

## Original Article

## A study of AgNOR count in FNAC lymphnode in case of lymphadenopathy

Vasava Kunjika T\*, Shah Nayana A\*\*, Katara Rajesh K\*\*\*, Desai Nandini J\*\*\*\*, Damor Virendra M\*\*\*\*\*

\*Consultant Pathologist, Apex heart institute, Thaltej-Ahmedabad, Gujarat, India

\*\* Tutor, Dept. of Pathology, GMERS, Sola, Ahmedabad, Gujarat, India.

\*\*\*Associate Professor, Dept. of Microbiology, GMERS, Gandhinagar, Gujarat, India

\*\*\*\*Professor, Dept. Of Pathology, Shri M.P.Shah Govt. Medical College Jamnagar, Gujarat, India.

\*\*\*\*\* Consultant General Surgeon, Mahavir multispecialty hospital, Ahmedabad, Gujarat, India.

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### ABSTRACT

**Background:** To study Argyrophilic Nucleolar Organizer Regions (AgNOR) count in FNAC lymph node in case of lymphadenopathy & to differentiate between malignant & non-malignant lesions

**Aim:** To prove the diagnostic & Prognostic role of AgNOR in tumour pathology & application of AgNOR techniques to non-neoplastic & neoplastic growth & evaluation of AgNOR in cytology of various lesions of lymph nodes.

**Materials and Methods:** Lymph node FNAC specimens were taken from 150 patients attending pathology department of Shri M.P.Shah Govt. Medical College & GGG hospital, Jamnagar, Gujarat, India during the period of January 2004 to February 2006. FNA of enlarged lymphnode was taken & smears were prepared & fixed in cytofix (50% ethyl alcohol) also all smears were stained by H & E (were confirmed) as well as silver nitrate stain. 100 nuclei were assessed for each smear by 100x oil immersion lenses.

**Result:** In this study a total number of 150 cases were included (80 benign & 70 malignant) in various lesions of lymphnode.

**Conclusion:** AgNOR count is directly proportional to the severity of neoplastic lesions which increases with ascending grades of malignancy. Also it is a good technique in cytology.

**Keywords:** AgNOR count, Lymphnode, AgNOR stain, Malignant lesions, Non-malignant lesions

### INTRODUCTION

Cell in a normal circumstance passes through a precisely formed cell cycle. So the production of new cell compensates the loss of cells in adults. The rate of cell division is very well coordinated with the demand for growth and replacements. Whenever this co-ordination is faulty then tissues either fail to grow properly or they over grow resulting in neoplasm [1].

The only way to access the cell cycle speed in situ on cytological smear consists of quantifying AgNOR protein. They found to be very well correlated with the cell proliferation like PCNA, Ki67, DNA flow cytometer [2].

Many reports have been published using the Argyrophilic Nucleolar Organizer Regions (AgNOR) technique in

-Pulmonary lesions [3]

-Proliferative activity of squamous intraepithelial and invasive lesions of cervix.

-Gall bladder [4]

-Lymph node [5]

Lymph nodes are most widely distributed and easily accessible component of lymphoid tissues are hence frequently affected by various inflammatory and neoplastic lesions [6].

Silver impregnation method offers promise as a simple easy and rapid method to cell proliferation [7].

### MATERIAL AND METHOD

Study duration & study area: Present study was carried out at department of Pathology, Shri M.P.Shah Govt. Medical College & GGG hospital, Jamnagar, Gujarat, India during period of January 2004 to February 2006. Study consists of cytological preparations of lymph node lesions.

Sample size: Total 150 cases were studied among which 80 cases are of benign lesions and 70 cases are of malignant lesions of lymph node.

### Methodology:

#### Staining methods

Haematoxyline and Eosin method [8, 9].

AgNOR method [10].

Silver Nitrate stain for AgNOR is prepared as below.

Solution A - 50% aqueous silver nitrate solution

Solution B - Gelatine solution (2%)

Gelatine 02 gm.

Formic acid 01 cc

Deionised water 99cc

Solution C –Working solution SolutionA-O2 parts and SolutionB-01 part

#### Steps

- 1) Smears were fixed by using cytofix for 30 minutes.
- 2) Smears were incubated with working solutions for 30 minutes in dark.
- 3) Smears were taken out of it and washed in running deionised water for 10-15 minutes
- 4) Smears were allowed to dry and mounted with DPX

#### Results of staining

Background - pale yellow and clear

Nuclear - Yellow and/or brown.

AgNOR site - Intracellular black dots.

100 nuclei were accessed for each smear using 100 X.

**Statistical analysis [11, 12]:** following types analysis were done

Mean (X), Range

Defines the normal limits of biological characteristics

Standard deviation (SD)

Standard Error (SE)

Probability (p)

It measures relative frequency of particular event happening by Chance in long run.

Suppose 95% confidence limit i.e.  $x \pm 2SD$  is considered.

This means that n 95% observations are included between the limit of  $x \pm 2SD$  hence the probability of a readings failing outside the 95% confidence limits is 1 in 20 that means  $p=0.05$ .

So if  $p < 0.05$ , significant

If  $p > 0.005$ , not significant

P is decided from distribution curves of observations.

Standard normal deviate

Statistically Z value is inversely proportional to the p value. If higher the Z value lowers the P value and it highly significant statistically.

Ethical consideration: all the samples were a part of routine diagnostic techniques, so ethical consideration is not necessary

### RESULT

In this study 150 cases of lymphadenopathy were studied. The FNA of all the lesions were taken & smears were prepared. All smears were stained by H&E staining method and AgNOR staining method by modified silver nitrate staining technique of Platon et al [10].

**Table 1: Distribution of cases**

Lesions	No of cases (% , n=150)
<b>Benign</b>	80 (53.33%)
<b>Malignant</b>	70 (46.67%)
<b>Total</b>	150 (100%)

Among 150 cases, 80 (53.33%) were of benign lesions, and 70 (46.67%) were of malignant lesions as shown in table-1.

From total 80 benign cases, 30 (37.5%) are of Koch's lymphadenitis, 50 (62.5%) of chronic non-specific lymphadenitis & reactive hyperplasia of lymphnode as shown in Table-2.

**Table 2: Distribution of Benign lesions**

Lesions	No .of cases (% , n=80)
<b>Koch's lymphadenitis</b>	30 (37.5%)
<b>Chronic non-specific lymphadenitis/ Reactive hyperplasia of LN.</b>	50 (62.5%)

**Table 3: Distribution of malignant lesions**

Lesions	No .of cases (% , n=70)
<b>Non-Hodgkin's lymphoma</b>	25 (35.71 %)
<b>Metastatic carcinoma</b>	45 (64.29 %)

From 70 malignant lesions 25 (35.71%) were of Non-Hodgkin's lymphoma, 45 (64.29%) were of metastatic carcinoma as shown in Table-3.

**Table 4: AgNOR count in benign & malignant lesions**

Lesions	AgNOR count Mean $\pm$ SD
<b>Koch's lymphadenitis</b>	1.04 $\pm$ 0.03
<b>Chronic non-specific lymphadenitis/ Reactive hyperplasia of LN.</b>	1.15 $\pm$ 0.04
<b>Non-Hodgkin's lymphoma</b>	12.66 $\pm$ 2.07
<b>Metastatic carcinoma</b>	5.10 $\pm$ 0.76

Among 80 cases of benign lesions the mean AgNOR count found in Koch's lymphadenitis was 1.04 +/- 0.03, and chronic non-specific lymphadenitis/reactive hyperplasia of lymph node was 1.15 +/- 0.04, and overall mean value of AgNOR count in benign lesions was 1.13 +/- 0.03 as shown in Table-4.

Among 70 malignant lesions the mean AgNOR count found in Non- Hodgkin's lymphoma was 12.66+/-2.07,& in metastatic carcinoma count was 5.10 ±0.76. So overall mean AgNOR count of malignant lesions was 8.88+/-1.42 as shown in Table-4.

From above the mean AgNOR count of benign lesions was 1.11+/- 0.03 and of malignant lesions was of 8.88+/-1.42, the difference between mean AgNOR count of benign and malignant lesions was significant statistically ( $P < 0.001$ ) as shown in table-5.

**Table 5: Comparison of Benign and Malignant lesions**

No. of cases of lesions		AgNOR count mean±mean SD		Z Value	P Value
Benign	Malignant	Benign	Malignant		
80 (53.33%)	70 (46.67%)	1.11±0.03	8.88±1.42	46.28	< 0.001

Among 150 cases most of the benign lesions (chronic non-specific lymphadenitis & Koch's lymphadenitis) were below 40 years of age. From which chronic non-specific lymphadenitis were common before 1<sup>st</sup> decade of life, Koch's is common in 2<sup>nd</sup> decade of life. All malignant lesions were common after 40 years of age, from which metastatic carcinoma were seen in 5<sup>th</sup> & 6<sup>th</sup> decade of life &, Non-Hodgkin's lymphoma were common after 6<sup>th</sup> decade of life.

## DISCUSSION

Nucleolar organizer regions are DNA loops that located on the five acrocentric chromosomes of D-13, 14, 15 & D-21, 22, in the nuclei of cells [12]. They are encoded for the ribosomal RNA & r-RNA molecules the main site of protein synthesis. Means that the no. of NORs is associated with certain proteins associated with the nucleolar organizer regions, shows that argyrophilic reaction. AgNOR on staining with silver nitrate in prescribed situation [12].

The AgNOR technique as a novel tool in the diagnosis is readily applicable to cytological specimens [13, 14].

The AgNOR method is easily performed on whole cell preparation providing excellent technical results [17]. This suggests that this method could be of value in the examination of cytological preparation. In the present study a wide range of cytological preparations of benign & malignant lesions of lymph node were studied. AgNOR has definite diagnostic values in these lesions of lymph node.

The present study was compared with two previous studies of Sagparia [13] & Bhalani [14]. It is seen that there is mean AgNOR count of both studies with present study are comparable. And there was less significant difference in mean AgNOR count of chronic non-lymphadenitis & Koch's lymphadenitis. In malignant lesions of lymphnode present study was compared to two previous studies of Sagparia [13], Bhalani [14]. Mean AgNOR count for malignant lesions for both studies were comparable. There were 30 cases (mean AgNOR count 8.87 ±1.03.) in study of Sagparia [13], 50 cases (mean AgNOR count 8.84 ±1.41) in study of Bhalani and 70 cases (mean AgNOR count 8.88 ± 1.43) in present study. So similar count was founds in all the three studies.

## CONCLUSION

Histology is the main basis of tumour pathology. Now days there are many recent developments taking place in diagnostic histopathology like immunohistochemistry, tumour markers which can aid the pathologists in diagnosis of the lesion. However these methods are expensive and require lot of expatriation. AgNOR count on the other hand, is a simple and cheap method which can act as an additional diagnostic tool in tumour histopathology as well as cytopathology.

Following are the conclusions of the present study. AgNOR count directly proportional to the severity of neoplastic lesions. Definitely it is higher in malignancy and increasing with ascending grades of malignancy.

Statistical suggest that the mean AgNOR within lesions of lymphnode is significantly higher in then the mean AgNOR count in benign lesions of lymphnode.

Regarding AgNOR they are small, round and regular and few in lymphadenitis, they increase in hyperplastic lesions while they are large, increased in number and often clumped in malignant lesions. Compared to other proliferative markers AgNOR staining can be easily performed, simple and inexpensive.

The AgNOR technique in cytological preparation provides excellent technical result.

AgNOR is having significant role in the laboratory with only basic infrastructure and limited recourses. Though it is rapid inexpensive and less laborious, it gives equivalent results to that of the sophisticated investigations like, DNA flow cytometer, Ki67 and morphological analysis.

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### Corresponding Author:

Vasava Kunjika T,  
45, Shyamsatya bungalow,  
Behind Satyamev hospital,  
Opposite: Parmeshwar bungalow,  
Chandkheda, Ahmedabad-382424, Gujarat  
Email: [dr.kunjika@gmail.com](mailto:dr.kunjika@gmail.com)

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