CYTOLOGICAL AND HISTOLOGICAL CORRELATION OF BENIGN AND MALIGNANT LESIONS WITH DIFFERENTIATION OF BENIGN AND MALIGNANT LESIONS BY AgNOR COUNTS

K. Mohini Rao[1], Aparna Behura[2]

[1]Consultant Pathologist and Assistant Professor, Department of Pathology, Hi-Tech Medical College, Bhubaneswar, Odisha.
[2]Consultant Pathologist and Associate Professor, Department of Pathology, KIMS Medical College, Bhubaneswar, Odisha.

ABSTRACT

BACKGROUND

Traditional approach to pathological diagnosis includes histopathological examination of paraffin sections stained with a variety of histochemical techniques. These routine approaches may be disappointing at times in differentiating benign from malignant lesions and occasionally fail to render a precise diagnosis.

There has been a growing interest in the study of DNA and proliferation markers. One of the most recent studies is on Nucleolar Organiser Regions (NORs) using a simple silver reduction technique (AgNOR) method.[1]

Aim of the Study- This study was conducted to correlate cytological and histopathological diagnosis of breast lumps, to categorise lesions as benign or malignant and to evaluate the AgNOR counts in differentiating benign and malignant tumours.

MATERIALS AND METHODS

Eighty-four patients referred to Department of Surgery in Jawaharlal Nehru Medical College, Aligarh were screened for this study. The study was conducted from April 2000 to September 2001 over a period of 17 months. Patients were counseled, and an informed consent was taken. Upon enrolment and clinical examination, the patients were subjected to FNAC of breast and lymph node, if present and surgical excision with histopathology wherever possible. The data is presented as mean and standard deviation. All the observations of AgNOR count were statistically evaluated by using students' 't' test.

RESULTS

In our study, 80 (95.2%) cases were female and remaining 4 (4.8%) cases were male. There were 32 (38.1%) cases of benign lesions and 52 (61.9%) were malignant lesions. The range of AgNOR counts in benign lesions were found to be 1.9-3.2 (2.3±0.26). The range of AgNOR counts in malignant lesions was found to be 4.9-6.8 (5.37±0.3). There was significant statistical difference ($p < 0.005$) between AgNOR counts in benign and malignant lesions.

CONCLUSION

This study concludes that AgNOR counts and dispersion pattern of AgNOR dots have a diagnostic value in distinguishing benign from malignant lesions of breast.

KEYWORDS

AgNOR Counts, Breast Tumour, Cytological Diagnosis, Histopathological Diagnosis.

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BACKGROUND

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Corresponding Author:
Dr. Aparna Behura,
Chandrama Complex,
C1/8, Kharvel Nagar, Unit- 3,
Bhubaneswar- 751001, Odisha.
E-mail: aparnabehura20@gmail.com
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There has been a growing interest in the study of DNA and proliferation markers. One of the most recent studies is on Nucleolar Organiser Regions (NORs) using a simple silver reduction technique (AgNOR) method.[1]

The NORs are loops of ribosomal DNA located in the short arms of acrocentric chromosomes 13, 14, 15, 21 and 22, transcribe to ribosomal RNA.[2] The NORs vary in size and shape according to nucleolar transcription. They are intimately related to the cell cycle and may be related to proliferation and ploidy. Recently, some studies showed that NORs are significantly higher in malignant cells than normal cells.[3]

The AgNORs are argyrophilic proteins. Binding of silver and protein occurs at carboxyl and sulfhydryl groups by colloidal precipitation of ionic silver. The carboxyl groups on the protein reduce the silver solution, forming micronuclei of silver. The larger aggregates of silver get deposited at

disulphide and sulfhydryl group sites. These are seen in light microscopy as black intranuclear granules.[4]

Most diseases of breast present as palpable masses, inflammatory lesions, nipple secretions or mammographic abnormalities. Lesions in the breast often pose a diagnostic dilemma. It has always created a great interest amongst surgeons, pathologists and radiologists. The primary challenge in the management of common breast lesions is to differentiate benign lesions from malignant ones.

The basic diagnostic methods for detection of breast cancer are cytopathological, histopathological studies and AgNORs. The number, size and clusters of AgNORs in tumour nuclei help in differentiating benign and malignant lesions, and also the grade of malignancy.

Aim of the Study

This study was conducted to correlate cytological and histopathological diagnosis in breast lumps, to categories lesions as benign or malignant and to evaluate the AgNOR counts in differentiating benign and malignant tumours.

MATERIALS AND METHODS

Eighty-four patients referred to Department of Surgery in Jawaharlal Nehru medical college, Aligarh were screened for this study. The study was conducted from April 2000 to September 2001 over a period of 17 months. The patients were counseled and an informed consent was taken. Upon enrolment and clinical examination, the patients were subjected to FNAC of breast and lymph node, if present and surgical excision with histopathology wherever possible. The data was presented as mean and standard deviation, which was calculated with the help of an electronic calculator. All the observations of AgNOR count were statistically evaluated by using students' 't' test.

RESULTS

This study was conducted on cytological smears and histopathological sections of breast tumours to determine the value of AgNOR count in differentiating benign from malignant tumours in Department of Pathology, of Jawaharlal Nehru medical college, Aligarh from April 2000 to September 2001.

Age Groups (yrs)	Total Cases	Benign (%)	Malignant (%)
11-20	4	4 (12.5)	0 (0.0)
21-30	17	12 (37.5)	5 (9.7)
31-40	30	11 (34.5)	19 (36.6)
41-50	11	1 (34.5)	19 (36.6)
51-60	18	3 (9.3)	15 (28.2)
61-70	3	1 (3.1)	2 (3.8)
71-80	1	0 (0)	1 (19)
Total	84	32 (100)	52 (100)

Table 1. Distribution of Cases in Relation to Age

Sex	Number of Cases	Percentage of Cases
Female	80	95.2
Male	4	4.8
Total	84	100

Table 2. Distribution of Cases in Relation to Sex

Sl. No.	Diagnosis	Total Cases	FNAC	Histopathology	Histopathological Diagnosis
1.	Nodular Adenosis	1	-	1	1 Nodular Adenosis
2.	Fibroadenoma	22	22	12	11 Fibroadenoma 1 Duct Ectasia
3.	Fibrocystic Disease	6	6	4	4 Fibrocystic Disease
4.	Phylloides Tumour	1	1	-	-
5.	Gynaecomastia	3	3	3	3 Gynaecomastia
6.	Ductal Carcinoma	48	48	44	40 Ductal Carcinoma 2 Lobular Carcinoma 2 Medullary Carcinoma
7.	Medullary Carcinoma	1	1	1	1 Medullary Carcinoma
8.	Sarcomatoid Carcinoma	1	-	1	1 Sarcomatoid Carcinoma
9.	Pylloides Tumour (Malignant)	1	-	1	1 Phylloides Tumour
10.	Ductal Carcinoma	1	1	1	1 Ductal Carcinoma
Total		85	82	68	

Table 3. Distribution of Cytopathologic and Histopathologic Diagnosis

One case of duct ectasia excluded as inflammatory lesion.

Sl. No.	Diagnosis	No. of Cases (%)	AgNOR Counts/Cell	
			Range	Mean ± SD
1.	Nodular adenosis	1 (3.1)	2.6	
2.	Fibroadenoma	21 (65.7)	1.9 - 2.6	2.06 ± 0.11
3.	Fibrocystic disease	6 (18.8)	2.5 - 3.2	2.78 ± 0.28
4.	Phyllodes tumour	1 (3.1)	3.2	
5.	Gynaecomastia	3 (9.3)	2.6 - 2.8	2.66 ± 0.6
Total		32 (100)	1.9 - 3.2	2.3 ± 0.26

Table 4. Distribution of AgNORs in Benign Lesions

Sl. No.	Diagnosis	No of Cases (%)	AgNOR Counts/Cell	
			Range	Mean±SD
1.	Ductal carcinoma	44 (84.6)	4.9 - 6.8	5.33 ± 0.35
2.	Lobular carcinoma	2 (3.9)	4.9 – 5.1	5.00 ± 0.2
3.	Medullary carcinoma	3 (5.8)	5.5 – 5.7	5.5 ± 0.5
4.	Sarcomatoid carcinoma	1 (1.9)	5.8	
5.	Phylloides tumour (malignant)	1 (1.9)	5.6	
6.	Ductal carcinoma (Male)	1 (1.9)	5.8	
	Total	32 (100)	4.9 – 6.8	5.37 ± 0.32

Table 5. Distribution of AgNORs in Malignant Lesions

Diagnosis	No. of Cases (%)	AgNORs / Cell		P values
		Range	Mean ± SD	
Benign	32 (38.9)	1.9 – 3.2	2.3 ± 0.26	< 0.005
Malignance	52 (61.9)	4.9 – 6.8	5.37 ± 0.32	
Total	84 (100)			

Table 6. Comparison of Mean AgNOR Counts of Benign and Malignant Tumours of Breast

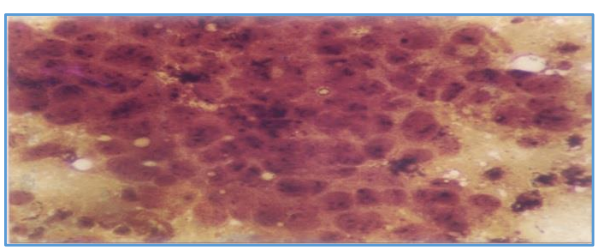

Figure 1. Fibroadenoma: Cy to. AgNOR x 500: - 1-2 Clusters and Satellite AgNOR Dots Regularly Distributed in Each Nucleus

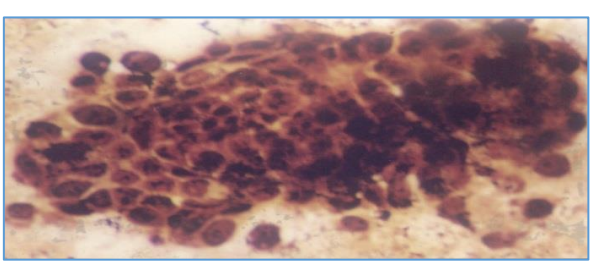

Figure 2. Fibrocystic Disease: Cy to. AgNOR x 500: - 1-2 Centrally Placed Large Clusters and a Few Satellite AgNOR Dots

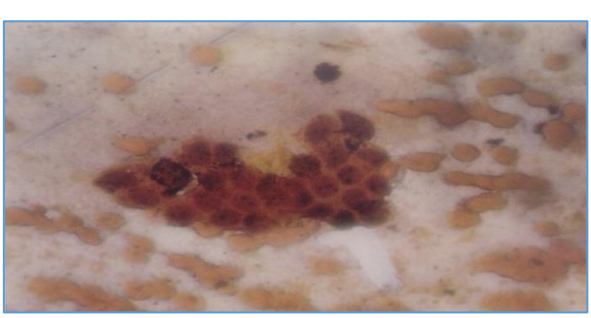

Figure 3. Phyllodes Tumour: Cy to. AgNOR x 500: 1-2 Clusters of AgNOR Dots with Uniform Satellite Dots. One Nucleus Shows 6 Clusters Distributed Evenly

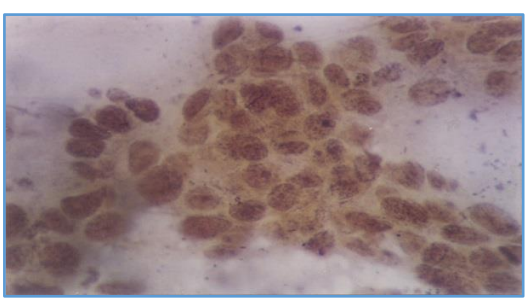

Figure 4. IDC (Grade I): Cy to. AgNOR x 500: Increased AgNOR Dots, Clusters and Moderate Variation of Satellite Size Seen in Nuclei

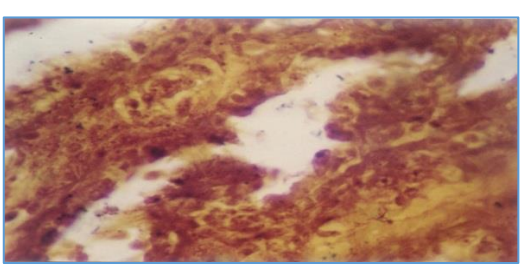

Figure 5. Infiltrating Lobular Carcinoma: Histo. AgNORx500: A Few Large AgNOR Clusters and Many Satellite Dots with Moderate Variation of Size

DISCUSSION

In our study, benign lesions showed a mean AgNOR count of 2.3±0.26, which was similar to the findings of Raymond et al. (1989) and Giri et al. (1989).[5,6] They observed a mean of 2.1±0.6 and 1.9±0.3 respectively. Chen et al. (1992), reported that benign lesions had AgNORs of <3 per nucleus. We found the total AgNOR count in Nodular adenosis with apocrine metaplasia (2.6) was higher than that of fibroadenomas (2.06±0.11). This was similar to the

observations of Smith and Crocker (1987), that the total AgNOR count of tumours with apocrine metaplasia was 6.7 and fibroadenoma was 4.9.[7] The AgNOR count didn't correlate probably due to a different counting system followed by them.

It was seen that the mean AgNOR count in fibrocystic disease (2.76±0.28), was slightly higher than fibroadenoma (2.06±0.11). Similar observations were made by Pande and Malhotra (1994), who found AgNOR count in fibroadenoma of (1.77±0.18) and fibrocystic disease of (2.32±0.26).[8]

The total AgNOR count of lone case of benign phyllodes tumour in the present study was 3.2, which was higher than the finding of Rajeevan et al. (1995), of 2.7.[9]

Dube and Govil (1995), found a mean AgNOR of 3.7 in gynaecomastia, which showed a higher value then our count of 2.66±0.6.[10]

We observed a mean AgNOR count of (5.37±0.32) in malignant lesions. This count was found statistically significant when compared to the benign lesions. Our count was similar to findings of Raymond et al. (1989) i.e. (5.5±2.3) and Chen et al. (1992), who observed that >5 AgNORs per nucleus was malignant.[5] The results were statistically closer, if not similar to the findings of Giri et al. (4.4±1.2).[6] We found a significant increase in AgNOR counts in malignant lesions. Our result was comparable to Meehan et al. (1992), even though their count was relatively higher i.e. (9.52±2.2).[11] Mourad et al. (1992) recorded a mean AgNOR count of 2.97 in malignant lesions, which was higher than mean AgNOR count of our benign lesions (2.3±0.26), but rather low as compared to the count in malignant lesions.[12] This variation in count is due to the subjective variation in counting technique by various authors.

We found that the mean AgNOR count in infiltrating ductal carcinoma (5.33±0.35), lobular carcinoma (5.0±0.1) and medullary carcinoma (5.5±0.5), were almost similar, although lobular carcinoma had a relatively low count. So, it was not possible to differentiate breast cancers histologically based on AgNOR count. Smith and Crocker (1989) found very high mean AgNOR count in infiltrating ductal carcinoma (16.9) and lobular carcinoma (6.9), with overlapping of range of AgNOR count.[7] Rajeevan et al. (1995) found that mean AgNOR count in infiltrating ductal carcinoma was (5.8), higher than lobular carcinoma (4.2) and medullary carcinoma (4.8).[11] They inferred that infiltrating carcinoma had a higher count than special type of carcinomas, in contrast to our findings.

In our study, the range of AgNOR counts in benign lesions were in the range of 1.9-3.2 and malignant lesions were 4.9-6.8. So, the diagnostic utility of AgNOR counts in individual cases is restricted by a broad zone (3.2-4.9), between benign and malignant lesions. This finding is statistically significant ($p < 0.005$).

In our study, the count of AgNOR dots, the size, shape and distribution were also studied in different lesions. In benign lesions, a mixture of uniform looking small regular dots, with occasional clusters and in fibrocystic disease 1-2 small centrally placed cluster were seen. In malignant lesions, the dots were mostly in clusters with a few satellite dots arranged at the periphery of the nucleus. Similar findings were observed by Meehan et al. (1994).[11] Giri et al. (1989), also described large central AgNOR dots with other regular dots in benign lesions with apocrine metaplasia.[6] So on comparison of benign and malignant lesions, the appearance of AgNOR dots revealed much variability in malignant lesions.

Increased AgNOR counts were seen in malignant lesions as compared to benign lesions. Smith & Crocker (1987), Giri et al. (1989), Raymond et al. (1989), Chen et al. (1992), Meehan et al. (1994) and Rajeevan et al. (1995) had similar findings.[5,6,7,9,11] We found no difference of AgNOR counts in various types of breast carcinomas. Smith and Crocker (1987) and Giri et al. (1989) had similar observations.[6,7]

CONCLUSION

This study concludes that AgNOR counts and dispersion pattern of AgNOR dots have a diagnostic value in distinguishing benign from malignant lesions of breast. Our study strongly suggests that, the quick and inexpensive AgNOR stain can be a useful adjunct to conventionally stained cytologic smears and histologic sections.

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