Original Research Article.

Diagnostic Role of Immunohistochemistry in Mimics of Prostatic Adenocarcinoma

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ABSTRACT

Objectives: To study and recognize different morphologic patterns of benign mimics of prostatic adenocarcinoma and couple it with use of immunohistochemistry for correct diagnosis and avoid false positive carcinoma interpretation.

Materials and Methods: Prostatic biopsy specimens were evaluated by histopathology using haematoxylin and eosin (H/E) staining. In suspicious cases for malignant lesions, immunohistochemistry (IHC) analysis using basal cell markers p63 and HMWCK 34βE12 were performed to confirm the final diagnosis.

Results: One hundred and ninety three prostatic biopsy specimens were received in department of pathology, 92(47.66%) were benign hyperplasia while carcinoma constituted 39(20.2%) cases. Among the remaining 62 specimens, 30 showed features of mimics while 32 specimens were suspicious of carcinoma. Of these 62 specimens, IHC analysis using basal cell markers like p63 and 34bE12 were performed. All the mimics were found negative of malignancy. But among 32 suspicious specimen of carcinoma, 8 cases were confirmed as prostate carcinoma while 24 cases were diagnosed as mimics of prostatic carcinoma.

Conclusion: In morphologically equivocal glandular architectural patterns and cytological features, immunohistochemistry highlighting the basal cell marker can help the pathologist to arrive to a correct diagnosis.

Key Words: Mimics, Prostate Adenocarcinoma, Immunohistochemistry, p63, HMWCK.

INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer in men worldwide.[1] It is the most frequently diagnosed cancer among men in more developed countries, where about two-thirds of all prostate cancer cases occur among just 17% of the world's male population.[1] With the shift towards a greater proportion of men diagnosed with localized disease, the proportion living more than 5 years after diagnosis has increased.[2] So correct diagnosis has a definite role to play. In case of smaller biopsies, diagnosis can be difficult due to the presence of either a small focus of cancer or due to the many benign mimickers of malignancy.[3] Chronic prostatitis, atrophy, partial atrophy, basal cell hyperplasia, clear cell hyperplasia, post atrophic hyperplasia, atypical adenomatous hyperplasia (adenosis), seminal vesicle, colonic mucosa, squamous metaplasia, prostatic intraepithelial lesions all can mimic the malignancy.[3] False-positive cancer diagnosis, although uncommon, may be rendered in some cases and may lead to serious clinical, psychological and medicolegal consequences.

Immunohistochemical (IHC) markers are often used as an aid in case of suspicious lesions to reach at a diagnosis.[4] The diagnosis is aided by IHC staining for basal cell layer markers, such as p63 (nuclear marker), cytokeratin 5/6 (CK 5/6), and high molecular weight cytokeratin (CK HMW) (cytoplasmic marker) as well as prostate-'specific' markers.[5] The loss of basal cells in prostate carcinomas is the most important diagnostic hallmark of malignancy, and basal cell markers has been the immunohistochemical cornerstone of prostate diagnostics for more than 25 years.[6]

The aim of this study is to recognize different morphologic patterns of benign mimics of prostatic adenocarcinoma and couple it with use of immunohistochemistry for correct diagnosis and avoid false positive carcinoma interpretation.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Pathology and Department of Urology, Shriram Chandra Bhanja Medical College and Hospital, Cuttack, Odisha from September 2013 to October 2015.

One hundred and ninety three patients with enlarged prostate diagnosed either by Digital Rectal Examination (DRE) or Ultrasonography (USG) were included in the study. Previously diagnosed cases of adenocarcinoma of prostate were excluded.

Haematoxylin and Eosin staining was performed in all biopsy samples. Paraffin blocks in suspicious cases were chosen for performing IHC using p63 and 34βE12. PathNSitu ready to use mouse monoclonal antibody (p63→4A4 clone, CKHMW-34βE12) were utilised for this purpose.

The process of IHC included a two-step process in which the tissue is treated with an unlabelled primary antibody, followed by application of a chromogen labelled secondary antibody (directed against the primary antibody). Its main advantage was an increased sensitivity.

Antibody concentration was optimized to obtain the strongest target staining with minimal background staining. The slides then were incubated sequentially with primary antibody, biotinylated secondary antibody, avidin-biotin complex. The primary antibodies were incubated for 45 minutes at room temperature and the secondary biotin-labelled antibody for 30 minutes for 34βE12 and 45 minutes for p63. The streptavidin-labelled streptavidin-biotin amplification method (PathNsitu) was carried out for 30 minutes, followed by peroxidase substrate/ chromogen for 34βE12 and p63. Slides were counterstained with hematoxylin.

The basic principle, as with any other special staining method was a sharp localization of target components in the cell & tissue, based on a satisfactory signal-to-noise ratio. Amplifying the signal, while reducing non-specific background staining (noise) was the major strategy to achieve a satisfactory & practically useful result.

The Primary Ab (mouse monoclonal Ab) with specificity against the Ag in the test was added to the section & the excess antibody was washed off. The labelled secondary Ab which has specificity against an antigenic determinant present on the primary mouse Ab was then added; it serves to label sites of tissue localization of Primary Ab. To overcome masking of the antigenicity of tissues by formalin fixation antigen retrieval was done before application of antibodies to expose the masked antigens in tissues. It was performed by steaming the slides for 15 minutes in a 10-mmol/L concentration of sodium citrate buffer, pH 6.0, in a microwave oven.

Non immunological binding of the applied antibody with the tissue under evaluation and presence of endogenous peroxidase in certain tissues which result in background staining that is minimized by pretreatment of tissues with hydrogen peroxide prior to application of primary antibody which eliminates endogenous peroxidise is called as blocking.

One positive control and negative control was set up with each batch of tissues undergoing IHC to validate the procedure and increase its specificity. In the present study, we used benign hyperplasia of prostate diagnosed in histopathology cases as positive control and for negative control, we used adenocarcinoma of prostate.

Differences in tissue processing & technical procedure may produce variable results. Hence controls used are fresh autopsy/surgical specimens processed in same manner as patient's sample. The controls slides were provided by PathnSitu manufacturers. Presence nuclear staining is considered as positive while absence of staining is considered as negative for p63. For cytoplasmic marker 34βE12 presence of cytoplasmic staining is considered positive while negative staining indicated by absence of cytoplasmic staining.

IHC stain in all cases were evaluated independently by two expert histopathologists and in case of any discrepancy the slides were subjected to concurrent review under pentahead microscope by both of them. Positive staining in suspicious foci was considered evidence of benignity and negative staining of an entire suspicious focus was considered presumptive evidence of a malignant process as long as the morphologic features were in agreement. Statistical analysis was done in SPSS software.

Table 1: Different Types of Surgical Specimens Received

Types of surgical specimen	No. of cases (N=193)	Percentage (%)
Trans-urethral resection of prostate (TURP)	141	73.05
Core needle biopsy	43	22.27
Enucleated prostatectomy	09	4.66
Total	193	100

Table 2: Distribution of Cases According To HP Diagnosis

HP DIAGNOSIS	NO OF CASES (%)
Fibromusculoglandular hyperplasia	92(47.66%)
Mimics of prostatic adenocarcinoma	30(15.54%)
Carcinoma prostate	39(20.20%)
Suspicious of malignancy	32(16.58%)
Total	193(100%)

Table 3: Result of Suspicious of Malignancy Cases After IHC

	FINAL DIAGNOSIS AFTER IHC ANALYSIS(n=32)			
	34βE12 (nuclear)		P63(cytoplasmic)	
Diagnosis before IHC analysis	Carcinoma	Mimics	Carcinoma	Mimics
Suspicious of malignancy (n=32)	08	24	08	24

Table 4: Comparison of the Association of IHC with Mimic and Suspicious Cases

HP DIAGNOSIS	IHC RESULT		TEST RESULTS
	POSITIVE	NEGATIVE	$X^2 = 6.53$
MIMIC	30	00	df=1
Suspicious of Carcinoma	24	08	p value<0.01

Table 5: Age Incidence in Different Mimics of Prostatic Adenocarcinoma

Age group	Mimics of prostatic adenocarcinoma (%)
41-50	01 (1.85%)
51-60	12 (22.22%)
61-70	34 (62.96%)
71-80	06 (11.11%)
81-90	01 (1.85%)
Total	54 (100%)

Table 6: Presenting Features in Different Prostatic Mimics

CLINICAL PRESENTATION	NO OF CASES (n=54)	PERCENTAGE (%)
Retention of urine	44	81.48 %
Frequency	32	59.25 %
Urgency	14	25.92 %
Nocturia	09	16.66 %
Dysuria	01	2.63 %

Table 7: Different Histological Mimics of Prostatic Adenocarcinoma

Mimics of Prostatic Adenocarcinoma	No. of cases (n=54)	Percentage (%)
CHRONIC PROSTATITIS	10	18.51
BASAL CELL HYPERPLASIA	08	14.81
CLEAR CELL CRIBRIFORM HYPERPLASIA	06	11.11
NON SPECIFIC GRANULOMATOUS PROSTATITIS	06	11.11
HIGH GRADE PIN	05	9.25
ATROPHIC PROSTATITIS	05	9.25
SEMINAL VESICLE	05	9.25
ATYPICAL ADENOMATOUS HYPERPLASIA	04	07.40
COLONIC MUCOSA	03	05.55
SCLEROSING ADENOSIS	02	03.70
TOTAL	54	100.00

Table 8: Correlation of Serum PSA with Different Mimics of Prostatic Adenocarcinoma

Range of serum PSA	Mimics of Prostatic Adenocarcinoma (%)
<4 ng/ml	33(61.11%)
4-10 ng/ml	18(33.33%)
>10 ng/ml	3(5.55%)
Total	54(100%)

RESULTS

Transurethral resection of prostate (TURP) was the most common surgical procedure performed and hence TURP bits were the commonest type of prostatic specimen received which is about 73.05% followed by core needle biopsy which was 22.27% (Table 1). The core needle biopsy is slowly gaining popularity recently. Prostatectomy specimen comprising of only 4.66% of the total was the least common procedure resorted to.

Out of total 193 cases of clinically diagnosed BHP, after histopathological analysis using H/E stain, 92 (47.66%) cases are confirmed as fibromusculoglandular hyperplasia (Table 2), 30(15.54%) cases as mimics of prostatic adenocarcinoma, 39(20.20%) cases as carcinoma prostate. 32(16.58%) cases could not be resolved using H/E stain and were interpreted as suspicious of malignancy and were subjected to immunohistochemical analysis. These 32 suspicious cases were subjected to immunohistochemical analysis using both p63 and HMWCK 34βE12. In 8 cases, both p63 and HMWCK 34βE12 were entirely negative in the atypical focus while positive in the adjacent surrounding benign glands, supporting the diagnosis of prostate carcinoma. Of the remaining 24 cases, both markers showed positive staining for basal cell layers confirming the diagnosis of the mimics of prostatic carcinoma. (Table 3)

Mimics of prostatic adenocarcinoma specimens were also subjected to immunohistochemistry. When compared between specimens suspicious of malignancy it showed a strong association (p < 0.01) which is shown in Table 4.

Out of total 54 cases of prostatic mimics biopsies studied, 62.96% of cases were in the 7th decade & 22.22% of cases were in the 6th decade followed by 11.11% in the 8th decade and only 1.85% cases in the 5th and 9th decade (Table 5).

Overlapping of clinical features is observed in most of the cases studied. The usual presenting features were retention of urine, followed by frequency, nocturia, urgency, dysuria, etc. Retention of urine was the commonest i.e. 81.48 % of cases (Table 6).

Being the most common mimic, chronic prostatitis comprising of 10 (18.51 %) cases (Table 7). This is followed by basal cell hyperplasia 8 (14.81%) cases, clear cell cribriform hyperplasia and nonspecific prostatitis each of 06 (11.11%) cases, high grade PIN, atrophic prostatitis and seminal vesicle each 5 (09.25%) cases, atypical adenomatous hyperplasia(adenosis) comprises 4 (7.40%) cases while colonic mucosa 3 (5.55%) and sclerosing adenosis being the least common one with 02 (03.70%) cases.

From the 54 cases of mimics studied, 61.11% of cases had a serum PSA below <4 ng/ml & 33.33% of cases had a serum PSA in range of 4-10 ng/ml and only 5.55% had serum PSA >10ng/ml (Table 8).

GROSS PICTURES

Fig 1A: Transurethral Resection Of Prostate (TURP) CHIPS

Fig.1B: Needle Biopsy Specimen
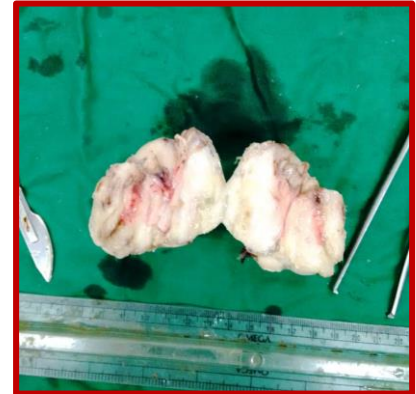
Fig 1C: Enucleated Prostatectomy Specimen

POSITIVE CONTROL- BENIGN HYPERPLASIA OF PROSTATE
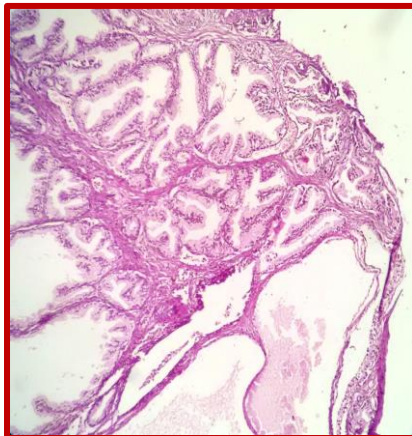
Fig. 2A: H & E (100X): Benign Hyperplasia Of Prostate
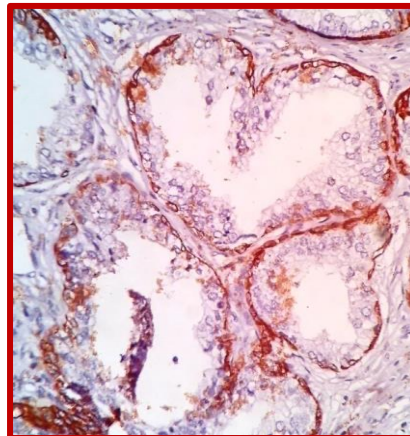
Fig. 2B: 34bE12(400X) : Continuous Cytoplasmic Stain Of Basal Layer
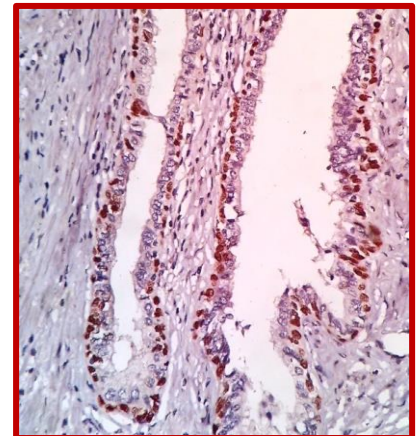
Fig. 2C: P63(400X): Continuous Nuclear Stain Of Basal Layer

NEGATIVE CONTROL- ADENOCARCINOMA OF PROSTATE
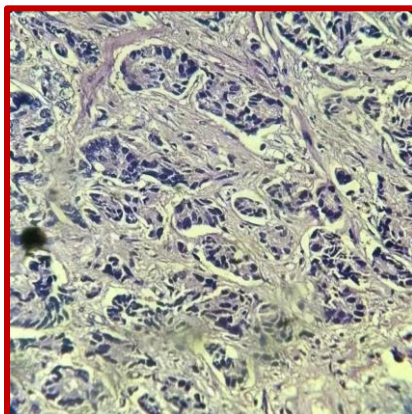
Fig. 3A: H & E (400X): Infiltrating Adenocarcinoma Prostate
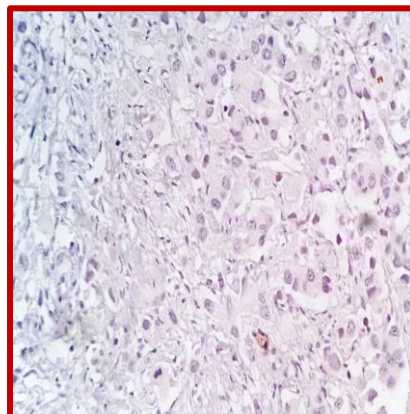
Fig.3B: 34bE12(400X): Absence Of Basal Cell Staining
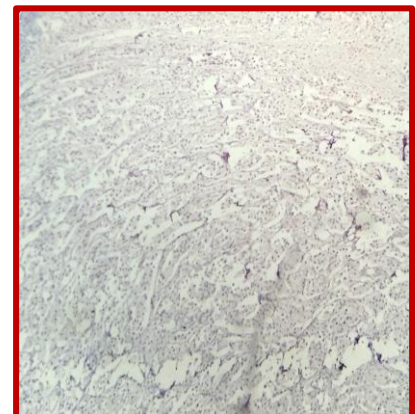
Fig.3C: P63 (100X): Absence Of Basal Cell Staining

BASAL CELL HYPERPLASIA
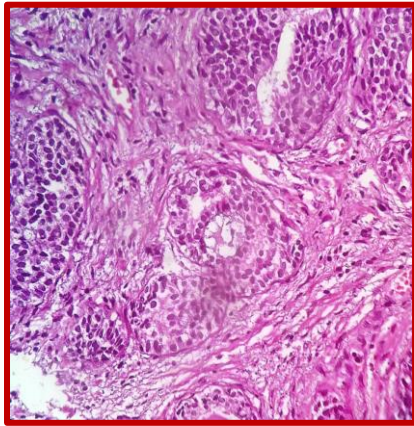
Fig.4A- H & E (400X): Proliferation Of The Basal Cell Layer
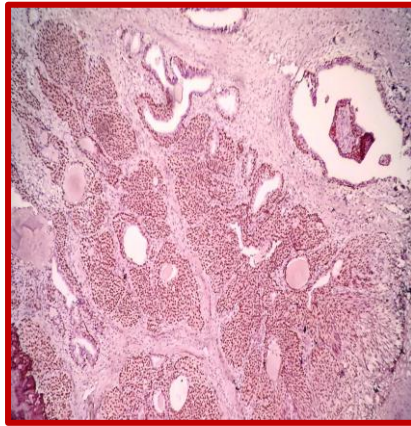
Fig.4B- P63(100X): Positive Staining Of Basal Cell Nuclei
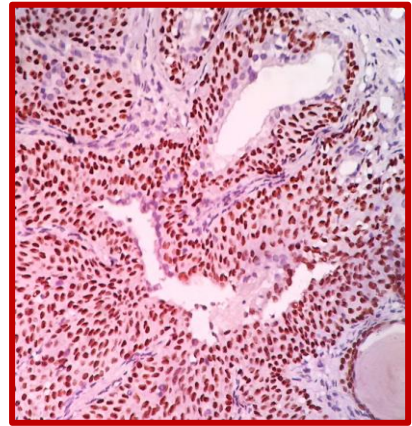
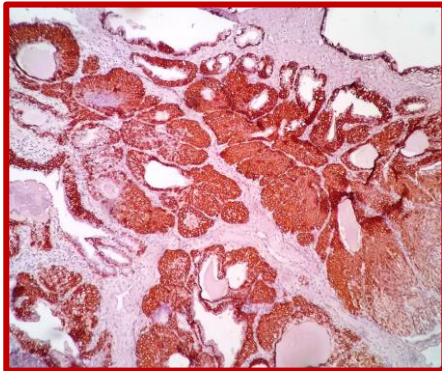**Fig.4C- P63 (400X): Positive Staining Of Basal Cell Nuclei**

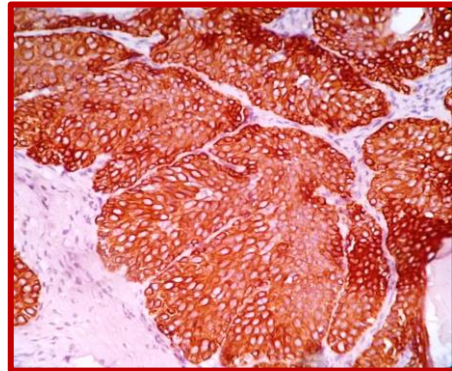**Fig.4D: 34bE12(100X):Intense And Diffuse Cytoplasmic Staining Of Basal Cell Layer**

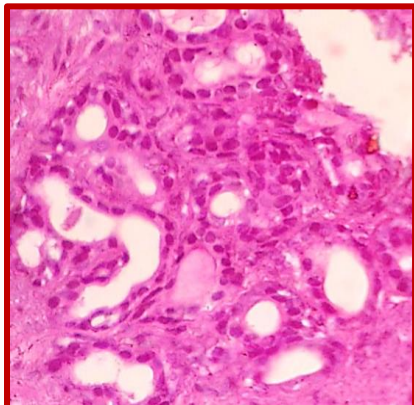**Fig.4E: 34bE12 (400X): Intense And Diffuse Cytoplasmic Staining Of Basal Cell Layer**

ADENOSIS

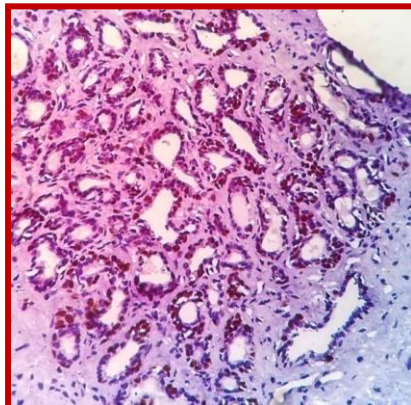**Fig.5A- H & E(400X): Adenosis Showing Proliferation Of Small Glands**

Fig.5B- P63 (100X): Positive Basal Cell Nuclear Staining
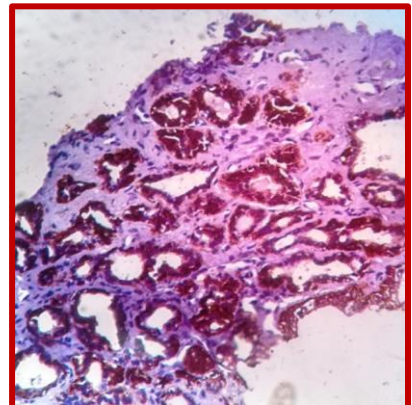
Fig.5C- 34bE12 (100X): Positive Basal Cell Cytoplasmic Staining

SQUAMOUS METAPLASIA
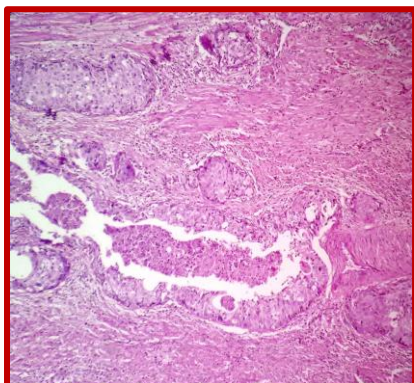
Fig 6A: H&E: Squamous Metaplasia Mimicking Squamous Carcinoma
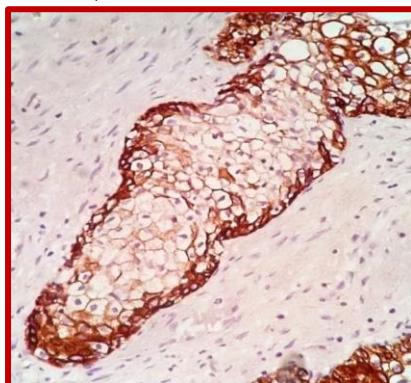
Fig.6B- 34bE12 (400X): Positive Basal Cell Cytoplasmic Staining
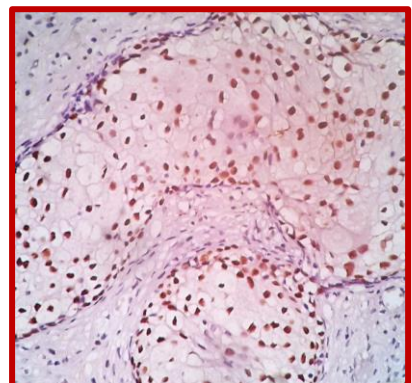
Fig.6C- P63 (400X):   Positive Basal Cell Nuclear Staining

SCLEROSING ADENOSIS
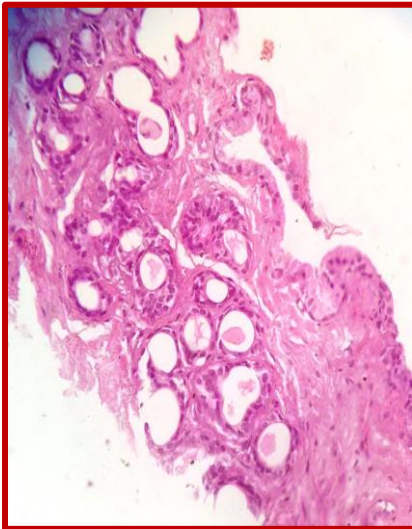
Fig.7A: H & E(100X): Proliferation Of Both Glands And Stroma Mimicking High Grade Invasive Adenocarcinoma
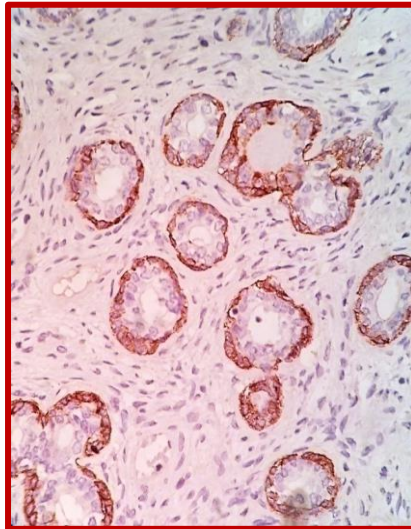
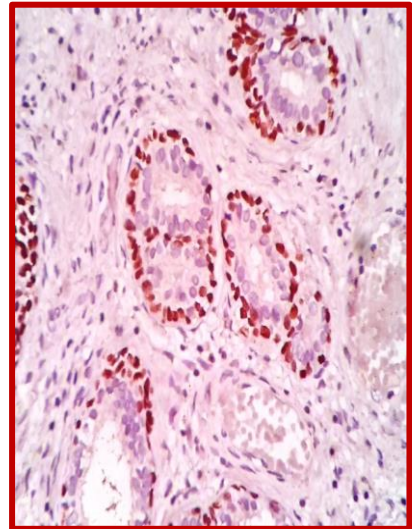**Fig.7B: 34bE12 (100X): Positive Basal Cell Cytoplasmic Staining**

Fig.7C: P63 (100X): Positive Basal Cell Nuclear Staining
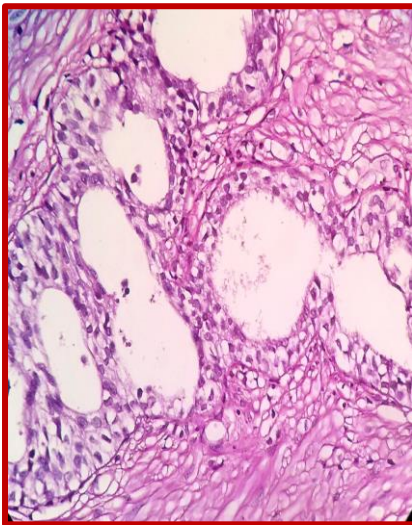
CLEAR CELL CRIBRIFORM HYPERPLASIA

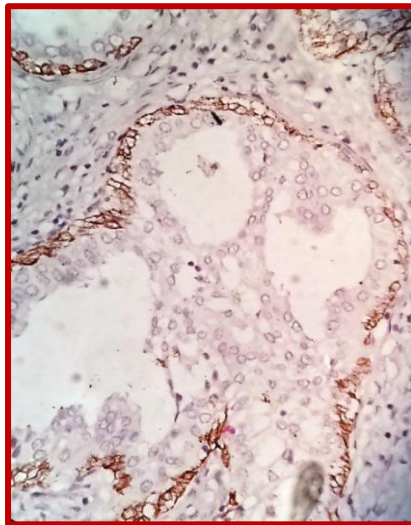**Fig.8A- H & E (400X): Complex Papillary Cribriform Hyperplasia Of Clear Cells Involving Acini**

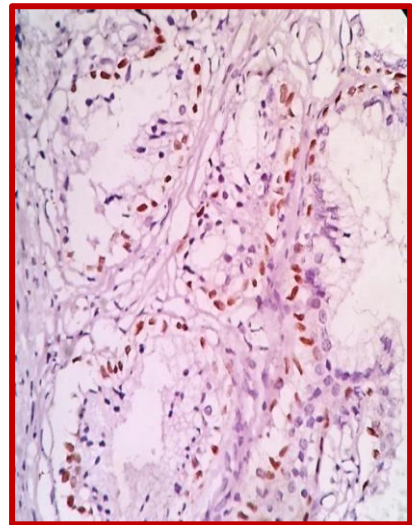**Fig.8B- 34bE12 (400X): Positive Basal Cell Cytoplasmic Staining**

Fig.8C- P63 (400X): Positive Basal Cell Nuclear Staining
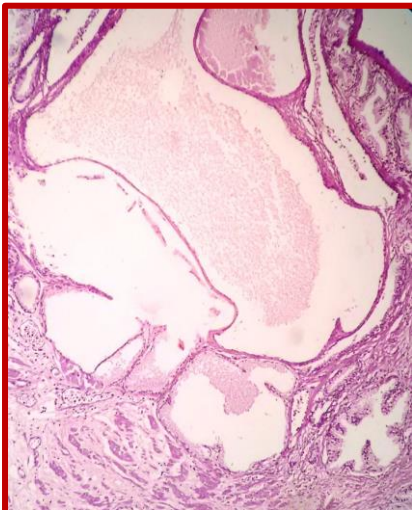
ATROPHIC PROSTATITIS

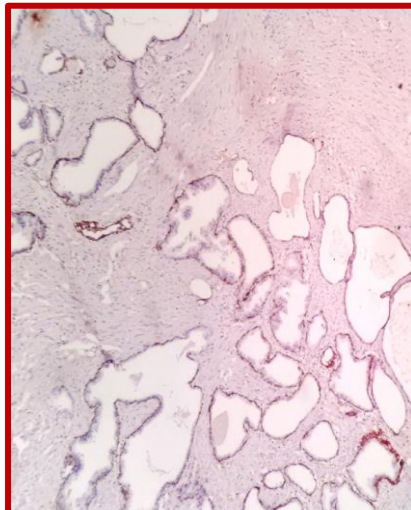**Fig.9A H & E: Atrophic Acini Lined By Small, Dark Nuclei, Scanty Cytoplasm.**

Fig.9B- P63 (100X): Positive Basal Cell Nuclear Staining
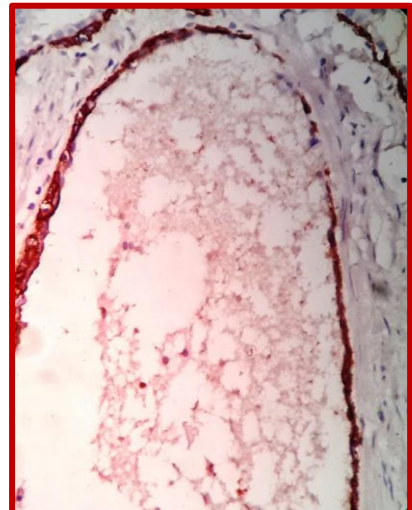
Fig.9C- 34bE12 (400X): Patchy Basal Cell Cytoplasmic Staining
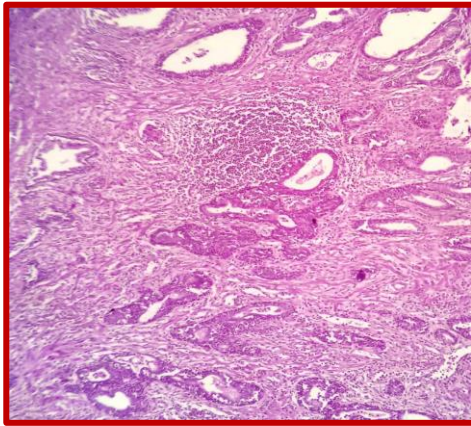
CHRONIC PROSTATITIS

Fig.10A- H&E(100X): BHP With Chronic Inflammatory Cells, Atypical Focus.
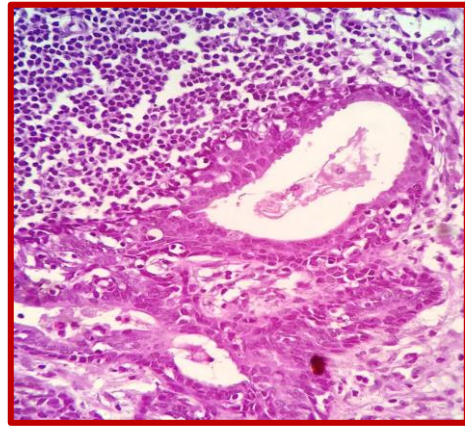

Fig.10B- H&E(400X): Chronic Inflammatory Cells, Atypical Focus Having Hyperchromatic Enlarged Nuclei
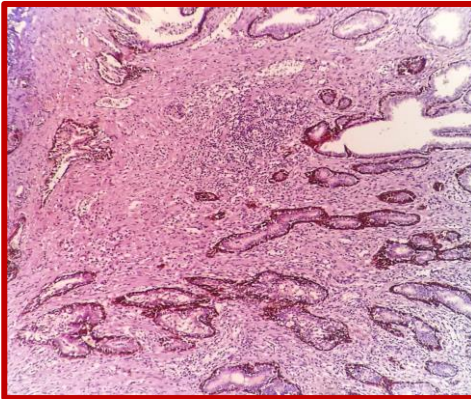

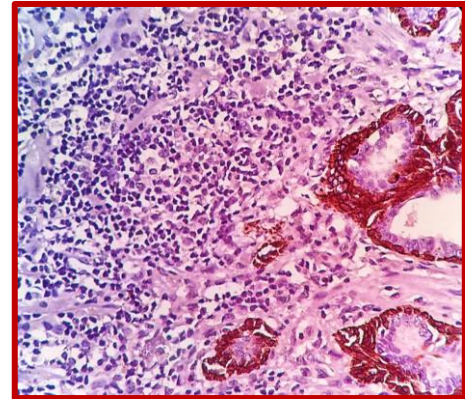**Fig.10C- P63 (100X): Positive Basal Cell Nuclear Staining**

Fig.10D- 34bE12 (400X): Positive Basal Cell Cytoplasmic Staining In The Atypical Focus Confirming It To Be A Benign Gland
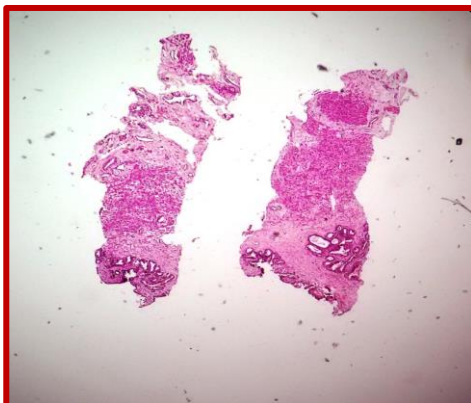
SEMINAL VESICLE

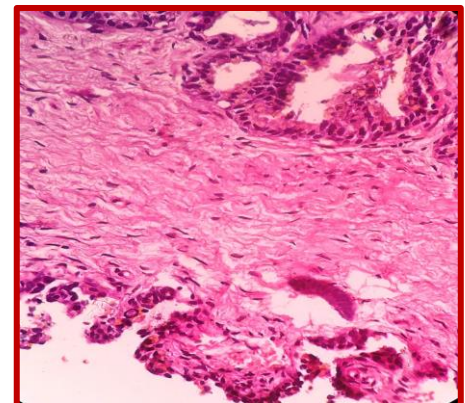**Fig.11A- H&E(100X): Seminal Vesicle In Needle Biopsy**

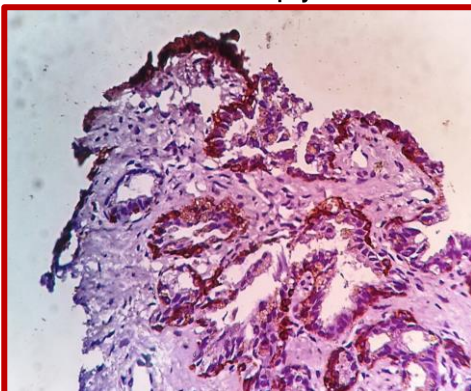**Fig.11B- H&E(400X): Seminal Vesicle, Atypical Looking Cells With Hyperchromatic Nucleus**

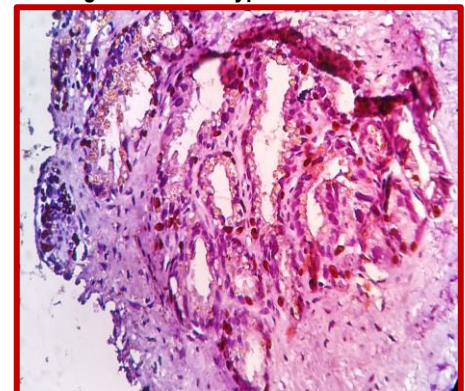**Fig 11C: 34bE12 (400X): Positive Basal Cell Cytoplasmic Staining**

Fig.11D: P63 (400X): Positive Basal Cell Nuclear Staining
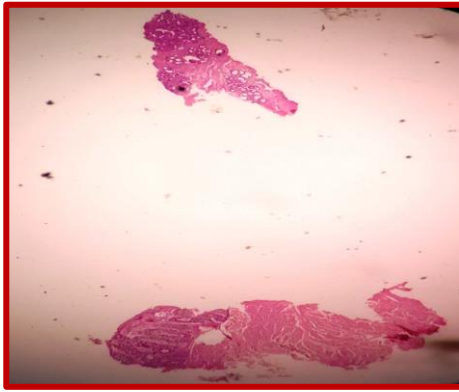
COLONIC MUCOSA

Fig.12A- H&E (100X): Colonic Mucosa In Needle Biopsy
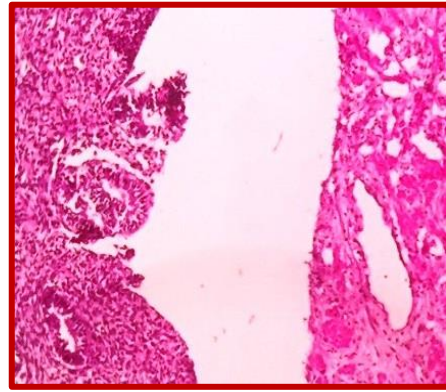
Fig.12B- H&E(400X): Prostate Tissue And Colonic Mucosa In Needle Biopsy
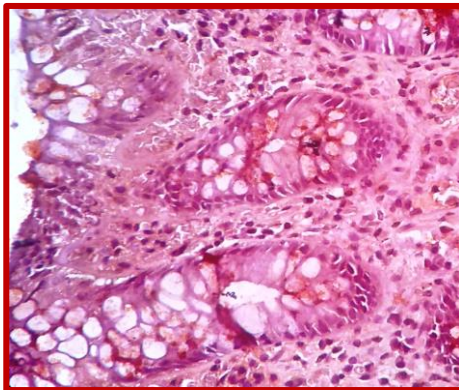
Fig.12C- 34bE12 (400X): Negative Basal Cell Cytoplasmic Staining
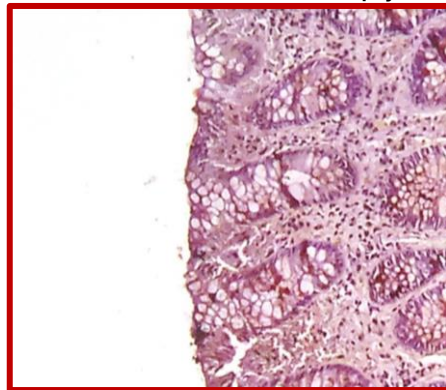
Fig.12D- P63 (100X): Negative Basal Cell Nuclear Staining

LOW GRADE PROSTATIC INTRAEPITHELIAL LESION
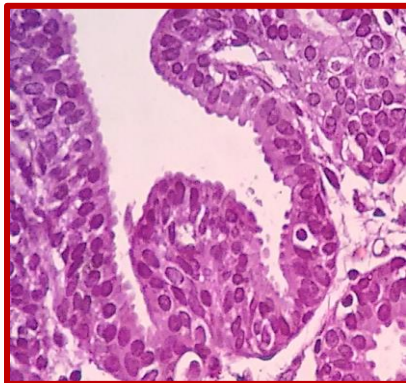
Fig.13A- H&E(400X): Low Grade Pin With Epithelial Cell Crowding & Stratification

Fig.13B- 34bE12 (400X): Continuous Basal Cell Cytoplasmic Staining

Fig.13C- P63 (400X): Continuous Basal Cell Nuclear Staining

HIGH GRADE PROSTATIC INTRAEPITHELIAL LESION

Fig.14A- H&E(400X): High Grade Prostatic Intraepithelial Lesion, Cribriform Pattern Of Architecture, Enlarged Dark Nuclei, Prominent Nucleoli.

Fig.14B- 34bE12(400X): Patchy Staining Of The Cytoplasm Of Basal Cell Layer

DISCUSSION

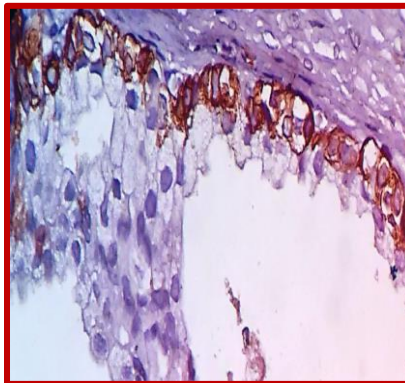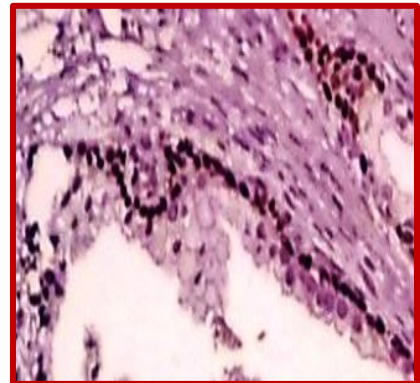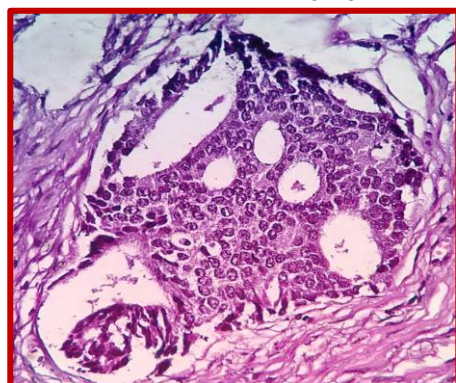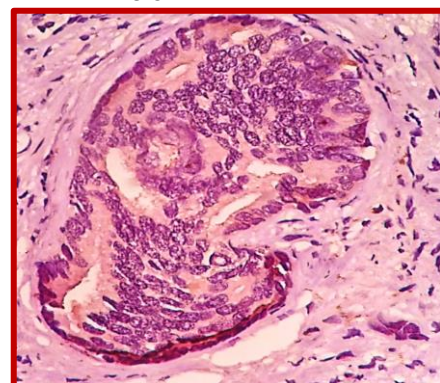In our study, BHP constitutes almost half number of cases while that of mimics and carcinoma constitutes about one fourth each which is similar to the study by Angurana et al.[7] In our hospital, transurethral resection of prostate (TURP) was the most common surgical procedure performed and hence TURP bits were the commonest type of prostatic specimen received which is about 73.05% followed by core needle biopsy which was 22.27% which is becoming more popular recently. Enucleated prostatectomy specimen comprising of only 4.66% of the total is performed mostly in very large glands and the cases are mostly coming from periphery and now-a-days it is being gradually replaced by the core needle biopsies.

Out of total 193 prostatic biopsy specimen, after histopathological analysis using H/E stain, 32(16.58%) cases could not be resolved using H/E stain and were diagnosed as suspicious of malignancy. After IHC, both p63 and HMWCK 34βE12 were entirely negative in 8 out of 32 specimens with atypical focus supporting the diagnosis of prostate carcinoma.

Of the remaining 24 cases, both markers showed positive staining for basal cell layers confirming the diagnosis of the mimics of prostatic carcinoma. We observed no differences in staining pattern between the two nuclear and cytoplasmic markers. This study does not correlates with the study of Wu et al[26] who observed that for diagnosing prostate carcinoma in the needle biopsies, p63 is as specific and sensitive as 34βE12 and therefore can be used as a complementary basal cell-specific stain for 34βE12 in difficult cases. This may be due different manufactures of the antibody and methods of antigen retrieval during immunohistochemical staining. We used the antibody from PathnSitu while Wu et al used that from DAKO.

Shah et al [27] used Laboratory vision anti-p63 antibody and a microwave for antigen retrieval and observed that 34βE12 and p63 are highly specific for basal cells and p63 is more sensitive than 34βE12 in staining benign basal cells, particularly for TURP specimens, offering slight advantage over 34βE12 in diagnostically challenging cases.

Mimics have a peak age incidence i.e. the 7th decade. The results of our study tallies with Ro et al[8] who stated that most of the mimics of cancer occurs in the same age as prostatic carcinoma. But the results of our study oppose with Harbitz et al [9] and Bostwick et al [10]. They noted a rise in incidence after the age of 40 years. This is due to lower life expectancy and negligent attitude towards very old people.

There was overlapping of clinical symptoms in most of the cases. They are retention of urine in 81.48% of cases, frequency of micturition in 59.25% of cases, urgency in 25.92%, nocturia in 16.66%, dysuria present in 2.63%. Similar findings were suggested by Steers et al[11] and Anderson et al[12]. J.Y. Ro et al[2] also noted that mimics do not have a specific clinical manifestation.

Being the most common mimic, chronic prostatitis comprising of 18.51% cases and is followed by basal cell hyperplasia in 14.81% cases. Our study is similar to Mittal et al[15] who studied 185 consecutive prostate specimens and found chronic prostatitis was frequently encountered (58%) cases associated with BHP. But there were differences with Billis A et al[16]who concluded partial atrophy was the most common mimic studied. In our study, prostatic atrophy was found in only 9.25 % cases. As prostatic

atrophy is located in peripheral zone it is less common in TURP specimens which may be the reason for low percentage.

We got all cases of basal cell hyperplasia in TURP specimens only and none were found in needle biopsies which tallies with Hosler et al[17] who recognized basal cell hyperplasia as a well-recognized entity in TURP specimens but an uncommon finding in prostatic needle biopsies. Nonspecific granulomatous prostatitis in our study was found in 6 (11.11%) cases. Only one of these cases had serum PSA less than 4ng/ml while rest all had serum PSA ≥4 ngm/ml. This study is in correlation with the study of Pavlica P et al[18] who studied 20 cases of nonspecific granulomatous prostatitis and found that 19 cases had serum PSA level ≥4 ngm/ml & only one case had less than 4(mean value was 13.3 ngm/ml). Another rare entity sclerosing adenosis which was seen in 2 (3.70%) cases, compared with Sakamoto et al[19] who reviewed sections of prostate from 263 patients and found only 5 cases of sclerosing adenosis with an incidence of 1.9%. This discrepancy is due to less number of cases in our study.

Clear cell cribriform hyperplasia had an incidence of 6(11.11%) cases in our study. All the 6 cases had positive basal cell layer staining with both the markers which correlates with the study of Elizabeth et al[20] who studied 15 cases of CCCH by immunohistochemistry.

A basal cell layer was demonstrated in all 15 CCCHs by the use of the 34 beta E12 anti-high-molecular-weight keratin antibody that reacts with the basal cells but not with the acinar cells of the prostate. Atypical adenomatous hyperplasia which accounted for 7.40% cases in our study correlated with Helpap B et al[21], who stated the incidence of AAH in TURP material between 4 to 15%.High grade PIN was found in 5(09.25%) cases in our study. This figure tallies with Troncoso et al[22] who noted 17.9% of PIN without carcinoma.

But according to Goudin et al [23], Pacelli A et al[24] and Bostwick DG et al[25] the frequency of high grade PIN in needle biopsies range to 5% to 16% and in TURP specimens between 2.3-4.2%. The raised percentage in our study is due to inclusion of mostly mimics in our study and exclusion of other benign entities and frank carcinoma from our study.

In our study, 61.11% had a serum PSA below <4 ng/ml & 33.33% had a serum PSA in range of 4-10 ng/ml. Only 5.55% cases had serum PSA >10ng/ml. This result can be correlated with Robbins et al[13] who had stated serum PSA can be elevated in conditions like chronic prostatitis, digital rectal examinations, transrectal ultrasonography, older age groups and in patients with enlarged hyperplastic prostate glands. Akhter et al[14] found higher levels of PSA (>20 ngm/ml) in 57.1% of patients of BHP with chronic prostatitis. Our study correlates with them, 2(50%) out of 10 cases of chronic prostatitis had serum PSA (>20ngm/ml).

CONCLUSION

This study was undertaken to create an awareness of the benign mimics which may simulate carcinoma. Within a short time span of 2 years all the available procedures were utilised. Out of total prostatic specimens received, suspicious lesions were given final diagnosis with additional help of IHC. Hence these mimics are often confusing but proper observation of their morphological patterns coupled with prudent use of IHC can lead to correct diagnosis.

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