CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1248867

Available online at: http://www.iajps.com Research Article

SIGNIFICANCE OF AGNOR STAINING IN DETERMINING MALIGNANT OR BENIGN PLEURAL EFFUSIONS

[1]Dr. Humaira Atta Ullah, [2] Dr. Muhammad Adnan Shahid, [3] Dr. Muhammad Awais Tahir

1Punjab Medical College Faisalabad, Pakistan.
2Independent Medical College Faisalabad, Pakistan.
3Punjab Medical College Faisalabad, Pakistan.

Abstract:

Objective: *The evaluation of the argyrophilic nucleolar regions (AgNORs) correlation and malignancy in malignant effusions and benign.*

Design: *Our research group was consisting of the peritoneal and pleural effusion samples which were obtained through the patients who were diagnosed from numerous malignant and benign diseases. We also studied the cytological smears through conventional eosin and hematoxylin including the AgNORs silver screening.*

Setting: *Research sample was taken from the patients hospitalized in the Allied Hospital, Shifa International Hospital, Aziz Fatima Hospital and District Headquarters Hospital, Civil Hospital Faisalabad.*

Subjects: *A total of 100 cases of peritoneal or pleural effusions were enrolled in the research sample. Fifty cases in the sample were positive in the malignant cells and remaining fifty were having reactive mesothelial cells. Major outcome results were AgNOR count assessment as a malignancy diagnostic marker.*

Results: *The count of the AgNOR was helpful for the differentiation of the malignant cells from benign. Count of the AgNOR in the malignant cells was observed as (10.62 ± 3.36) & (3.04 ± 0.64) in the reactive mesothelial cells.*

Conclusion: *We observed that the AgNOR count was rapid, easily reproducible technique for the differentiation of the malignant cells and reactive mesothelial cells.*

Keywords: *Cytology; AgNOR count and Effusions.*

Corresponding author:
Dr. Humaira Atta Ullah,
Punjab Medical College,
Faisalabad, Pakistan

QR code

Please cite this article in press Humaira Atta Ullah et al., ***Significance of Agnor Staining In Determining Malignant or Benign Pleural Effusions,*** *Indo Am. J. P. Sci, 2018; 05(05).*

INTRODUCTION:

Identification of reliability of the tumor cells in the pleural and peritoneal effusions is very well-known issue of the diagnostic. Cells distinguishing is not possible of neoplastic cells from macrophages specially in the case of reactive mesothelial cells on the pure morphological basis. Variety of the testing includes the flow of the DNA cytometry, Restricted Enzyme Fragment Length Polymorphism (RFLP), monoclonal, PCR Sequencing and poly clonal antibodies that have been employed for the distinguishing the neoplastic cells benign. We used a comparative simple method for this objective that was nucleolar organizer regions (NORs) silver staining. Inter phase AgNORs are functional and structural nucleolus units those containing all the required and essential elements to synthesize the ribosomal RNA [1]. In the karyotype of human, location of the NORs can be found in each of short acrocentric chromosomes arms as 13^{th}, 14^{th},15^{th}, 21^{st} and 22^{nd}. Two argyrophilic proteins those linked with the transcription of rRNA and in the processing are nucleophosmin and nucleolin. These proteins are easily sustained and argyrophilic through sliver staining.

After silver staining, identification of the NORs was seen in the shape of black dots, which were observable throughout nucleolar region. Size and number of the NORs shows activity of the cells, transformation and proliferation help which distinguishes malignant cells from benign. Quantitative distribution evaluation of AgNORs has been applied in the pathology of tumor for both the prognostic and diagnostic purposes. There have been numerous research studies which studies the types of the tumor that demonstrate malignant cells which are present frequently in the count of AgNOR than the non-malignant correspondent cells [2].

Our research applies the same method for the differentiation of the malignant cells from reactive mesothelial cells in peritoneal and pleural effusions.

PATIENTS AND METHODS:

We selected a total of hundred effusions cases. Fifty cases in the sample were positive in the malignant cells and remaining fifty were having reactive mesothelial cells. Major outcome results were AgNOR count assessment as a malignancy diagnostic marker. Staining of the smears was carried out by silver staining, the purpose of the staining was the determination of the smears were stained by H&E and silver stains. We carried out H&E staining for the differentiation of the reactive mesothelial cells and frankly malignant cells.

To stain the AgNOR staining, we dissolved gelatin in formic acid (1%) to make a solution of (2%) [3]. After that we added fifty percent of the aqueous silver nitrate with a ratio of 1:2 for the making of a working solution. to obtain the working solution. We also post-fixed the smears in a proportion of 3:1 ethanol and a mixture of acetic acid. Graded alcohol was used to bring them and we also covered them with the filter paper after that soaked into a working solution [4].

The smears were stored for thirty minutes in the dark area, this was actually a chamber that was humid, we also washed it with dehydrated, deionized water which was also taken to xylene & mounted. Count of the AgNORs was made in 100 malignant nuclei or malignant mesothelial cells and respectively benign effusions, and also counted the mean AgNOR [5]. Their variation and distribution in terms of size was also documented with the help of standard criteria as proposed by Ahsan. We also performed Student's T – test for the outcomes statistical analysis and graded the variation in the size as under:

0 = Less uniform in size, 1+ = Two varying sizes, 2+ = More than 2 different sizes but not as in 3+, 3+ = All sizes and grades including 2-minute count. AgNORs distribution in nuclei were graded as under: 0, 1+, 2+ and 3+ respectively limited to nucleoli, occasional dispersion outside nucleoli, moderate dispersion outside nucleoli, widely dispersed throughout nucleus.

RESULTS:

The appearance of the AgNORs was in the shape of black discrete dots which were also observed in pale yellow nucleus staining. It is shown in Table – I that mean count of the AgNOR in the malignant effusions was high significantly when compared to the counts of AgNOR in the effusions of the benign.

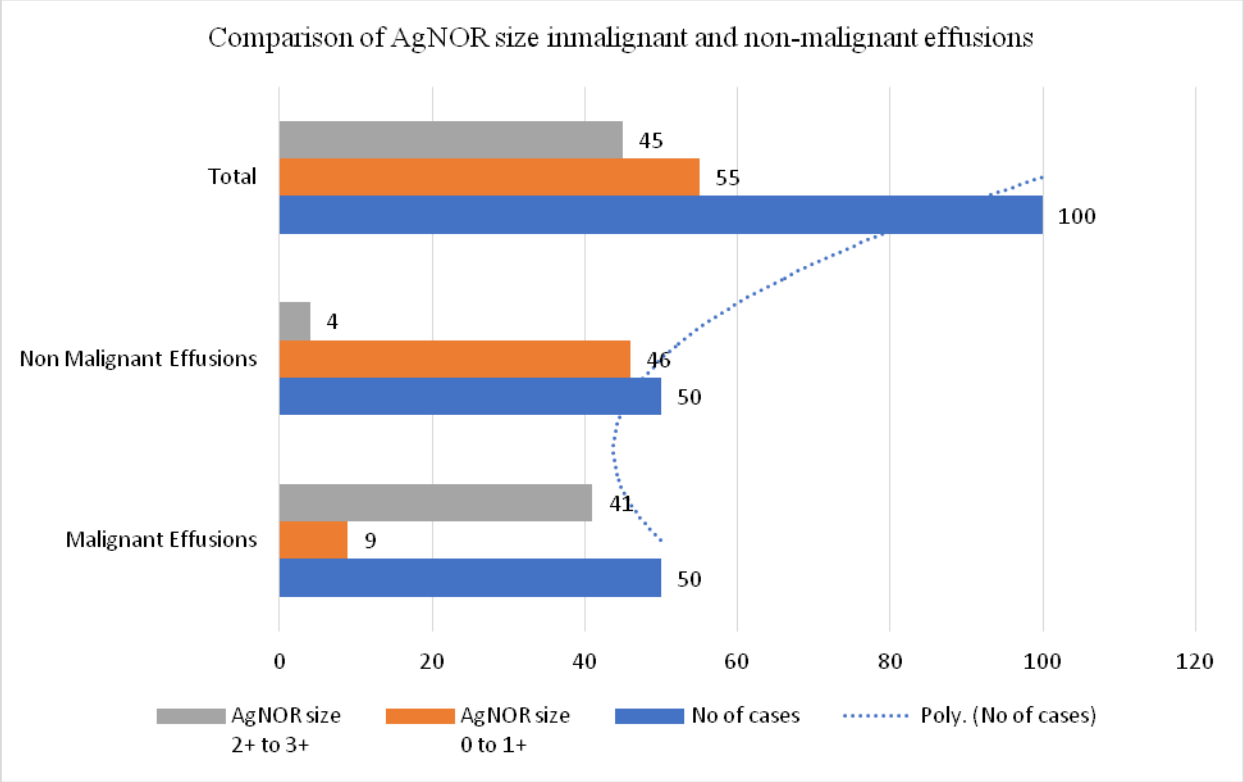
Table – I: Comparison of AgNOR counts in malignant & non-malignant effusions

Groups	*Mean Ag NOR 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 in comparison to the non-malignant effusions)
AgNOR distribution and size were of significant higher grade with a p-value as (< 0.001) in the malignant peritoneal and pleural effusions in comparison to the non-malignant effusions. (Tables – II & III).

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

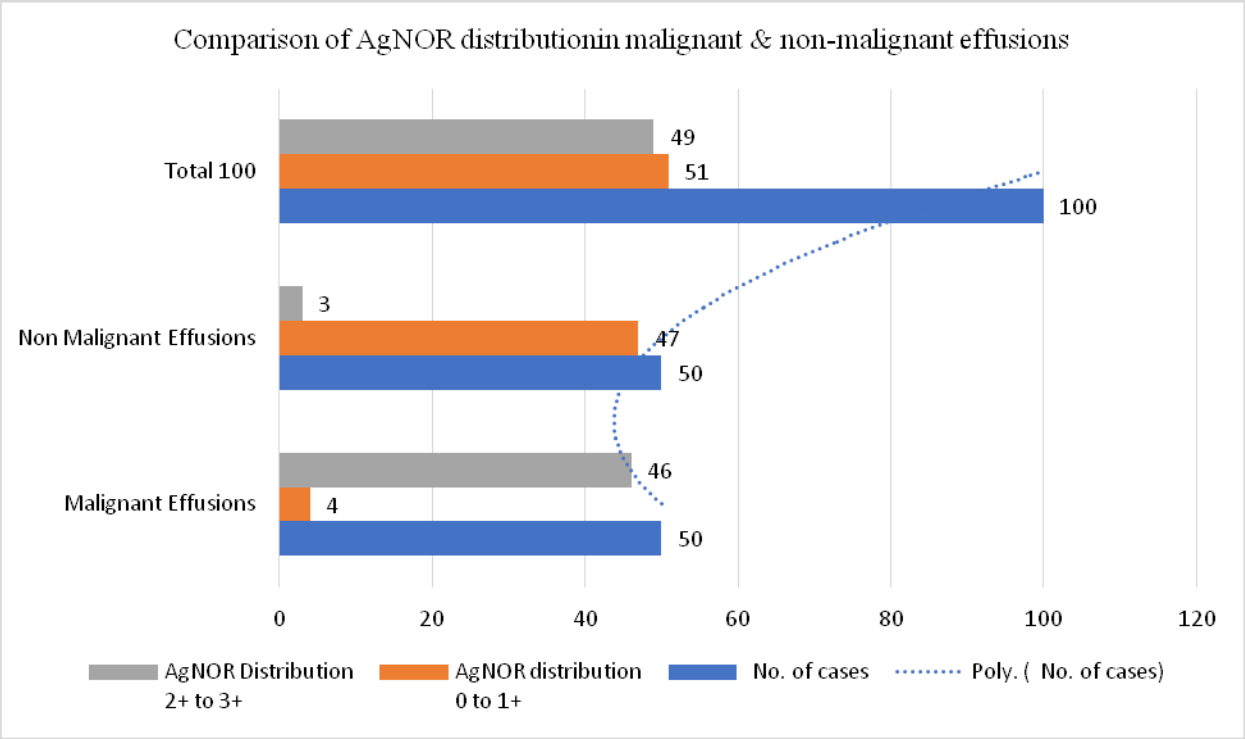
Groups	No of cases	*AgNOR size 0 to 1+*	*AgNOR size 2+ to 3+*
Malignant Effusions	50	9	41
Non-Malignant Effusions	50	46	4
Total	100	55	45



*p<0.001 (Significant in comparison to the nonmalignant 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	100	51	49



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

DISCUSSION:

Numerous research studies have evaluated the count of AgNORs on the varying malignant tissues and benign of a human body, it was demonstrated through malignant cells frequently which presents a huge number of AgNOR against benign cells [6]. Furthermore, there is an increased attention that focuses the count of the AgNOR in cells which are present in the effusions and also in the various methods that are devised for their evaluation. Silver staining of the AgNOR was carried out on the cells embedded with paraffin of malignant and benign effusions [7]. Other involved techniques which are an alternative to the visual evaluation methods such as back scattered electron imaging also employed for the parameters study that relates to AgNORs, it also includes the total AgNOR area calculation [8]. A significant difference is yielded through this method among the malignant and benign cells, having a mean area of the AgNOR of malignant cells greater than the area of the benign cells [9].

We also observed that staining of AgNOR and the visual count proved much simple method against the above quoted process which was adopted by various authors in their research studies. A research studied the pleural effusions and it states that mean count of AgNOR in malignant cells was also observable in the pleural effusions which was significantly high in comparison to the benign mesothelial cells. Similar outcomes have been observed by other research works in their studies [9].

Our outcomes reflect that AgNORs number permits to draw a clear line in the benign and malignant cells. Less number of the AgNORs was observed in the reactive mesothelial cells in comparison to the malignant cells [10]. AgNORs assessment has proved and explained the importance of the size and nucleus distribution. In the guidelines of the criteria proposed by Ahsan we observed that AgNORs in the malignant cells was greater in number when it was compared in the large numbers, an irregular distribution through heterogeneous and nucleus in size. Whereas, on the other hand, mesothelial cells are characterized through a less number of the small, regularly clustered and homogenously sized AgNORs [11].

Our research also indicated the staining of the AgNORs as an inexpensive malignancy diagnosis strategy, which can be employed as a separate diagnostic instrument for the pleural and perinatal sample of the fluids when cytological diagnosis is associated with a problem [12].

CONCLUSIONS:

We observed that the AgNORs count was rapid, easily reproducible technique for the differentiation of the malignant cells and reactive mesothelial cells.

REFERENCES:

1. Hwang, H.C., et al., Utility of BAP1 immunohistochemistry and p16 (CDKN2A) FISH in the diagnosis of malignant mesothelioma in effusion cytology specimens. The American journal of surgical pathology, 2016. 40(1): p. 120-126.
2. Hamasaki, M., et al., Low homozygous/high heterozygous deletion status by p16 FISH correlates with a better prognostic group than high homozygous deletion status in malignant pleural mesothelioma. Lung Cancer, 2016. 99: p. 155-161.
3. Angeleri, A.A., et al., Biochemical Parameters in Pleural Fluids: New Contributions in the Etiologic Diagnosis of Body Fluids. 2016.
4. Ribeiro, C.V., A.C. Vasconcelos, and J.d.S. Andrade Filho, Apoptosis and expression of argyrophilic nucleolus organizer regions in epithelial neoplasms of the larynx. Brazilian journal of otorhinolaryngology, 2015. 81(2): p. 158-166.
5. JAFFER, R., et al., PROLIFERATION MARKERS,—AgNORs, CORRELATION WITH GRADE OF INVASIVE DUCTAL CARCINOMA OF BREAST. Biomedica, 2017. 33(2): p. 120-123.
6. Chai, S.M. and C. Van Vliet, Cytological diagnosis of malignant pleural mesothelioma. Current Pulmonology Reports, 2017. 6(1): p. 1-8.
7. Sharma, R. and G. Kumar, Significance of Silver Binding Nucleolar Organizer Regions in Oral Squamous Cell Carcinomas. INTERNATIONAL JOURNAL OF SCIENTIFIC STUDY, 2016. 3(12): p. 189-193.
8. Fagere, M.O.M., Diagnostic Utility of AgNORs Staining of Serous Effusion among Sudanese Patients. International Journal of Science and Technology, 2016. 5(1): p. 36-42.
9. Hiroshima, K., et al., Cytologic differential diagnosis of malignant mesothelioma and reactive mesothelial cells with FISH analysis of p16. Diagnostic cytopathology, 2016. 44(7): p. 591-598.
10. AA, I. and S. SM, COMPARISON OF AGNORS SIZE AND DISPERSION WITH ER STAINING IN INVASIVE DUCTAL CARCINOMA OF BREAST. Biomedica, 2016. 2(3).
11. AHTESHAM, K., et al., COMPARISON OF AGNORS SIZE AND DISPERSION WITH ER STAINING IN INVASIVE DUCTAL CARCINOMA OF BREAST. Biomedica, 2016. 32(3): p. 195.
12. Anttila, S., Malignant mesothelioma: pathology, in Occupational Cancers. 2014, Springer. p. 285-298.