

USE OF AgNOR INDEX IN GRADING AND DIFFERENTIAL DIAGNOSIS OF ASTROCYTIC LESIONS OF BRAIN

Mulazim Hussain Bukhari,¹ Shahida Niazi,² Ihsanullah Hashmi,³ Samina Naeem,⁴
Abdul Khalik Abro,⁵ Mohammad Tayyab,⁶ Naseer Ahmad Chaudhry⁷

ABSTRACT

Objective: This study was conducted to see the importance of AgNOR staining in grading and differential diagnosis of Astrocytic lesions.

Methodology: It was a descriptive and prospective study conducted in Department of Pathology King Edward Medical University, from June-December 2002. AgNOR staining was performed on 60 randomly selected brain specimens of Astrocytic lesions including Astrogliosis and Astrocytoma.

Results: AgNOR count, size and dispersion were normal in Astrogliosis, low in Pilocytic Astrocytoma, high in grade II, higher in grade III and highest in grade IV. AgNOR counts of different grades of astrocytoma (2.97 ± 0.96 , 3.97 ± 0.43 , 6.01 ± 2.74 and 8.01 ± 3.56) were significantly ($P < 0.01$) greater as compared with counts of normal brain (0.40 ± 0.01), and reactive gliosis (0.60 ± 0.01). There was no statistical difference in normal brain tissues and inflammatory lesions of the brain. AgNOR size and dispersion were of higher grade in significantly greater proportion in malignancy as compared to benign conditions. ($P < 0.05$). AgNOR size and dispersion were normal in Astrogliosis.

Conclusions: Typing of AgNOR count, size and dispersion was found to be an important marker in grading and differential diagnosis of Astrocytic lesions, especially in Astrogliosis and low grade Astrocytoma.

KEY WORDS: AgNOR, Astrocytoma, Astrogliosis, Glioblastoma Multiforme.

Pak J Med Sci April 2007 Vol. 23 No. 2 206-210

1. Mulazim Hussain Bukhari,
 2. Shahida Niazi,
 3. Ihsanullah Hashmi,
 4. Samina Naeem,
 5. Abdul Khalik Abro,
 6. Mohammad Tayyab,
 7. Naseer Ahmad Chaudhry
- 1-4: Department of Pathology,
King Edward Medical University,
5-7: University of Health sciences and
Postgraduate Medical Institute,
Lahore - Pakistan.

Correspondence

Dr. Mulazim Hussain Bukhari, DCP, M.Phil, FCPS
Associate Prof Pathology,
King Edward Medical University, Lahore
26-MOF, GOR-3, Shadman,
Lahore - Pakisatn.
E-mail: drmhbukhari@yahoo.com

- * Received for Publication: July 3, 2006
- * Revision Received: August 23, 2006
- * 2nd Revision Received: January 15, 2007
- * Revision Accepted: January 18, 2007

INTRODUCTION

Nucleolar organizer regions (NORs) are specific portions of DNA, called rDNA that, by using the enzyme RNA-polymerase-1, code for the transcription of ribosomal RNA (rRNA). This rRNA inside the ribosomes is responsible for protein synthesis of the cell. Protein synthesis is a necessary step in the process of cell proliferation. Therefore a relation between NORs and cell proliferation is suggested. With the use of silver colloid impregnation, described by Goodpasture and Bloom in 1975,¹ modified by Ploton et al in 1986,² NORs can be identified much easier.

By using the silver nucleolar organizer region (AgNOR) impregnation technique the number, size and shape of NORs can be studied in a fast and simple way, not only in fresh frozen

tissue specimens but also in formalin fixed paraffin embedded material. The amount of silver deposit in a cell, reflecting the amount of NORs that are involved in protein-synthesis, is thought to be related to the proliferative capacity of that cell. The exact relationship between proliferation, protein-synthesis and expression of AgNORs is, however, not yet well understood. But the expression of AgNOR is either causally or indirectly coupled to DNA-synthesis and thus AgNOR can be considered as a cell proliferation marker.³⁻¹¹

Counting the number of AgNORs is subject to intra- and interobserver variability, which is often regarded as a limitation to the reliability of the results. However, when the interobserver variation was tested, it appeared that there was a statistical significant correlation between the results found by two observers,^{14,15} but the correlation coefficient was higher in counting AgNOR-areas than in AgNOR numbers.^{14,15} To make the counting more specific, the silver-deposit inside the nucleolus only instead of the whole nucleus can be regarded. In one study this led to the conclusion that measurement of the whole nucleolar size has the same relevance as AgNOR scoring in these nucleoli.¹⁶ Another study found a positive correlation between AgNOR number and nuclear size¹⁷ but both had no value in predicting survival of the patient. These results were contradicted by Tosi et al. who found that the number of AgNORs had a significant predictive value in patient outcome, but form, shape and size of the nucleus did not.¹⁸

The mean number of AgNOR dots per cell, or per nucleus, is often referred to as mAgNOR. The mAgNOR scores in cells with slow proliferation may be in the range between 0.5 and 1.5, however there are only very few reports giving "normal-values" of mAgNOR for different types of human tissues. The mAgNOR scores are higher in fast proliferating tissues. Most AgNOR studies focus on the difference in AgNOR counts among tumors of different pathological grades and tissues in different

stages of neoplasia, i.e. dysplasia, in situ carcinoma or invasive carcinoma.

The pAgNOR refers to the percentage of cells in a tumor or tissue slice that harbors more than a certain number of AgNORs per cell (mostly more than 5), this is also called the AgNOR distribution score and sometimes the AgNOR proliferation index. Some studies, correlating the results of flowcytometry and BrdU-labeling with AgNOR staining, demonstrated that pAgNOR correlates with percentage of cells in S-phase of the cell cycle, or with proliferative activity, whereas mAgNOR correlates with ploidy.¹⁹⁻²³

PATIENTS AND METHODS

Sixty patients of Astrocytic lesions were included in this study. Adult patients of both sexes were taken into consideration. History of the patients along with relevant investigations were recorded. Particular stress was given on the age, sex and clinical findings of the patient. The CT Scan report was also added to the history of the patient. The specimens were collected in properly labeled jars, containing 10% formal saline. Detailed gross examination of each specimen was carried out and recorded. Representative tissue sections were taken. The tissues were processed in an automatic processor. Embedding of tissues was done in paraffin wax using L-shaped metal moulds. Each block was cut into multiple sections 3-4 micrometer thick on a rotary microtome.

Sections were taken on albumenized slides. Sections of all the cases were stained with Haematoxylin and Eosin and sections of all cases were also stained by AgNOR stain.

RESULTS

These AgNORs counts were high in all 40 cases of Astrocytoma of grade I-IV and normal in normal brain tissue (0.40 ± 0.01) low in reactive gliosis (0.60 ± 0.01). The AgNORs count increased as the grade of astrocytoma varied i.e I to IV. There was slight increase in AgNORs count in Pilocytic astrocytoma (2.97 ± 0.96), moderately much more increased

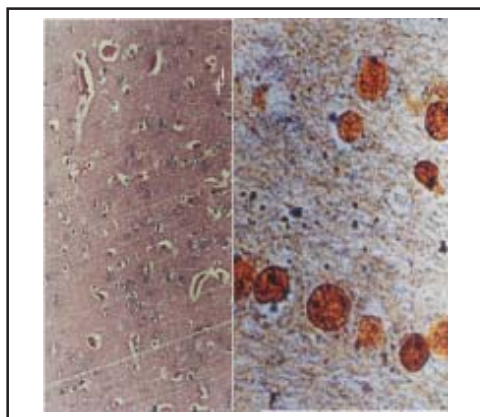


Fig-1: Photomicrograph of normal brain tissue stained with H& E on left side and AgNOR on right side. AgNORs (black dots) count, size and dispersion are normal.

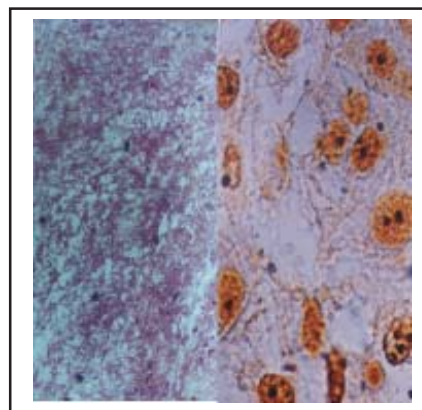


Fig-2: Photomicrograph of Pilocytic Astrocytoma stained with H& E on left side and AgNOR on right side. AgNORs (black dots) count, size and dispersion is increased.

in Astrocytoma grade II (3.97 ± 0.43), increased in Astrocytoma grade III and Astrocytoma grade IV (6.01 ± 2.7 and 8.01 ± 3.56) respectively. This difference is significant. ($P < 0.05$), pAgNOR counts were high in increasing grades of astrocytoma; 25% in Pilocytic astrocytomas, 50% in all astrocytoma grade II, 65% in all grade III and 72% in all grade IV Astrocytoma (Fig: 1-3). It was less than 5% in normal brain tissue and 8% in reactive gliosis. This count was believed to represent proliferative activity. Tumors having a pAgNOR count of 8% or more were considered to display high proliferative activity. The AgNOR count in malignant cells was significantly higher than that in resting cells and reactive cells ($p < 0.01$), and the count significantly increased with tumor grade ($p < 0.01$). AgNOR count of resting cells was close to reactive cells ($p > 0.05$). Varia-

tion in AgNOR size in Grade I-II Astrocytoma was also observed. There was non-to mild variation in the size of AgNOR dots in low grade Astrocytoma (mean 0.25 ± 0.19) and moderate to high variation in the size of AgNOR dots in high grade Astrocytoma (mean 1.86 ± 0.80). The statistical difference between the variations of AgNOR dot size was significantly high between low grade and high grade Astrocytic Glioma $P < 0.05$. The mean dispersion of AgNOR per cell in normal tissue (and reactive gliosis was nil, while it was low in low-grade astrocytoma and high in high-grade astrocytoma. This difference was significantly high between low to high-grade astrocytoma ($p < 0.05$). The mean dispersion of AgNORs per cell in high grade Astrocytoma was high (mean 1.56 ± 0.44 in grade IV) and statistically significant ($p < 0.05$) (Table-I).

Table-I: Comparison of Mean AgNOR Count, Size, and Distribution per Cell between in different lesions

Lesions	No. Of Cases	AgNOR count		AgNOR variation in Size Per cell (Mean±SD)	AgNOR Dispersion Per cell (Mean±SD)
		mAgNOR Count Per cell (Mean±SD)	pAgNOR Proliferative Index (Percentage of Nuclei with e'5 AgNOR dots/Nucleus)		
Normal brain tissue	10	0.40 ± 0.01	5%	0.20 ± 0.15	0.18 ± 0.68
Reactive Gliosis	10	0.60 ± 0.01	8%	0.23 ± 0.23	0.20 ± 0.54
Pilocytic Astrocytoma	10	2.97 ± 0.96	25%	0.25 ± 0.19	0.29 ± 0.13
Astrocytoma grade II	10	3.97 ± 0.43	50%	0.45 ± 0.18	0.43 ± 0.24
Astrocytoma grade III	10	6.01 ± 2.74	65%	1.24 ± 0.26	0.93 ± 0.42
Astrocytoma grade IV	10	8.01 ± 3.56	72%	1.86 ± 0.80	1.56 ± 0.44

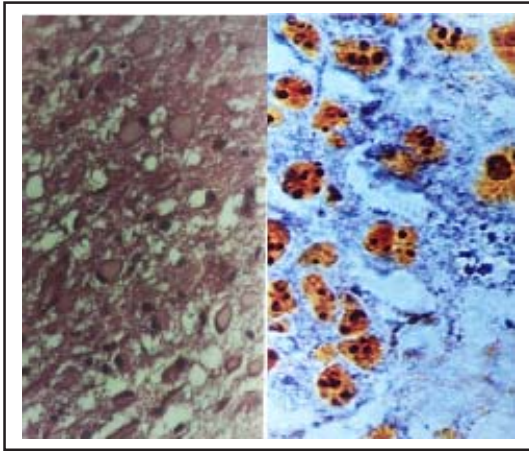


Fig-3: Photomicrograph of Grade II Astrocytoma stained with H& E on left side and AgNOR on right side indicating high AgNORs (black dots) count, size and dispersion increased.

DISCUSSION

AgNOR is a simple, cheap and valuable marker in grading and staging of Astrocytic lesions of the brain and important in differentiating benign and malignant tumours. In our study AgNOR index (count, size and dispersion) was low in Astrogliosis as compared to low grade astrocytoma e.g., Pilocytic Astrocytoma. This index rises as the grade and stage of the tumor increases. In our study it was low in Pilocytic astrocytoma while high in astrocytoma grade II, higher in astrocytoma grade III and highest in astrocytoma grade IV. The difference was significant ($P < 0.05$).

An AgNOR count is another parameter to be considered in grading and differential diagnosis of Astrocytoma and reactive conditions. It rises according to the grades of astrocytoma. We have seen that $pAgNOR$ was 25% in Pilocytic astrocytoma, 50% in all astrocytoma grade II, 65% in all grade III and 72% in all grade IV Astrocytoma. It was less than 5% in normal brain tissue and 8% in reactive Astrogliosis. The AgNOR count in malignant cells was significantly higher than that in resting cells and reactive cells ($p < 0.01$), AgNOR count of resting cells was close to reactive cells and the difference was non significant ($p > 0.05$). These results are consistent with the findings

of Khan et al,²⁴ who concluded that typing of AgNOR size and dispersion is a more reliable and reproducible alternative to traditional AgNOR counts for differentiating malignant from non-malignant effusions.

Our results are in accordance with studies of Pich et al. showing an association between AgNOR count and histological grade of differentiation^{25,26} and with a study of Fonseca showing that the AgNOR count is low in benign lesions as compared to malignant lesions of the oral cavity.²⁷ Our findings are also consistent with the local investigators and international studies like the reports of Helpap et al,²⁸ Khan et al,²⁴ Hashmi et al²⁹ and Parveen et al.³⁰

REFERENCES

1. Goodpasture C, Bloom SE. Visualization of Nucleolar Organizer Regions in mammalian chromosomes using silver staining. *Chromosoma* 1975;53:37-50.
2. Ploton D, Menager M, Jeannesson P, Himber G, Pigeon F, Adnet JJ. Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer region at the optical level. *Histochem J* 1986;18:5-14.
3. Crocker J. Nucleolar Organizer Regions. In: Current topics in pathology. Ed. JCE Underwood. Springer Verlag, New York, 1990.
4. Plate KH, Ruschoff J, Mennel HD. Application of the AgNOR technique to neurooncology. *Acta Histochem Suppl* 1992;42:171-8.
5. Derenzini M, Sirri V, Trere D, Ochs RL. The quantity of nucleolar proteins nucleolin and protein B23 is related to cell doubling time in human cancer cells. *Lab Invest* 1995;73:497-502.
6. Derenzini M, Sirri V, Pession A, Trere D, Roussel P, Ochs RL, et al. Quantitative changes of the two major AgNOR proteins, nucleolin and protein B23, related to stimulation of rDNA transcription. *Exp Cell Res* 1995;219:276-82.
7. Ofner D, Riedmann B, Maier H, Hittmair A, Rumer A, Totsch M, et al. Standardized staining and analysis of argyrophilic nucleolar organizer region associated proteins (AgNORs) in radically resected colorectal adenocarcinoma, correlation with tumour stage and long-term survival. *J Pathol* 1995;175:441-8.
8. Lohr CV, Driemeier D, Teifke JP. Polyethylene glycol-thiosulfate (PEG-Th) staining a modification of the AgNOR method. *Zentralbl Pathol* 1995;140:465-8.
9. Li Q, Hacker GW, Danscher G, Sonnleitner-WU, Grimelius L. Argyrophilic nucleolar organizer regions. A revised version of the Ag-NOR-staining technique. *Histochem Cell Biol* 1995;104:145-50.

10. Vidal B de C, Mello ML. Re-evaluating the AgNOR staining response in Triton X-100-treated liver cells by image analysis. *Anal Cell Pathol* 1995;9:39-43.
11. Yekeler H, Erel O, Yumbul AZ, Doymaz MZ, Dogan O, Ozercan MR, et al. A sensitive staining method for NORs. *J Pathol* 1995;75:449-52.
12. Lindner LE. Improvements in the silver-staining technique for nucleolar organizer regions (AgNOR). *J Histochem Cytochem* 1993;41:439-45.
13. Rowlands DC, Ayres JG, Crocker J. The effect of different fixatives and length of fixation time on subsequent AgNOR staining for frozen and paraffin-embedded tissue sections. *Histochem J* 1993;25:123-32.
14. Johnson GC, Miller MA, Ramos-Vara JA. Comparison of argyrophilic nucleolar organizer regions (AgNORs) and mitotic index in distinguishing benign from malignant canine smooth muscle tumors and in separating inflammatory hyperplasia from neoplastic lesions of the urinary bladder mucosa. *J Vet Diagn Invest* 1995;7:127-36.
15. Agarwal PK, Mehrotra A, Chandra T. Diagnostic relevance of silver stained nucleolar organizer region (AgNORs) in benign and malignant breast lesions. *Indian J Exp Biol* 1995;33:715-20.
16. Losi L, DiGregorio C, Fante R, Migaldi M, Roncucci L, Pedroni M, et al. Argyrophilic nucleolar organizer regions and bromodeoxyuridine and ³[H]-thymidine labeling indices in colorectal cancer. *Cell Prolif* 1995;28:471-80.
17. Trere D, Migaldi M, Trentini GP. Higher reproducibility of morphometric analysis over the counting method for interphase AgNOR quantifying. *Anal Cell Pathol* 1995;8:57-65.
18. Derenzini M, Farabegoli F, Trere D. Relationship between interphase AgNOR distribution and nucleolar size in cancer cells. *Histochem J* 1992;24:951-6.
19. Eskelinen M, Lipponen P, Syrjanen K. Nucleolar organiser regions (AgNORs) related to histopathological characteristics and survival in prostatic adenocarcinoma. *Anticancer Res* 1992;12:1635-40.
20. Tosi P, Cintonino M, Santopietro R, Lio R, Barbini P, Ji H, et al. Prognostic factors in invasive cervical carcinomas associated with human papillomavirus (HPV). Quantitative data and cytokeratin expression. *Pathol Res Pract* 1992;188:866-73.
21. Mourad WA, Connelly JH, Sembera DL, Atkinson EN, Bruner JM. The correlation of two argyrophilic nucleolar organizer region counting methods with bromodeoxyuridine-labeling index: a study of metastatic tumors of the brain. *Hum Pathol* 1993;24:206-10.
22. Carbajo S, Orfao A, Vicente-Villardón JL, Carbajo-Perez E. Expression of silver-stained nucleolar organizer regions is coupled to cell cycle in rat thymic cells. *Cytometry* 1993;14:46-52.
23. Mourad WA, Erkman-Balis B, Livingston S, Shoukri M, Cox CE, Nicosia SV, et al. Argyrophilic nucleolar organizer regions in breast carcinoma. Correlation with DNA flow cytometry, histopathology, and lymph node status. *Cancer* 1992;69:1739-44.
24. Khan SA, Chaudhry NA, Khalid AW, Akhtar GN, Ibne-Rasa SN. Patterns of argyrophilic nucleolar organiser regions in pleural and peritoneal effusions. *J Coll Physicians Surg Pak* 2006;16:412-5.
25. Pich A, Chiusa L, Marmont F, Cappello N, R. Navone R. Argyrophilic nucleolar organizer region counts in multiple myeloma: A histopathological study on bone marrow trephine biopsies. *Virchows Archiv* 1992;421:143-7.
26. Pich A, Chiusa L, Comino A, Navone R. Cell proliferation indices, morphometry and DNA flow cytometry provide objective criteria for distinguishing low and high grade bladder carcinomas. *Virchows Archiv* 1994;424:143-8.
27. Fonseca LM, do Carmo MA, do Carmo MA. AgNORs in hyperplasia, papilloma and oral squamous cell carcinoma. *Braz Dent J* 2000;11:105-10.
28. Helpap B, Loesevitz L, Bulatko A. Nucleolar and argyrophilic nucleolar organizer region counts in urothelial carcinomas with special emphasis on grade II tumors. *Virchows Archiv* 1994;425:265-69.
29. Hashmi IU, Bukhari MH, Tayyab M, et al. Evaluation of AgNOR in normal brain tissue and Astrocytic Glioma. *Biomedica* 2006;22:41-7.
30. Parveen S, Bukhari MH, Khan SA, et al. AgNOR stain in normal, cirrhotic and carcinomatous liver. *Biomedica* 2006;22:59-61.