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ORIGINAL ARTICLE

Study of Immunohistochemistry in Prostatic Lesions with Special Reference to Proliferation and Invasiveness

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Abstract Prostatic lesions on routine staining sometimes cause diagnostic dilemma especially in premalignant lesions like atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia. Benign small acinar lesions also may be difficult to differentiate from small acinar adenocarcinoma. An important differentiating point is the loss of basal cell layer in adenocarcinoma and its presence in benign lesions. Basal cell markers (e.g. 34βE12 cytokeratin) & proliferative markers (e.g. AgNOR and PCNA) can help in this regard. Total 60 cases of different prostatic lesions studied. After history taking, clinical examination, radiological & other investigations were done. Routine H&E staining, immunohistochemical staining against 34βE12 cytokeratin & proliferative markers (AgNOR & PCNA) was performed. Statistically significant differences found in expression of 34βE12 cytokeratin and proliferative markers between benign, premalignant and malignant prostatic lesions. Basal cell markers and proliferative markers are important parameters to distinguish between different benign, premalignant and malignant prostatic lesions.

Keywords Prostate · Immunohistochemistry · 34βE12 · AgNOR · PCNA

Abbreviations

BHP	Benign hyperplasia of prostate
AAH	Atypical adenomatous hyperplasia
PIN	Prostatic intraepithelial neoplasia
Pca	Prostatic adenocarcinoma
PSA	Prostate specific antigen
H&E	Hematoxylin and Eosin stain
34βE12	Monoclonal antibody against high molecular weight cytokeratin 34βE12
AgNOR	Silver staining nucleolar organizer regions
PCNA	Proliferating cell nuclear antigen
DRE	Digital rectal examination

Introduction

The pathologic processes which affect the prostate gland with sufficient frequency are inflammation, benign nodular hyperplasia and tumors. Nodular hyperplasia is an extremely common in men over age 50; adenocarcinoma of prostate is the most common form of cancer in men and second leading cause of cancer death [1]. There are a number of benign small acinar lesions in the prostate gland that may be difficult to differentiate from small acinar adenocarcinoma [2].

Prostatic lesions on routine Haematoxylin & Eosin (H&E) staining sometimes cause diagnostic dilemma between benign and malignant lesions and especially in

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Table 1 Showing distribution of age of the patients (n=60)

Group	≤60 years	61–70 years	71–80 years	>80 years
BHP	7	19	11	3
AAH	2	2		
PIN		2		
Pca	4	9		1

Table 3 Showing provisional diagnosis according to histopathological examination prior to staining by antibody to 34βE12 cytokeratin (n=60)

Diagnosis	Number of cases	Percentage
BHP	42	70
AAH	6	10
PIN	3	5
Pca	9	15

premalignant lesions like atypical adenomatous hyperplasia (AAH) and prostatic intraepithelial neoplasia (PIN). An important diagnostic criterion in the differentiation is the loss of basal cell layer in adenocarcinoma and its presence in the benign lesions. Several immunohistochemical stains have been used to stain the basal cells of prostate against their markers, e.g. high molecular weight cytokeratin (34βE12), p63 etc [2–4]. The proliferative activity also signifies the nature of the cells. Proliferative markers e.g. silver staining nucleolar organizer regions (AgNOR), proliferating cell nuclear antigen (PCNA) etc are also of great help in this grey zone [5–10].

Our study was performed to evaluate the role of basal cell markers and proliferative markers in different benign and malignant lesions of prostate and especially in the premalignant lesions like atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia so far the diagnosis is concerned.

Material and Method

Our study population was the patients attending urology/surgery OPD having the clinical features of BHP, PIN or adenocarcinoma like increased frequency of micturition, dysuria, nocturia, difficulty in starting and stopping the stream of urine, urinary retention, over flow dribbling and low back pain due to matastasis to vertebrae [1]. Detailed history, clinical findings especially digital rectal examination (DRE), prostate specific antigen (PSA), radiological and other investigation findings were noted. The surgical specimens were taken from transurethral prostatectomy (TURP), trans-rectal ultrasono-guided biopsy (TRUS) and open prostatectomy.

The specimens were examined for gross findings and the tissue obtained were fixed in formalin, processed and embedded in paraffin wax block. One section of three micron thickness from each block was affixed on egg albumin coated slide and three sections of three micron thickness from each block were affixed on poly-l-lysine coated slides. The former slide was stained by H&E staining and the later group were used for cytokeratin 34βE12 study, PCNA labelling index study and AgNOR count. H&E stained slides were examined thoroughly and a provisional diagnosis of each case was made.

For immunohistochemical staining by antibody against 34βE12 cytokeratin and proliferating cell nuclear antigen (PCNA), the kit literature of the manufacturer was followed [11–14]. Expression of 34βE12 cytokeratin was considered as cytoplasmic positivity of the basal cells of the prostatic epithelium. Continuity of basal cells staining was assessed. For PCNA labelling index study, at least 1000 nuclei were counted under 400× magnification and the results expressed as stained to total nuclei counted in percentage (PCNA labelling index i.e. L.I. %). All immunostained nuclei independent of intensity were scored positive.

AgNOR staining was done with 50% silver nitrate solution and gelatin solution [14]. The nuclei were

Table 2 Showing PSA values in different prostatic lesions (n=60)

Prostate lesions	PSA values		
	<4 ng/ml	4–10 ng/ml	10 ng/ml
BHP	26	10	4
AAH	1	3	
PIN		2	
Pca			14

Table 4 Showing staining pattern of basal cell using high molecular weight cytokeratin (34βE12) antibody in prostate lesions (n=60)

Histological Diagnosis	Staining of Basal Cell	No. cases
BHP	Continuous	40
	Discontinuous	2
AAH	Continuous	4
	Discontinuous	2
PIN	Continuous	2
	Discontinuous	1
Pca	Continuous	0
	Discontinuous	9

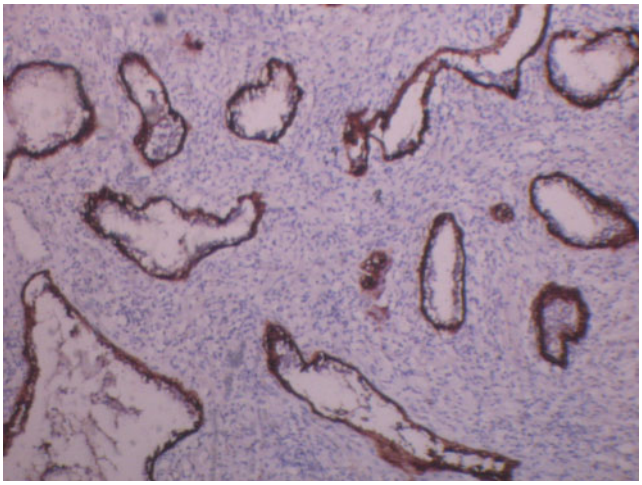

Fig. 1 Photomicrograph showing benign hyperplasia prostate (monoclonal antibody against high molecular weight cytokeratin 34βE12 × 100)

examined under 1000× magnification. The nucleolar organizer regions were seen as black dots in yellow background. They were counted as number per nuclei and an average count were noted.

After provisional diagnosis by H&E stained slides, final diagnosis was made by assessing the basal cell staining by 34βE12 cytokeratin and proliferative markers (PCNA & AgNOR). Statistical analysis was done by unpaired Student's't' test and P values were obtained. The study was done as per the criteria of institutional ethics committee (no. Inst/IEC/459) and the papers are ready for submission.

Results

Total 60 cases were studied, all patients were aged (Table 1). Most of them presented with the lower urinary tract symptoms. In two cases symptoms like bone pain and in one case weight loss were noted. On digital rectal examination (DRE) findings like smooth firm enlargement of prostate, palpable median sulcus and free rectal mucosa were suggestive of benign lesions. In malignant prostate lesions, suggestive DRE findings were nodular hard enlargement, obliterated median sulcus and fixed rectal mucosa. In two suspected cases, features of metastasis like bone pain was recorded.

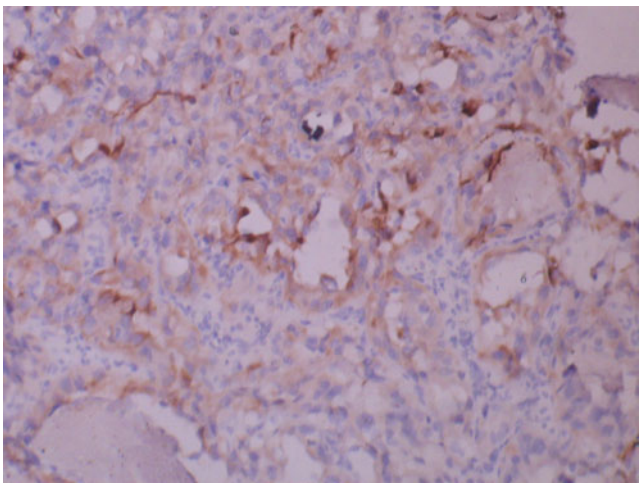

Fig. 2 Photomicrograph showing adenocarcinoma prostate (monoclonal antibody against high molecular weight cytokeratin 34βE12 × 100)

Table 5 Showing histopathological and final diagnosis after 34βE12 staining (n=60)

Cases	Histopathological Diagnosis (before 34βE12 staining)	Final Diagnosis (after 34βE12 staining)
BHP	42	40
AAH	6	4
PIN	3	2
Pca	9	14

Prostate specific antigen (PSA was <4 ng/ml in 26 cases of BHP and in one case of AAH. It was 4–10 ng/ml (in the 'grey zone') in 10 cases BHP, 3 cases AAH and in all the cases of PIN. It was >10 ng/ml in 4 cases of BHP and in all the cases of prostatic carcinoma (Table 2).

Histopathologically on H&E staining (prior to staining by antibody to 34βE12 cytokeratin), 42 cases were found to be benign hyperplasia of prostate (BHP), 6 cases were atypical adenomatous hyperplasia (AAH), 3 cases were of prostatic intraepithelial neoplasia (PIN) and 9 cases were of prostatic adenocarcinoma (Pca) (Table 3).

Basal cells of prostate was stained with high molecular weight cytokeratin antibody 34βE12 (Table 4, Figs. 1 and 2) and diagnosis of some prostatic lesions was to be modified. Final diagnosis was made accordingly (Table 5).

Tables 6 and 7 show the PCNA labelling index (LI %) and AgNOR count per nucleus respectively (Figs. 3, 4, 5 and 6). The P values in the comparison of different lesions are shown in Table 8.

Table 6 Showing PCNA labelling index (%) of different prostate lesions (n=60)

Final diagnosis	No. of cases	Range (%)	Mean (%)
BHP	40	2–8	5.8
AAH	4	17–35	25
PIN	2	38–42	40
PCa	14	54–82	65.5

Table 7 Showing distribution of AgNOR count (n=60)

Final Diagnosis	No. of cases	AgNOR count/nucleus (Range)	AgNOR count/nucleus (Mean)
BHP	40	0.4–2.5	1.3
AAH	4	1.5–3.2	2.1
PIN	2	4.5–4.9	4.7
Pca	14	4.5–5.4	4.91

Discussion

In our study all the patients were in older age group (Table 1). They mainly presented with symptoms like increased frequency, nocturia, retention of urine etc.

Majority of cases of BHP had normal P.S.A. value (<4 ng/ml). 10 cases had values falling in the 'grey zone' of 4–10 ng/ml. 4 cases had significantly raised P.S.A. levels (>10 ng/ml). One case of AAH had normal P.S.A. value less than 4 ng/ml and 3 cases had values in 'grey zone' in between 4–10 ng/dl. All cases of P.I.N. had P.S.A. values in 'grey zone'. And all cases of Pca had significantly elevated level of serum P.S.A. values (11–41 ng/ml) (Table 2).

BHP was the most common finding after interpretation of H&E staining in the provisional diagnosis (Table 3) followed by prostatic adenocarcinoma, atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia respectively.

Cytokeratin 34βE12 study (Table 4) showed continuous staining of basal cell in benign and premalignant lesions whereas discontinuous staining in malignant prostatic lesions (Figs. 1 and 2). Two cases of BHP (on provisional diagnosis) showed discontinuous staining of basal cells. Similarly two cases of AAH and one case of PIN (on H&E staining) showed discontinuous staining of basal cells. So the final diagnosis (Table 5) in these cases was revised to adenocarcinoma (Pca).

Two cases of BHP which were finally diagnosed as Pca after 34βE12 cytokeratin study had PSA values 12.3 ng/ml & 11.8 ng/ml respectively, PCNA labelling index 60% & 57% and AgNOR count 4.8/nucleus & 4.6/nucleus respectively. Similarly two cases of AAH which were finally diagnosed as Pca had PSA values 11.2 ng/ml & 13.5 ng/ml, PCNA labelling index 73% & 71% and AgNOR count 5.1/nucleus & 4.7/nucleus respectively. One case of PIN which was finally diagnosed as Pca had PSA value 14 ng/ml, PCNA labelling index 64% and AgNOR count 4.7/nucleus. These five cases were suspicious because in these cases PSA, PCNA labelling index & AgNOR count were high.

From the above parameters we see that on H&E staining, prostatic adenocarcinoma may be under diagnosed as PIN & AAH, even as BHP. Staining using antibody against basal cells to see its continuity and other investigations like PSA, PCNA labelling index study, AgNOR count along with clinical features can help in the proper diagnosis of benign, premalignant and malignant lesions.

Low PCNA (Table 6) values ranging from 2–8% were observed in BHP (Fig. 3). AAH had PCNA values ranging from 17–35%. Thus PCNA values had no overlapping between BHP and AAH. PIN had moderately high level of PCNA values 38–42%. Very high PCNA values (54–82%) were seen in Pca (Fig. 4). Interestingly it was found that adenocarcinoma, with higher Gleason's grade, had compara-

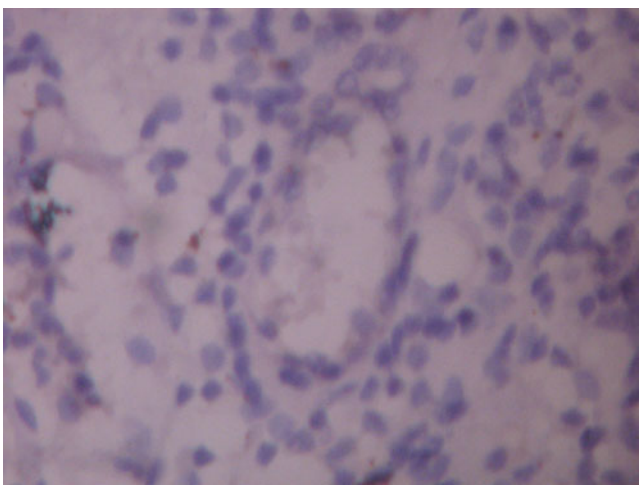

Fig. 3 Photomicrograph showing benign hyperplasia prostate (monoclonal antibody against PCNA × 400)

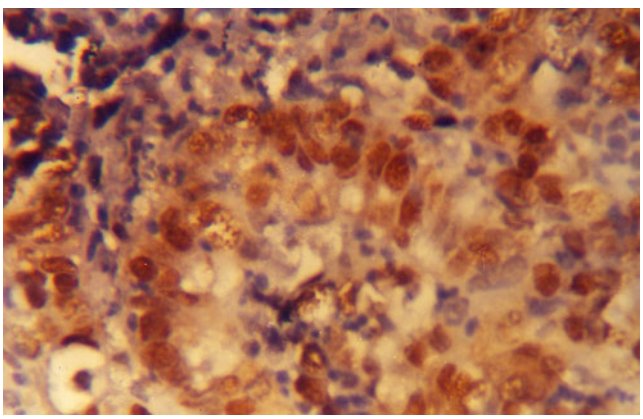

Fig. 4 Photomicrograph showing adenocarcinoma prostate (monoclonal antibody against PCNA × 400)

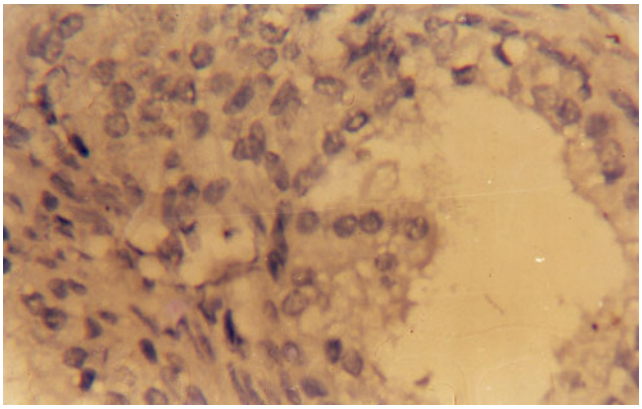

Fig. 5 Photomicrograph showing benign hyperplasia prostate (silver staining nucleolar organizer region × 1000)

tively higher PCNA values than that of lower Gleason's grade lesions. However this point was not highlighted due to less number of cases of prostatic adenocarcinoma.

AgNOR count in BHP was between 0.4–2.5/nuclus (Table 7, Fig. 5). AAH had AgNOR count between 1.5–3.2/nuclus. Thus some overlapping in AgNOR values were noted between BHP and AAH. The focus of PIN showed higher AgNOR count ranging from 4.5–4.9/nuclus. Pca had AgNOR count between 4.5–5.1/nuclus (Fig. 6).

Considering the values of PCNA and AgNOR, we see that PCNA LI% is superior to AgNOR count as a proliferative marker because values for interpretation are wider and there is no overlapping of the values in different lesions.

P values (Table 8) of the difference between two groups revealed PCNA LI% is significantly higher in Pca than BHP, AAH & PIN. It is also significantly higher in AAH than BHP. AgNOR value is significantly higher in Pca than BHP & AAH. It is higher in Pca than PIN but not

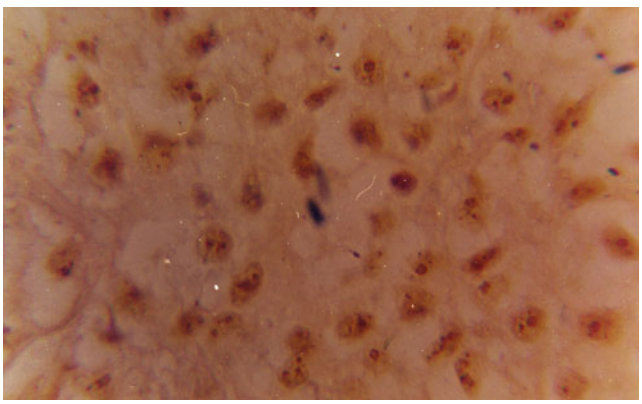

Fig. 6 Photomicrograph showing adenocarcinoma prostate (silver staining nucleolar organizer regions × 1000)

Table 8 Showing P values of the difference between two groups

Groups	AgNOR	PCNA
BHP & AAH	0.006320769	1.56895×10^{-17}
BHP & PIN	1.54883×10^{-10}	0.068206134
BHP & Pca	5.65956×10^{-29}	2.19417×10^{-40}
AAH & PIN	0.00540912	0.011640916
AAH & Pca	7.83782×10^{-10}	3.94693×10^{-07}
PIN & Pca	0.155379695	1.87644×10^{-07}

significant statistically. No significant difference was found in both the AgNOR count and PCNA LI% in AAH & PIN.

Our study showed identical results with the study done by Ghosh J [5]. Kawase N found AgNOR counts were higher in carcinoma (4.2+/−1.57) than in benign lesions (1.9+/−0.24) and more PCNA positive cells were identified in cancer areas [7]. Helpap B found lowest PCNA index and AgNOR score in prostatic hyperplasia and atypical adenomatous hyperplasia, while maximum values were in carcinoma of high malignancy [3]. Leed RD showed AgNOR cluster size was dependant on proliferative activity in normal and neoplastic tissues [15]. Wael A. Sakr found that AgNOR study is helpful for assessing tumour proliferation. The values were compared flow cytometrically and with PCNA patterns [16].

Our study on 34βE12 cytokeratin also showed similar results with studies done by Zhou M et al. They showed absence of basal cells in an atypical lesion supports a diagnosis of prostatic carcinoma [4]. Rennac et al found 34βE12 cytokeratin show the presence or absence of basal cells in a benign or malignant lesion respectively [8]. Ramos Soler D found that discontinuous or heterogenous reactivity of the basal compartment are indicative of malignancy [17]. Totten RS found that absence of basal cells in prostatic epithelium was key to the diagnosis of malignancy of atypical microglangular lesions of prostate [18]. Yang XJ found prostate cancer rarely expresses high molecular weight cytokeratin (34βE12) [19]. Yan-gao Man found that cytokeratin 34*β*E12 has been routinely used to elucidate prostate basal cells for differentiation between non-invasive and invasive lesions [20].

Samaratunga H. found that differentiation of high-grade PIN from prostatic adenocarcinoma is difficult and presence of a basal cell layer favours the diagnosis of the former [21].

So we can conclude that both proliferative activity and invasiveness increases from benign to malignant end in the spectrum of prostatic lesions. Cocktail use of antibody to high molecular weight cytokeratin (34βE12), PCNA labelling index and AgNOR count with clinical & biochemical findings was found useful in the diagnosis

of prostatic lesions especially which fall in the grey zone and create difficulty in the diagnosis in routine histopathological study.

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