Original Article

AgNOR STAINING IN MALIGNANT AND BENIGN EFFUSIONS

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ABSTRACT

Objective: To evaluate the correlation of argyrophilic nucleolar regions(AgNORs) and malignancy in benign and malignant effusions.

Design: The study group consisted of pleural and peritoneal effusion samples obtained from patients suffering from various benign or malignant diseases. The cytological smears were studied by conventional haematoxylin & eosin(H&E) and silver staining for AgNORs.

Setting: The samples were obtained from patients admitted in Mayo Hospital, Services Hospital, Gulab Devi Chest Hospital and Institute of Nuclear Medicine and Oncology (INMOL).

Subjects: One hundred cases having either pleural or peritoneal effusions were selected. Fifty of these cases were positive for malignant cells and fifty had reactive mesothelial cells in them.

Main Outcome Measures: Assessment of AgNOR count as a diagnostic marker of malignancy.

Results: AgNOR count was helpful in differentiating benign from malignant cells. AgNOR count in malignant cells was 10.62 ± 3.36 and 3.04 ± 0.64 in reactive mesothelial cells.

Conclusion: AgNOR count is a rapid, easily reproducible method of differentiating reactive mesothelial cells and malignant cells.

KEYWORDS: Cytology; Effusions; AgNOR count.

Pak J Med Sci January-March 2004 Vol. 20 No. 1 29-32

INTRODUCTION

Identifying tumour cells reliably in peritoneal and pleural effusions is a well known diagnostic problem.[1] It is not always possible to distinguish neoplastic cells from macrophages and particularly, reactive mesothelial cells on purely morphological grounds.[2] A number of tests including DNA flow cytometry, Restriction Enzyme Fragment Length Polymorphism (RFLP), PCR Sequencing, monoclonal and polyclonal antibodies have been employed to distinguish the benign from the neoplastic cells.[3,4,5]

A comparatively simpler technique used for this purpose is the silver staining of nucleolar organizer regions (NORs).

Interphase AgNORs are the structural and functional units of the nucleolus which contain all the essential components for the synthesis of ribosomal RNA. In the human karyotype, NORs are located in each of the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22.[6] The two argyrophilic proteins which are associated with rRNA transcription and processing are nucleolin and

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* *Received for publication:* July 31, 2003

Accepted: October 31, 2003

nucleophosmin.[7] These proteins are argyrophilic and are easily stained by silver stains. After silver-staining, the NORs can be identified as black dots present throughout the nucleolar area. The number and size of NORs reflect cell activity, proliferation and transformation and help to distinguish benign from malignant cells.[8] Evaluation of the quantitative distribution of AgNORs has been applied in tumour pathology both for diagnostic and prognostic purposes. A number of studies carried out in different tumour types demonstrated that malignant cells frequently present a greater AgNOR count than corresponding non-malignant cells.[9,10]

In the present study, this technique has been applied to differentiate malignant cells from reactive mesothelial cells in pleural and peritoneal effusions.

PATIENTS AND METHODS

One hundred cases of effusions were selected. Fifty of these were positive for malignant cells and the other set of 50 contained reactive mesothelial cells. The smears were stained by H&E and silver stains. H&E staining was undertaken to differentiate between frankly malignant cells and reactive mesothelial cells.

For AgNOR staining, gelatin was dissolved in 1% formic acid to make a 2% solution. 50% aqueous silver nitrate was then added in a proportion of 1:2 to obtain the working solution. The smears were postfixed in 3:1 ethanol:acetic acid mixture. They were brought to water through graded alcohols, covered with filter paper and soaked drop-wise by the working solution. The smears were kept in the dark for 30 minutes in a humid chamber, washed with deionised water, dehydrated, taken to xylene and mounted.

The AgNORs were counted in 100 nuclei of malignant or mesothelial cells in malignant and benign effusions respectively, and the mean AgNOR counts were recorded. Their distribution and variation in size was also recorded by using the criteria of Ahsan et al.[11] Student's t-test was applied for the statistical analyses of the results.

Size Variation was graded as follows:

0 =More or less uniform in size.
1+ =Two different sizes.
2+ =More than two different sizes (but not those of 3+).
3+ =All grades and sizes including two minute to be counted.

Distribution of AgNORs in the nuclei were graded as follows:

0 =Limited to nucleoli.
1+ =Occasional dispersion outside nucleoli.
2+ =Moderate dispersion outside nucleoli.
3+ =Widely dispersed throughout the nucleus.

RESULTS

The AgNORs appeared as black discrete dots in a pale yellow staining nucleus. Table-I shows that the mean AgNOR counts in malignant effusions was significantly higher as compared to the AgNOR counts in benign effusions.

Table-I: Comparison of AgNOR counts in malignant & non-malignant effusions

Groups	*Mean AgNOR Counts / Cell*		
	Range	*Mean*	*± SD*
Malignant Effusions	4.04-19.82	10.62*	± 3.36
Non Malignant Effusions	2.12-4.62	3.04	± 0.64

*p< 0.001 (Significantly higher as compared to non malignant effusions)

AgNOR size and distribution were of a significantly higher grade (p<0.001) in the malignant pleural and peritoneal effusions as compared with non-malignant effusions. (Tables II & III).

Table-II: Comparison of AgNOR size in malignant and non-malignant effusions

Groups	*No. of cases*	*AgNOR size 0 to 1+*	*AgNOR size 2+ to 3+*
Malignant Effusions	50	9	41*
Non Malignant Effusions	50	46	4
Total	100	55	45

*p<0.001 (Significant as compared to non malignant effusions)

Table-III: Comparison of AgNOR distribution in malignant & non-malignant effusions

Groups	*No. of cases*	*AgNOR distribution 0 to 1+*	*AgNOR distribution 2+ to 3+*
Malignant Effusions	50	4	46*
Non Malignant Effusions	50	47	3
Total	100	51	49

*p<0.001 (Significant as compared to non malignant effusions)

DISCUSSION

Many studies for the evaluation of AgNORs have been conducted on different benign and malignant tissues of the human body, demonstrating that malignant cells frequently present a greater AgNOR number than benign cells.[12,13] Furthermore, a great deal of attention has been focused on AgNOR count in cells present in effusions and different methods have been devised to evaluate them. AgNOR silver staining technique has also been employed on paraffin embedded cell blocks of benign and malignant effusions. Other techniques, alternative to visual assessment, like back scattered electron imaging have also been utilized to study a number of parameters pertaining to AgNORs, including calculation of the total AgNOR area. This technique also yielded a significant difference between the benign and malignant cells, with the mean AgNOR area of malignant cells being greater than that of benign cells.[14]

It was seen that AgNOR staining and visual counting proved to be a much simpler technique than the above mentioned procedures and was adopted in a number of studies. A study conducted on pleural effusions reported that the mean AgNOR count in malignant cells present in pleural effusions was significantly higher than that in benign mesothelial cells.[15] Other workers have also documented similar findings.[16,17]

The results of our study, show that the AgNOR number permits a clear distinction to be made between malignant and benign cells. Reactive mesothelial cells have a lesser number of AgNORs as compared to malignant cells. The evaluation of AgNORs has been further elaborated by taking into account their size and distribution in the nucleus. Following the criteria devised by Ahsan et al,[11] we found that the AgNORs in malignant cells were greater in number, irregularly distributed throughout the nucleus and heterogenous in size. On the other hand, mesothelial cells were characterized by a lesser number of small, homogenously sized, regularly clustered AgNORs.

This study also indicates that AgNOR staining is a rapid, simple and inexpensive method for the diagnosis of malignancy. It can be employed as an additional diagnostic tool for use in peritoneal and pleural fluid samples when the cytological diagnosis poses a problem.

CONCLUSIONS

AgNOR count is a rapid, easily reproducible method of differentiating between reactive mesothelial and malignant cells.

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