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Diagnostic Utility of Modified Method of AgNOR Staining in the Evaluation of Benign and Malignant Effusions

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ABSTRACT

An exact identification of malignant cells in fluid by cytological examination is a well-known diagnostic challenge. One of the common problems is to distinguish reactive mesothelial cells from malignant cells. Conventional smears reported as 'suspicious for malignancy' indicate that the suspicious cases could not be classified with certainty as to whether they were reactive mesothelial cells or malignant cells. It poses problem in clinical staging of tumor, treatment and prognosis of malignancy. The purpose of the study was to determine the role of modified method of AgNOR staining in the evaluation of benign and malignant effusions. This cross-sectional study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka, from July 2019 - June 2021. A total of 115 cases of effusion were included. All the samples were centrifuged and then smears were prepared from the deposit followed by staining with Hematoxylin & Eosin stain, Papanicolaou stain and AgNOR stain. At first the diagnosis was made on conventional smear method. Then the findings were compared and analyzed by modified AgNOR staining method. In malignant cells, the mean AgNOR count was 5.59±1.05 (±SD) and the AgNORs were multiple and irregular in shape. On the other hand, in benign cells the AgNORs were comparatively larger, single dots with a mean count of 1.31±0.48.The AgNOR count method has definite role in differentiating benign from malignant effusion. This method has supportive value which can be utilized in differentiating malignant effusions from the benign ones, especially in suspicious cases.

Keywords: Conventional smear, AgNOR stain, Effusion, Benign, Malignant, and Suspicious cytology.

INTRODUCTION:

Cytological examination of fluid is often helpful to differentiate between benign and malignant effusions (Sujathan *et al.,* 1996). But there are various major diagnostic issues in cytologic interpretation of effusions. They commonly contain abundant reactive mesothelial cells, histiocytes, and lymphocytes. If the effusion contains rare malignant cells, those can be often obscured by the relative overabundance of other cellular elements and may not be readily detectable on microscopic examination. Malignant cells are often exfoliated as single cells or minute tissue fragments and thus, a relative lack of cellular architecture may hamper an accurate cytologic assessment (Sujathan *et*

al., 1996). The exfoliated foreign cells and the mesothelial cells in the fluid may mimic one another. Moreover, neoplastic cells may significantly change their appearance after a prolong time suspension in fluid. Cells may appear more rounded and cytoplasm may develop pseudo vacuoles. So, most of their morphologic resemblance to the primary tumor could be lost. Determining the primary cancer based on only effusion analysis becomes a challenging task due to inadequate patient's history or information about prior malignancy.

Due to morphologic similarities, cytomorphologic distinction between reactive mesothelial cells, malignant mesothelioma, and metastatic adenocarcinoma can often be extremely difficult. The majority patients with suspected cancers or known malignancies routinely undergo cytological evaluation. Cytologic examination of serous cavity fluid is a very useful diagnostic tool and considered to be a highly accurate diagnostic procedure. Sensitivity of conventional cytology for the detection of malignant cells varies from 50-78%. Several ancillary diagnostic methods have been proposed to increase the diagnostic accuracy for detection of malignant cells (Karki *et al.,* 2012). If cytomorphology is combined with immunocytochemistry, the sensitivity increases from 84 to 94%, and the specificity increases from 92 to 100% (Ali & Cibas, 2012; Mohammad *et al*., 2021).

Ancillary techniques like cell block, image analysis and flow cytometry have proved to be useful in the detection of benign and malignant effusion. But they are not readily available in most of the laboratories (Fagere, 2016). Interest therefore has focused on identifying dependable methods for supplementing conventional smear method to differentiate malignant cells from benign. One such area under investigation is nucleolar organizer regions (NORs) (Sujathan *et al.,* 1996). NORs are chromosomal loops of DNA that involved in ribosomal synthesis. A comparatively simpler technique used for this purpose is the silver (Ag) staining of nucleolar organizer regions (NORs). Interphase NORs are the structural and functional units of the nucleolus. It contains all the essential components for the synthesis of ribosomal RNA. NORs are located in each of the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22.6. NORs are argyrophilic because they are associated with two argyrophilic proteins. Nucleolin and nucleophosmin are argyrophilic proteins 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 that help to distinguish benign from malignant cells. Evaluation of the quantitative distribution of AgNORs has been applied in tumor pathology both for diagnostic and prognostic purposes. A number of studies carried out in different tumor type's demonstrated that malignant cells frequently present a greater AgNOR count than corresponding non-malignant cells (Akhtar *et al.,* 2004). Now a day, in countries like us only conventional smears are made in almost all of the laboratories. Cell blocks are particularly used when the cytological abnormality is misleading. Cell block is definitely a good technique that usually requires some extra work which is not needed in conventional smear method and AgNOR staining method. The study has been undertaken to assess the utility of AgNOR staining in the cytological diagnosis of suspected malignant effusions and compare the diagnostic efficacy of conventional cytological method with AgNOR count method in effusion.

MATERIAL AND METHODS:

This cross-sectional observational study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka from July 2019 to June 2021. In this study 115 samples of effusion were included. All the samples were centrifuged and then smears were prepared from the deposit followed by staining with Hematoxylin and Eosin stain, Papanicolaou stain and AgNOR stain. At first the diagnosis was made on conventional smear method. Then the findings were compared and analyzed by AgNOR staining method. AgNOR count, variation in size and dispersion of AgNOR dots as well as proliferative index (pAgNOR) were graded and compared in malignant and non-malignant effusions. Relevant clinical data including age, sex, site of effusion and known history of benign or malignant neoplasm were collected and recorded in a predesigned data collection sheet. Statistical analysis was carried out as required. Ethical practice was ensured in every step of the study.

Sample selection criteria

Inclusion criteria

Sample of effusion received in the Department of Pathology during the study period.

Exclusion criteria

- Patients unwilling to participate in the study.
- Scanty fluid samples when cell block preparation cannot be done.
- Poorly preserved material

Laboratory Methods

After receiving the fluid sample, without agitation it was decanted in a 60 mL beaker and the remaining fluid was saved in another container. Then 60 mL fluid was distributed in 3 tubes. The effusion samples were centrifuged. From the deposited cells smears were prepared and fixed according to the method of staining.

A. Conventional Smear Preparation

- After transferring the fluid from to centrifuge tube labeled with the specimen identifier and then was centrifuged for 5 minutes at 2000 rpm.
- Supernatant fluid was discarded and the sediment was taken on the slide with the help of glass rod and spread by thick and thin method.
- Three smears were prepared.
- Two slides were fixed in 95% ethanol and stained with Papanicolaou and H & E stain. Remaining one smear was further processed to stain with AgNOR.

B. AgNOR staining Procedure

a. Materials for AgNOR staining

- Silver nitrate
- Gelatin
- Formic acid
- 3:1 mixture of absolute alcohol: acetic acid
- Deionized water
- Distilled water
- Xylene

b. Making of AgNOR solution

Solution A: 50% aqueous silver nitrate

Solution B: 2 gm gelatin and 1 mL formic acid in 100 mL deionized distilled water.

AgNOR solution

2 parts of solution A+ 1 parts of solution B

Method of staining

The smear was post-fixed in 3:1 ethanol: acetic acid mixture. It was brought to deionized distilled water through graded alcohols, covered with filter paper. Smears were covered with 10 drops of working silver staining solution in a dark humidity chamber at room temperature for 30-40 minutes. Smears were washed by covering them with a layer of distilled water 3 times, 5 minutes for each. Dehydrated through alcohol series, clear in xylene and mount in DPX. AgNOR stained smear was examined under the light microscope. The nuclei stained light yellow. The AgNORs were visualized as brown black dots of variable size within the nuclei. Only nuclei of mesothelial, epithelial or malignant cells were evaluated. Inflammatory cells (PMNs, lymphocytes and macrophages) were excluded. AgNOR counting was performed under 100x objective using oil immersion.

Scoring system

- **Mean AgNOR (mAgNOR)**
  The mean number of AgNORs in 100 cells was calculated.
- **Proliferative index (pAgNOR)**
  The percentage of nuclei exhibiting 5 or more AgNOR dots/ nucleus/ 100 cells called proliferative index (pAgNOR). The size variation and distribution of AgNORs was performed by the following criteria

Size variation grading

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 too minute will have to be counted.

AgNOR dispersion in the nuclei

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

Data Processing and Analysis

Statistical analysis was performed applying the SPSS-PC package, version 23 (Statistical Package for Social Science). P values less than 0.05 were considered significant. Analysis of the results of methods (con-

ventional smear and AgNOR staining) were performed using Chi square test, Unpaired t test, ANOVA test, Mann-Whitney U test and McNEMAR test. The results were published in tables, pie charts and bar diagrams.

RESULTS:

Table 1: Age distribution of the study cases (n=115).

Age (years)	No. of cases	Percentage (%)
11-20	2	1.7
21-30	3	2.6
31-40	12	10.4
41-50	19	16.5
51-60	44	38.3
61-70	27	23.5
>70	8	7.0
Total	**115**	**100.0**
Mean ± SD	55.20 ± 12.76	
Range of age (Min.-Max.)	11-82	

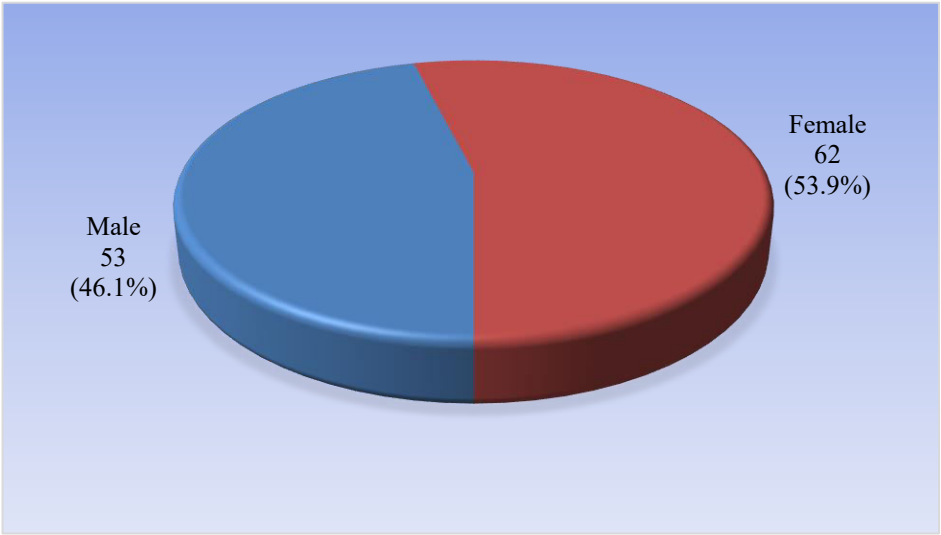

Fig. 1: Pie chart showing sex distribution of study cases.

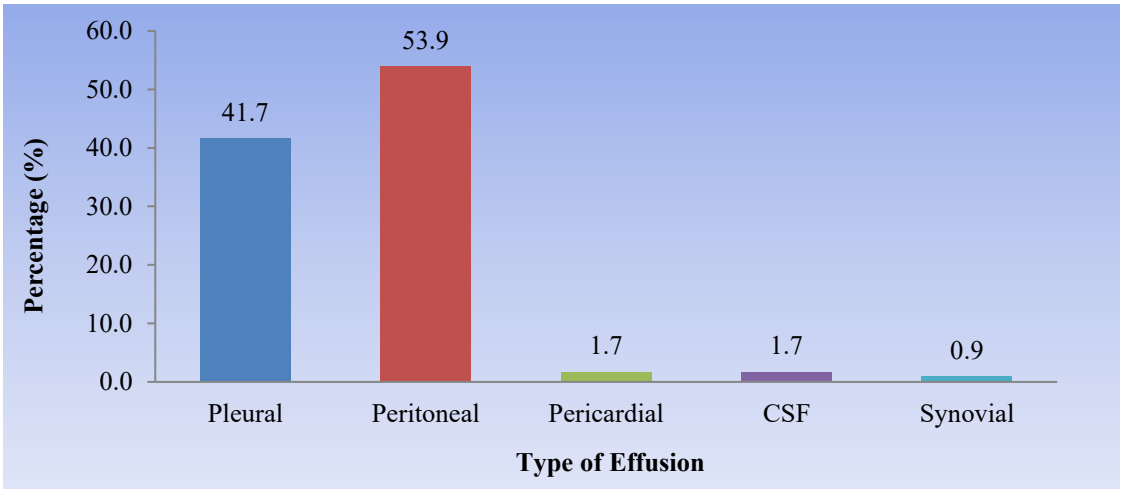

Fig. 2: Bar diagram showing distribution of study cases according to type of effusion.

Table 2: Mean AgNOR count (mAgNOR) of different groups of study cases diagnosed by conventional smear method (n=115).

Conventional smear	No. of cases	Range	Mean ± SD (mAgNOR)
Malignant	58	3.10-8.10	5.59 ± 1.05
Benign	48	1.00-2.90	1.31 ± 0.48
Suspicious	9	1.00-6.20	4.54 ± 1.49
p value*			<0.001

ANOVA test was carried out to measure the level of significance.

Table 3: Comparison of study cases diagnosed by conventional smear and AgNOR count method (n=115).

Conventional smear	n	AgNOR count method	
		Malignant No. of cases (%)	Benign No. of cases (%)
Malignant	58	56 (96.6)	2 (3.4)
Benign	48	1 (2.1)	47 (97.9)
Suspicious	9	8 (88.9)	1 (11.1)

* Figure within the parentheses indicated in percentage

Table 4: Comparison of suspicious cases diagnosed by conventional smear and AgNOR count method (n=9).

NO. of suspicious cases in conventional smear	AgNOR count method	
	Benign No. of cases	Malignant No. of cases
9	1	8

Table 5: AgNOR dispersion of different groups of study cases (n=115).

Cell block	AgNOR dispersion				p value*
	2+ to 3+	No. of cases (%)	0 to 1+	No. of cases (%)	
Malignant	63(98.4)		1 (1.6)		<0.001
Benign	3 (5.9)		48 (94.1)		

*Chi Square test was carried out to measure the level of significance

** Figure within the parentheses indicated in percentage

Table 6: AgNOR proliferative index (pAgNOR) of different groups of study cases (n=115).

	No. of cases	Median of pAgNOR	Mean ± SD of pAgNOR
Malignant	66	75.5	68.67 ± 22.87
Benign	49	0	2.90 ± 12.17
p value		<0.001	

*Mann-Whitney U test was carried out to measure the level of significance.

Table 7: Validity test of the study cases according to conventional smear diagnosis &AgNOR count method (n=115).

Variables	Conventional smear	AgNOR count method
	% (95% CI)	% (95% CI)
Sensitivity	90.6 (85.2-90.6)	96.9 (91.0-99.4)
Specificity	100.0 (93.2-100.0)	94.1 (86.7-97.2)
PPT	100.0 (94.0-100.0)	95.4 (89.6-97.8)
NPT	89.5 (83.4-89.5)	96.0 (88.5-99.2)
Accuracy	94.8 (88.7-94.8)	95.7 (89.1-98.4)

CI = Confidence Interval

PPT = Positive Predictive Value

NPT = Negative Predictive Value

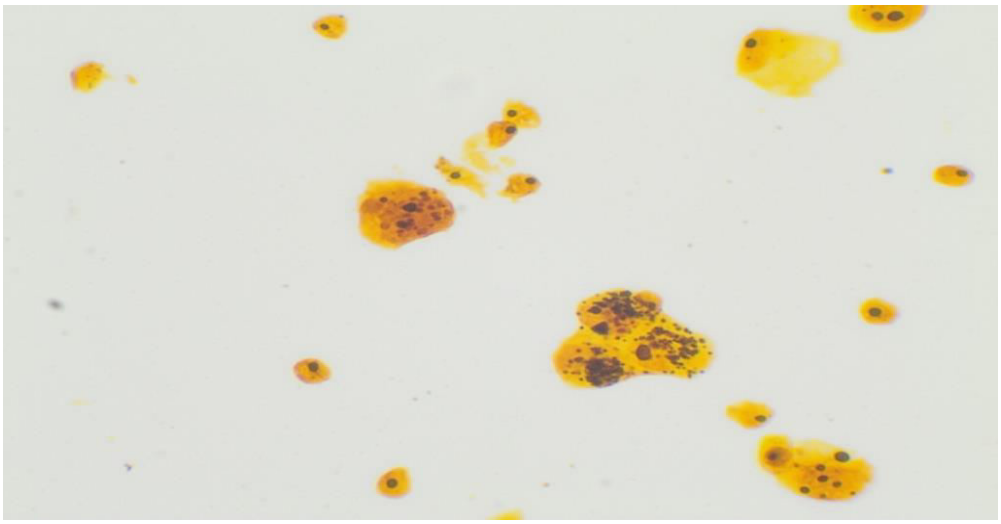

Fig. 3: (case no-109) Photomicrograph showing malignant cells with numerous, minute AgNOR dots and surrounded by benign cells containing few (1-2 dots/cell), regular AgNOR dots (AgNOR stain 100x).

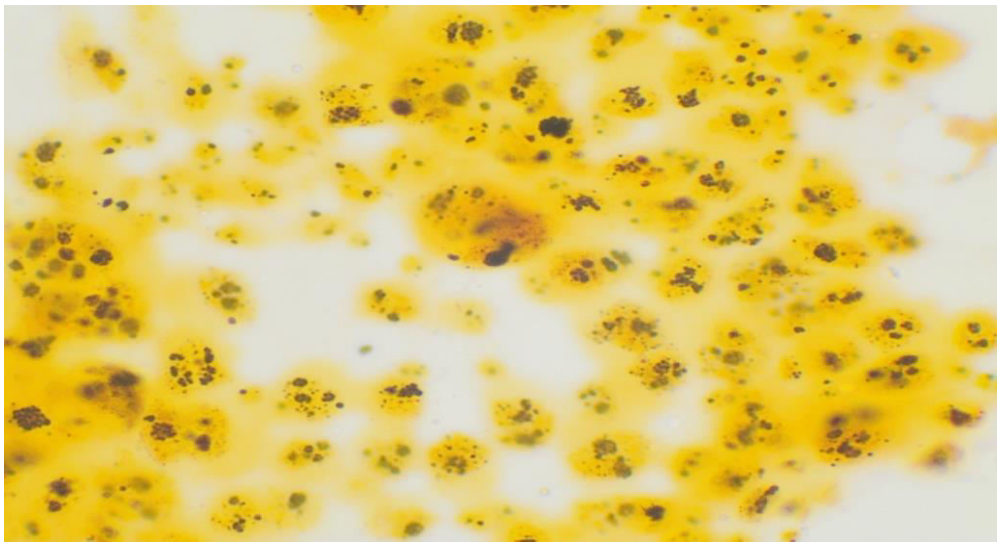

Fig. 4: (case no-11) Photomicrograph of malignant cells showing numerous (>3 dots/cell), irregular black AgNOR dots (AgNOR stain 100x).

DISCUSSION:

The diagnosis of malignancy in effusion is often difficult. The challenge is either differentiating malignant cells from macrophage and reactive mesothelial cells or due to subtle cytomorphological features of some malignant neoplasm. Indeed, it is not always possible to distinguish neoplatic cells from reactive mesothelial cells on purely morphologic features. The problem becomes more compounded due the artifacts which are caused by fixation, preparation, or staining techniques (Gill *et al.,* 2011). The age range in the current study population was from 11 to 82 years with a mean age of 55.20±12.76 years. The dominant age of the study population was among the age group of 51-60 which constituted 44 (38.3%). This findings are similar to the studies performed in Nepal and Sudan in which the mean age were 52.7 (ranging from 1-88 years) and 52 (ranging from 20-72 years) respectively (Shulbha and Dayananda, 2015). In the current study, most common fluid was peritoneal fluid 62 (53.9%) followed by pleural 48 (41.7%), peri-cardial 2 (1.7%), CSF 2 (1.7%) and synovial 1 (0.9%). This data was consistent with the other studies performed in India, Nepal and Sudan (Shulbha and Dayananda, 2015; Karki *et al.,* 2012 and Fagere, 2016). In our study, 115 cases of effusions were

evaluated by conventional smear which comprised of benign effusions (48 cases), malignant effusions (58 cases) and a third group consisting of suspicious for malignancy (9 cases). In the latter group the suspicious cases could not be classified with certainty as to whether they were reactive mesothelial cells or malignant cells.

This is similar to the study done by Sujathan and her colleagues in India where 37 benign cases, 55 malignant cases and 8 atypical cases were encountered. In the study of Gill *et al.* (2011), the number of benign to malignant cases was reversed; i.e. 57 cases were benign and 28 cases were malignant, whereas 15 cases were atypical. The present study validates the diagnostic utility of AgNOR staining of serous effusion. All effusions were subjected to AgNOR staining. The benign group consisted of cells showing 1 to 2 dots which were regular in size and shape. In the malignant group, more than three irregular dots as many as more than twenty dots were observed per cell distributed within the nucleus. The dots had variable size, shape and irregular contours. In suspicious group, the reactive mesothelial cells showed 1 to 2 dots and malignant cells showed 3 to 4 irregular dots. In this way clear separation could be achieved between reactive mesothelial cells and malignant cells. Similar observational views had been put forwarded by Crocker *et al.* (1989). After AgNOR counting of three different groups of study cases (diagnosed by conventional smear), the mean ± SD (AgNORs count /100 cells) in malignant effusion cases (5.59 ± 1.05) were found to be higher than benign effusion cases (1.31 ± 0.48) and atypical/ suspicious cases (4.54 ± 1.49). A statistical significant difference is found among the Means of different groups. Sujathan *et al.* (1996) observed a mean value of 1.92 ± 0.23 for benign, 4.72 ± 0.76 for malignant and 3.74 ± 1.50 for atypical cases. In a similar study, Gill *et al.* (2011) found a mean count of 1.53 ± 0.15 for benign cases, 4.03 ± 0.38 for malignant cases and 3.39 ± 0.59 for atypical cases. Karki *et al.* (2012) reported the mean count of 2.12 ± 0.54 in benign effusion, 10.43 ± 0.73 in malignant effusion and 8.77 ± 2.97 in atypical cases. When compared our findings are in accordance to with the findings of other researchers.

Table 8: Mean AgNOR count (mAgNOR) in benign and malignant effusion in different studies.

Author, year	Country	Study population no.	Benign (mAgNOR)	Malignant (mAgNOR)
Current study, 2020	Bangladesh	115	1.46 ± 0.95	5.52 ± 1.03
Ullah *et al.,* 2018	Pakistan	100	3.04 ± 0.64	10.62 ± 3.36
Sharma *et al.,* 2018	India	65	<4	>4
Fagere, 2016	Sudan	83	4.16 ± 0.86	13.52 ± 4.21
Karki *et al.,* 2012	Nepal	174	2.12 ± 0.54	10.43 ± 0.73
Gill *et al.,* 2011	India	100	1.53 ± 0.15	4.03 ± 0.38
Akhter *et al.,* 2003	Pakistan	100	3.04 ± 0.64	10.62 ± 3.36
Nezhad *et al.,* 2002	Iran	94	2.328 ±0.502	4.747 ± 0.657
Sujathan *et al.,* 1996	India	100	1.92 ± 0.23	4.72 ± 0.76
Ayres, 1988	Birmingham	30	1.04	5.43

Conflicting data exist about the mean AgNOR count in benign and malignant effusion. Significantly high mean AgNOR counts were reported in studies done by Akhter *et al.* (2003); Ullah *et al.* (2018); Karki *et al.* (2012) and fagere, (2016). The reason for this may be due to lack of standardization of the scoring system of AgNOR method. It was noticed by Sharma *et al.* (2018) that the mean AgNOR counts increases significantly as we move from well differentiated carcinomas to poorly differentiated carcinomas. This finding can be explained on the basis of the speculations given by Crocker *et al.* (1988), who observed a linear correlation between the mean AgNOR per nucleolus and percentage of S phase cell. Sharma *et al.* (2018) observed that different cytogenetics explained differently about the causes of increased AgNOR counts in malignancy. It may be due to increase in the chromosome number, increasing the chromosome arms containing NORs, gene amp-

lification. But most significantly it is correlated with proliferation.

In our study, when AgNOR size was compared using the criteria cited by Ahsan *et al.* (1992), 63 (98.4%) cases of malignant effusions were between 2+ to 3+ and the remaining one malignant case was 1+. On the other hand, 47 (92.2%) cases of benign effusion were within 0 to 1+ and four cases were within 2+ to 3+. The difference was highly statistically significant. This result is consistent with Akhter *et al.* (2003); Ullah *et al.* (2018); Fagere (2016); Karki *et al.* (2012); Ibnerasa *et al.* (2005) and Sujathan *et al.* (1994). Following the criteria devised by Ahsan *et al. (*1992), when AgNOR dispersion was compared in our study, 63 cases of malignant cases were between 2+ to 3+ and the rest one malignant case was 1+. On the other hand, 48 cases of benign effusion were within 0 to 1+ and remaining three cases were within 2+ to 3+. AgNOR dispersion was of significantly higher grade in the malignant effusion as compared with benign effusion. It means AgNORs were more dispersed in malignant effusions than in nonmalignant effusions. Similar result was found in the studies done by Akhter *et al.* (2003); Ullah *et al.* (2018); Fagere, (2016); Karki *et al.* (2012); Ibnerasa *et al.* (2005); Khan *et al.* (2006) and Sharma *et al.* (2018).

AgNOR size and AgNOR dispersion parameters are two most widely used methods besides AgNOR count. In many studies, it has been found that increased AgNOR size and wide areas of AgNOR dispersion correlate well with the proliferative activity, malignant potential and prognosis (Khan *et al.,* 2006). Again Sharma *et al.* (2018) mentioned that increase in the AgNOR count is not an total increase in the number, but rather an index of dispersion. In present study, the cases evaluated by another parameter labeled as AgNOR proliferative index (pAgNOR) which shows the proliferative activity of malignant cells. pAgNOR was high in malignant effusions. There is a statistically significant difference between benign and malignant cases. This result is consistent with Mourad *et al.* (1997); Bukhari *et al.* (2007) and Khan *et al.* (2006). After evaluating the study cases by AgNOR count method and other parameters (AgNOR size, AgNOR dispersion and AgNOR proliferating index), it was interrelated that out of 58 confirmed cases of malignancy, 56 were malignant and remaining 2 were benign in AgNOR method. On the other hand, out of 48 confirmed benign cases, 47 cases came out to be benign and the remaining 1 case was malignant. Nine cases were diagnosed as suspicious after using conventional smear method. By the AgNOR count method, eight cases showed presence of malignant features and one was benign. Two cases showed features of malignancy by AgNOR staining method. The overall study showed that there was statistical significance among the different test methods (conventional smearand AgNOR count method). The statistically significant difference was found between conventional smear &AgNOR count method. So, the diagnostic efficacy of conventional smear and AgNOR count method are not the same. The overall accuracy of AgNOR count method in case of interpretation of cytological diagnosis was 95.7%. On the other hand, cytological diagnosis made by conventional smear encountered overall accuracy of 94.8%. The accuracy of AgNOR count method is slightly higher than the conventional smear method. Thus, it could be concluded that though AgNOR count method have a definite role in differentiating benign from malignant effusions, they do not supersede the value of routinely used conventional smear as there is very little difference in their accuracy. Also, it has been observed in our study that eight out of nine cytologicaly diagnosed suspicious cases had high AgNOR counts and higher AgNOR size and dispersion grade as well as high proliferative index. Hence, AgNOR staining is depicted as a useful technique in detecting malignant cells in effusions where cytological diagnosis is difficult. So, our study illustrates that AgNOR staining have supportive value which can be utilized in differentiating malignant effusions from benign ones, especially in suspicious cases. It can be employed as an inexpensive and rapid additional diagnostic tool for effusions when the cytological diagnosis poses a dilemma.

CONCLUSION:

A wide variety of abnormalities can produce effusions. Most often effusions caused by tumor or inflammation of the serous membrane. The role of cytopathology is most significant in the evaluation of fluid for the

malignant cells. The presence of malignant cells in effusions has an important therapeutic and prognostic implication. Malignant serous effusions are a commonly encountered early clinical manifestation of metastatic disease and often only a clinical clue of an unknown primary. Therefore, assessment effusion has been routinely used in suspected cases of malignancies. The diagnosis of malignancy in effusion is often critical. The challenge is either differentiating malignant cells from macrophage and reactive mesothelial cells or due to fine cytomorphological features of some malignant neoplasm. Indeed, it is not always possible to distinguish neoplastic cells from reactive mesothelial cells on purely morphologic features.

The problem becomes more critical due the artifacts which are caused by fixation, preparation, or staining techniques. The AgNOR count method has role in differentiating benign from malignant effusion, they do not supersede the value of routinely used conventional smear as there is very little difference in their accuracy. This method has supportive value which can be utilized in differentiating malignant effusions from the benign ones, especially in suspicious cases. So, our study illustrates that AgNOR staining have supportive value which can be utilized in differentiating malignant effusions from benign ones, especially in suspicious cases. It can be employed as an inexpensive and rapid additional diagnostic tool for effusions when the cytological diagnosis poses a dilemma.

ETHICAL CLEARENCE:

Prior to the commencement of this study the thesis protocol was submitted to the Institutional Review Board (IRB) of BIRDEM General Hospital, Dhaka for approval.

AUTHOR CONRIBUTIONS:

N.R. performed laboratory work, N.R.; and M.R. prepared the manuscript, Z.S.U completed data analysis and made the framework.

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CONFLICTS OF INTEREST:

Authors declare that no competing interest exists to publish the present research work.

REFERENCES:

1) Ali, S. Z., & Cibas, E. (2012). Serous cavity fluid and cerebrospinal fluid cytopathology, **11**, *Springer Science & Business Media*.
2) Bukhari, M. H., Niazi, S., & Hasan, M. (2007). Modified method of AgNOR staining for tissue and interpretation in histopathology. *Inter j. of experimental pathology*, **88**(1), 47-53. https://doi.org/10.1111/j.1365-2613.2006.00522.x
3) Crocker, J., Boldy, D. A., & Egan, M. J. (1989). How should we count AgNORs? Proposals for a standardized approach. *The J. of pathology,* **158**(3), 185-188.
4) Fagere, M. O. M. (2016). Diagnostic utility of AgNORs staining of serous effusion among Sudanese patients. *Inter J. of Science and Technology*, **5**(1), 36-42.
5) Gill, M., Singh, U., & Sen, R. (2011). Role of argyrophilic nucleolar organizer region staining in identification of malignant cells in effusion. *J. of cytology*, **28**(4), 191-195.
6) Gul Naz, A., Naseer Ahmed, C., & Saeed Akhtar, K. (2004). AgNOR staining in malignant and benign effusions. https://pesquisa.bvsalud.org/portal/resource/pt/emr-68051
7) Ibnerasa, S. N., Chaudhry, N. A., & Khan, S. A. (2018). AgNOR proliferative index in malignant pleural and peritoneal effusions. *International Journal of Pathology*, 86-91. https://applications.emro.who.int/imemrf/Int_J_Pathol/Int_J_Pathol_2005_3_2_86_90.pdf
8) Karki, S., Jha, A., & Sayami, G. (2012). The role of argyrophilic nucleolar organizer región (AgNOR) study in cytological evaluation of fluids, especially for detection of malignancy. *Kathm. Univ. Medical Journal*, **10**(1), 34-39.
9) Khan, S. A., Chaudhry, N. A., & Ibne-Rasa, S. N. (2006). Patterns of argyrophilic nucleolar organiser regions in pleural and peritoneal effusions. *J Coll Physicians Surg Pak*, **16**(6), 412-5. http://dx.doi.org/10.13005/bbra/3036
10) Mohammad Zakerin Abedin, Laila Jarin, Rubait Hasan, *et al*. (2021): Analysis of the Dengue

Infection, Occurrence and Hematological Profile of Dengue Patients in Dhaka City, *J Microbiol Pathol*. **5**: 115. https://www.hilarispublisher.com/open-access/analysis-of-the-dengue-infection-occurrence-and-hematological-profile-of-dengue-patients-in-dhaka-city.pdf

11) Mourad, W. A., Vallieres, E., & Alrobaish, A. (1997). Cell kinetics analysis of surgically resected non-small cell carcinoma of the lung using the AgNOR silver stain. *Annals of Saudi medicine*, **17**(2), 161-166.
Sharma, R., Sharma, V., & Goyal, N. (2018). Evaluation of Serous Effusions using AgNOR Count-A Study in a Tertiary Care Centre of Saharanpur. *J. of Evol. of Med. and Den. Sci.*, 7(47), 5857-5863. http://dx.doi.org/10.1002/cre2.870

12) Shulbha, V. S., and Dayananda, B. S. (2015). Cytology of body fluids-an aid to primary diagnosis. *Indian J Pathol Oncol*, **2**(2), 81-3. https://doi.org/10.17511/jopm.2019.i01.08

13) Sujathan, K., Kannan, S., & Nair, K. (1996). Significance of AgNOR count in differentiating malignant cells from reactive mesothelial cells in serous effusions. *Acta cytologica*, **40**(4), 724-728.

14) Ullah, H.A., Shahid, M.A.S. and Tahbir, M.A. (2018). 'Significance of AGNOR Staining in Determining Malignant or Benign Pleural Effusions', *Indo American J. of Pharmaceutical Sciences,* **5**(5), pp.3864-3868.

15) WA, M. (1992). Argyrophilic Nncleolar Organizer Regions in Breast Carcinoma. *Cancer*, **69**(7), 1739-1744.

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