Original Article

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A Study of AgNOR Scores on FNAC of Breast Lumps and Its Correlation with Histopathological Grading

Rumana Tasneem[1]*, Manjunath M R[2] and Muralidhar Bhat Y[2]

[1]Department of Pathology, Mysore Medical College, Mysore, Karnataka

[2]Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka

Abstract

Background: Breast cancer is one of the leading cancers in women both in developed and developing countries. Nucleolar Organizer Regions (NORs) are loops of DNA related to protein synthesis and ribosomal activity. Argyrophilic Nucleolar Organizer Region (AgNOR) counts act as a reliable cell proliferative marker as they aid in identifying cell proliferation activity and aggressiveness of a lesion, and thereby helps in differentiating benign and malignant breast lesions.

Aim: To evaluate the role of AgNOR count in differentiating benign and malignant breast lumps.

Methods: All breast lump specimens collected for Fine Needle Aspiration Cytology (FNAC) and histopathology, for a period of one year, were included in the study. A total of 69 specimens were included. All FNAC and histopathology slides were stained with AgNOR stain, and the score was recorded.

Result: A total of 69 cases were studied, both non-neoplastic and neoplastic breast lesions. All FNAC cases correlated histologically. Out of the 69 cases, 38 were malignant. The mean AgNOR counts in non-neoplastic, benign and malignant breast lesions were found to be 2.19, 2.88 and 5.94 respectively ($p<0.05$). Other parameters such as proliferative AgNOR index, AgNOR size variation, AgNOR distribution within the nucleus and the Subjective AgNOR Pattern Assessment (SAPA) scores also showed statistically significant difference between benign and malignant lesions.

Conclusion: AgNOR scores showed good correlation in differentiating benign and malignant breast lesions, they can be used as a reliable tool to aid in the diagnosis.

Keywords: *AgNOR, Breast lesions, FNAC, Histopathological grading, Non-neoplastic, Neoplastic*

Introduction

Breast cancer is the leading cancer in women both in developed and developing countries.[1] In India, out of 1,44,937 women diagnosed with breast cancer, 70,218 deaths were recorded in 2012.[2] As per the Indian Council of Medical Research - Population Based Cancer Registry (ICMR-PBCR) data, more than 30% of cases constitute the breast cancer in female urban registries of Delhi, Mumbai, Ahmedabad, Kolkata and Trivandrum (National Cancer Registry Programme, 2001).[3] According to population-based cancer registry of 2012-2014, breast cancer accounts for 53.2% of women of younger age group.[4]

The primary tool for screening of breast cancer is breast self-examination (BSE). Adjuvant methods such as mammography, ultrasound examination of breast, fine needle aspiration cytology (FNAC) of breast lumps, magnetic resonance imaging (MRI), computed tomography (CT) scan should be applied to arrive at a proper diagnosis.[5]

Argyrophilic Nucleolar Organizer Region (AgNOR) staining is an easy, rapid and cost-effective method compared to other cell proliferative markers and is immensely helpful in the rapid diagnosis of breast lesion.[6]

This study was conducted to establish the role of AgNOR score as a cell proliferation marker to assess proliferative activity in breast lesions. This study also intended to look at the feasibility of the NORs as a tumour marker and prognostic indicator of neoplastic cells.

Material and methods

A prospective study was conducted over a period of one year (2019 - 2020). All patients with palpable breast lumps,

irrespective of age and gender, were included in the study. Autolysed specimens and post-treatment patients presenting with recurrence were excluded. Breast lump specimens collected primarily for Fine Needle Aspiration Cytology (FNAC) and histopathology were studied. All FNAC and histopathology slides were stained with AgNOR stain, and the score was recorded.

All FNAC and histopathology slides were stained with the AgNOR stain. AgNOR staining was performed as per the procedure proposed by Crocker and Ploton, and the score was recorded.

Cytological grading for breast carcinoma was done as per the grading proposed by Robinson et al. [7] Bloom and Richardson was followed for histopathological grading of breast carcinoma.[8] The original system was based on tubule formation, pleomorphism of nuclei and mitotic figures. This grading system was later modified by Nottingham as modified Scarff-Bloom-Richardson grading. Institutional Ethics Committee approval was obtained prior to the start of the study.

Nucleolar organizer regions (NORs) are chromosomal loops of DNA and proteins which transcribe to RNA. They are located at the ends of each of the acrocentric chromosomes 13, 14, 15, 21 and 22. They can be identified as black dots called 'Argyrophilic Nucleolar Organizer Regions (AgNORs)' on silver staining.[6]

AgNOR counts are related to cell cycle and are directly proportional to the cell proliferative activity. The number of AgNOR dots rise with increase in proliferative activity of cells. The number of AgNORs will therefore be significantly higher in malignant cells than in normal cells.[6] During the interphase, AgNORs are seen as clumped one to two nucleoli. Visualisation of AgNORs in a cell depends on number of AgNOR bearing chromosome & RNA transcriptional activity.[9]

Various methods have been used in tissue sections to know the proliferative index like DNA flurocytometry, in-situ hybridization, monoclonal antibodies etc, but these methods are time-consuming and are less cost effective.[10]

Analysis of AgNOR dots was done by estimating mean AgNOR count (mAgNOR), AgNOR proliferative index (pAgNOR), AgNOR size variation and distribution grading and Subjective AgNOR Pattern Assessment (SAPA). Mean AgNOR count (mAgNOR) estimation helps in assessing the proliferative activity. It is the mean count of the number of NORs present in the nucleus of 100 neoplastic cells. It is calculated as: mAgNOR = AgNOR count in 100 cells ÷ 100. AgNOR proliferative index (pAgNOR) is used to assess whether the cells are in synthetic phase. It is calculated as a percentage of neoplastic cells exhibiting more than five NORs within the nucleus of the 100 counted cells. Formula used is: pAgNOR = percentage of cells showing >5 nuclei in 100 cells.

Table-1 shows the grading system was used for recording AgNOR size variation and distribution grading.

Table 1: AgNOR size and distribution grading [11]

Size	Distribution	Grading
More or less uniform in size	Limited to the nuclei	0
Two different sizes	Occasional dispersion outside nucleoli	1
More than two different sizes	Moderate dispersion outside nucleoli	2
All grades and sizes including too minute to be counted	Widely dispersed throughout the nucleus	3

Subjective AgNOR Pattern Assessment (SAPA) scoring is shown in Table-2.

Table 2: Subjective AgNOR Pattern Assessment (SAPA) [12]

Criteria	SAPA SCORE
Number of dots per cell	
less than 2	1
2-5	2
more than 5	3
Variation of satellite size and shape	
Uniform	1
Moderate variation	2
Marked variation	3
Variation in cluster and shape	
Uniform	1
Moderate variation	2
Marked variation	3

To enhance visualization of AgNOR staining, 7% nitric acid was used for pre-treatment of slides which improved the image quality by decreasing background staining. [13] It has also been seen that better results could be obtained by incubating the slides in 1% gold chloride solution. [14]

Statistical analysis: Data was collected and entered in Microsoft Excel software. Data was analysed using Statistical Package for Social and Sciences (SPSS). Statistical tests included descriptive statistics and Chi-square test. Significance level was set at 0.05 ($p < 0.05$) at 95%

confidence interval. Results are presented in frequency distribution tables and statistical significance has been shown where applicable.

Result

Specimens from a total of 69 patients were included in the study. Majority of cases were in the age group of 36-55 years. Females constituted about 91.3% of the patients. Out of the 69 breast lesion specimens, 38 (55.1%) were malignant in nature, benign lesions constituted 23 (33.3%) and the rest (11.6%) were non-neoplastic lesions.

About 1/3rd (33.3%) of the breast lesions were in the upper outer quadrant and the least affected was the lower inner quadrant (11.6%). Table-3 shows the distribution of cases based on FNAC and histopathology diagnosis. There was very good correlation between FNAC and histopathology diagnosis in the broader classification – non-neoplastic / benign / malignant. However, the specific diagnosis showed some variation i.e., one case was diagnosed as breast carcinoma on FNAC, but was reported as ductal carcinoma in-situ on histopathology examination.

Table 3: Distribution of cases based on FNAC and histopathology diagnosis

	Type	FNAC	Histopath
Non-neoplastic	Gynaecomastia	5	3
	Gynaecomastia with FCD	1	2
	Fibrocystic disease (FCD){ fig-1}	1	2
Benign	Fibroadenoma	18	10
	Fibroadenoma with FCD	3	10
	Cellular fibroadenoma	2	3
	Phyllodes	4	4
Malignant	Malignant phyllodes {Fig-2}	1	1
	Ductal carcinoma in-situ	0	1
	Ductal carcinoma {Fig-3}	37	36
Total		69	69

In cytology, the average AgNOR count was 2.27 [Minimum - 1.64 / Maximum - 3.53] for non-neoplastic, 2.93 [1.36-4.95] for benign cases, and 5.94 [3.96-7.9] for malignant cases.

In histopathology, the mean AgNOR count was 2.19 (1.56-3.4) for non-neoplastic lesions (Fig: 1 &2), while the mean increased to 2.88 (1.46-3.89) for benign, and further increased to 5.94 (3.08-7.83) for malignant cases (Fig: 3, 4, 5 & 6). The AgNOR dots morphology was homogenous, symmetric and had regular contours in both FNAC and histopathology slides of benign breast lesions. However, in malignant breast lesions, the dots were asymmetric and had irregular contours. They were aggregated, smaller and more scattered.

The results also showed that there is no statistically significant difference in the AgNOR values of non-neoplastic / benign / malignant lesions from both FNAC and histopathology studies. It can therefore be inferred that AgNOR scores can be used as a cell proliferation and tumour marker in FNAC and histopathology examinations (Table-4).

These results show that all AgNOR parameters were elevated in malignant lesions compared to benign lesions. Also, all AgNOR parameters showed a statistically significant difference between benign and malignant lesions ($p < 0.05$).

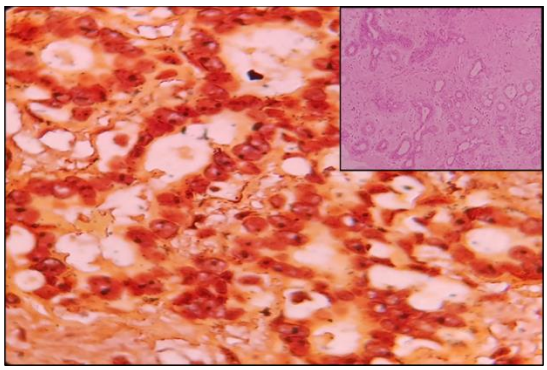

Figure 1: Fibrocystic disease, AgNOR, X100 (Insert- H& E)- Histopathology

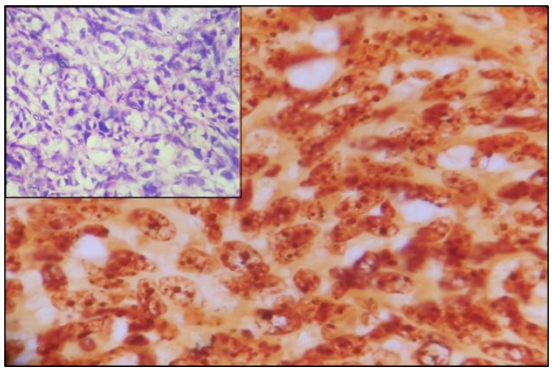

Figure 2: Malignant phyllodes, AgNOR X100 (Insert- H&E)- Histopathology

Table 4: AgNOR Parameters of Benign v/s Malignant Breast Lesions

AgNOR parameters	Benign lesions			Malignant lesions			P value
	Mean	SD	Range	Mean	SD	Range	
mAgNOR	2.88	0.66	1.46-3.89	5.94	0.98	3.08-7.83	<0.05*
PAgNOR	16.08	5.41	7-24	46.36	9.71	29-68	<0.05*
AgNOR size variation	0.61	0.70	0-3	2.13	0.41	1-3	<0.05*
AgNOR Distribution	0.61	0.70	0-3	2.13	0.41	1-3	<0.05*
SAPA score	3.22	0.66	2-8	5.91	1.35	2-8	<0.05*

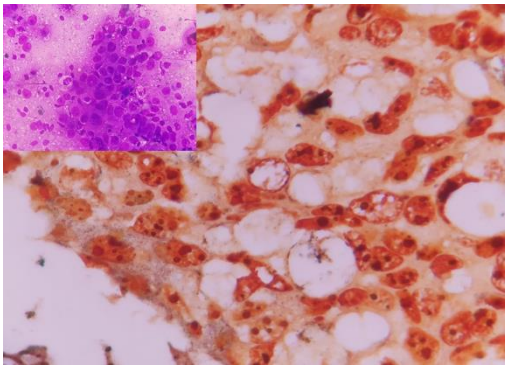

Figure 3: Infiltrating ductal carcinoma, AgNOR, X100 in histopathology (Insert- FNAC, MGG STAIN)

Discussion

Diagnosis and management of breast cancer is influenced by several morphological and biological aspects viz. type and grade of tumour, metastasis to lymph node, extent of atypical and presence or absence of specific tumour markers. Several studies have been carried out to establish the role of AgNOR score in cancerous lesions of breast, oral cavity, pancreatic head and glioblastoma etc, and have found significant correlation between aggressiveness and mitotic index of tumour with AgNOR counts. [15-18] . In this study, results from a total of 69 cases have been reported. Increase in AgNOR dots may be considered to have diagnostic and predictive significance while analysing the pathology of a tumour because of its direct relationship to the frequency of cell proliferation.

In the studies conducted by Nepal N et al, [6] Madhavi B V et al, [19] and Hasan T B et al, [11] mAgNOR scores on benign and malignant breast lesions were found to be 1.694 ± 0.329, 2.45 ± 0.05, 2.18 and 4.263 ± 0.5991, 4.76 ± 0.17, 5.64 respectively. Hasan T B et al [11] also reported mAgNOR scores of 1.52 on non-neoplastic breast lesions. Similar findings were seen in this study wherein mAgNOR in non-neoplastic, benign and malignant lesions were found to be 2.19, 2.88 and 5.94 respectively.

Hasan T B et al[11], Ansari H A et al[20], Darad D et al[21] reported pAgNOR scores of 4.98 ± 3.68, 4.0 ± 1.42, 20.47 ± 14.8 and 21.4 ± 13.99, 6.1 ± 2.16, 65.1 ± 15.1 in benign and malignant breast lesions respectively. In the present study pAgNOR values of benign and malignant lesions were 16.08±5.41 and 46.36 ± 9.71 respectively, which correlated well with other studies reported in the literature.

SAPA score in benign and malignant breast lesions has been reported as 4.57 ± 0.50, 3.72 ± 0.79 and 7.62 ± 1.06, 6.7 ± 0.8 respectively in studies conducted by Nepal N et al[6] and Joseph S A et al[22]. The results obtained in the present study also show similar SAPA scores i.e., 3.22 ± 0.66 in benign lesions and of 5.91 ± 1.35 in malignant lesions.

the present study, co-infection rate in our institute was 4%. Karaba S et al reported 1.1% rate of bacterial respiratory co-infection. Non-respiratory co-infections were 5% and urinary tract co-infection was the commonest in their study [8]. This was comparable to our findings and

Conclusion

In this study, AgNORs showed higher values in proliferative breast lesions than non-proliferative breast lesions. It may therefore be concluded that the mean AgNOR counts and other AgNOR parameters correlate with cell proliferative markers indicating benign / malignant potential of breast lesions. The cytology and histopathology grades correlated well with those of the AgNOR values. Based on these results, the use of AgNOR scores for assessing benign / malignant status of breast lesions, particularly in resource-constrained settings, may be recommended.

It should be noted that one of the limitations of this procedure is its inability to differentiate suspicious lesions of breast and may require a considerable time and effort to complete the entire process. Inter-observer variability also is a concern as that plays a major role in arriving at a final result.

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Corresponding Author:

Dr. Rumana Tasneem

2469, 5th Cross Gandhinagar

Mandya – 571401, Karnataka

drrumana.mims@gmail.com

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