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.

DOI: 10.5455/jrmds.20153315

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% ethly 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 washedin 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 - Intranuclear 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 ± 2SD is considered.

This means that n 95% observations are included between the limit of x±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 nirate staining technique of Platon et al [10].

Table 1: Distribution of cases

Lesions	No of cases (%, n=150)
Benign	80 (53.33%)
Malignant	70 (46.67%)
Total	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)
Koch's lymphadenitis	30 (37.5%)
Chronic non-specific lymphadenitis/ Reactive hyperplasia of LN.	50 (62.5%)

Table 3: Distribution of malignant lesions

Lesions	No .of cases (%, n=70)
Non-Hodgkin's lymphoma	25 (35.71 %)
Metastatic carcinoma	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± SD
Koch's lymphadenitis	1.04 ±0.03
Chronic non-specific lymphadenitis/ Reactive hyperplasia of LN.	1.15 ± 0.04
Non-Hodgkin's lymphoma	12.66±2.07
Metastatic carcinoma	5.10±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 was1.13 +/- 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 1st decade of life, Koch's is common in 2nd decade of life. All malignant lesions were common after 40 years of age, from which metastatic carcinoma were seen in 5th& 6th decade of life &, Non-Hodgkin's lymphoma were common after 6th decade of life.

DISCUSSION

Neucleolar 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 techinique 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 this 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.

REFERENCES

1. Kapoor V, Suri RK. Basic Human Genetics, 2nd Edition, Jaypee Brother Medical publishers Ltd., New Delhi;1991. p. 5-20.
2. Vatsala Misra, SP Misra, Manisha Dwivedi, Suresh C Gupta. AgNOR counts are not altered in alcoholic cirrhosis. Indian J. Pathol microbiology. 2003;46(2):170-2.
3. Archana Gulati, Daishree Sharma, BB Sharma, Rajni Kaushik, Surinder Kashyap. Evaluation of AgNORs in Pulmonary lesions. A cyto-histopathological correlation. Indian J. Pathol. 2002;45(3):459-461.
4. Nalini Gupta, Amarjit Kaur, Surinder Pal, K K Aahlo. Study of Nucleolar organizer regions (NORs) in lesions of gall bladder. Indian J. Pathol Microbiol 2003;46(3):371-4.
5. J Crocker, J .Macartney, PJ Smith. Correlation between DNA flowcytomericgrand nucleolar organizer regions data non- Hodgkins lymphoma. J.Pathol 1988;154 (2):151-156.
6. Robbins. Pathologic Basis of Diseases Lymphnodes, 6th Edition, Harcourt India private Ltd. New Delhi;1999. p. 645
7. Sangeeta Pahuja, Monishaq Choudhary, Usha Gupta. Proliferative activity in squamous intraepithelial and invasive lesions of Cervix, analysis by AgNORStaining. Indian Jol. Pathol 2003;45(4):573-5.
8. FA Culling. Handbook of histopathological technique including museum technique 2nded, London, Butterworth;1963. p. 413-415
9. Luna GL. Manual of histopathological staining method of the armed force institute of pathology, 3rd edition, The blackstone division, MC Graw, Hill book company, New York 1979; 34-36.
10. Park JK, Park K. textbook of preventive and social medicine 13th edition. M/s bansaridas bhanot publishers, Jabalpur. 1991.455-61.
11. Mahajan BK. Methods in biostatistics for medical students and research workers. 5th edition. Jaypee brothers, New Delhi. 1991.135-43.
12. DD Giri, JF Nottingham, J Lawry, SAC Cundas, JCE Underwood. Silver binding nucleolar organizer regions (AgNORs) in benign and malignant breast lesions, correlation with ploidy and growth phase by DNA flow cytometry. J. Pathol 1989;157(4):307-18.
13. HM Sagparia. Application of AgNOR count in cytological examination of lymphnode for lymphadenopathy. Dissertation submitted to Gujarat University in 1998(unpublished data).
14. AD Bhalani. A study of AgNOR count in cytology of lymphnode for lymphadenopathy. Dissertation submitted to the Saurastra University in 1999.(unpublished data)
15. A Mahotra, PK Agrawal, T Chandra. Cytopathology and AgNOR count in Fine needle aspiration cytology smears of thyroid lesions. H. diagnostic cytopathol 1998;19(4):238-43.
16. J Crocker, Parmagitnar: Nucleolar organizer regions in lymphomas. J Pathol 1987;151(2):111-8.
17. AJ Howat, DD Giri, A L Wright, JCE Underwood. Silver stained nucleoli and nucleolar organizer region counts. J Pathol 1998; 156 (3):227-32.

Corresponding Author:

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

Date of Submission: 05/07/2015
Date of Acceptance: 20/08/2015

How to cite this article: Vasava KT, Shah NA, Katara RK, Desai NJ, Damor VM. A study of AgNOR count in FNAC lymph node in case of lymphadenopathy, J Res Med Den Sci 2015;3(3):225-28.

Source of Support: None
Conflict of Interest: None declared