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Original Article

Evaluation of AgNoR scores in aspiration cytology smears of breast lesions and their correlation with histopathology

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Keywords:

AgNOR;
SAPA;
Carcinoma;
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ABSTRACT

Background: Breast cancer is the leading cause of cancer-related deaths in Asia. The number of intra-nuclear silver stained structures, termed AgNORs, is significantly higher in malignant cells than in normal, reactive or benign cells. The main purpose of this study was to evaluate the AgNOR scores in aspiration cytology smears of breast lesions and their correlation with histopathology.

Materials and Methods: This was a prospective study conducted over a period of 24 months from October, 2009 to 2011. A total of 40 cases were included in the study, including fine needle aspiration and biopsy. AgNOR stain was done in both cytology and histopathology slides and scoring was done and analyzed.

Results: The mean AgNOR counts were 1.734 for benign cases and 4.508 for malignant cases. Statistically significant 'P' value < 0.05 for both benign and malignant cases was observed. The AgNOR dots morphology was homogenous, symmetric with regular contours in both FNAC and histopathology slides of benign breast lesion. In malignant breast lesions, the dots were asymmetric with irregular contours and were aggregated, smaller and more scattered.

Conclusion: The mAgNOR counts were significantly higher in smears from malignant breast lesions than in those from benign. These results were similar to those obtained for tissue sections and were comparable to established data. Both AgNOR counts and SAPA score gave similar results done in this study, indicating that SAPA is also as convenient, reproducible and rapid method of AgNOR evaluation.

INTRODUCTION

Breast cancer is the leading cause of cancer-related deaths in Asia and in recent years is emerging as most common female malignancy in the developing Asian countries, overtaking cancer of the uterine cervix.

Nucleolar organizer regions (NORs) are loops of DNA that encode ribosomal RNA. They are considered important in the synthesis of protein which are related to cell proliferation rate and transcriptional. The number of AgNOR dots rises with the increasing proliferative activity of cells. So, the number of AgNORs, should be significantly higher in

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Table 1: Descriptive values of AgNORs in both benign and malignant cases of FNAC

Type	Cases (n)	Minimum	Maximum	Mean	SD
FNA in Benign cases	11	12	2.16	1.734	0.29077
FNA in Malignant cases	29	3.3	6.04	4.508	0.9810

Table 2: Descriptive values of AgNORs in both benign and malignant cases of Histopathology.

Parameter	Cases (n)	Minimum	Maximum	Mean	SD
Benign Cases	7	1.2	2.16	1.7314	0.28115
Malignant Cases	20	3.3	5.84	4.263	0.5991

Table 3: Comparative studies of AgNORs in both benign and malignant cases of FNAC. (t-Test).

Cases	Numbers	Mean	Standard Error Mean	Standard deviation	P value
Benign Cases	11	1.736	0.0877	0.2908	0.001
Malignant Cases	29	3.3	0.1822	0.9810	

malignant cells than in normal, reactive or benign cells.

The development and continued growth of cancers involves altered rates of cell proliferation. In early breast cancer, measurement of proliferation can be used in conjunction with tumor size, grade, nodal status and steroid receptor status as a prognostic indicator. Proliferation rates can provide useful information on prognosis and aggressiveness of individual cancers and can be used to guide treatment protocols in clinical practice. Various techniques have been developed to evaluate and quantify proliferation rates in the laboratory such as mitotic activity, the S-phase fraction (SPF), Ki67 labeling index, MIB, proliferating cell nuclear antigen (PCNA), and other methods such as argyrophilic nucleolar organiser regions.[1] The main purpose of this study was to evaluate the AgNOR scores in aspiration cytology smears of breast lesions and their co-relation with histopathology.

MATERIALS AND METHODS

This was a prospective cross-sectional hospital based study conducted in the department of Pathology, Manipal Teaching Hospital, Pokhara over a period of 24 months from October, 2009 to 2011. A total of 40 cases were included in the study, which included fine needle aspiration and biopsy. All women above the age of 15, with proper clinical history and adequate material on fine needle aspiration cytology (FNAC) and biospy (lumpectomy/mastectomy) were included in the study. In all cases of FNAC samples, the slides were stained with Leishman stain and silver staining for AgNORs. Similarly histological specimens were processed routinely and stained with H&E and silver staining for AgNORs. AgNOR stained slides were analyzed for AgNOR dots.

Table 4: Comparative studies of AgNORs in both benign and malignant cases on Histopathology: (t-test).

Cases	Numbers	Mean	Standard Error Mean	Standard deviation	P value
Benign	7	1.694	0.1242	0.3286	0.001
Malignant	20	4.344	0.2247	1.0047	

Table 4: Comparative studies of AgNORs in both benign and malignant cases on Histopathology: (t-test).

Cases	Numbers	Mean	Standard Error Mean	Standard deviation	P Value
Benign	7	1.694	0.1242	0.3286	0.001
Malignant	20	4.344	0.2247	1.0047	

Table 6: AgNOR count and SAPA score in benign and malignant breast lesion

	AgNOR Count		SAPA score	
	FNAC	BIOPSY	FNAC	BIOPSY
BENIGN				
Mean ± SD	1.736± 0.2908	1.694±0.329	4.687± 0.403	4.5738±0.501
Range	(1.2-2.16)	(1.2-2.16)	(4-8)	(4-8)
MALIGNANT				
Mean ± SD	4.508± 0.981	4.263±0.5991	7.625±1.060	7.204±0.702
Range	(3.3-6.04)	(3.5-5.84)	(6-11)	(6-11)

Comparisons of the cytological and histopathological findings were made with AgNOR dots and were evaluated and counted in the slides taken as 50 cells' mean number and pattern. The mean number of dots was counted in both malignant and benign cells of both FNA and biopsy slides. The AgNOR dots morphology were divided into four groups:

a) Spherical, irregular, b) aggregate and c) scattered. SAPA score was also performed and every case was scored according to the scoring system.[2] Scores were assigned by keeping in mind the estimated number of dots, the size, and shape of dots, clusters and their variations form cell to cell. SAPA was estimated and found to be higher in malignant cases as compared to benign breast lesions.

RESULTS

A total 40 cases of breast lesions were included in the study. The age range was from 17 to 90 years. Highest number of breast cancer was observed in the group of 40-49 years, followed by 50-59 years. There were 29 cases of malignant breast tumors and 11 cases benign breast tumors. Benign cases included fibroadenoma (n=7, 73%), fibrocystic disease (n= 3, 20%) and acute mastitis (n= 1, 7%).

Of the 29 malignant cases diagnosed in FNAC, 20 cases were operated and sent for histopathological evaluation. Amongst the 20/29 cases of malignant breast tumors, 18 cases were of Invasive carcinoma, No special type, 1 case of mucinous

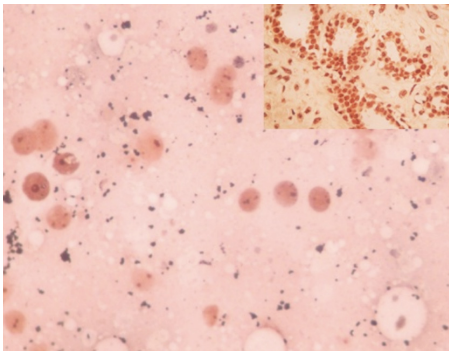

Figure 1: Photomicrograph showing FNAC and biopsy slide (inset) of fibroadenoma. Note the AgNOR dot with symmetrical and regular contour. (AgNOR stain, X100).

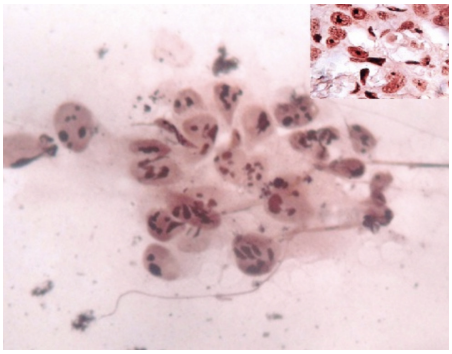

Figure 2: Photomicrograph showing FNAC and biopsy slide (inset) of invasive ductal carcinoma. Note the increase in numbers of AgNOR dots with scattered and irregular contour. (AgNOR stain, X100).

carcinoma and 1 case was of medullary carcinoma. In all 18 cases 100 % correlation was observed in both histological and FNAC. Amongst the 19 histopathological graded cases, grade 1 was the highest (n= 11), followed by grade 2 (n= 7) grade 3 (n=1).

Of the 11 cases of benign breast lesions in FNACs, 7 cases had histopathological correlation; fibroadenoma (n=5), fibrocystic changes and acute mastitis (each n=1).

AgNOR counts were performed in 40 cases of FNAC out of which 29 cases of malignant breast lesions and 11 cases of benign breast lesions. AgNOR counting's were also performed in 7 cases of benign breast lesions (fig. 1) and 20 cases of biopsy slides of malignant breast (fig. 2).

The mean AgNOR counts were 1.734 for benign cases and 4.508 for malignant cases. The descriptive values of AgNoRs dots in FNAC and Biopsy slides are shown in table 1 and table 2 respectively. Statistically significant 'P' value < 0.05 for both benign and malignant cases in FNAC as shown in table 3.

Similarly, table 4 illustrates the comparative study of AgNORs in both benign and malignant cases of HP (t-Test) which was statistically significant.

Statistically significant 'P' value < 0.05 for both benign and malignant cases in FNAC.

There were a total of 19 malignant cases graded according to Bloom-Richardson's guidelines. 11 cases were of grade-1, 7 cases of grade-2 and 1 case of grade-3. All descriptive values were possible for grade-1 and grade-2, but it was not possible for grade-3 because only 1 case was included in the study. Comparing the grades, we can see that in grade-1 the mean AgNOR counts were 4.2709 with 4.6971 in grade-2 which shows that in higher grade the mean AgNOR count was higher.

The AgNOR dots morphology were homogenous symmetric and had regular contours in both FNAC and histopathology slides of benign breast lesion. In the case of malignant breast lesions, the dots were asymmetric and had irregular contours. They were aggregated, smaller and more scattered. SAPA score was also performed and every case was scored according to the scoring system and was found to be significant in differentiating benign and malignant lesions (Table 6).

DISCUSSION

AgNOR technique is implicated to show the difference between benign and malignant cells of the particular lesions as they can be easily demonstrated in routinely processed cytological and histological sections.[3] AgNOR staining in aspiration smears of the breast is simple and feasible technique. There is very good discrimination between benign and malignant lesions.[4]

Majority of the dots were homogenous symmetric and had regular contours in both FNA and HP slides. The mAgNOR value obtained in our study was 1.734. There was not much difference in AgNOR counts of different types of benign lesions. We observed a slight increase in the AgNOR scores in cases of fibrocystic disease as compared to fibroadenoma and acute mastitis. The mAgNOR values obtained in our study are in concordance with the observations of other authors.[5,6]

In a study done by Ansari H et al observed a slight increase in cases of fibrocystic breast disease in comparison to fibroadenoma.[5,6] Dube MK and Govil A[7], in their study have also reported a similar AgNOR count of 1.2 with the range of 1.0-1.5, whereas Rajeevan K et al[4], have reported AgNOR count of 2.8 with the range of 0.7-3.0. Their study stated that distribution of AgNOR counts in different types of benign lesions did not show significant differences.

In the case of malignant breast lesions, the dots were asymmetric and had irregular contours. They were aggregated, smaller and more scattered. The AgNOR counts tend to increase with increasing nuclear grades of malignancy. In our study the AgNORs were significantly higher in the malignant cases, being 4.508 with the range of 3.3 -6.04.

Ansari et al[8] also have reported mean AgNOR count of of 4.0. Simha A et al[9], Kim A et al[10] and Kumar A et al[11], have also reported a mean AgNOR count for malignant lesion as 3.5, 5.09 and 6.57 respectively. These studies show a definite discrimination between benign and malignant lesions, but some studies have reported the overlap of the values.[4] The overlap was reported to be due to occasional malignant cases with low AgNOR counts and occasional benign cases with high counts.[4] However we had no overlap of AgNOR count in our study.

Amongst the different histological types of carcinoma in our study, medullary carcinoma showed lower mAgNOR of 3.5 as compared to IDC showing highest of 5.68.

In a comparative study of benign and malignant breast lesions in FNAC, there was a significant difference of mAgNOR with a p value of less than 0.001 which was statistically significant. Similar finding was observed by other study.[12]

Similarly in histopathology specimens the mean AgNOR counts for benign cases were 2.16 whereas it was 5.84 for malignant breast lesions. Similar findings were observed by other studies.[4,12] In this present study, AgNOR counts were correlated with tumor grade in malignant breast lesions. The mean AgNOR count for grade-I was 4.2079 and grade-II was 4.6971.

Kazuhiko H et al[13],carried out a study and analyzed AgNOR in a total of 76 patients with breast carcinoma that included 33 cases of IDC in which the mean AgNOR count for grade-I was 4.71 +/- 1.17, in grade-II it was 4.38 +/- 1.41 and in grade-III it was 5.42 +/- 1.63. No significant difference between the various grades was in his and our studies.

Regarding the polymorphism of AgNOR dots, in our study, the shape of AgNOR dots in benign and malignant lesions showed different patterns. Dots were homogenous, symmetric and had regular contours in the cases of benign breast lesions. Whereas, in the case of malignant breast lesions, the dots were asymmetric and had irregular contours, they were aggregated, smaller and more scattered.

Khanna AK et al studied 24 cases of carcinoma and found that SAPA as an important diagnostic tool to differentiate between benign and malignant lesions. SAPA scoring for normal was 5.2 whereas for carcinoma it was 8.0.[2] In another study done by Akhtar GN, SAPA score was helpful in differentiating benign from malignant cells.[14]

The average area and distribution of the dots were more discriminating between the benign and malignant lesions than the simple dot count. In benign lesions, there was uniform small compact centrally placed dot and large irregular marginally located dot in infiltrating ductal carcinoma.[15] In contrast, counting of every single AgNOR dot is laborious and less reproducible. Analysis of the AgNOR dots using computer assisted image analysis has been introduced. Image analysis provides information about the size and distribution of AgNOR. It seems that, the addition of size or area measurements using image analysis gives improved diagnostic and prognostic specificity. Image analysis proved to be advantageous over AgNOR counting alone as it facilitated the standardization of the AgNOR technique itself.[16-18]

CONSLUSION

The mAgNOR counts were significantly higher in smears from malignant breast lesions than in those from benign. These results were similar to those obtained for tissue sections and were comparable to established data. Both AgNOR counts and SAPA score gave similar results done in this study, indicating that SAPA is also as convenient, reproducible and rapid method of AgNOR evaluation.

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