically diagnosed as fibroadenoma, nine cases (17.3%) as fibroadenosis and eight cases (15.38%) were labeled as inflammatory lesions. There was only a single case of gynaecomastia (1.91%).

Out of 24 malignant cases, eight (33.3%) were in premenopausal group, while 16 (66.67%) were in postmenopausal group. All the 24 cases were diagnosed as infiltrating duct carcinoma and confirmed histologically.

The age group in breast lesion ranged from 15-60 years. Maximum numbers of cases were found in the age group of 20 to 30 years, followed by age group of 30 to 40 years. For 24 malignant breast cases, age ranged between 32 to 70 years, with maximum numbers of cases (17 cases) was older than 40 years.

Out of 52 benign cases 51(98.08%) were females and there was only a single case of Gynaecomastia (1.92%). Out of 24 malignant breast lesions, 23 (98.08%) cases were female and only one (1.92%) was male. (Table. 1)

Table 1: Age wise distribution of 79 breast lesions

Breast lesions	Cases (%)	Age group in years					
		11-20 (%)	21-30 (%)	31-40 (%)	41-50 (%)	51-60 (%)	61-70 (%)
Benign	52 (65.82)	06 (11.53)	27(51.92)	16(30.76)	02(3.84)	01(1.92)	-
Fibroadenoma	34 (65.38)	04 (12.5)	16 (45.45)	12(33.33)	02 (6.06)	-	-
Fibroadenosis	09 (17.38)	-	05 (6.25)	04 (37.5)	-	-	-
inflammatory	08 (15.38)	-	04 (42.85)	01 (14.28)	-	01 (14.28)	-
Gynaecomastia	01 (1.92)	-	01(100)	-	-	-	-
Suspicious of malignancy	03 (3.79)	-	-	02 (66.67)	-	-	01(33.33)
Malignant	24 (30.37)	-	01 (4.16)	06 (25)	07(29.16)	06(25)	04(16.66)

AgNOR silver staining technique was applied in all the 79 cases and mAgNOR, pAgNOR and percentage mAgNOR score were calculated. Table 2 shows the values of AgNOR counts in different breast lesions, where it is apparent that the AgNOR counts of malignant lesion were higher than benign lesions.

The AgNOR dots in benign breast lesions were mainly small, round, uniform, compact, and regular. While the AgNOR dots in Malignant breast lesions were mainly large sized show irregularity in size and shape, angulations and were arranged in spiky clusters.

Table 2: Values of different AgNOR counts in breast lesions

Breast lesions	Cases (%)	Mean AgNOR/cell		PAgNOR % of cells with >5 AgNOR dots/nucleus		% Mean AgNOR/Cell Score	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Benign	52 (65.8)	3.45 ± 1.19	1.24 - 5.76	20.47 ± 14.8	0- 56	9.25 ± 3.30	1.40 – 18.2
Malignant	24 (30.3)	9.54± 2.53	4.68 -14.85	65.12 ± 15.12	32- 89	26.56 ± 6.98	14.50 – 40.22
Suspicious of malignancy	3 (3.8)	7.53± 2.9	3.48- 10.5	44.33±18.26	20- 75	18.32 ± 6.34	9.46- 23.99

Table 3: Comparison of p values in benign &malignant breast lesions

Breast lesions	Cases	Mean AgNOR p value	PAgNOR p value	% Mean AgNOR score p value
Benign	52	< .001	< .001	< .001
Malignant	24	Highly significant	Highly significant	Highly significant

There was a statistical significant difference in the values of AgNOR counts of Benign and Malignant lesions. As per shown in table 3, the p value between the two was also found to be <0.001 which is highly significant.

In the present study three cases were diagnosed cytologically as suspicious of malignancy, but their AgNOR counts were in the malignant range. Table 4 shows the comparison of p values of benign and suspicious malignant breast lesions, which is also statistical highly significant (p value <0.001). Thus, AgNOR technique is quite useful in such cases.

In present study, different groups of benign breast lesions were assessed for AgNOR counts. Table 5 shows the comparison of AgNOR counts among various benign breast lesions.

Table 4: Comparison of p values in benign &suspicious of malignant breast lesions

Breast lesions	Cases	Mean AgNOR p value	PAgNOR p value	% Mean AgNOR score p value
Benign	52	< .001	< .001	< .001
Suspicious of Malignancy	03	significant	significant	significant

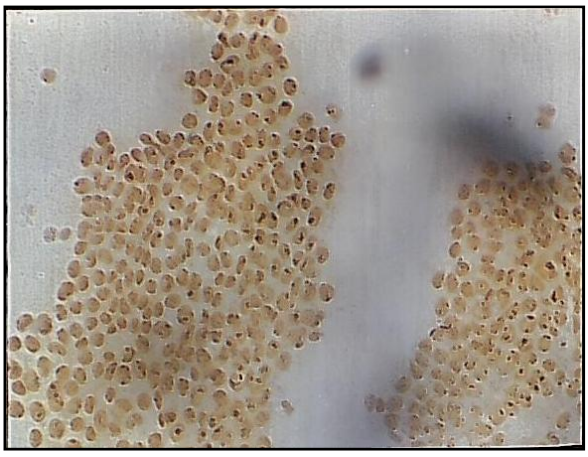

Figure 1: Cellular smear of fibroadenoma- showing small, one-two, uniform AgNOR dots

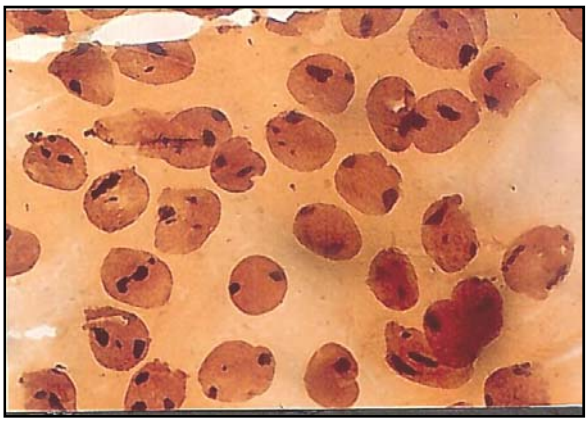

Figure 2: Cellular smear of fibroadenosis- showing one-two, regular, uniform AgNOR dots

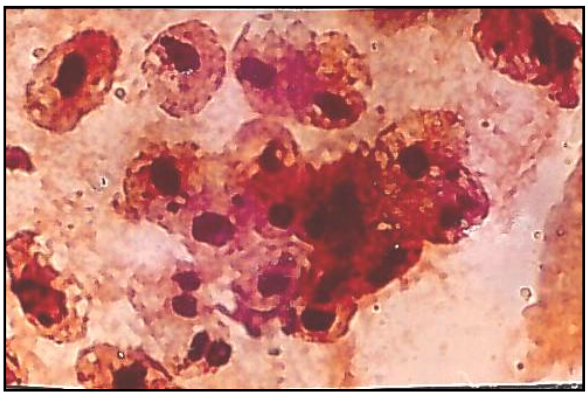

Figure 3: FNA of non specific inflammation- shows large AgNOR dots, single dot per cell, with foamy cytoplasm.

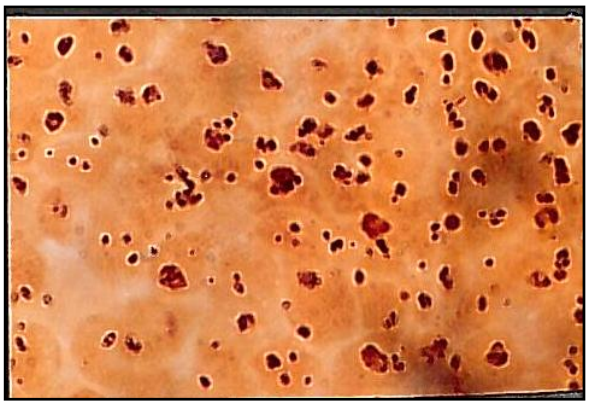

Figure 4: FNA of invasive breast carcinoma- showing numerous, irregular AgNOR dots, at places in clumps.

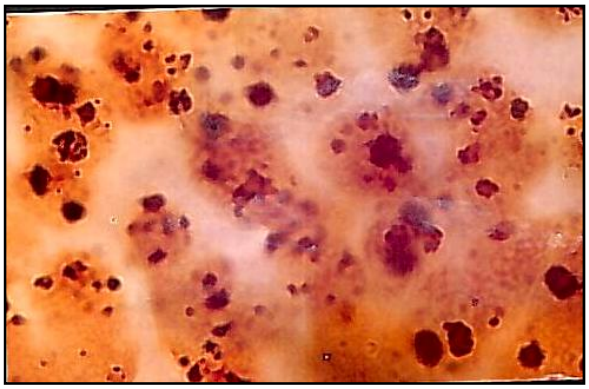

Figure 5: FNA of invasive breast carcinoma- shows huge irregular clusters of intracellular and extracellular clumps of AgNOR dots.

It was also observed that the AgNOR dots in inflammatory lesions were one-two in number, small sized, uniform and compact. The AgNOR dots of fibroadenoma ranged from one-six in number, uniform, regular small to medium sized where as those of fibroadenosis were two-five in number, small sized and medium sized, uniform irregular.

It was also observed that the combined AgNOR counts of fibroadenoma+ fibroadenosis were quite higher than inflammatory lesions and the difference is statistically significant (Table 6). Thus AgNORs can discriminate between these two groups of lesions.

Though fibroadenoma showed slightly higher AgNOR counts than fibroadenosis, the 'p' value was >0.05 which is statistically not significant. Thus the AgNORs cannot be used to distinguish between fibroadenoma and fibroadenosis.

Table 5:Values of different AgNOR counts in benign breast lesions.

Breast lesions	Cases	Mean AgNOR/cell		PAgNOR % of cells with >5 AgNOR dots/nucleus		% Mean AgNOR/Cell Score	
		Mean ± SD	Range	Mean ±SD	Range	Mean ±SD	Range
Fibroadenoma	34	3.92± 0.9	1.93- 5.76	24.9 ± 14.08	4 – 56	10.06 ± 2.78	4.75 – 16.0
Fibroadenosis	09	3.61 ± 1.15	2.2- 5.27	22.25 ± 19.7	6 -48	10.10 ± 4.02	5.48 – 18.29
inflammatory	08	1.97 ± 0.37	1.24- 2.56	2.00 ± 2.13	0 -6	05.07 ± 1.81	1.40 – 6.40
Gynaecomastia	1	2.75	-	28.0 ± 2.13	-	07.13	-

Table 6: comparison of p value of fibroadenoma, fibroadenosis & inflammatory lesions

Breast lesions	Cases	Mean AgNOR/cell	Mean AgNOR p value	pAgNOR	pAgNOR p value
Fibroadenoma +fibroadenosis	43	3.76 ± 1.05	< .001	23.57 ± 14.39	< .001
Inflammatory lesions	08	1.97 ± 0.37	Highly significant	2.00 ± 2.13	Highly significant

AgNOR counts of premenopausal and postmenopausal groups were compared, which showed higher counts in premenopausal group, but the 'p' value is found to be >0.05 which is statistically not significant.

DISCUSSION

AgNOR staining technique in aspiration smears of the Breast is simple and feasible technique. The present study is done on nucleolar organizer regions in Breast cytology material.

In the present study, a total of 90 cases of breast lumps in the age group (15-70 yrs) were aspirated, after relevant clinical history and clinical examination, in the cytology OPD. Out of these, in 79 (87.77%) cases smears were satisfactory, representative and diagnosed on H & E stain, while 11 (12.23%) cases were unsatisfactory and excluded from the study. The causes of unsatisfactory smears might be due to aspirations were taken from non-representative area, scanty cellularity and/or poor quality of AgNOR staining.

Table 7: Evaluation of AgNOR counts of different studies

Name of author	lesions	Cases	mAgNOR (mean ± SD)	pAgNOR (mean ± SD)	% Mean AgNOR (mean ± SD)	P value
Rajeevan & Arvindam (1995)[4] (FANC)	Benign Malignant	48/84 (57.4%) 36/84 (42.8%)	2.8 (0.7-0.3) 5.4 (5.0 – 5.9)	-	5.3 (4.9-5.7) 11.2 (10.2–12.2)	<0.01
Wolfson et al (1995)[5] FNAC)	Benign Malignant	67/112 (59.8%) 45/112 (40.2%)	1.78 ± 0.53 2.69 ± 0.88	-	-	<0.0001
Mehrotra & Chandra (1998)[6] (FNAC)	Benign Malignant	31/95 (72.6%) 64/75 (67.4%)	3.081± 1.53 (1.0-4.0) 7.10 ± 1.54 (4.1 – 11.5)	1.00% 75%	-	<0.139
Mehrotra et al (1997)[7] (FNAC)	Benign Malignant	48/75 (64%) 27/75 (7.4%)	1.53 ±0.37 6.70±0.91	14 ±2.19 82.03±7.68		<0.0002
Agrawal et al (1995)[8] Tissue section	Benign Malignant	18/44 (40.4%) 26/44 (59%)	3.4 ± 1.0 (1.8± 5.5) 9.1±1.4 (7-12.3)	-	-	<0.0001
S Simha et al (1996)[9]	Benign Malignant	55/200 (27.5%) 140/200 (70%)	1.8 3.5	0.00 18.7		<0.0001
Present study (FANC)	Benign Malignant	52/79 (65.8%) 24/79 (30.4%)	3.45 ± 1.9 (1.2– 5.8) 9.54±2.53 (4.7-14.8)	20.47±14.8 (0-56) 65.1±15.1 (32 – 89)	9.25±3.3 (1.4-18.2) 26.6±6.9 (14.5–40.2)	<0.001

AgNOR counts were evaluated in the Benign and Malignant breast lesions and their results were evaluated and compared with those of other workers under the following headings- mAgNOR, pAgNOR, and % mean AgNOR score and p value among the benign lesions, their subtypes, malignant lesions and miscellaneous lesions. Table 7 shows these details, which shows that, in the present study the AgNOR counts are quite comparable to other studies.

In the present study, mAgNOR in 24 malignant cases was 9.54±22.53 (Range: 4.68-14.85). This is comparable to Agrawal et al (1995).[8]

We found p AgNOR of 65.12±15.1 (Range: 32 – 89) in 24 malignant cases. These findings reflect quite a high rate of cell proliferation. This is somewhat lower than that of Mehrotra et al (1997) and Mehrotra & Chandra (1998).

We also found, the % Mean AgNOR score was 26.57±6.98 (Range: 14.5–40.2) in 24 malignant cases which indicates a larger % of cells in every malignant lesions studied showed evidence of increased cell proliferation.

The difference in above discussed parameter might be due to Quality of the AgNOR preparation; whether the AgNOR technique applied on block or slide; and the number of visible AgNOR dots per cell varies depending upon whether the cells are in S phase or metaphase.

The present study also showed a statistically significant difference between the AgNOR counts of benign and

malignant breast lesion, with the P value (<0.001). There was no overlap between the absolute AgNOR counts of benign and malignant lesions, thus AgNORs act as true diagnostic factor. Even a mAgNOR value of <4 goes in favors of benign lesions. Thus, the cut off value of "Four" can be used to discriminate benign from malignant lesions.

The difference between AgNOR counts of fibroadenoma and fibroadenosis was not statistically significant (i.e. the 'p' value was >0.05). This finding is quite comparable with other workers.[1,7,9]

CONCLUSION

AgNOR technique is easy to establish in any pathology laboratory with minimal basic facilities. The only prerequisite are provision of scrupulously clean glassware and fresh silver nitrate solution. The AgNOR counting procedure is time consuming and has to be through.

FNA smears are ideal for AgNOR demonstration and absolute counting as they show AgNOR dots clearly, in a monolayer without overlapping.

AgNOR count can be used as independent prognostic factor, as different parameters related to AgNOR count can give a clear discrimination between benign and malignant breast lesions. Besides this, there is a difference in number, size, shape and distribution of the AgNOR dots in benign and malignant lesions. The role of AgNOR technique in identification of individual benign breast lesion is not very effective. As statistically significant difference was not found between fibroadenoma and fibroadenosis.

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