Argyrophilic Nucleolar Organizer Regions (AgNORs) as a Proliferative marker in various prostatic lesions

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ABSTRACT
Aims and objectives:
  1. *To establish the role of AgNOR in differentiating the benign from the premalignant and the malignant lesions of the prostate.*
  2. *To establish the significance of AgNOR count in differentiating the grades of malignancy.*

Materials and methods: *A prospective study was conducted on 197 prostatic lesions from 2011- 2013. Two sections from each case were stained with haematoxylin and eosin and the other with AgNOR stain. The data was analysed by using SPSS-17.0.*

Results: *The mean AgNOR count of prostatic adenocarcinoma was significantly higher than the benign and premalignant lesions of prostate with P<0.001. The mean AgNOR count in benign lesions is1.6 +/- 0.2 and the AgNOR dots tend to be large, regular and homogenously stained. The mean AgNOR count in malignant lesions was between 4.7 +/- 0.1 – 5.7+/- 0.9 and the dot appear large, irregular in size and shape with gaint and bizarre clusters.*

Conclusion: *AgNOR staining can be considered as a useful adjunct to diagnostic pathology. The study was helpful in evaluating the importance of AgNORs in differentiating the benign, premalignant and malignant lesions of prostate can be considered as a valuable tool in analysing the proliferative activity of the cell.*

Keywords: *AgNORs, Prostatic lesions, Proliferation markers*

INTRODUCTION

Prostatic carcinoma has become one of the common malignant tumours among men[1]. Early diagnosis and prompt treatment of prostatic carcinoma is very essential for good prognosis, however it is challenging for the uropathologist to diagnosis due to smaller size of the biopsy available due to minimal invasive technique. A prostatic lesion on routine haematoxylin and eosin (H&E) reveals various problems in differentiating between benign, grey zone and malignant lesions of prostate. The routine histotechniques do not show all the features which are of diagnostic and prognostic significance. The criteria available for the diagnosis and prognosis of prostatic cancer such as histological grade, clinical stage, prostatic specific antigen (PSA) levels and deoxy ribose nucleic acid(DNA) content often do not sufficiently predict the outcome of the disease [2,3].Hence it is eminent to develop adjunct procedures which can diagnose malignancy at the earliest and with accuracy.

Studies have revealed the correlation between nuclear function, size, and the cell doubling time in human cancer cell lines, which has stimulated a revolution of the importance of the nucleus in tumour pathology[4]. The nucleus plays an essential role in the control of proliferation and protein synthesis. Various proliferative markers like argyrophilic nucleolar organising regions(AgNORs), Ki-67 and estimation of S- phase cells and mitotic cells are used to know the proliferation rate of the nucleus in the cell.

The nucleolar organising regions (NORs) are loops of ribosomal DNA which occur in the nucleoli of the cells on the short arm of acrocentric chromosomes 13,14,15,21 and 22[5].Due to high affinity of NORs for silver ions they are called as AgNOR. Studies have revealed that, the sliver staining technique have showed the variability in the number of NORs from cell to cell[6]. AgNOR as a proliferative marker correlates with the rate of proliferation as estimated by Ki-67 and the percentage of S phase cells and the mitotic cells[5]. The present study was undertaken to analyse the AgNOR count of various prostatic lesions and the use of AgNOR count in differentiating the benign from the pre-malignant and the malignant lesions of the prostate. We have also correlated the AgNOR count with the histological grade and Gleason score of prostatic carcinoma.

MATERIALS AND METHODS

The present study is a prospective type of study carried out during the period of July 2011to October 2013. A total of 197 prostatic specimens were received during the study period.

Eligibility criteria adopted in our study.

1. **Inclusion criteria:** All types of prostatic specimens like transurethral resection of prostate (TURP), needle biopsies (NB) and prostatectomy specimens were included.
2. **Exclusion Criteria:** Inadequate biopsies and poorly preserved prostatic specimens were excluded.

The biopsy specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Two serial sections were cut from each block. The first section (5µ thick) was stained with routine haematoxylin and eosin (Hand E) stain for histopathological diagnosis. The second section (3µ thick) was stained with silver nitrate to visualise the Nucleolar Organizer regions (NORs) using Howel's and Black rapid 1-step method [7].

AgNOR counting Procedure: The number of AgNORs which were present in each nucleus was counted in 100 nuclei by using a 100X oil immersion lens. The NORs are visualized as a black/purple dots arranged both in clusters, clumps and as individual 'satellites' within the cell nucleus. The results were obtained by the counting procedure which were tabulated, analysed and subjected for statistical analysis using SPSS 17.0. Results are presented as mean +/- SD. Mann – Whitney test was used to assess the statistical differences between AgNOR counts of benign and malignant lesions of prostate.

Grading of AgNOR: The grading of size variation and dots dispersion was performed according to criteria cited by Ahsan et al [8]

The score of distribution for AgNOR dots are given as below

    0 -   More/less uniform in size
    1+ - Two different sizes of AgNOR dots
    2+ -> two different sizes of AgNOR dots.
    3+ - Including all grades and sizes

The score of distribution for AgNOR dots dispersion are given as below:

    0 – Limited to nucleoli
    1+ - Occasional dispersion outside nucleoli
    2+ - Moderate dispersion outside nucleoli.
    3+ - Widely dispersed through the nucleolus

RESULTS

A total of 197 specimens were received during the study period constituting 2.1% of total specimens received to the department of the pathology. Demographic details of the received prostatic lesions are shown in graph 1. The distributions of prostatic lesions with respect to specimens are given in table 1. There was some overlapping in AgNOR values between BPH (figure 1A and 1B). And AAH. High grade prostatic intraepithelial neoplasia (HGPIN) had mean AgNOR count of 2.3 +/- 0. None of these cases show overlapp with AgNOR count of BPH.

In adenocarcinoma of prostate the mean AgNOR count was between 4.7+/- 0.1 – 5.7+/- 0.9 (figure 5A and 5B). It was observed that mean AgNOR count increases with increase in the grade of malignancy. In our study we observed that the AgNOR dots tend to be large, homogenously stained and regular in the nuclei of benign lesions, whereas in the malignant lesions the AgNOR dot shows irregular in size and shape with giant and bizarre clusters seen. Hence by seeing the number and morphology of AgNOR dots one can differentiate between benign and malignant lesions of prostate.

The pooled mean AgNOR count for the benign prostatic lesions was 1.60±0.2 which was significantly lower than that of the prostatic adenocarcinomas (P<0.001) which was highly significant. Mann – Whitney test was used to assess the statistical differences between AgNOR counts of benign and malignant lesions with t=53.9, Z=7.23 and P<0.001 which was considered highly significant.
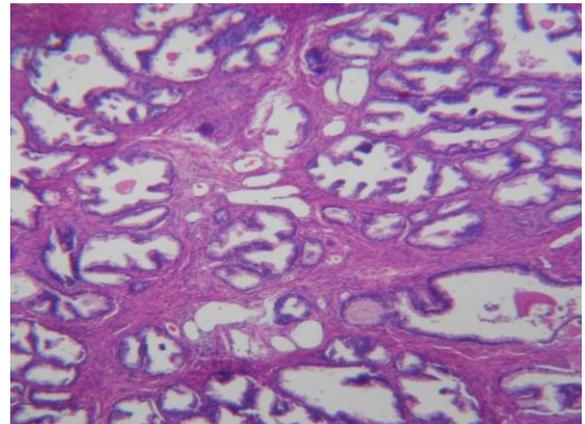

Fig. 1A: Nodular hyperplasia showing hyperplastic glandular and stromal components (H&E stain, x 10).
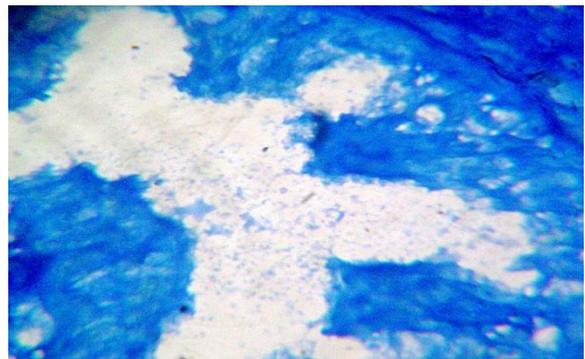

Fig. 1B: Benign prostatic hyperplasia with mean AgNOR count of 1.6+/-0.2 (AgNOR stain, x 100 oil immersion).
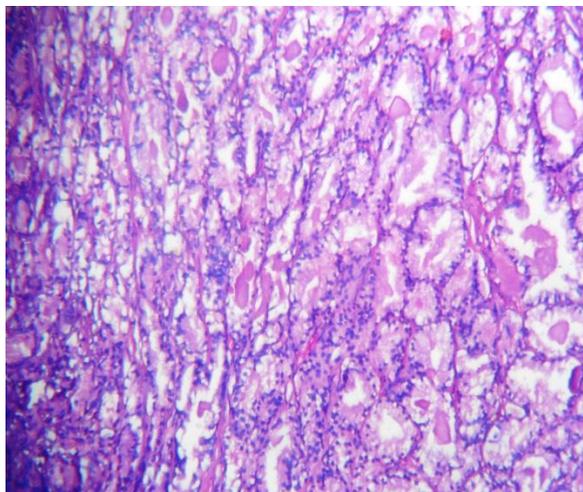
Fig. 2A: Atypical adenomatous hyperplasia(AAH) showing circumscribed cluster of glands (H&E stain, x 10).
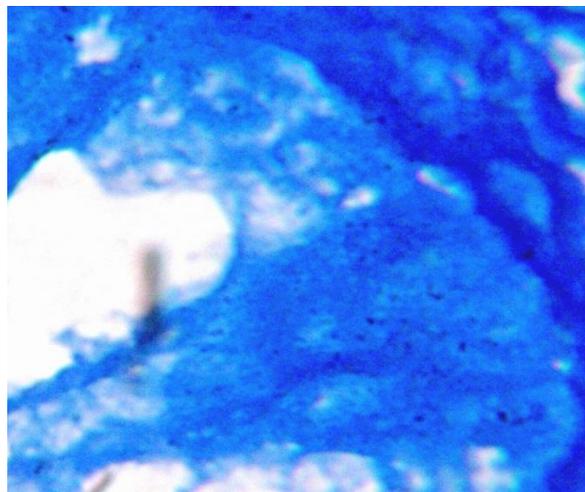

Fig. 3B: High grade prostatic intraepithelial hyperplasia (HGPIN) with mean AgNOR count of 2.3+/- 0 (AgNOR stain, x100 oil immersion).
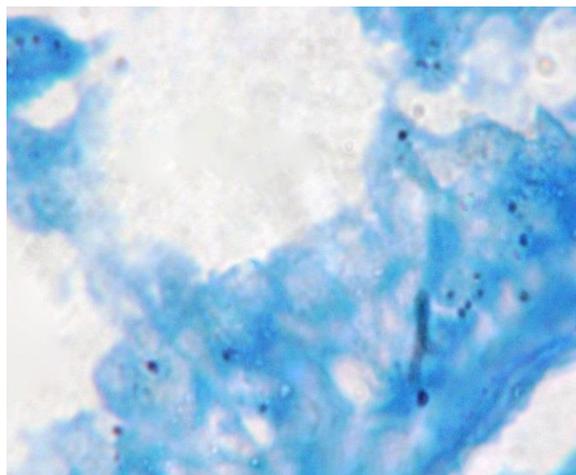

Fig. 2B: Atypical adenomatous hyperplasia (AAH) with mean AgNOR count of 2.2+/- 0.1 (AgNOR stain, x100 oil immersion).
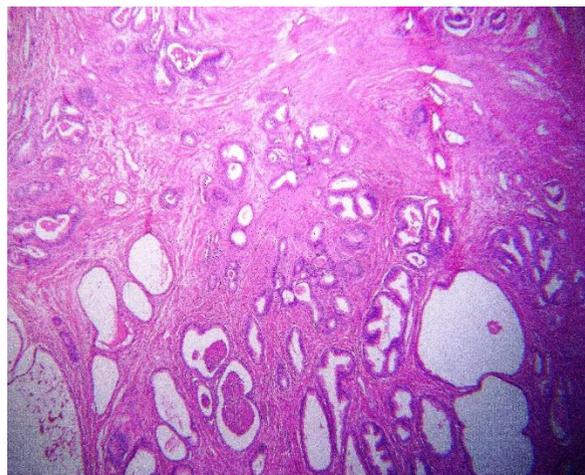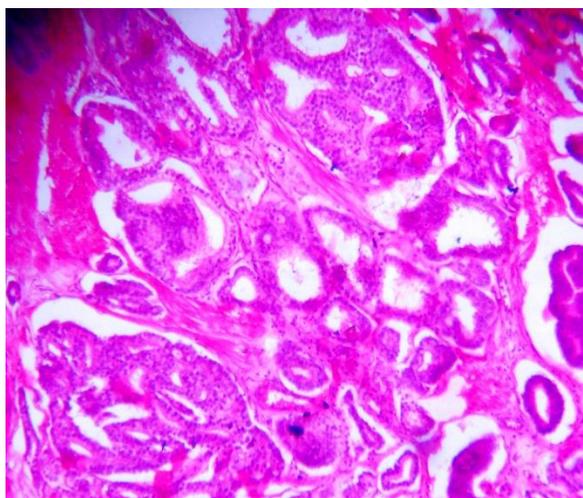

Fig. 4A: Proliferative inflammatory atrophy (PIA) showing areas of closely packed small acini lined by atrophic epithelium with stroma showing areas of fibrosis, elastosis (H&E stain, x10).

3A. HGPIN showing cribriform pattern with epithelial cell stratification, crowding with enlarged nucleus and prominent nucleoli   (H&E stain, x40).

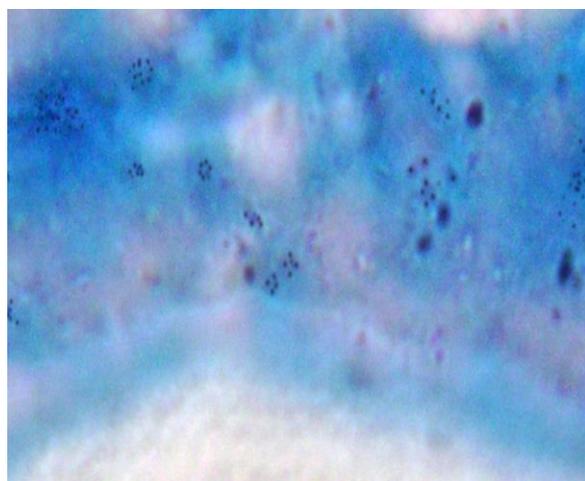

Fig. 4B: Proliferative inflammatory atrophy (PIA) with mean AgNOR count of 2.4+/- 0   (AgNOR stain, x100 oil immersion).
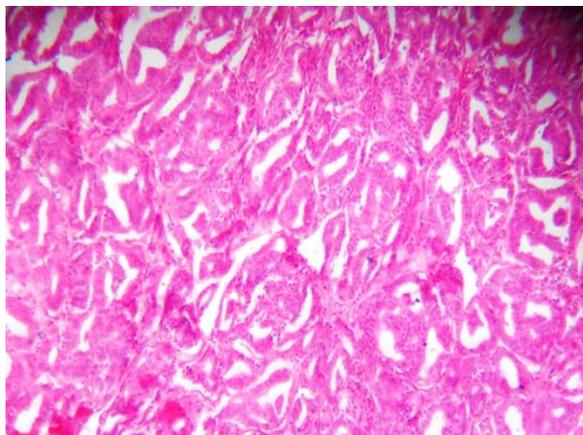
Fig. 5A. Prostatic adenocarcinoma with Gleason's score 3+4=7 (H&E stain, x10).
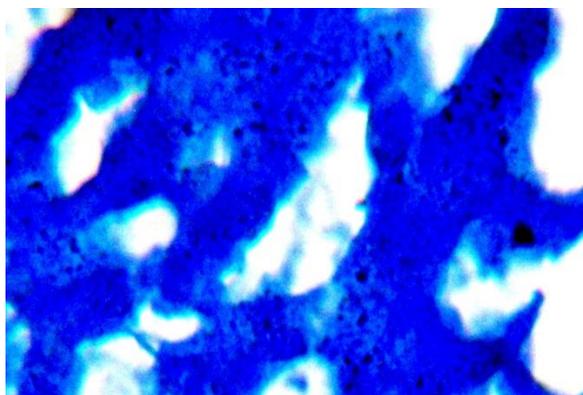

Fig. 5B: Prostatic adenocarcinoma with mean AgNOR count of 4.7+/-0.1 (AgNOR stain, x100 oil immersion).

DISCUSSION

Histological grade is the most important parameter for predicting prognosis of patients with prostatic carcinoma [9]. Various methods are proposed for grading prostatic tumours, out of which most frequently used is the Gleason's grading system[10]. However, the criteria used for histological grading are subjective. Hence the present study was conducted to evaluate the importance of AgNORs in differentiating benign, premalignant and malignant lesions of prostate. The AgNOR count increases with increased cell ploidy and with increased transcript-tional activity in the stage of active cell proliferation [11]. Variations in the size and/or number of the AgNOR dots may depend on the stage of the cell cycle, metabolic activity of the cell or the number of NOR-bearing chromosomes in the karyotype. Of the various newer techniques in assessing the tumour tissue based on nuclear studies, AgNOR staining by a silver compound is the one which has become popular for its

- Simplicity
- Easy to use
- Low Cost

- Good Correlation with other proliferative markers[5]
- No repeated antibody incubation as in case of immunochemistry and
- Gives idea of tumour aggressiveness [6].

In our study age range of the patient was between 61-70 years in benign lesions and between 71-80 years in malignant lesions. Similiar findings were noted by Mittal et al [12] and Anushree C.N etal [13]. Among the prostatic specimens TURP chips was the most common specimen accounting for 83.2% of the cases. Above findings are in consistent with Sadia Hameed et al[14], Anushree C.N etal[13], Chandanwale S etal[15]. In the present study benign lesions were most common accounting for 90.4% of cases followed by prostatic adenocarcinoma accounting for 9.6% of cases. Our findings are in concordance with Mittal et al[13], F. Di Silverio et al[16], Anushree C.N et al[13] and Chandanwale S et al[15].

All the cases of prostatic adenocarcinoma were graded using Gleason's pattern and scoring system. In our study Gleason's pattern four was the most common primary pattern accounting for 63.1% of the cases and pattern three and four was the second common secondary Gleason's pattern observed. Gleason's score 7 and 8 accounting for 31.6% of the cases was common followed by Gleason's score9 constituting for 21.1% of the cases, was noted which is belonging to moderately- poor differentiation category. Gleason pattern 1 and 2 were not encountered as most of the carcinoma was detected predominantly on needle biopsies. Similiar findings were noted by Anushree C.N et al[13] and Chandanwale S et al [15].

The value of mean AgNOR count of various prostatic lesions in the present study (Table 3) showed a linear and significantly increasing trend from benign to malignant lesions of prostate (P< 0.05). The mean AgNOR count also showed significant difference between the histological grades of the tumour. The findings are in consistent with the study done by Ghazizadeh et al [18] as shown in table 5. In our study all efforts were made to standardize the tissue processing and staining techniques. During AgNOR staining each tissue section, had its internal control like lymphocytes and connective tissue. Limitations of our study include

- Resolution of individual AgNORs within relatively small nucleolus.
- Affinity of the nucleolus for silver stain which obscures the individual AgNORs in cases of intense staining
- A variable degree of overlap between high and low grade tumours.

The other limitations include subjective, influenced by sampling, processing and staining procedures. To overcome these limitations the technique of image cytometry characterized by high precision, reproduce-bility and good inter-observer correlation in determination of AgNOR count per cell[20] could be employed.

The role of prognostic marker of prostatic adenocarcinoma of prostate was evaluated in our study by follow up examination of the patient.

CONCLUSION

With inherent limitations of the present study, we conclude AgNOR staining procedure is the simple, cost- effective, needs lot of dedication, standardization and meticulous bench work to achieve accurate results. The AgNOR staining technique can definitely be used as a supportive tool to the routinely performed haematoxylin and eosin staining which helps in making the decision of therapeutic and prognostic value in a case of prostatic adenocarcinoma.

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