# Correlation of mean AgNOR count with the histological grade of high grade gliomas-A five year study

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**Abstract:**
**Background**-Staining for Nucleolar organizing regions can be utilized as a modality to grade the glial tumours.AgNOR has advantage of being simple, ease of use, low cost and its good correlation with other proliferative markers, as their frequency within nucleus are significantly higher in malignant cells than in normal cells.
**Objectives**-To correlate the AgNOR's mean number with the histological type and grade of high grade glial tumors.
**Methods**-257 high grade glial tumors (140 Anaplastic Astrocytomas and 145 Glioblastomas)specimens received in the Pathology Deptt. G.R Medical College Gwalior (India) was studied. Sections were stained with routine H & E stain & with AgNOR stain.All Glial tumours were categorized and graded histologically. The mean AgNOR values (mAgNOR) per nuclei were determined.
**Results**-The average values of mean AgNOR i.e. mAgNOR/nucleus presented a linear increase with increasing grade of malignancy from 2.74 for Anaplastic Astrocytoma (GIII), to 3.58 for Glioblastoma (GIV).
**Conclusion**- The malignancy grade of Glial tumors can be established both on histological features of the conventional stained sample and on the average number, the shape and the distribution of AgNORs within tumoral nuclei. The no. of AgNORs increased with increasing grade of malignancy .
**Keywords**: Glial tumours, Anaplastic Astrocytomas, nucleor organizer region

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## I.  Introduction

The annual incidence of CNS tumors ranges from 10 to 17 per lakh persons for intracranial tumors and 1 to 2 perlakh persons for intraspinal tumors; about 50-75%are primary tumors, and the rest are metastatic.

Tumors of the CNS make up a larger proportion of Pediatric cancers, accounting for as many of 20% of all pediatrictumors. Pediatric CNS tumors differ from those in adults in both location and histologic subtype. In childhood,tumors are located in the posterior fossa; in adults, they are mostly supratentorial.[1]

**AgNOR**

This technique has the advantage of being simple, easy to perform and quick procedure. It can be performed on routinely fixed paraffin wax embedded specimens. Paraffin blocks can also be utilized for retrospective studies. The usefulness of the method has recently been increased by means of a modification allowing the AgNOR reaction to run at room temperature rather than at conventional $60^0C$[2].

The technique of AgNOR has been utilized for prognostic value and in the grading of a tumor and is a well-established method of estimation the proliferative activity of the tumor. Nucleolar organizer regions (NORs), which are loops of DNA (rDNA) encoded for ribosomal RNA (rRNA) production [3, 4].

The arrangements studied in this technique are argyrophilic non-histone proteins (AgNORs) whose silver stainability serves as an indicator for transcriptional activity of NORs [5]. NORs can now be demonstrated relatively easily by routinely processed histological sections, thus the technique is obviously of potential value in diagnostic histopathology.

## II.  Material and Methods

**Selection of cases**
- This study was conducted for a period of five years (Feb 2014-March 2018) on 285 specimen of high grade gliomas ie astrocytoma grade III and Glioblastoma submitted to the Department of Pathology, Gajra Raja Medical College and J.A Group of Hospitals, Gwalior.
- Patients of both sex were taken into consideration.

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- History of the patients along with relevant investigations was recorded. Particular stress was given on the age, sex and clinical findings of the patient
- Thin sections were cut and stained with routine hematoxylin and eosin stain. All slides are thoroughly evaluated for histopathological features.
- All glial tumours are categorized histologically (morphologically ) into Astrocytoma grade III and Glioblastomas, and graded according to WHO Criteria 2016.
- The sections are stained with AgNOR stain and All glial tumours are graded depending upon histological and AgNOR count.
- The mean AgNOR values (mAgNOR) per nuclei were determined
  **AgNOR Staining metho**d

- Sections were dewaxed in xylene, hydrated through alcohols to water.
- Then the sections were rinsed with deionized water 3 times.
- Sections were incubated with working staining solutions (freshly prepared) for 60 minutes under dark room temperature condition.
- Sections were rinsed in deionized water for 10-15 minutes.
- Sections were dried cleared in Xylene and mounted in DPX.

## III. Observation And Results
**Table 1 :Mean values of AgNORs in tumoral nuclei of glial tumours according to their histological subtypes and grades**.

| Malignancy grade | Average number of AgNORs/nucleus | |
|---|---|---|
| | Range | Group (Mean±SD) |
| **Anaplastic astrocytoma (G III)** | 2.6-3.1 | 2.74±0.11 |
| **Glioblastoma (G IV)** | 3.1-4.2 | 3.58±0.36 |

**P value is <0.00001** .The result is significant at P<0.05.

The average values of mean AgNORs/nucleus (mAgNOR/nucleus) presented a linear increase with increasing grade of malignancy. In Anaplastic astrocytoma (GIII) mAgNOR/nucleus ranged from 2.6-3.1 with a group mean of 2.74±0.11 SD. In Glioblastoma Multiforme (GIV)mAgNOR/nucleus ranged from 3.1-4.2 with a group mean of 3.58±0.36 SD.

**Table 2 : distribution of glial tumours and its distribution**

| Astrocytoma grade III | 140 | 49.12% |
|---|---|---|
| Glioblastoma Multiforme | 145 | 50.88% |
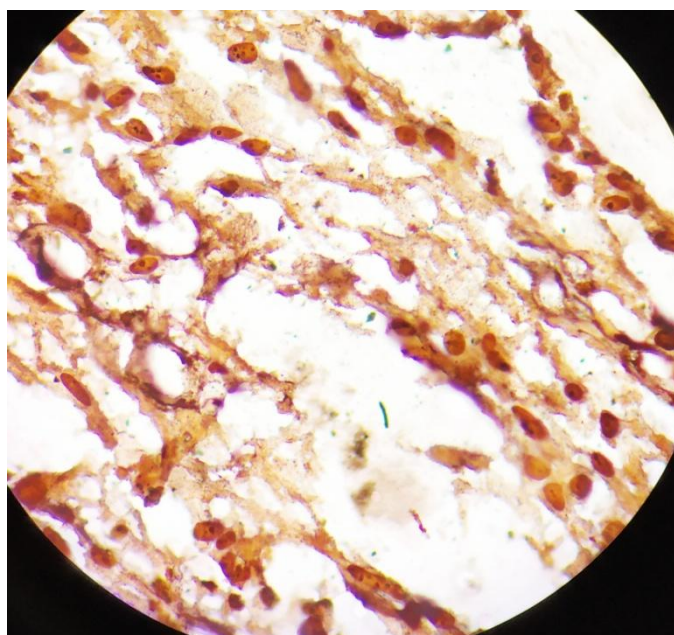


**Figure 1:** Astrocytoma grade III AgNOR indicating black dots in tumoral nuclei (silver impregnation method,magnification x100).
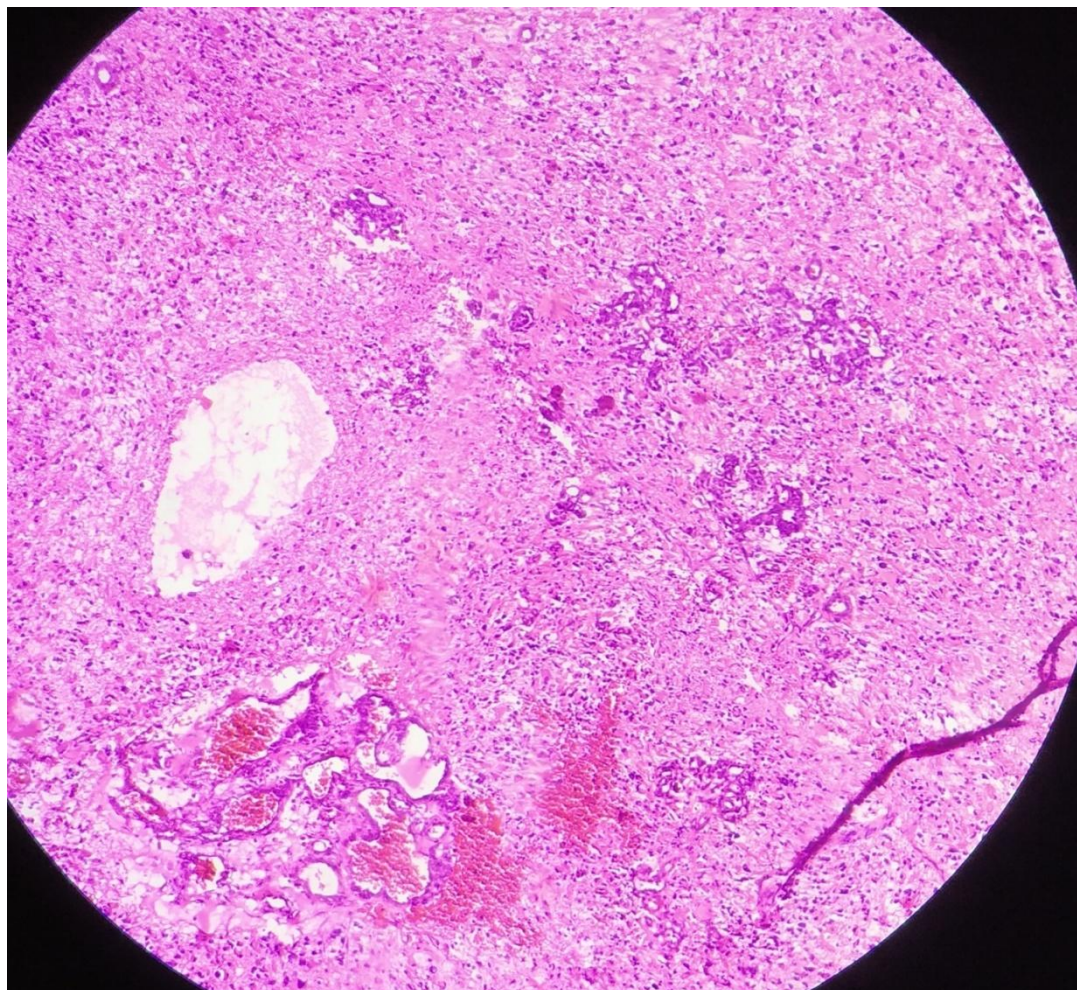
**Figure 2:** Glioblastoma Multiforme (Endothelial cell proliferation) and necrosis(40X,H&E)

### IV. Discussion

The requisite information was collected from the patient's requisition forms which were sent from Bhagwat Sahay Hospital Department of neurosurgery Jayarogya group of hospital Gwalior (Madhya Pradesh).

Various studies have been carried out on AgNOR in Glial tumors in the past. We have tried to compare our results with the advancement and scientific achievement in the modern era, our knowledge on the histolopathogical and various modifications in the classification & staging of the Glial tumours has been progressed to present stage as a natural evolutionary process.

Analysis of the AgNOR's mean number is a widely accepted method for diagnosis of a wide range of tumors, both on cytological and histological preparations.

**In 1986, Ploton et al.**[6] have used and improved the silver impregnation method brought in histopathological practice by Goodpasture and Bloom in 1975[7]. Ploton et al. [6] suggested that the number of AgNORs/cell correlates with the proliferative activity of a cell and may be an indicator of malignancy, because larger the number of AgNORs more active is the proliferation, and thus a more malignant cell.

AgNORs have been found to be markers of proliferation in brain Gliomas because from 1990 onwards some articles have shown linear correlations between the number of AgNORs, binding index of Ki-67, Proliferating Cell Nuclear Antigen (PCNA), or histological grade and the mitotic rate[8].

**Janczukowicz (2003)**[9]found a significant relationship between the number of AgNOR and histological grade in astrocytic tumors, but the main stress was on the presence of a small overlapping of extreme values between GII and GIII Astrocytomas and between GIII and GIV[10]

**Gabriela – Florenta Dumitrescu et al. (2010)**[11]concluded that the presence of an increased mean number of mAgNOR/vascular nucleus in Anaplastic Astrocytomas (GIII) and, especially,in Glioblastomas Multiforme (GIV) suggest that the proliferative processes of vascular cells and tumor cells are interconnected.

In our study the average values of mean AgNORs/nucleus (mAgNOR/nucleus) presented a linear increase with increasing grade of malignancy. In Anaplastic astrocytoma (GIII) mAgNOR/nucleus ranged from

2.6-3.1 with a group mean of 2.74±0.11 SD.In Glioblastoma Multiforme (GIV)mAgNOR/nucleus ranged from 3.1-4.2 with a group mean of 3.58±0.36 SD.

We also observed some overlapping of the extreme values of the mean AgNOR/nucleus between Anaplastic astrocytomas (GIII) and Glioblastomas (GIV) probably because some of the samples which were considered Anaplastic astrocytomas were in fact infiltration areas of a Glioblastoma.

## V.  Conclusion

The present study comprised of the prospective histopathological study of 100 cases of glial tumors from 1st December 2016 to 30th may 2018 in the department of pathology, Gajra Raja Medical College, Gwalior(M.P.).

The mean number of AgNORs/nucleus (mAgNOR/nucleus) showed a straightforward increase with increasing grade of malignancy.

AgNOR proved to be a useful method for histological grading as it supplemented the information which was obtained with the conventional histological assessment.

The grade of Glial tumors can be established both on histological features of the conventional H& E stained sections and on the average number and the distribution of AgNORs in the nuclei of tumoral cells

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