

Histopathological spectrum of 364 prostatic specimens including immunohistochemistry with special reference to grey zone lesions

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Purpose: Prostatic lesions on routine staining sometimes cause a diagnostic dilemma, especially when malignant tissue is limited and is mixed with benign prostatic glands or because of the presence of benign mimickers of carcinoma. The application of immunohistochemistry contributes a valuable differential diagnosis. This study aimed to evaluate a complete spectrum of various prostatic lesions and to supplement the histopathological diagnosis with immunohistochemistry in suspicious or atypical cases.

Methods: A total of 364 consecutive prostatic specimens were evaluated. Routine hematoxylin and eosin staining and immunohistochemical staining against 34 β E12 cytokeratin and proliferative marker (alpha-methylacyl-CoA-racemase, AMACR) were performed by use of the peroxidase antiperoxidase method.

Results: Benign prostatic hyperplasia was the most frequent finding and involved 285 patients (78.3%). Prostatitis (majority nonspecific) formed the predominant subgroup in nonneoplastic lesions (n = 119, 32.7%). The incidence of carcinoma was low (n = 73, 20.1%). Of the 26 atypical or suspicious cases, 18 cases were positive for high molecular weight cytokeratin (high molecular weight cytokeratin, HMWCK) only, 4 cases were positive for AMACR only, and 4 cases showed positivity for both HMWCK and AMACR.

Conclusions: Biopsy remains the gold standard. However, as an adjunct to biopsy, proliferative markers and basal cell markers have value for resolving suspicious or atypical cases.

Keywords: Prostate, Malignancy, Immunohistochemistry, Alpha-methylacyl-CoA racemase, Cytokeratin

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common urological condition in men. The prevalence of BPH increases from 20% at 40 years of age to 90% by the eighth decade of life [1]. Prostatic carcinoma is globally the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males [2]. In India, it constitutes about 5% of all male cancers [3]. Prostate-specific antigen (PSA), digital rectal examination, and transrectal ultrasound are the tools most commonly

used to screen for prostate cancer. However, biopsy remains the gold standard for final diagnosis. Histological diagnosis of prostatic cancer is usually based on morphological features such as growth pattern, nuclear atypia, and absence of basal cells. However, this can be challenging, particularly when the malignant tissue is limited and is mixed with benign prostatic glands, or because of the presence of benign mimickers of carcinoma [4]. Therefore, the application of immunohistochemistry (IHC) contributes valuable diagnostic information.

In recent years, several new markers, including the basal

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cell marker high-molecular-weight-cytokeratin (HMWCK, clone 34 β E12) and the prostate biomarker alpha-methylacyl-CoA-racemase (AMACR), have been used as an adjunct to morphology in these diagnostically challenging cases with very high sensitivity and specificity. The accurate pathological evaluation of prostatic lesions is essential, because the subject of prostatic disease is fraught with doubts, uncertainties, and apparent contradictions [2,5].

This study was undertaken to evaluate the complete spectrum of various prostatic lesions and to study the role of 34 β E12 and AMACR in different benign and malignant lesions of the prostate, especially in suspicious or atypical cases whenever possible.

MATERIALS AND METHODS

All prostatic specimens received over a period of 18 months (January 2010 to June 2011) were analyzed with reference to light microscopic findings. The surgical specimens were taken through transurethral prostatectomy, transrectal ultrasound-guided biopsy, and open prostatectomy. The histology slides were prepared from original paraffin blocks and were stained by hematoxylin and eosin (H&E). The histological features were correlated with clinical profile and PSA levels. H&E-stained slides were examined thoroughly and a provisional diagnosis of each case was made.

For immunohistochemical staining, by antibodies against 34 β E12 and AMACR, the kit literature of the manufacturer (Dako, Glostrup, Denmark; AMACR IS060-Clone-13H4; HMWCK IS051-M0630, Clone-34 β E12, Isotype-IgG1, kappa) was followed. Expression of 34 β E12 cytokeratin was considered as cytoplasmic positivity of the basal cells of the prostatic epithelium, and the continuity of basal cells was assessed. AMACR showed continuous dark, diffuse cytoplasmic staining of the glandular epithelium. After the provisional diagnosis, a final diagnosis was made by the assessment of basal cell staining by 34 β E12 and proliferative activity by AMACR.

RESULTS

The present study constituted a total of 364 cases. All prostatic specimens were broadly classified into nonneoplastic and neoplastic. Each category was then subclassified into specific types according to the standard classification systems. The age distribution of nonneoplastic and neoplastic lesions is depicted in Table 1.

BPH was the most frequent finding and was observed in 285 of the 364 cases (78.3%). Maximum numbers of lesions

Table 1. Age distribution of the patients

Age (yr)	Nonneoplastic, n (%)	Neoplastic, n (%)
≤50	6 (2.11)	4 (5.06)
51–60	53 (18.60)	12 (15.19)
61–70	111 (38.95)	26 (32.91)
71–80	87 (30.53)	27 (34.18)
81–90	24 (8.42)	9 (11.39)
>90	2 (0.70)	1 (1.27)
Not recorded	2 (0.70)	0 (0)
Total	285 (78.30)	79 (21.70)

were seen in the age group of 61 to 70 years with an average age at presentation of 68.6 years. BPH alone was seen in 126 cases (34.6%), whereas BPH was associated with acute and chronic prostatitis in 119 cases (32.7%), granulomatous prostatitis in 13 (3.6%), basal cell hyperplasia (BCH) in 14 (3.9%), infarct in 2 (0.6%), squamous metaplasia in 3 (0.8%), and low-grade prostatic intraepithelial lesions in 2 (0.6%). Six cases of atypical adenomatous hyperplasia (AAH) and 2 of atrophy constituted 1.7% and 0.6% of the total cases, respectively. No case of tubercular prostatitis was seen during our study period. This practically nonexistent tubercular prostatitis in our series was attributed to early diagnosis and better availability of the drugs.

The majority of cases in the neoplastic group were encountered in the age group of 71 to 80 years with a mean age of 68.7 years. Histologically, of 79 neoplastic lesions, prostatic carcinoma formed the bulk with 73 of the total cases (20.1%), followed by 2 cases each of prostatic intraepithelial neoplasia (PIN) and rhabdomyosarcoma and 1 case each of leiomyoma and cystadenoma.

Prostatic adenocarcinoma accounted for almost all neoplasms of the prostate gland, accounting for 73 of 79 neoplastic lesions (92.4%). All cases of prostatic adenocarcinoma exhibited different growth patterns and were categorized depending on the primary, secondary, or tertiary pattern. The most common pattern seen in this study was angulated glands in 45 cases (61.6%), followed by a fused glandular pattern in 24 cases (32.8%) and a cribriform pattern in 22 cases (30.1%). A sheeting pattern was observed in 17 cases (23.3%), a hypernephroid pattern in 15 cases (20.6%), comedonecrosis in 9 cases (12.3%), and a single separate uniform glandular pattern in 4 cases (5.5%). Perineural invasion was seen in 31 cases (42.5%).

Of the 73 cases of prostatic adenocarcinoma, Gleason grading was done in 68 cases. The majority of cases were of an intermediate grade ($n=35$, 51.5%), followed by low grade ($n=19$, 27.9%) and high grade ($n=14$, 20.6%). No grading and scoring was done in 5 cases because of the paucity of tissue and lack of specific pattern. The tumor cells showed features such as

Table 2. Showing histopathological and final diagnosis after IHC

Histopathological diagnosis	HMWCK	AMACR	Final diagnosis (after IHC)
Proven BPH (n=20)	20	-	BPH
Proven carcinoma (n=20)	-	20	Carcinoma
Grey zone			
BPH with suspicious focus (n=2)	1	-	BPH
	-	1	Carcinoma
BCH (n=14)	11	-	BCH
	-	3	Carcinoma
AAH (n=6)	4	-	AAH
	2	2	PIN
Atrophy (n=2)	2	-	Atrophy
LGPIN (n=2)	2	2	PIN

IHC, immunohistochemistry; HMWCK, high molecular weight cytokeratin; AMACR, alpha-methylacyl-CoA-racemase; BPH, benign prostatic hyperplasia; BCH, basal cell hyperplasia; AAH, atypical adenomatous hyperplasia; PIN, prostatic intraepithelial neoplasia; LGPIN, low grade prostatic intraepithelial neoplasia.

nuclear enlargement and hyperchromasia with prominent nucleoli at places, and these cells were seen infiltrating the stroma.

Of the 26 atypical or suspicious cases, 18 were positive for HMWCK only, 4 cases were positive for AMACR only, and 4 cases showed positivity for both HMWCK and AMACR. Thus, on the basis of the IHC, of 2 cases of BPH with suspicious focus, one turned out to be BPH and the other adenocarcinoma. In the other cases the diagnostic error was due to benign mimickers of prostate cancer, which included AAH, BCH, and atrophy. Of 6 cases of BPH with AAH, 4 were finally categorized as AAH and 2 turned out to be PIN. Of 14 cases of BCH, 11 cases were labelled as BCH and 3 as carcinoma, respectively. No discrepancy was noted in cases of atrophy and PIN. Twenty cases each of BPH and carcinoma were selected for IHC, keeping the cost factor in mind, and the results were concordant (Table 2). After IHC application, the diagnosis was modified and is shown in Table 3.

Serum PSA levels were measured in 185 cases, of which 132 were benign and 53 were malignant. PSA levels >20 ng/mL were seen in 17 benign cases (12.9%) and 45 malignant cases (84.9%). On comparison of PSA levels of >20 ng/mL in non-neoplastic versus neoplastic lesions, a *P*-value of 0.005 was obtained, which is highly significant. Thus, we conclude that a PSA value of >20 ng/mL is highly suggestive of carcinoma of the prostate.

In our study the most common clinical diagnosis in non-neoplastic lesions was BPH (272 cases), with a positive predictive value of 90.1%. The diagnosis of carcinoma of the prostate was made in 88 cases, with a negative predictive value of 94.5%

Table 3. Showing histopathological and final diagnosis after IHC (n=66)

Case	Histopathological diagnosis (before IHC)	Final diagnosis (after IHC)
BPH	20	21
BCH	14	11
AAH	6	4
Atrophy	2	2
PIN	2	4
Carcinoma	22	24

IHC, immunohistochemistry; BPH, benign prostatic hyperplasia; BCH, basal cell hyperplasia; AAH, atypical adenomatous hyperplasia; PIN, prostatic intraepithelial neoplasia.

and a low positive predictive value of 67.1%.

DISCUSSION

Prostatic specimens constitute a good percentage of the surgical pathology workload. This study was undertaken to evaluate the various histological lesions in prostatic specimens.

The age of the patients in our study ranged from 7 years to 93 years; however, the predominant population was in the 6th to 7th decade with a mean age of 68.6 years. No significant difference was noted in the mean age of the nonneoplastic and neoplastic groups. The results of the present study agree with the studies by George and Thomas [6], in which the mean age was 66.81 years, and by Barakzai et al. [7], in which the mean age was 66.9 years. The decline in the number of cases beyond the age of 80 years reflects the average life span of people in our country.

In our study, of 364 cases, 285 (78.3%) were nonneoplastic lesions, whereas neoplastic lesions constituted 79 of the total cases (21.7%). This agrees with the studies conducted by Mittal et al. [8], Anjorin et al. [9], and George et al. [6], in which nonneoplastic lesions formed the bulk of the cases. These results are shown in Table 4. However, the incidence of neoplastic lesions was relatively higher in our study than in the other studies. Various factors can contribute to this disparity. For example, in recent times, the incidence rates of prostate carcinoma have been influenced by the diagnosis of cancer in early stages. Moreover, this study was carried out in a tertiary health institution, which puts a high selective index on the data because the health center acts as a referral center.

Prostatitis formed the predominant subgroup in the non-neoplastic lesions with 132 of 364 cases (36.3%). Mittal et al. [8] in a study of 185 prostatic specimens reported that prostatitis constituted 38.4% of the cases, of which 26.3% of cases were chronic prostatitis, and acute prostatitis with or without

Table 4. A comparison of the findings of the current study with other studies

Histopathological diagnosis	Mittal et al. [8]	Anjorin et al. [9]	George and Thomas [6]	Present study
Total no. of cases	185	801	1,163	364
Nodular hyperplasia	40.00	71.6	88.5	34.62
With prostatitis	38.39	11.2	-	32.69
Granulomatous prostatitis	1.62	-	-	3.57
Nonspecific				3.57
Tubercular				0
Basal cell hyperplasia	5.4	-	-	3.85
Infarct	-	-	-	0.55
Squamous metaplasia	3.24	-	-	0.82
Atypical adenomatous hyperplasia	2.16	-	-	1.65
Atrophy	1.63	-	-	0.55
Prostatic intraepithelial neoplasia	-	-	0.6	0.55
Adenocarcinoma	7.02	17.2	10.9	20.05
Leiomyoma	0.54	-	-	0.27
Rhabdomyosarcoma	-	-	-	0.55
Cystadenoma	-	-	-	0.27

Values are presented as percentage unless otherwise indicated.

abscess formation was noted in 12.1% of cases.

Granulomatous prostatitis was seen in 13 of 364 cases (3.6%). One of 13 cases showed granulomas with Langhans' giant cells and small foci of necrosis; however, Ziehl Nelsen (20%) staining in this case was negative for acid fast bacilli and no history of Bacillus Calmette–Guérin vaccine instillation could be elicited. Also, the granulomas were seen in close proximity to acini and thus were finally labeled as a nonspecific type. In all other cases, nonspecific granulomas composed of a collection of epithelioid cells were present around the ruptured acini. No granuloma was thus labelled as tubercular. The practically nonexistent tubercular prostatitis in our series could be explained by the fact that the incidence of tubercular prostatitis has been reduced because of early diagnosis and better drugs available for treatment. Also, our center caters to a better socioeconomic group of society; hence, tuberculosis is rarely seen as a disseminated disease.

BCH was the second most common entity in the nonneoplastic group and accounted for 3.9% (14 cases) of the total cases. All cases had associated BPH. Thorson et al. [10] studied 500 needle core biopsy samples and found the incidence of BCH in the setting of usual nodular hyperplasia to be in the range of 3.1% to 8.9%.

The present study showed squamous metaplasia in only 3 cases, accounting for 0.8% of the cases. Similar results were seen in a study done by Abdollahi and Ayati [11], who found

squamous cell metaplasia in 4 cases (0.3%) in a study of 1,566 prostatic specimens.

In the present study, 6 cases of AAH and 2 cases of atrophy were observed, constituting 1.7% and 0.6% of the sample, respectively. This is comparable to the reported incidence of less than 1% by Hameed and Humphrey [12], who stated that AAH is invariably an incidental histological finding, usually localized in the transition zone, and is thereby seen more often in transurethral prostatectomy chips. The peculiarity of these two processes is that they may be confused with the diverse patterns of prostatic adenocarcinoma. In general, routine microscopy helps us reach the correct diagnosis; however, ancillary IHC studies (HMWCK) may sometimes be needed to demonstrate the presence of basal cells in keeping with the benign nature of these two lesions.

The age-specific prevalence of PIN is similar in almost all populations. High-grade PIN is generally accepted as a precursor lesion of carcinoma [13]. This statement holds true for our study because all the cases of high-grade PIN were seen with adenocarcinoma and no association was observed with BPH. However, owing to the paucity of cases of high-grade PIN in the present study, we cannot conclusively comment on this finding.

The prevalence of prostatic carcinoma in the present study was 20.1%, i.e., 73 of 364 cases. Similar results were obtained by Anjorin et al. [9] and Rekhi et al. [14]. Most cases of prostate cancer are diagnosed after 50 years of age, but prostate cancer can be seen in younger adults. The frequency increases with age. In the present study, the age range was widespread (44–93 years) with a mean of 68.7 years. However, the majority of cases (n=26, 35.6%) were seen in the age group of 71 to 80 years, closely followed by the age group of 61 to 70 years. Two cases were seen below the age of 50 years. The time trends in detection of carcinoma at an early age have been greatly affected because more and more latent cases are diagnosed with the increasing use of PSA, which can explain higher percentage of cases in the age group of 61 to 70 years (Table 4).

Histological grade has been recognized as a powerful prognostic predictor of prostate cancer. Various other studies including ours highlight a score of 7 to 8 as a predominant score [6,15].

Perineural invasion is regarded as pathognomonic of prostate cancer if there is circumferential or intraneural invasion by the tumor cells [16]. A study conducted on 302 needle biopsies by Bastacky et al. [17] showed perineural invasion in 20% of biopsies with cancer. This correlates well with the present study in which perineural invasion was found in 31 of the malignant cases (42.5%) and was not observed in any of the

benign cases studied.

The correlation of PSA levels with nonneoplastic and neoplastic lesions was analyzed. PSA levels were available in 185 cases (132 nonneoplastic and 53 neoplastic) only. This is because prostate biopsy in our institution is performed in symptomatic patients as an outpatient procedure in a significant number of patients and PSA testing is not requested in these cases.

The IHC panel for prostatic carcinoma usually includes at least one basal cell-specific marker and the prostate cancer-specific marker AMACR. The most commonly used basal cell-specific markers in prostate cancer are HMWCK and clone 34 β E12.

Cytokeratin 34 β E12 shows continuous, intact, circumferential staining of basal cells in benign and premalignant lesions but discontinuous staining in malignant lesions [18,19]. Negative staining must be interpreted with caution because cytokeratin is formalin sensitive and a progressive loss of immunoreactivity can be seen in prolonged formalin fixation. AMACR showed a continuous dark diffuse cytoplasmic staining or circumferential apical granular staining pattern in malignant prostatic lesions but little or no immunoreactivity

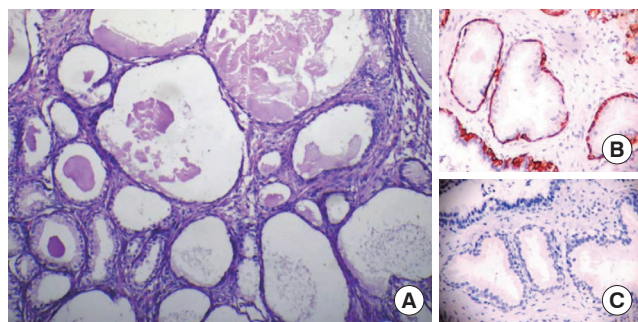


Fig. 1. (A) Benign prostatic hyperplasia (BPH) of the prostate with corpora amylacea (H&E, $\times 100$). (B) BPH high molecular weight cytokeratin. (C) BPH alpha-methylacyl-CoA-racemase.

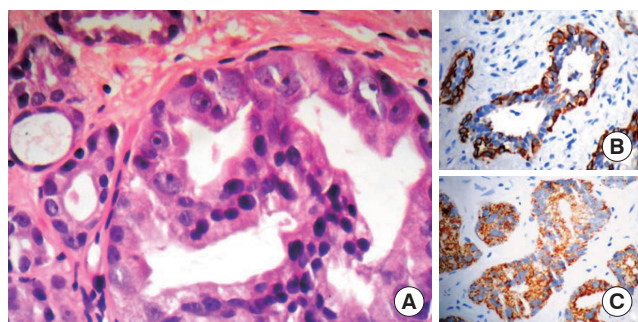


Fig. 2. (A) High-grade prostatic intraepithelial neoplasia (PIN) showing cribriform pattern of the gland. Note the prominent nucleoli and intact basal cell layer (H&E, $\times 400$). (B) PIN high molecular weight cytokeratin. (C) PIN alpha-methylacyl-CoA-racemase.

in benign lesions [20] (Figs. 1–3).

IHC markers (HMWCK and AMACR) were done in 66 cases in our study because the clinicians ordered markers in 26 suspicious or atypical cases. The other reason was the cost factor of the markers.

HMWCK was positive in all cases of BPH (100%), 1 case of BPH with suspicious focus, 11 cases of BCH, 4 cases of AAH, and 2 cases of atrophy, whereas AMACR was positive for all cases of prostate cancer (100%), 1 case of BPH with suspicious focus, and 3 cases of BCH. On the other hand, cases of BPH did not show AMACR positivity and HMWCK was completely negative in cases of prostate cancer. Jiang et al. [19] reported 100% AMACR positivity in a study of 137 cases of prostate cancer. Lesions like BPH and adenosis were completely negative for this marker.

In 6 of 26 suspicious or atypical cases, the diagnosis was changed on the basis of the IHC markers (HMWCK and AMACR). In 5 of the 6 cases the diagnosis was changed from nonneoplastic to neoplastic, whereas in 1 it was changed from neoplastic to nonneoplastic (BPH with suspicious focus to BPH). Immunostaining with both markers resolved these cases; thus, we inferred that both HMWCK and AMACR increase the diagnostic efficacy.

We conclude that proliferative activity and invasiveness increases from the benign to the malignant end in the spectrum of prostatic lesions. IHC plays an important role in the diagnosis of prostatic lesions and helps to differentiate malignant glands from benign lesions, especially for lesions in the grey zone in routine histopathological study. Although the number of cases included in this study was 364, only 26 atypical or suspicious cases were encountered, which forms a small subgroup and is not very significant for reaching a definite conclusion.

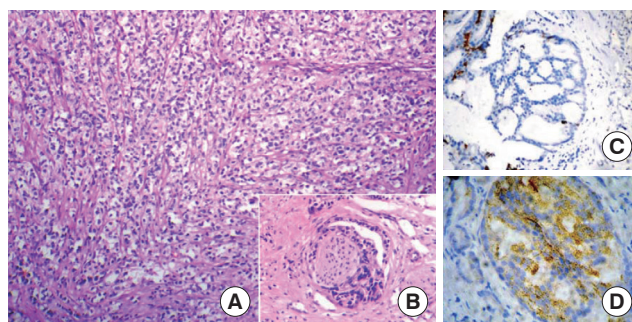


Fig. 3. (A) Gleason pattern 4 showing cells with clear cytoplasm (hypernephroid). (B) Inset showing perineural invasion (H&E, $\times 100$). (C) High molecular weight cytokeratin in cancer. (D) Cytoplasmic staining of alpha-methylacyl-CoA-racemase in epithelial cells in a case of prostate cancer.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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