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Role of Ag NOR staining in benign and malignant lesions of prostate at a tertiary hospital

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Abstract

Background: Nucleolar organizing regions (NOR) can be located by staining with silver nitrate under prescribed conditions; the structures thus demonstrated termed as `AgNORs'. Nucleolar organizing regions (NOR) counts might be of helpful specifically in conditions where histological distinction of prostatic cancer is difficult. Present study was aimed to study role of AgNORs in differentiating benign from malignant lesions of prostate. **Material and Methods:**Present study was single-center, prospective, observational study, conducted in samples of prostatic biopsies. The diagnosis in all cases were made on H & E and subsequently taken up for study.All prostatic biopsies were stained by the modified AgNOR technique. All silver-stained structures, both intra and extra nucleolar were counted.**Results:** Based on the histopathological examination of prostatic biopsies, 87 were diagnosed as benign (BPH) and 13 cases were malignant (Carcinoma prostate). Maximum no. of cases of BPH are in the age group of 51 - 60 years (40.2 %) followed by 61-70 years (39.1 %). Majority cases of carcinoma prostate were from age group of 51 - 60 years (38.46 %). In the present study we have applied AgNOR staining procedure to histopathologically diagnosed cases of BPH and Ca prostate. In BPH showed a mean AgNOR count of 2.38 ± 0.31, while biopsies from carcinoma prostate had a mean AgNOR count of 5.39 ±0.408. In our study BPH constituted majority of the lesions (87% of cases). Among BPH lesions, BPH with prostatitis constituted (29 %) which obscures the AgNOR staining. Ca prostate constituted (13%). **Conclusion:** Prostatic disease is responsible for significant morbidity and mortality in men throughout the world. AgNOR is a simple, cost effective and easy stain to evaluate the proliferative activity of different prostatic lesions.

Keywords: Prostate lesions, AgNOR- Silver-stained nucleolar organizing regions (NORs), BPH, carcinoma prostate.

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Introduction

The biological behavior of a neoplasm is determined to large extent by the proliferative activity of its cellular population. Attempts to accurately estimate proliferative activity have met with mixed results, Mitotic figure counts, AgNOR counts, flow cytometric estimation of the `S' phase fraction, determination of incorporated radiolabelled thymidine and immunohistochemical methods-PCNA, Ki 67 Antibody estimation represent current methods to assess proliferative activity.[1,2]

While immunohistochemistry and flow cytometry are accurate, their expensive methodology and stringent standardization requirements make these methods unaffordable for general laboratory use. On the other hand, the mitotic figure count is highly subjective, with poor reproducibility, making AgNOR count estimation a more attractive method of assessing proliferative activity.[2,3]

NORs are chromosomal segments in which ribosomal RNA (rRNA) is encoded and they are thus responsible for the development of the RNA containing nucleolus or nucleoli into which the NORs project on large loops of DNA. In the Human Karyotype, NORs are located on each of the short arms of the Acrocentric chromosomes 13,14,15,21 and 22. NORs can also be located by staining with silver nitrate under prescribed conditions; the structures thus demonstrated termed — `AgNORs'[3,4]

Nucleolar organizing regions (NOR) counts might be of helpful specifically in conditions where histological distinction of prostatic cancer is difficult. Prostatic intra epithelial neoplasia is the precursor of adenocarcinoma originating from the ducts and Acini. The morphological continuum that results in early invasive adenocarcinoma is now divided into two grades low grade and high-grade replacing the previous three-grade system. Therefore, there is need for cost effective and simple supplementary methods which would reduce much of the ambiguity in reporting prostatic intraepithelial neoplasia and prostatic cancer. Present study was aimed to study role of AgNORs in differentiating benign from malignant lesions of prostate.

Material And Methods

Present study was single-center, prospective, observational study, conducted in department of pathology, at Osmania General Hospital, Hyderabad, India. Study duration was of 18 months (June 1997 to December 1999). Study approval was obtained from institutional ethical committee.

Samples of prostatic biopsies from surgery department were considered for present study These include cases of Benign prostatic hyperplasia and cases of carcinoma of prostate. Biopsy material received, was fixed in 10% buffered formalin. After adequate fixation the tissues were processed by conventional met hods. Sections were cut at 4 to 6 microns thickness and stained by H & E technique. Sections were cut at 3 microns thick ness for AgNOR staining.

The diagnosis in all cases were made on H & E and subsequently taken up for study.All prostatic biopsies were stained by the modified AgNOR technique.

Colloidal developer solution was prepared by dissolving 2 gms of powdered gelatin in 100 ml double glass distilled water to which 1 ml analytical grade formic acid is added. 50% aqueous silver nitrate solution is prepared by dissolving 5 gms of analytical grade silver nitrate in 10 ml of double glass distilled water.

- Saturated solution of glycine in 99% Ethanol.
- 10% Nitric Acid solution.

STAINING PROCEDURE: The slides cut at 3 microns thick ness were immersed in a coplin Jar containing the saturated glycine solution for 15 minutes.The sections were hydrated to

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distilled water through xylene (thorough cleaning of slides around the sections is essential at this stage to remove the whitish material formed) Acetone and Alcohol. The slides were then placed on butter paper in a row over a hot plate with the temperature regulated to between 40°C - 50°C.3 ml of the colloidal developer solution and 6 ml of 50 % aqueous silver nit rate solution were then mixed in a small beaker. This silver colloidal developer solution was warmed gently till light brown colour developed. The sections were then covered with the mixture and left over the hot plate. The slides were allowed to develop for 30 minutes & then gently, but thoroughly washed in flowing distilled water. The slides were immersed in 10% nitric acid solution for 30 seconds and washed again in flowing distilled water. The slides were finally dehydrated and mounted with DPX. No counter stain was employed .

The Argyrophilic proteins were observed using a X 100 objective 100 nuclei were studied in each case and the mean number of AgNORS per nucleus calculated. The cells in the Basal layers were considered and the counting done. All silver-stained structures, both intra and extra nucleolar were counted.AgNOR counts were subjected to statistical analysis for the Data collected. Statistical analysis was done using descriptive statistics.

Results

Based on the histopathological examination of prostatic biopsies, 87 were diagnosed as benign (BPH) and 13 cases were malignant (Carcinoma prostate). We have not come across any Prostatic intraepithelial neoplasia (PIN) cases. Maximum no. of cases of BPH are in the age group of 51 - 60 years (40.2 %) followed by 61-70 years (39.1 %). Majority cases of carcinoma prostate were from age group of 51 - 60 years (38.46 %).

Table 1: Age distribution

Age groups (in years)	BPH		Carcinoma prostate	
	No. of patients	Percentage	No. of patients	Percentage
31-40	1	l .14%	0	0%
41-50	9	10.3%	1	7.69%
51-60	35	40.2%	5	38.46%
61-70	34	39.1%	3	23. 1%
71-80	6	6.9%	3	23.1%
>81	2	2.31%	1	7.69%
Total	87	100%	13	100%

In the present study we have applied AgNOR staining procedure to histopathologically diagnosed cases of BPH and Ca prostate. In BPH showed a mean AgNOR count of 2.38 ± 0.31, while biopsies from carcinoma prostate had a mean AgNOR count of 5.39 ±0.408.

Table 2: Comparison of AgNOR counts

Histological Grading	AgNOR range	Mean	SD value	(t) Value	(P) Value
BPH	1.82 - 3.16	2.38	0.31	1.72	Significant
Carcinoma prostate	4.24 -5.80	5.39	0.408		

In our study BPH constituted majority of the lesions (87% of cases). Among BPH lesions, BPH with prostatitis constituted (29 % of cases) which obscures the AgNOR staining. Ca prostate constituted (13% of cases)

Table 3: Histological typing

Histological diagnosis	No. of cases	Percentage
BPH	53	53

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BPHwithprostatitis	29	29
Basalcellhyperplasia*I*cribriform hyperplasia	5	5
Caprostate	13	13

Figures

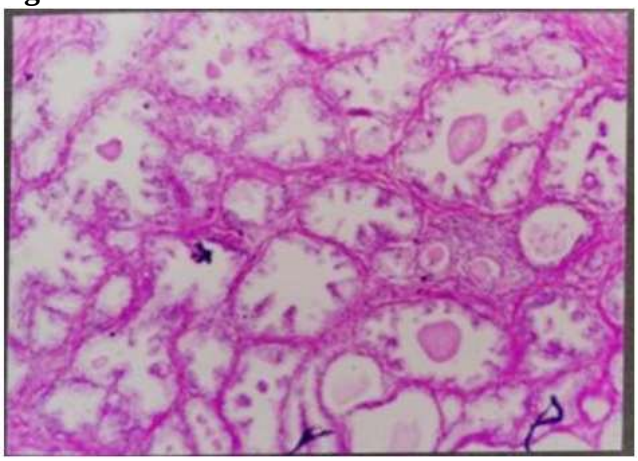

Figure 1: Benign Prostatic Hyperplasia HE

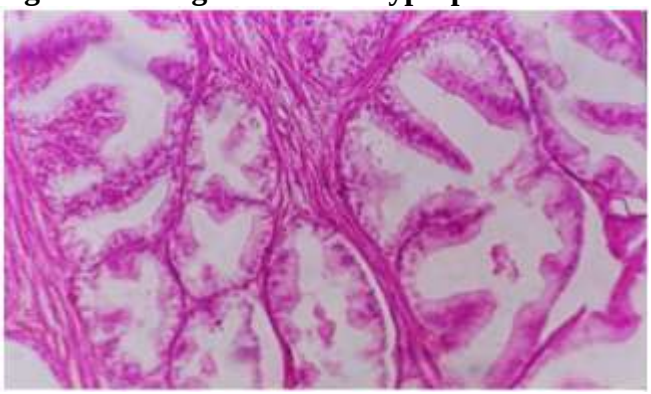

Figure 2: BPH HE (x400)

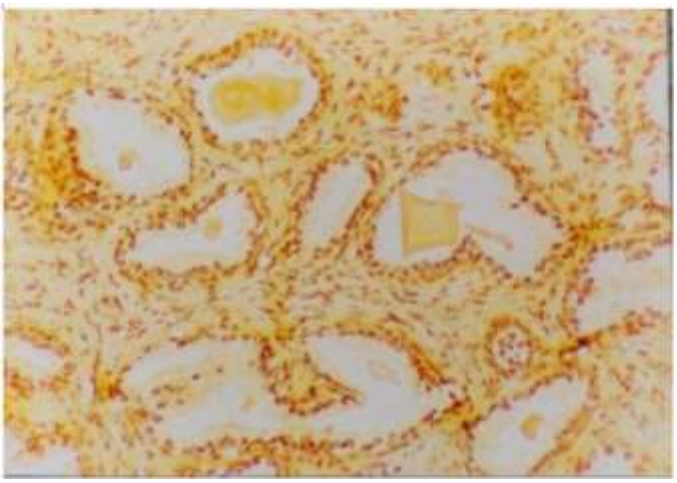

Figure 3: BPH AgNORs (x100)

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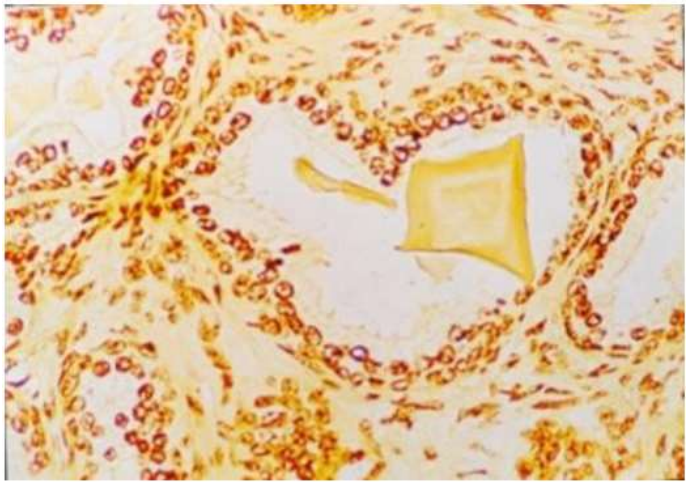

Figure 4: BPH AgNORs (x200)

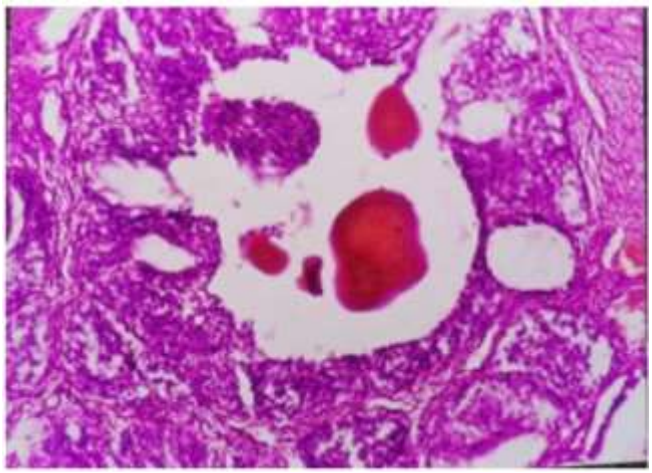

Figure 5: BPH - Basal cell Hyperplasia HE (x50)

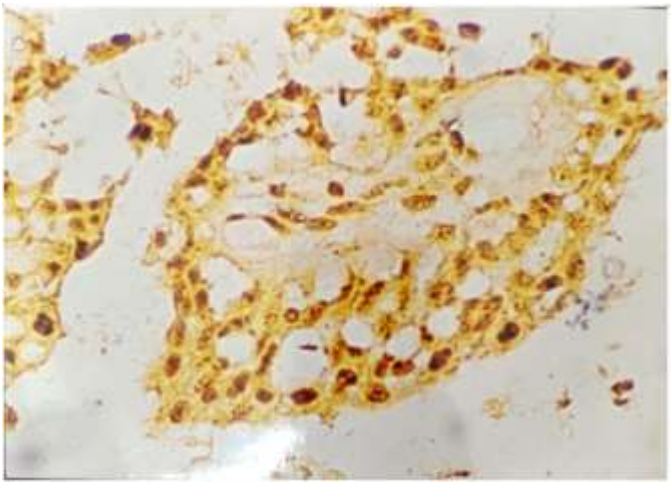

Figure 6: BPH - Basal cell Hyperplasia Ag NORs (x200)

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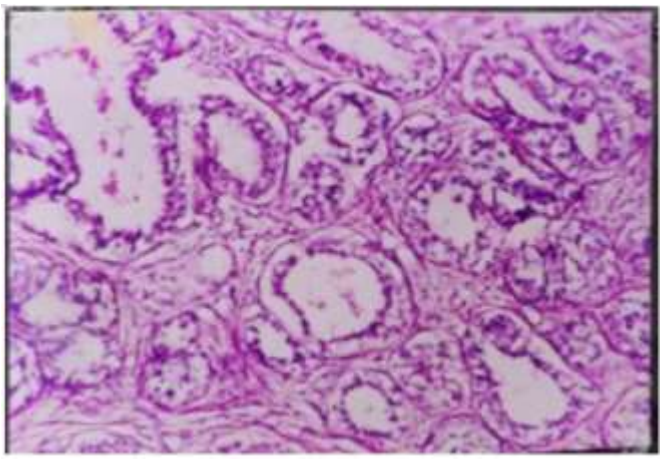

Figure 7: Carcinoma Prostate Well Differentiated HE (x100)

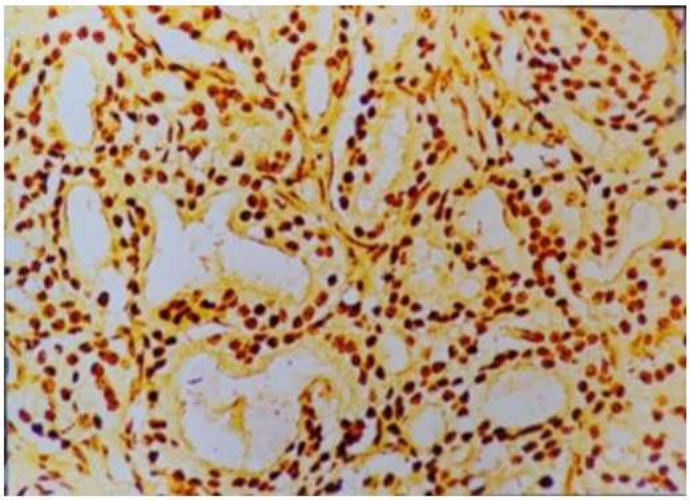

Figure 8: Carcinoma Prostate Well Differentiated AgNORs (x100)

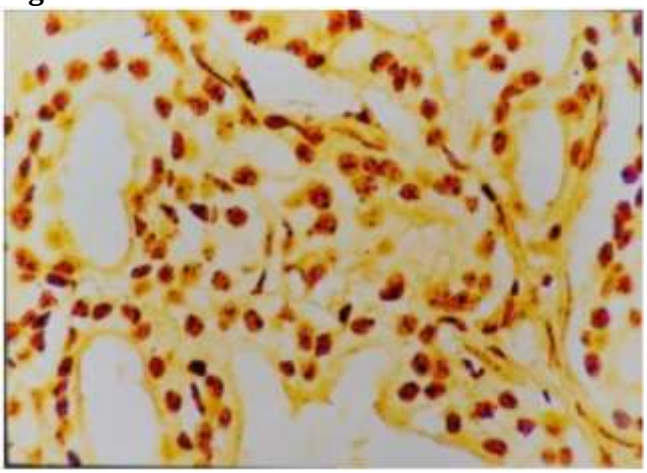

Figure 9: Carcinoma Prostate Well Differentiated AgNORs (x400)

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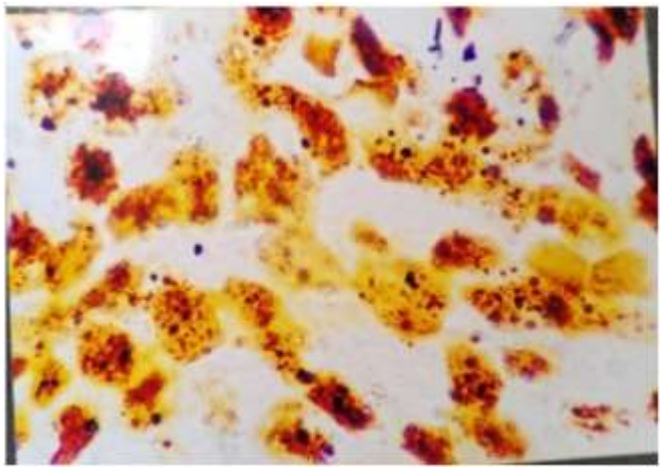

Figure 10: Ca Prostate Poorly Differentiated AgNORs (x1000)

Discussion

Nucleolar organizer regions (NORs) are ribosomal DNA loops. They are located on the short arms of the acrocentric chromosomes (13,14, 15,21 and 22). Silver binding to nucleolar organizer regions is attributable to proteins associated with these sites. The silver binding is attributed to acidic, non-histone constituents. The AgNOR technique has been used on chromosomal preparation to study the chromosomes in genetic disorders including trisomy 21, and in Leukemias and other neoplasms such as testicular tumors and meningiomas.[5,6]

Although the detection of advanced malignant lesions is not difficult , the early detection of malignant lesions remains a challenge to the scientific community. Ongoing search for new methods and early detection of malignancy lead to the categorization of prostatic intraepithelial neoplasia as premalignant lesion on light microscopic examination. One of the methods in the direction of early cancer detection is identification and scoring of nucleolar organizing regions (NORs). NOR's are chromosomal segments in which ribosomal RNA is encoded and are the interphase equivalent of Pars Amorpha in the nucleolus. These NORs when detected by silver staining are called AgNORs.

Khanna et al.,[7] reported that there was a statistically significant difference between AgNOR value per nucleus of benign lesion compared to a malignant lesion. The mean number of silver-stained nucleolar regions, such as AgNOR count per nucleus, was observed to be 2.1 in BPH, of which lowest value was 1.2,and the highest value was 3.0. In another hand, the average AgNOR count per nucleus in malignant specimens was 5.15, ranging in 3.8 as the lowest value and 7.1 as the highest value. The mean of AgNOR count per nucleus was shown to be highest in poorly differentiated prostatic adenocarcinoma.

In study by Gupta V et al.,[8] mean AgNOR count in cases of prostatic intraepithelial neoplasia was high compared with cases of benign prostatic hyperplasia (BPH) but lower than that of carcinoma cases. The intensity of cyclin D1 expression was high in carcinoma. A total of 14 cases (46.67%) showed strong positivity. No significant correlation was found between the intensity of cyclin D1 expression, AgNOR count, and histologic grades of prostatic carcinoma, whereas a significant correlation was observed between intensity and

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percentage expression of cyclin D1 in BPH and carcinoma (P<0.01). Nuclear as well as cytoplasmic positivity was seen among various grades of carcinoma.

Chiusa et al.,[9] concluded that AgNOR counts not only reflect cell proliferation but also the degree of cellular differentiation. Therefore, a combination of histological grading and AgNOR counts allows the stratification of low- and high-risk groups. Ghazizadeh et al.,[10] also observed that the mean AgNOR count significantly increases with grade and clinical stage of tumor. They concluded that AgNOR counting may contribute to making a conventional diagnosis and prognosis of carcinoma of the prostate.

NORs can serve as an independent indicator of differentiation in malignant tumours, and/or as prognostic factor. AgNOR staining can be performed on routine paraffin sections and is inexpensive. When compared with immunohisto-chemistry, AgNOR can give information related to the proliferation status of the tumours inferred by ki67, p53, Bcl-2, and other proliferation and apoptotic markers.[11]

Conclusion

Prostatic disease is responsible for significant morbidity and mortality in men throughout the world. AgNOR is a simple, cost effective and easy stain to evaluate the proliferative activity of different prostatic lesions. AgNOR can be used as an additional test which will be of immense value in identifying and grading of prostatic carcinoma.

Conflict of Interest: None to declare
Source of funding: Nil

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