

Silver Stained Nucleolar Organizing regions [AgNOR] in Metastatic Carcinoma to peritoneal cavity

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Summary:

Background : AgNOR parameters are well known to pathologists as a proliferation marker with advantage over other proliferation markers of being cheaper, simpler and able to assess proliferation speed as well as state , AgNOR stainability was found to be well preserved in smears kept for up to 2 years , studies have shown that AgNOR values can serve as a useful prognostic parameter and a marker for tumour progression in different carcinomas (1) This study was conducted to see the importance of AgNOR staining in the peritoneal fluid cytopathological examination

Patient and Methods: It was a descriptive and prospective study conducted in Department of cytopathology in the medical city and Department of pathology in the medical college of Baghdad University, from September 2003 to June 2007. AgNOR staining was performed on 50 peritoneal fluid specimens having malignant cells and 20 other peritoneal fluid specimens as control cases

Results: AgNOR count, size and dispersion were normal in benign mesothelial cells (the proportion of cells with 1 or 2 AgNOR dots ranged from 32% to 98% with a median of 82% and a mean of 80.7%, S.D. 15.4 the proportion of cells with clusters ranged from 0% to 12% with a median of 0% and a mean of 1.3%, S.D. 2.5) , higher in the malignant cells (the proportion of cells with 1 or 2 AgNOR dots ranged from 15% to 75% with a median of 30.5% and a mean of 28.9%, S.D. 21.1, the proportion of cells with clusters ranged from 23% to 88% with a median of 67.6% and a mean of 56.2%, S.D. 36.7). AgNOR counts in the malignant cells were significantly greater as compared with counts of normal mesothelial cells .

Conclusion: Typing of AgNOR count, size and dispersion was found to be an important marker in differentiation between normal mesothelial cells and malignant cells of metastatic adenocarcinoma

Key Words: AgNOR Argyrophilic nucleolus organizer region, Pap stain Papanicolau stain ,no. number ,vs. versus, NOR nucleolar organizing regions, S.D. Standard Deviation

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Introduction:

Nucleolar organizer regions (NORs) are specific regions located in the secondary constriction areas of the short arms of each of the five acrocentric chromosomes 13, 14, 15, 21 and 22 (on metaphase chromosomes which cause the formation of a nucleolus or nucleoli in interphase) i.e. they are the regions on chromosomes that contain r-RNA genes (2) , the silver stainability of active nucleolar organizer regions is the property of the following acidic non-histone proteins that associate with rRNA genes (C23, B23, RNA polymerase 1, UBF transcription factor), the active groups involved in the staining reactions are the carboxyl groups, sulphhydryl and disulphide groups. (3) The diagnostic possibilities inherent in AgNORs first attracted attention 15 years ago. Numerous experiments have shown that the number of AgNORs is significantly higher in malignant tumors than in physiologic, reactive, or benign

processes. It has been demonstrated that the number of AgNORs per nucleus can be regarded as one of the hallmarks of proliferation and that mean AgNOR counts are good indicators of the degree of malignancy. (4) . The results obtained by various research groups consistently show that a rising AgNOR count is indicative of an increasing tendency toward proliferation; however, there are differences between the values obtained. (5) Using histologic or cytologic samples, the AgNOR technique is suitable for distinguishing malignancy cases according to prognosis (poor or better) as reported by several authors. (6) According to some authors, however, it is the increase of the total area of AgNORs within the nucleus, rather than the number of AgNORs, that shows a close correlation with the rate of proliferation and the prognosis. (7)

Patients and methods:

Fifty patients diagnosed as metastatic carcinoma to the peritoneal cavity with ascites were enrolled in this study; specimens were collected in the cytopathology departments in the teaching

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laboratories in the medical city in the period between September 2003 and June 2007. Twenty patients had ascites without malignant cells in the ascitic fluid were enrolled in this study and considered as control cases. Ten smears were aspirated from ascitic fluids of the patients enrolled in this study, alcohol fixed samples were stained by pap stain, two morphologically different cells were observed on examination of the smear, these are malignant cells in the peritoneal fluid of patients with metastatic carcinoma to the peritoneal cavity and reactive mesothelial cells in the control cases, the defining cytological features for each of the above mentioned cells are listed in the table (1)

One hundred fifty to 200 intact cells were examined in all smears. (7) The number, size, and dispersion of AgNORs (blackish-brown staining dots) were determined for each nucleus. Silver precipitates, if any, were not counted as AgNORs. The mean nuclear AgNOR count per nucleus was determined (6)

AgNOR studies :The smears were spread on clean dust free and grease free slides

Cytological specimen staining :Each cytologic sample was stained with pap stain. These samples were subjected to routine cytological examination.

AgNOR staining and counting: AgNOR staining was performed according to the description of Crocker and Nar (8) with some modification: cytological samples were fixed with 70% ethanol for 10 minutes. The staining solution was always prepared fresh from two components: 2% gelatin solution (Sigma-Aldrich) and 50% silver nitrate solution (Sigma-Aldrich). The silver nitrate solution was mixed with the gelatin-containing mixture in a 2 : 1 ratio immediately before staining and under conditions of reduced room light. Efforts were made to ensure maximum dissolution of the silver nitrate crystals. One to two drops of the resulting mixture were placed on the smears for 30 minutes in the dark. After the incubation period (30 minutes) , slides were washed in distilled water and then allowed to air-dry. Smears were examined light microscopically.

There are certain criteria for differentiation between benign and malignant cells as seen in table no. 1 .Peritoneal fluid from cases of metastatic malignancy and other cases without malignancy are stained with AgNOR stain and cells having different numbers of dots and clusters (figures 3 and 4) were involved in this study .

SPSS ver. 10 software was used for statistical analysis. Cytological and AgNOR data are presented as median, mean, and S.D. , ANOVA test and Spearman correlation were used for analyzing parametric data, while kruskal-Wallis test and Spearman correlation were used for analyzing non parametric data. The level < 0.01 was considered as significant. a picture is often seen in "resting" lymphocytes and other cells (e.g., mesothelial).

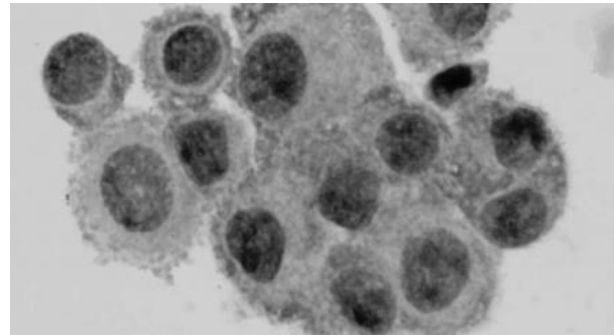


Figure 1: Normal mesothelial cells in the peritoneal fluid (pap stain x 400)

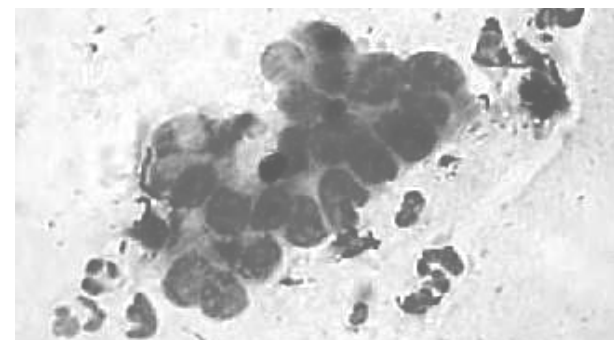


Figure 2 : Neoplastic cells from adenocarcinoma in peritoneal fluid (pap stain x 400)



Figure (3): Normal mesothelial cells stained with AgNOR technique (x1000)

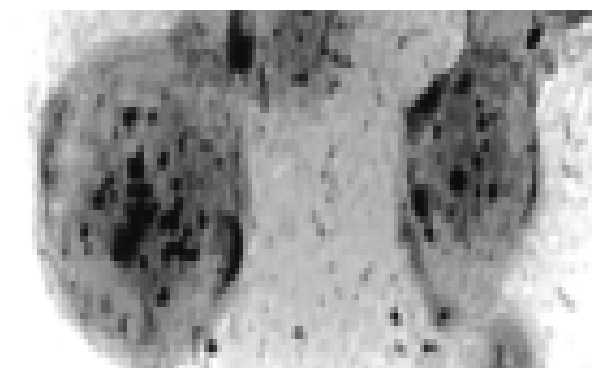


Figure (4): Neoplastic cells from adenocarcinoma in peritoneal fluid showing a high number of AgNOR particles (x1000)

Table no. 1 Morphologic differences between normal and malignant cells: (9)

Cytological feature	Benign cell	Malignant cell
Cell size	Variable within physiological limits	Variables beyond physiological limits
Cell shape	Variabl within physiological limits	Abnormal shape
Nuclear size	Variable within limits of cell cycle	Significantly variable "anisonucleosis"
Nuclear shape	Generally spherical or kidney shaped	Aberration of shape and configuration
Chromatin	Finly granular "trasperant"	Coarsely granular"opaque"
Nucleoli	small regular in shape and limited in number	Enlarged , increased in number and of irregular configuration
Adhesiveness	Good	Poor because loss of cadherin
Mitosis	Bipolar	Aberrant forms
Mitotic rate	As needed for replacement	Elevated

The difference is significant at the level of < 0.01

Table no. (2) AgNOR in relation to Ascites with malignancy vs. Ascites without malignancy

Ag NOR parameters	Ascitic fluid with malignancy			Ascitic fluid without malignancy			Significance
	Median	Mean	SD	Median	Mean	SD	
Cells with 1 or 2 dots (%)	30.5	28.9	21.1	82	80.7	15.4	0.0021*
Cells with 3dots (%)	2.8	3.9	7.1	0.2	3.1	4.2	0.609
Cells with 4 dots (%)	3.9	4.6	6.8	0	1.4	2.6	0.403
Cells with ≥ 5 dots (%)	4	5.7	6.9	0	1.7	7	0.489
Cells with cluster (%)	67.6	56.2	36.7	0	1.3	2.5	0.0012*

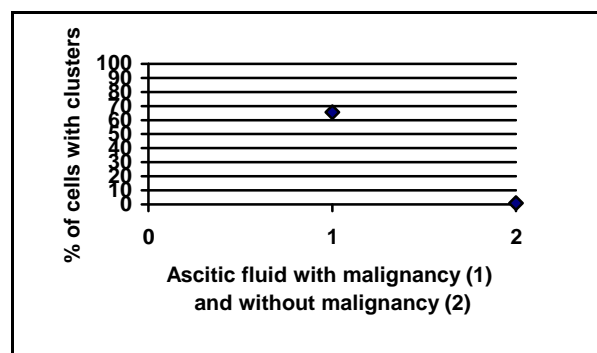


Figure no.(5) Ag NOR in relation to Ascites with malignancy vs. Ascites without malignancy Results:

Results :

Fifty cases are diagnosed as metastatic carcinoma to the peritoneal cavity by cytological examination of the ascetic fluid stained with pap stain (figure 2) , twenty cases having ascites without malignancy are diagnosed also by cytological examination of the peritoneal fluid stained with pap stain (figure 1) .The possible configurations of AgNORs included the following groups.(6)

Group 1. AgNORs are fully aggregated and form a homogeneous silver staining structure corresponding to the nucleolus. When using a short incubation period (30 minutes), multiple AgNORs are not visible in the nucleolus. Such a picture is often seen in "resting" lymphocytes and other cells (e.g.,mesothelial). This category includes also the arrangement in which the nucleus contains multiple nucleoli, provided that each nucleolus contains a maximum of one AgNOR.

Group 2. When the cell is dividing the nucleoli are not seen , AgNORs relate to acrocentric chromosome only.

Group 3. Numerous small AgNORs can be seen outside the nucleolus in the nucleoplasm. This is typical of highly malignant tumor cells.

In some cases the arrangement of precipitates is poorly evident, which renders it difficult to make an unambiguous evaluation of AgNORs. When the Ascites with malignancy group (in the malignant cells the proportion of cells with 1 or 2 AgNOR dots ranged from 15% to 75% with a median of 30.5% and a mean of 28.9%, S.D. 21.1, the proportion of cells with clusters ranged from 23% to88% with a median of 67.6% and a mean of 56.2%, S.D. 36.7) was compared with the ascites without malignancy (In benign mesothelial cells the proportion of cells with 1 or 2 AgNOR dots ranged from 32% to 98% with a median of 82% and a mean of 80.7%, S.D. 15.4 the proportion of cells with clusters ranged from 0% to12% with a median of 0% and a mean of 1.3%, S.D. 2.5) using AgNOR study depending on the number of the dots in the nuclei of the cells , significant difference (P value <0.01) was found between the two groups regarding cells with 1-2 dots and cells with clusters , and no significant difference(P value > 0.01) was

found between the two groups regarding cells having more than 2 dots without clusters as seen in the table no. 2 and figure no. 5

In poorly differentiated carcinoma some malignant cells have large giant dots with satellite of small dots were noticed.

Discussion:

The AgNOR parameters observed in malignant effusion in this study suggest that most cells are quiescent and contain one or two compact nucleoli and that there is a smaller but highly variable fraction of cells with AgNOR clusters entering the cell cycle .Most of the significant correlations were

found with the proportion of cells with a cluster. In some cells, large giant dots with satellite of small dots were also noticed especially in poorly differentiated carcinoma, Similar observations have been made by Crocker J et al (10), Derenzini (11), Giri et al (12), Khanna et al (13). With the exception of few reports that found, no overlapping of NOR values between neoplastic cells and reactive cells in serous fluid effusion (14). AgNOR parameters can not be considered to represent a specific tool for the cytological diagnosis of malignancy (15). AgNOR stain can be used to differentiate between mesothelial cells and malignant cells of metastatic adenocarcinoma. Normal mesothelial cells have one NOR, two NORs and less commonly three NORs while reactive mesothelial cells may have clustered NORs but not more than one. Cells with more than one cluster of NOR or with a cluster and dots are malignant cells. Investigating the changes of AgNOR before and after treatment may provide information about response to treatment, treatment selection and overall survival. For prognosis of malignant effusion using AgNOR studies requires follow up studies to find if there is correlation between a high proliferative rate and survival.

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