

ORIGINAL ARTICLE

SILVER STAINED NUCLEOLAR ORGANIZER REGION COUNT (AGNOR COUNT) – VERY USEFUL TOOL IN BREAST LESIONSJaymin Bhatt¹, Gaurishanker Shrimali², Tarak Patel¹, Sadhna Sarvaiya³, Darshna Modha⁴, Maitrey Gajjar⁵

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ABSTRACT

Introduction: Accurate histopathological typing, grading and staging of a tumour is of proven value in clinical management as well as prognostic outcome of cancer patients. But many times histopathological assessment correlates poorly with clinical outcome. These limitations have motivated development of new technique so as to improve accuracy and reproducibility of prognostication.

Method: One of the newer techniques is “Silver stained nucleolar organizer regions (AgNORs)” which is cheap, simple used to assess its role in histopathological diagnosis and prognosis of disease of breast lesion cases.

Results: There is high AgNOR count in malignant breast tumours as compared to benign lesions. Characteristic morphological changes are observed in benign and malignant lesions. Morphological variation can also be used as diagnostic as well as prognostic parameter.

Conclusion: The newly invented simple technique of AgNOR staining and its proven predictability may become a trustworthy milestone.

Key Words: AgNOR, NOR Proteins, Benign Breast Lesions, Malignant Breast lesions

INTRODUCTION:

Histopathological typing, grading and staging of a tumour is of proven value in the clinical management as well as prognostic outcome of the cancer patients. But many times histopathological technique may not reveal all markers of the prognostic importance.

These limitations have motivated development of new technique like “Silver Stained Nucleolar Organiser Regions (AgNOR) for histopathological diagnosis as well as prognosis of the diseases¹.

Crocker and Nar in 1987² had directed attention to this novel tool for diagnostic histopathology. Nucleolus plays a vital role in the control of cell proliferation and protein synthesis. Rapidly dividing cells and cells with high mitotic activity have prominent nucleoli. Malignant cells possess large irregular nucleoli.

Nucleolar Organiser Regions (NORs) -segments of DNA, closely associated with nucleoli containing coding gene for Ribosomal RNA and contribute to the regulation of the cellular synthesis.

Recent modification of a silver staining technique allows NORs to be visualized in conventional histopathological sections-where they are called as “Argyrophilic Nucleolar Organizer Regions (AgNORs)”.

Malignant cells contain more AgNORs than in normal, benign or reactive cells. The size and number of the AgNORs might reflect cell proliferation, transformation and even overt malignancy.

MATERIAL AND METHOD

The present study consist of the prospective examination of 100 consecutive cases of benign breast lesions both benign and malignant. 10 normal breasts were also included as control obtained from the autopsy. The selection of the patient was on the basis of admission in tertiary care hospital in Gujarat during the period from 1st January 2012 to 31st December 2012. Lumpectomy was done and tissue was sent to the pathology department. The routinely formalin fixed tissue were processed to the paraffin embedding technique and H & E staining and histopathological reporting was done. The blocks were also cut with very sharp knife to get 3 micron sections for the Silver Staining Technique. We followed the staining procedure of Ploton et al.1986.

AgNOR Method

Principal: Acidic proteins are present on the active

ribosomal gene of interphase nucleoli as well as on the NORs of metaphase chromosomes. These proteins are selectively stained with silver.

The specificity of this Argyrophilia of NORs had been confirmed by means of ultra structural studies by fluorescent probes. There is identical localization of argyrophilia and fluorescent tagged antibodies to certain NORs.^{3,4}

Technical Method^{5,6}:

(1)The routinely processed 3 micron sections were de-waxed by two changes of Xylene, 15 minutes each and rehydrated.

(2)The slides were downed to double glass distilled water for 8 to 10 minutes.

(3)AgNOR staining solution:

Solution A: 50 % Aqueous Silver nitrate solution was prepared by dissolving 5 gms of aqueous silver nitrate in 10 cc of double glass distilled water .It was filtered through 0.22 micron Millipore Filter and placed in dark before it was used to prepare working solution .

Solution B: Gelatin 2 gm% w/v was prepared by dissolving 2 gms gelatin in 99 ml of double glass distilled water in a water bath at 60 to 70o C. One ml pure formic acid was added to above prepared gelatin solution.

Working Solution: Finally the working solution was prepared by mixing both the solution in Coplin Jar in the following proportion - Solution A: 2 parts and Solution B: 1 part. This mixture was kept in dark till light brown colour developed.

(4)The section were then incubated in working solution for 30 to 35 minutes in dark. Then sections were washed with running double glass distilled water for 10 to 15 minutes, dehydrated and mounted with DPX.

Finally the AgNOR count was done counting nuclei of 100 cells under oil immersion lens. In this clusters of black dots within nucleoli were counted as one AgNOR and dispersed dots throughout the nucleus were counted as discrete AgNORs.

In each case, the mean number of total AgNOR dots per case was calculated. The results were assessed by analysis^{7,8} to decide the significance.

OBSERVATION

In this study total 100 cases were taken (Non-neoplastic breast lesions 14, Benign breast tumours 27, Malignant breast tumours 59) along with 10 control cases (in form of normal breast tissues-samples derived from the autopsy cases).

Table 1: Distribution of Cases included in Present Study

	Cases in various AgNOR Count Groups						Total	
	0-0.99	1-1.99	2-2.99	3-3.99	4-4.99	5-5.99		6-6.99
Control Group	05	05	-	-	-	-	10	
Non-neoplastic Group	-	1	10	3	-	-	14	
Benign Tumors	-	1	15	8	3	-	27	
Malignant Tumors	-	-	-	-	20	24	15	59

There is an overlap of AgNOR count in the region of 4-4.99, between benign and malignant tumour cases, where determination of the type of lesion purely on the basis of AgNOR count becomes difficult.

Result of benign and malignant breast lesions have been tested as per “Z” test which showed that observed difference in the mean value of benign and malignant breast lesions is statistically highly significant... Results of malignant breast lesions with and without metastasis also have been tested as per student’s test-which showed that observed difference in mean AgNOR count of malignancy with and without metastasis is statistically significant.

DISCUSSION

NORs have been applied to histopathology for investigating malignant tissue in man. It has been found that the number of AgNORs in nuclei may reflect grade of malignancy.

Flowcytometric DNA analysis and other techniques can not be adopted routinely as prognostic determinants⁹ as

they require costly equipments. AgNOR staining technique is simple and useful method, in the diagnosis, grading and assessment of prognosis of neoplastic lesions for the onco-pathologist.

We have applied the AgNOR staining, to study the scores in breast tumours.

This consists of small discrete intra nuclear dots and large structures resembling nucleoli which may stain uniformly or be seen as aggregates of dots within them.

Smith and Crocker¹⁰ in 1988 tried to enumerate the number of AgNOR clusters per nucleus, the mean number of AgNORs per clusters and the number of satellite AgNORs in breast lesions and derived total-AgNOR count. Morphologically inseparable clusters were considered as a single dot.

AgNOR count in Malignant tumours with metastasis were average 5.55 (SD 0.56) and p value is significant (<0.05) while AgNOR count in Malignant tumours without metastasis were average 4.82 (SD 0.68) and p value is significant (<0.05).

Malignant tumours with metastasis show high AgNOR

score in comparison to those without metastasis.

In contrast to the small round regular AgNOR, typically seen in benign lesions, large, irregular, often angulated, AgNOR were frequently observed in malignant tumours.

CONCLUSION

From the present study it is concluded that the number of AgNOR count steadily increases from normal breast to the various lesions. There is strikingly high AgNOR count in malignant breast tumours as compared to benign breast lesions and the count is in higher range of malignant group having affected lymph nodes and difference obtained in the values are statistically significant. The AgNOR count increases as the grade of malignancy increases. Hence it is having predictable prognostic significance. Observed characteristic morphological variations can also be used as prognostic parameter. The newly invented simple technique of AgNOR staining and its proven predictability may become a trustworthy milestone. However still much is to be learned about this new enigmatic organel.

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