Original Research Article

Role of Argyrophilic nucleolar organiser regions [AgNOR’s] in preneoplastic lesions of prostate

Meenakshisundaram K1*, Rajeswari K2, Rajalakshmi V3

1Associate Professor, 2Assistant Professor, 3Head of the department, Department of Pathology, ESIC Medical College & PGIMSR, KK Nagar, Chennai 600078

*Corresponding Author:
E-mail: drkms75@gmail.com

Abstract
Aims and objectives: 1. To demonstrate the patterns in different premalignant lesions of prostate. 2. Correlating AgNOR counts with various grades of PIN.

Materials and methods: This study was conducted in 83 cases of different prostatic biopsy specimens from 2013 -2015. All biopsy samples were routinely processed and stained with both haematoxylin and eosin and with AgNOR stain.

Results: The mean AgNOR count and score of high grade prostatic intraepithelial neoplasia was significantly high when compared to low grade intraepithelial lesion. High grade prostatic intraepithelial lesions associated with nodular hyperplasia or with carcinoma was the same.

Conclusion: AgNOR is a simple, cost effective and easy stain to evaluate the proliferative activity of the cell. It can be used as an additional test which will be of immense value regarding the progression of preneoplastic lesions in prostate.

Keywords: AgNOR, low grade PIN, high grade PIN.

Introduction
The incidence of prostatic cancer has risen dramatically in the past decade. It has emerged as one of the common forms of cancers among men partially owing to early detection and increased longevity. Two lesions in the prostate have been proposed as being premalignant namely the Prostatic intraepithelial neoplasia (PIN) and Atypical adenomatous hyperplasia (AAH). PIN is the precursor of prostatic adenocarcinoma originating from the ducts and acini particularly those of peripheral zones of the prostate gland. PIN is of two types – Low grade and high grade. Proliferation has been determined in prostate by evaluating PCNA, MIB 1, Ki67, mitoses and AgNORs. High grade PIN has a high predictive value as a marker for carcinoma and identification in biopsy specimen warrants further search for synchronous invasive cancer. The risk of adenocarcinoma in subsequent biopsies is 15 times greater in patients with high grade PIN. So based on these facts we proceeded to document the role of AgNOR’s in evaluating PIN and their association and progress to prostatic adenocarcinoma.

Aims
This study is aimed at
1. Demonstrating AgNOR patterns in different prostatic premalignant lesions
2. Correlating AgNOR counts with various grades of PIN and AAH.
3. To compare and contrast AgNOR proliferation index in cases showing synchronous lesions of PIN and prostatic carcinoma
4. To evaluate the diagnostic utility of AgNOR’s in routine prostatic biopsy specimens

Materials and methods
The study material composed of eighty three cases of prostatic adenocarcinoma including needle biopsy, transurethral resected specimen and radical prostatectomy specimens from our hospital during the period of May 2013 to May 2015. The diagnosis of benign prostatic hyperplasia was made in sixty eight cases and prostatic adenocarcinoma in fifteen cases in routine hematoxylin and eosin (H&E) staining. Later associated foci of low grade PIN, high grade PIN and atypical adenomatous hyperplasia were noted in each case and slides were grouped accordingly. Fresh sections of 4 microns thick were made and AgNOR staining was done.

Staining protocol: 50% silver nitrate solution was prepared by adding silver nitrate 50 gms to 100 ml of distilled water. Gelatin solution was prepared by adding 2gm of gelatine with 100 ml of distilled water to which 1 ml of formic acid is added. Working solution was prepared by mixing silver nitrate solution and gelatin solution in the ratio of 2:1 and was used immediately.
Staining procedure: Sections were dewaxed in xylene, hydrated in alcohols and brought to distilled water. Slides are then incubated in silver nitrate solution for 35 minutes in dark room at room temperature. Then slides were washed in distilled water. Then the slides were dried, cleared in xylene and mounted in DPX. The sections were examined in oil immersion objective.

Counting: Counting is done in AgNOR stained sections in the foci of low grade PIN, high grade PIN and atypical adenomatous hyperplasia with corresponding foci in H&E stained sections. AgNOR’s are visible as black intranuclear dots. [Figures 1,3,4,5] They are counted in 100 nuclei in a particular foci. Each dots were classified as small, medium and large according to its size. A small dot is defined as just visible but distinct one. Dots about three times the size of small one were classified as medium and those five times or more were classified as large. In each foci the mean AgNOR count was noted by counting the number of dots and finding the average.

AgNOR scores were calculated by multiplying the number of small dots by a factor of one, medium dots by a factor of three and number of large dots by a factor of five and adding up the three and finding the average. Then in each foci AgNOR count was done in basal cells and luminal cells separately. AgNOR scores were calculated in the similar manner for both basal cells and luminal cells and the results were tabulated.

Results
Out of these 83 cases, sixty eight cases (81.9%) were diagnosed as benign nodular hyperplasia and rest fifteen(18.1%) were diagnosed as carcinoma. Regarding the distribution of preneoplastic lesions in association with either benign nodular hyperplasia or carcinoma, we had eighty four foci of preneoplastic lesions in association with other primary lesions. We had thirty six cases of low grade PIN (42.8%), thirty five cases of high grade PIN (41.7%) and thirteen cases of atypical adenomatous hyperplasia (15.5%).

Twenty five cases of benign nodular hyperplasia (36.8%) were associated with low grade PIN only. Sixteen cases of benign nodular hyperplasia (23.5%) were associated with high grade PIN only. Only one case (1.5%) of benign nodular hyperplasia was associated with both low grade and high grade PIN lesion. Thirteen cases (19.1%) of benign nodular hyperplasia were without any associated preneoplastic focus. None of the benign nodular hyperplasia were associated with atypical adenomatous hyperplasia alone. Five cases (7.4%) of benign nodular hyperplasia were associated with AAH and low grade PIN and eight cases (11.8%) of benign nodular hyperplasia were associated with AAH and high grade PIN. Five cases (33.3%) of carcinoma were associated with low grade PIN and ten cases (66.7%) of carcinoma were associated with high grade PIN.

AgNOR count and score was done on 68 cases of benign nodular hyperplasia followed by AgNOR count on basal cells and luminal cells separately. The total AgNOR count was found to have a mean value of 2.09. AgNOR score was found to have a mean value of 2.52. The mean AgNOR count and score in basal cells (2.13 & 2.35) were significantly higher to mean count and score in luminal cells(1.81 & 1.95) with a p value of <0.01.

Total AgNOR count and score was done on 36 cases of low grade PIN followed by AgNOR count on basal cells and luminal cells. The total AgNOR count was found to have a mean value of 2.25. AgNOR score was found to have a mean value of 2.76. The mean AgNOR count and score in basal cells (2.26 & 2.94) were significantly higher to mean count and score in luminal cells (2.03 & 2.39) with a p value of<0.05.

Total AgNOR count and score in 15 cases of carcinoma of prostate show a mean count of 3.24 and 5.19 respectively which was significantly higher than that of high grade PIN.

We have compared the AgNOR count and score in low grade PIN associated with benign nodular hyperplasia and low grade PIN associated with carcinoma. In low grade PIN irrespective of its association with benign nodular hyperplasia or carcinoma, the mean AgNOR count and score, basal cell AgNOR count and score, luminal cell AgNOR count and score remains the same with a p value of >0.05.

We have compared the AgNOR count and score in high grade PIN associated with benign nodular hyperplasia and high grade PIN associated with carcinoma. In high grade PIN irrespective of its association with benign nodular hyperplasia or carcinoma, the mean AgNOR count and score, basal cell AgNOR count and score, luminal cell AgNOR count and score remains the same with a p value of >0.05.

We have compared the total AgNOR count in benign nodular hyperplasia, low grade PIN, high grade PIN and AAH. Low grade PIN had significantly higher AgNOR count when compared to benign prostatic hyperplasia with a p value of <0.01. High grade PIN had significantly higher AgNOR count when compared to low grade PIN with a p value of <0.01. AAH had significantly higher AgNOR count when compared to high grade PIN with a p value of <0.01.

We have compared the total AgNOR scores in benign nodular hyperplasia, low grade PIN, high grade
PIN and AAH. Low grade PIN had significantly higher AgNOR score when compared to benign prostatic hyperplasia with a p value of <0.01. High grade PIN had significantly higher AgNOR score when compared to low grade PIN with a p value of < 0.01. AAH had significantly higher AgNOR score when compared to high grade PIN with a p value of <0.01.

Fig. 1: Photomicrograph showing AgNOR staining pattern in benign nodular hyperplasia prostate (x1000)

Fig. 2: Photomicrograph showing low grade prostatic intraepithelial neoplasia (H&E x 400)

Fig. 3: Photomicrograph showing AgNOR staining pattern in low grade prostatic intraepithelial neoplasia (x400)
Fig. 4: Photomicrograph showing AgNOR staining pattern in high grade prostatic intraepithelial neoplasia (x1000).

Fig. 5: Photomicrograph showing AgNOR staining pattern in prostatic adenocarcinoma (x1000).

Table 1: Mean AgNOR count and score

<table>
<thead>
<tr>
<th></th>
<th>Mean total count</th>
<th>Mean score</th>
<th>Basal cell count</th>
<th>Basal cell score</th>
<th>Luminal cell count</th>
<th>Luminal cell score</th>
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<td>Nodular hyperplasia</td>
<td>2.09</td>
<td>2.52</td>
<td>2.13</td>
<td>2.35</td>
<td>1.81</td>
<td>1.95</td>
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<td>Low grade PIN</td>
<td>2.25</td>
<td>2.76</td>
<td>2.26</td>
<td>2.94</td>
<td>2.03</td>
<td>2.39</td>
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<tr>
<td>High grade PIN</td>
<td>2.60</td>
<td>3.57</td>
<td>2.40</td>
<td>3.43</td>
<td>2.18</td>
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<td>Carcinoma</td>
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<td>5.19</td>
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Table 2: AgNOR count and score in Low grade PIN associated with nodular hyperplasia (NH) and carcinoma.

<table>
<thead>
<tr>
<th></th>
<th>NH (Count / Score)</th>
<th>NH (Count / Score)</th>
<th>NH (Count / Score)</th>
<th>NH (Count / Score)</th>
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<tr>
<td></td>
<td>Mean 2.25/2.79</td>
<td>Mean 2.275/2.575</td>
<td>Mean 2.26/2.94</td>
<td>Mean 2.275/2.925</td>
<td>Mean 2.03/2.39</td>
<td>Mean 2.025/2.375</td>
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<td></td>
<td>SD 0.20/0.27</td>
<td>SD 0.12/0.19</td>
<td>SD 0.30/0.42</td>
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<td></td>
<td>Z 0.38/2.19</td>
<td>Z 0.197/0.17</td>
<td>Z 0.0735/0.036</td>
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<tr>
<td>p value</td>
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<td>&gt;0.05/ &lt;0.05</td>
<td>&gt;0.05/ &lt;0.05</td>
<td>&gt;0.05/ &lt;0.05</td>
<td>&gt;0.05/ &lt;0.05</td>
<td>&gt;0.05/ &lt;0.05</td>
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Table 3: AgNOR count and score in High grade PIN associated with nodular hyperplasia (NH) and carcinoma.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>NH</th>
<th>Carcinoma</th>
<th>NH</th>
<th>Carcinoma</th>
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<tbody>
<tr>
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<td>Basal cell</td>
<td>Count / Score</td>
<td>Count / Score</td>
<td>Count / Score</td>
<td>Count / Score</td>
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<tr>
<td>Mean</td>
<td>2.61/3.57</td>
<td>2.60/3.42</td>
<td>2.39/3.43</td>
<td>2.425/3.45</td>
<td>2.18/2.87</td>
<td>2.275/2.85</td>
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<tr>
<td>SD</td>
<td>0.24/0.33</td>
<td>0.24/0.15</td>
<td>0.32/0.42</td>
<td>0.15/0.39</td>
<td>0.30/0.41</td>
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<tr>
<td>Z</td>
<td>0.112/1.85</td>
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<tr>
<td>p value</td>
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**Discussion**

Prostate biopsy is indicated in all patients having clinical suspicion of prostate cancer. Recently we encounter more of TURP specimens and needle biopsies of which needle biopsy is of paramount importance since the material available is too little.

Prostatic intraepithelial neoplasia (PIN) is the currently used term for a process involving prostatic ducts and acini, also described as intraductal or ductal acinar dysplasia. It was initially divided as three grades PIN1, PIN2 and PIN3. Now PIN is divided into two grades – low grade and high grade. PIN 1 is considered as low grade. High grade includes PIN2 and PIN3. Atypical adenomatous hyperplasia (AAH) is a localised proliferation of small glands in apex and transition zone.

In our work we studied low grade PIN, high grade PIN and AAH in association with benign nodular hyperplasia and prostatic adenocarcinoma. We had 36 cases of low grade PIN as associated lesion out of which 31 cases (86.1%) were seen along with benign nodular hyperplasia and 5 cases (13.9%) were seen with carcinoma. The degree of cytologic alteration particularly nuclear and nucleolar changes in high grade PIN are analogous to those seen in invasive carcinoma. All studies of differentiation marker indicate that high grade PIN is more closely related to carcinoma than to benign epithelium.

High grade PIN has a high predictive value marker for carcinoma. The risk of adenocarcinoma in subsequent biopsies is 15 times greater in patients with high grade PIN. Studies to date have not determined whether PIN remains stable, regresses or progresses, though the implication is that it can progress.

Several studies have shown a statistical association between high grade PIN and adenocarcinoma in the sense that PIN has been found in 59% to 100% of step sectioned radical prostatectomy specimens.

In our study we had 35 cases of high grade PIN as an associated lesion out of which 25 cases (71.4%) were seen along with benign hyperplasia and 10 cases (28.6%) were seen along with carcinoma. We had 13 cases of AAH as associated lesion with nodular hyperplasia.

Cell proliferation has been determined in prostate tissue by evaluating PCNA, MIB1, Ki67, mitoses and silver stained nucleolar organiser regions (AgNOR’s). AgNOR cluster size as represented by their visible diameter was consistently related to proliferative status of cells. AgNOR protein area measurement is proposed as a simple inexpensive and reliable method of evaluating the proliferative activity in routinely processed tumour samples.

Basal cell layer of prostatic ducts and acini contains cells able to divide and form the proliferative compartment. On the other hand luminal cells have limited capacity for proliferation since they mostly comprises of cells in post mitotic phase and they constitute the differentiated compartment.

In our study we tried to elucidate this hypothesis by studying the AgNOR count and AgNOR score in basal cells and luminal cells in various proliferative disorders of prostate ranging from benign to malignancy.

Our study clearly depicts that the proliferative activity is significantly high in basal zone. In nodular hyperplasia the mean AgNOR count was 2.13 in basal cells when compared to 1.81 in luminal cells. In low grade PIN it was 2.26 in basal cells and 2.03 in luminal cells. In high grade PIN it was 2.40 in basal cells and 2.18 in luminal cells. These observations clearly points to the fact that there is significant expansion of the proliferative compartment of the prostatic glandular epithelium comprising the basal cells and is correlated well with observations made by Montironi et al.

An analysis of AgNOR count in low grade PIN and high grade PIN deserves special value. The AgNOR counts and scoring was done and results analysed. The total count had a mean value of 2.25 in low grade and 2.60 in high grade PIN. The scoring also reflected a similar picture with 2.76 in low grade and 3.57 in high grade PIN.

Sakr WA and Sarkar et al in 1993 established that the mean AgNOR values in PIN was 3.12 whereas invasive tumour nuclei showed mean AgNOR value of 4.73. Our study found that high grade PIN irrespective of its association with nodular hyperplasia or carcinoma, the mean AgNOR count and score remain the same with significant probability value.
Bostwick et al in 1996 pointed out that high grade PIN was a synchronous lesion with invasive cancer. They also stated that high grade PIN has a high predictive value as a marker for carcinoma. Our study also shows that most cases of high grade PIN are seen in association with carcinoma when compared to low grade PIN.

**Conclusion**

AgNOR is found to be of utility in giving an insight into the proliferative capacity of cells. It is simple, reliable, cost effective proliferative marker. Proper evaluation of AgNOR sections using standard criteria for mean AgNOR value and score gives reliable and fairly accurate results.

**Acknowledgement**

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**References:**