
ORIGINAL ARTICLE**Mucin Histochemistry Study of the Prostate in Normal and Malignant Lesions***Manoj P.Ambali^{*}, Megha A. Doshi¹, Pratibha P. Patil¹, Shweta H. Chavan¹*¹*Department of Anatomy, Krishna Institute of Medical Sciences, Karad-415110 (Maharashtra) India*

Abstract:

Background: There is an increase in the incidence of the prostate cancer by 1% yearly which has been recorded in the last three years. Early diagnosis of prostate cancer is important and differentiation between normal and malignant lesions of prostate has been a diagnostic dilemma. There is unavailability of modern techniques in rural areas as well as in developing countries. **Aims and Objectives:** To evaluate the usefulness of mucin stains in differentiating normal and malignant lesions of prostate. **Material and Methods:** The study was done on 33 specimens of normal (n=11) and malignant (n=22) prostates which were collected from postmortem and surgically resected specimens in Krishna Institute of Medical Sciences and Krishna Hospital Medical Research Central. Normal prostate specimens were used as controls with carcinoma of prostate as test. The type of study was case control study. Routine Hematoxylin / Eosin and special stains as PAS, PAS-Diastase, PAS-Phenyl Hydrazine, Alcian Blue pH-2.5 and 1, Aldehyde Fuchsin, combined AB-PAS and AF-AB were performed. **Results:** Results were tabulated according to colour intensity into different grades ranging from -ve to +++. Neutral mucins were observed in normal prostate and in prostate carcinoma acid mucins were seen predominantly present. **Conclusion:** Mucin histochemistry may provide a valuable and cost-effective tool for the diagnostic histopathology of prostate lesions.

Keywords: Prostate, Mucin, Histochemistry

Introduction:

Carcinoma of the prostate is one of the most common internal malignancies in males which is responsible for 10% of cancer related deaths [1].

As the age increases the incidence of Prostatic Carcinoma (PC) rises progressively especially, after the age of 50 years with a peak incidence in the age group of 75 years and above [2]. Presence of PC during autopsy varies between 15-70 % [1]. Prostatic needle biopsy is commonly used for its diagnosis. But in this procedure limited amount of tissue is available for diagnosis. Sometimes, benign hyperplasia may mimic adenocarcinoma of prostate. Serum Prostate Specific Antigen (PSA) is used as an adjunct for diagnosis of the prostate malignancy. However PSA can be raised in benign hyperplasia, prostatitis, and trauma and even after prostatic massage. The diagnosis of carcinoma of prostate is one of the most challenging areas of surgical pathology [3]. For precise diagnosis of prostate carcinoma there is a need of a marker which is specific, cost effective and can be useful in rural area where the advance techniques are not available.

In mammalian tissue different types of mucins are present. The term “mucosubstances” is used to denote all tissue components, which are present in connective tissue or as secretion of certain epithelial cell except glycogen [4] Connective tissue muco-substances are called “mucopolysaccharides”, while those secreted by epithelia are referred as “mucins” [5]. Mucins perform variety of functions like lubrication, protection against acids etc. Mucosubstances are classified into two types: A) Neutral mucins and B) Acidic mucins.

Neutral mucins are slightly alkaline in nature and mainly help in reducing the pH and toxicity of substances. Acidic mucins are sub classified into weakly acidic and strongly acidic [5-7].

Histochemistry is defined as any technique in which a chemical reaction is involved in coloring tissue, be it staining with dyes. The designation of a stain as special may be arbitrary but generally any stain other than H and E is regarded as special stain. They are used in an attempt to identify cell and tissue components by virtue of their specific chemical reactions [8, 9].

Specific chemical composition of mucosubstances is documented by various researchers with the help of new histochemical methods with special stains but there have been few studies of human prostate mucosubstances [10]. In the present study, combination of special stains such as P.A.S. Diastase, P.A.S.- Phenyl hydrazine, Alcian Blue-P.A.S., Aldehyde fuchsin-Alcian blue were used.

Material and Methods:

The present study was conducted in the Department of Anatomy, Krishna Institute of Medical Sciences, Karad from Dec 2013 to Dec 2017 after taking permission from Institutional Ethics Committee of KIMS, Karad. The study was designed as a control study. The study was carried out on 33 specimens of normal (n=11) and carcinoma prostate (n=22) collected from postmortems and surgically removed specimen from Krishna Hospital. Normal prostate specimens were used as controls with carcinoma prostate as test.

The tissues were fixed in 10% formal saline with 2% calcium acetate and a pinch of phosphotungstic acid to help for preservation of mucins. The tissues were embedded in paraffin and blocks were prepared by histopathological technique and cut at

5-6 microns. Sections were stained with Hematoxylin and Eosin (H&E), and the following histochemical methods were performed on paraffin- embedded sections for the characterization of different mucosubstances as PAS, PAS diastase, PAS- Phenyl hydrazine, Alcian Blue (AB) – pH 1 and 2.5, Aldehyde Fuchsin(AF), combined AB-PAS and combined AF-AB.

1. P.A.S. -- Periodic acid Schiff reagent stains all carbohydrates including mucosubstances. Therefore mucosubstances are P.A.S. positive.
2. P.A.S. Diastase -- Diastase dissolves glycogen like carbohydrates, but mucin remains unaffected. This stain is used for confirmation of mucosubstances.
3. P.A.S. Phenyl hydrazine -- Phenyl hydrazine dissolves neutral mucosubstances only and hence to prove their presence.
4. Alcian blue -- This stain can be used at various pH levels.
5. a) AB pH 1 -- This stain is highly acidic and stains sulphomucins only.
b) AB pH 2.5 -- This stain is weakly acidic and stains both carboxylated and sulphomucins.
6. Aldehyde Fuchsin – This stain only stains sulphomucins and confirms their presence.
7. Combined AB-PAS – This staining procedure will stain all different types of mucin. Neutral –Magenta, Carboxylated –Blue, Sulphated --Purple.
8. Combined AF-AB -- This staining procedure helps for differentiation and confirmation of carboxylated and sulphatedmucins. Carboxylated--Blue, Sulphated--Purple

Table 1: Mucin Histochemistry of Normal Human Adult Prostate

| Stain | Glandular Acini | | |
|--------------------|---------------------------|----------------|---|
| | Colour | Intensity | Inference |
| H&E | Nucleus: Blue Tissue-pink | — | Identified and Confirmed |
| PAS | Magenta | +++ | PAS positive substance |
| PAS D | Magenta | +++ | No glycogen. Mucosubstances are present. |
| PAS PH | Magenta | -ve | Neutral mucin |
| AB (pH 2.5) | Blue | -ve | No Acid mucin |
| AB (pH 1) | Blue | -ve | No Sulphomucin |
| AF | Violet/Purple | -ve | No Sulphomucin |
| AB -PAS | Blue and Magenta | M +++ B -ve | Predominantly Neutral mucin Acid mucin-Nil |
| AF -AB | Violet/purple and Blue | B -ve P -ve | Sulpho and Sialomucin- Nil |

H&E: Hematoxylin and Eosin; PAS: Periodic Acid Schiff; PAS D: PAS Diastase; PAS PH: PAS Phenyl Hydrazine; AB: Alcian Blue; AF: Aldehyde Fuchsin; M: Magenta; B: Blue; P: Purple.

+++ : Strong reaction; ++ : Moderate reaction; + : Weak reaction; ± : trace; - ve: Negative reaction.

Table 2: Mucin Histochemistry of Prostatic Cancer

| Stain | Glandular Acini | | |
|--------------------|---------------------------|--------------|---|
| | Colour | Intensity | Inference |
| H&E | Nucleus: Blue Tissue-pink | — | Identified and Confirmed |
| PAS | Magenta | +++ | PAS positive substance |
| PAS D | Magenta | +++ | No glycogen. Mucosubstances are present. |
| PAS PH | Magenta | ++ | Neutral mucin and acid mucin present |
| AB (pH 2.5) | Blue | ++ | Acid mucin present |
| AB (pH 1) | Blue | + | Few Sulphomucin |
| AF | Violet/Purple | + | Few Sulphomucin |
| AB -PAS | Blue and Magenta | M ++ B ++ | Neutral mucin and Acid mucin are present |
| AF -AB | Violet/purple and Blue | B ++ P + | Predominately Sialomucin with few Sulphomucin |

H&E: Hematoxylin and Eosin; PAS: Periodic Acid Schiff; PAS D: PAS Diastase; PAS PH: PAS Phenyl Hydrazine; AB: Alcian Blue; AF: Aldehyde Fuchsin; M: Magenta; B: Blue; P: Purple.

+++ : Strong reaction; ++ : Moderate reaction; + : Weak reaction; ± : trace; - ve: Negative reaction.

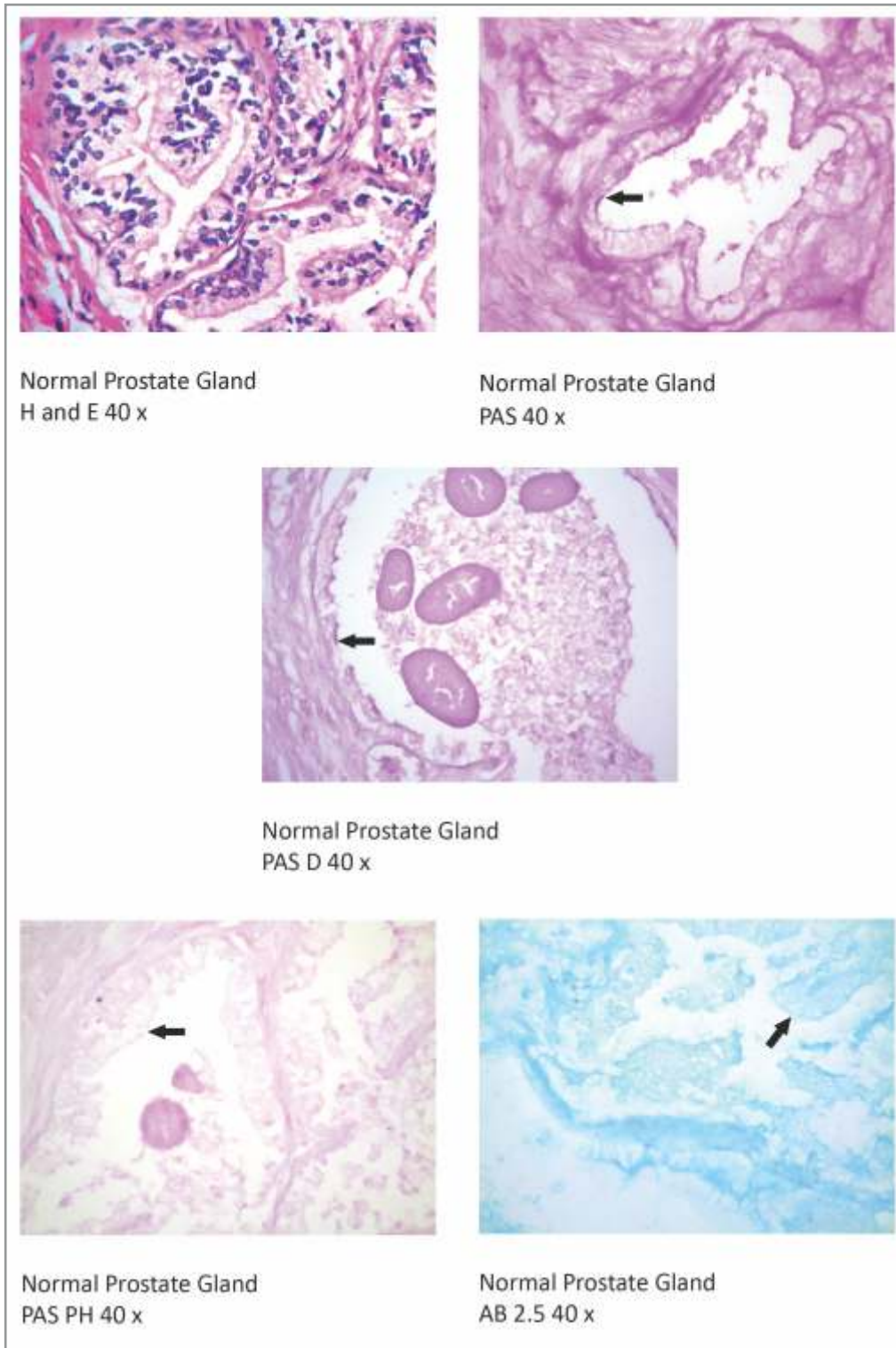


Fig. 1: Mucin Histochemistry of Normal Prostate Using Different Stains

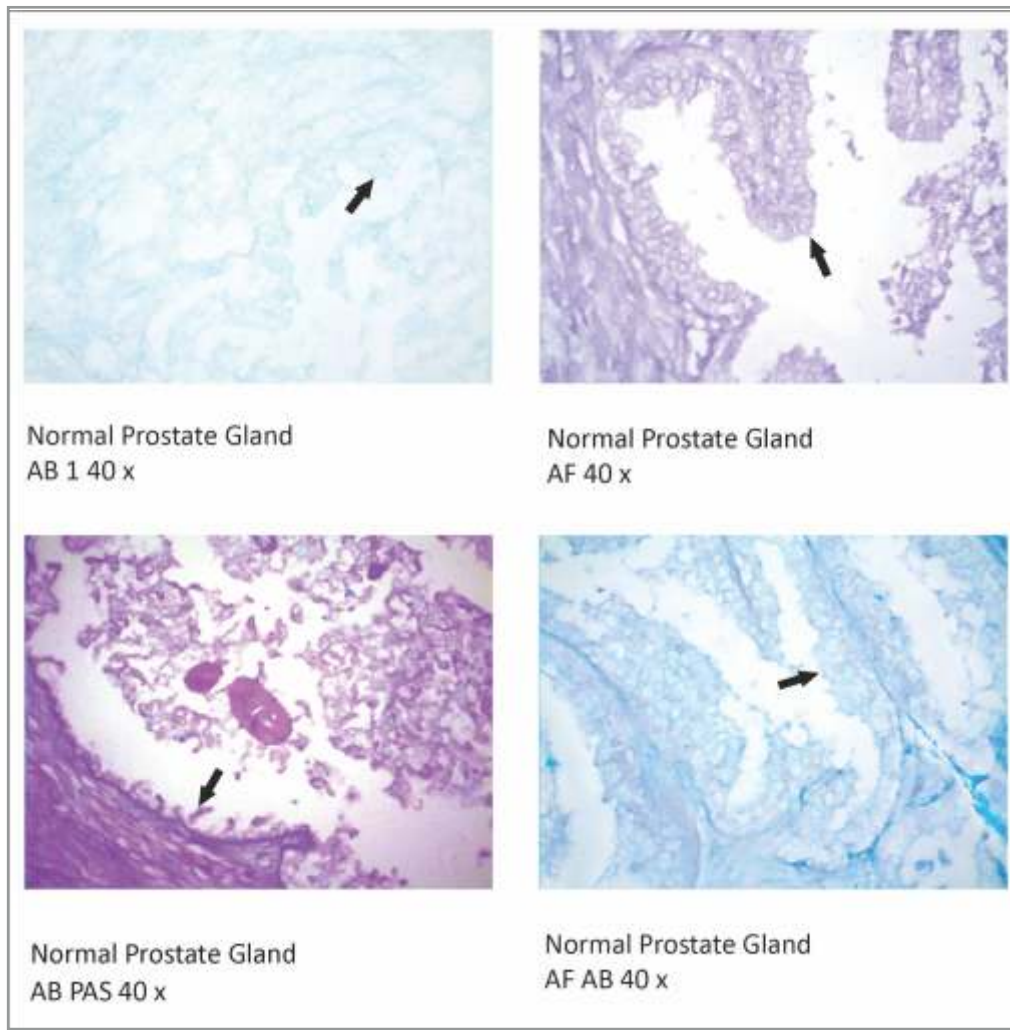


Fig. 2: Mucin Histochemistry of Normal Prostate Using Different Stains

All the results obtained were tabulated according to colour intensity into different grades ranging from -ve to +++. [10]

Results:

All the results were tabulated according to colour intensity into different grades ranging from -ve to +++ [10, 11].

Colour index [10, 11]:

- 1) +++: Strong reaction.
- 2) ++: Moderate reaction

- 3) +: Weak reaction
- 4) ±: trace
- 5) -ve: Negative reaction

According to the below table the acinar cells are showing presence of neutral mucins only. No acid mucins are detectable (Table 1 and Fig. No. 1 - 2).

It is seen that in prostate carcinoma both neutral and acid mucins are seen. In acidic mucins sialomucins are more than sulphomucins (Table 2 and Fig. No. 3 - 4).

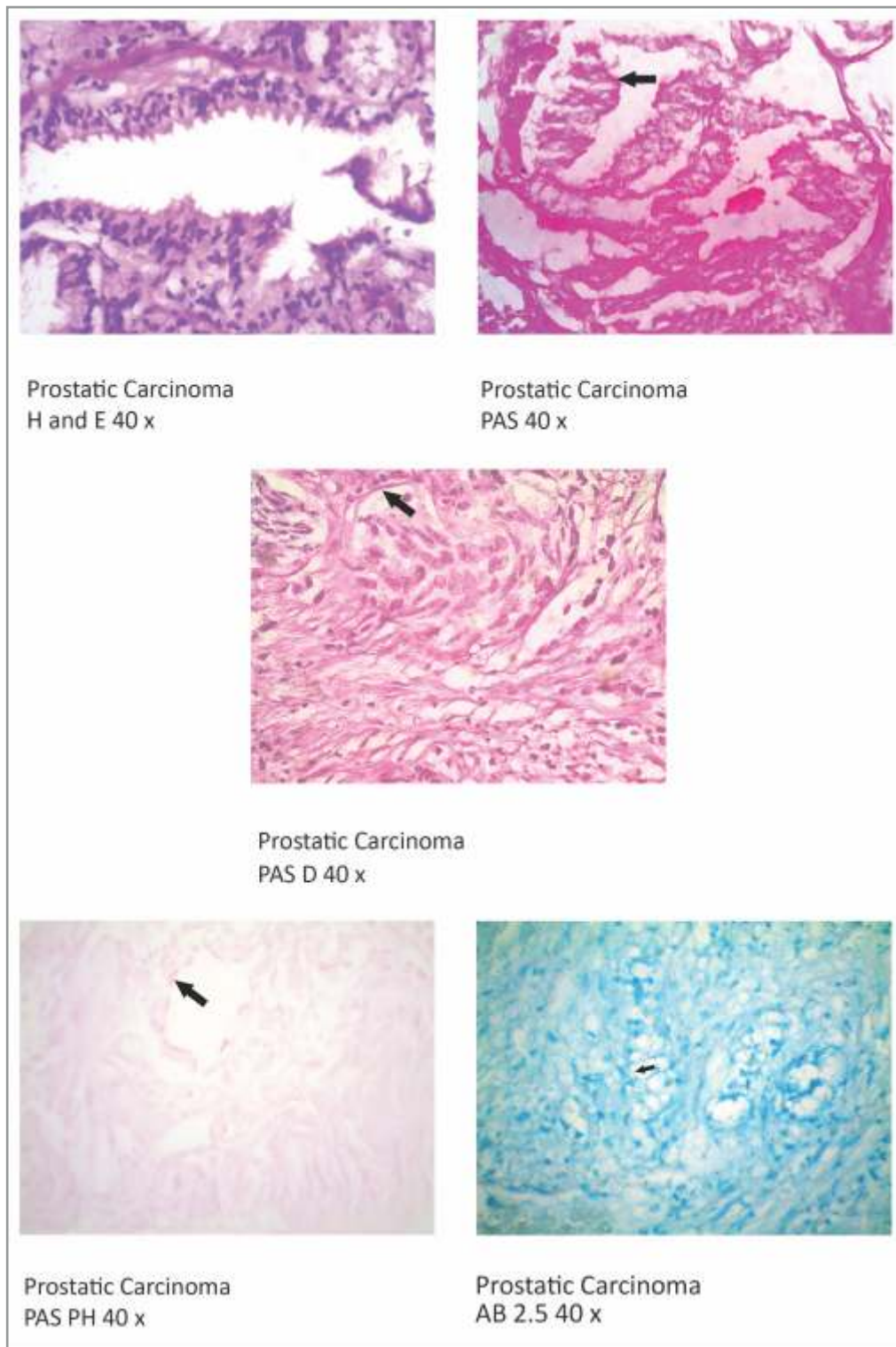


Fig. 3: Mucin Histochemistry of Prostatic Carcinoma Using Different Stains

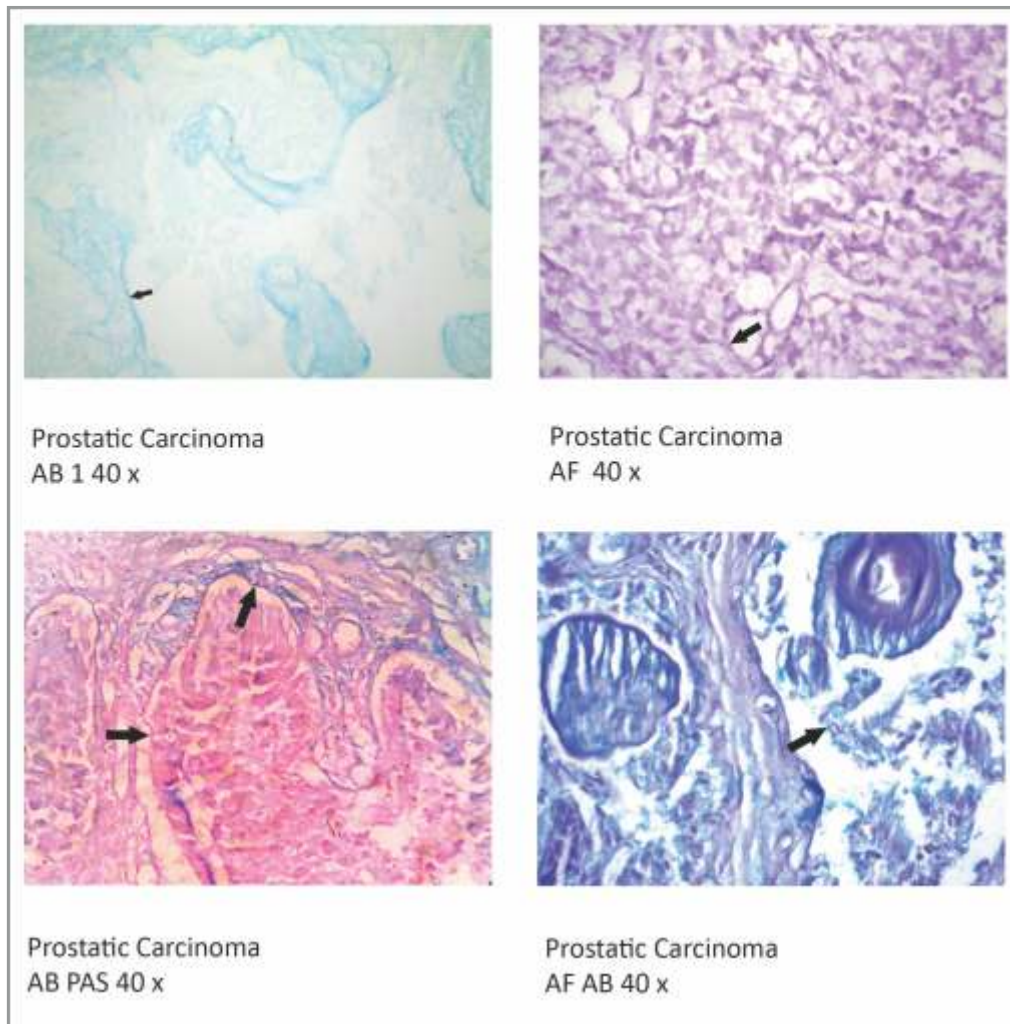


Fig. 4: Mucin Histochemistry of Prostatic Carcinoma Using Different Stains

Discussion:

Prostatic malignancy shows wide spectrum of appearances ranging from anaplastic tumors to highly differentiated neoplasms which are distinguished from non-neoplastic glands with great difficulty [12, 13].

Frank *et al.* observed PAS positive in normal prostate, negative in PAS-PH and red in AB-PAS [14]. Our findings also go along with them. We observed the presence of neutral mucins only in normal prostate (Table 1). Also, our findings of

acidic mucin positivity in normal prostate (0%) are in accordance with the findings of Pinder *et al.* (0%) but McMahon *et al.* (5%), Arora *et al.* (33.3%) and Mathur *et al.* (16%) reported higher values which may be due to higher sample size.

In cases of prostatic carcinoma, we observed positivity for acidic mucin in 100% of samples. When compared with other authors their reported values were on the lower side (Table 4).

In our study positivity for acidic mucin in well

Table 3: Comparison between Normal and Carcinoma

| Stains | Normal | Carcinoma |
|-------------|----------------|--------------|
| H&E | Confirmed | Confirmed |
| PAS | +++ | +++ |
| PAS-D | +++ | +++ |
| PAS-PH | -ve | ++ |
| AB (pH 2.5) | -ve | ++ |
| AB (pH 1) | -ve | + |
| AF | -ve | + |
| AB-PAS | M +++ B -ve | M ++ B ++ |

H&E: Hematoxylin and Eosin; PAS: Periodic Acid Schiff; PAS D: PAS Diastase; PAS PH: PAS Phenyl Hydrazine;
AB: Alcian Blue; AF: Aldehyde Fuchsin; M: Manganite; B: Blue.

+++ : Strong reaction; ++ : Moderate reaction; + : Weak reaction; ± : trace; -ve: Negative reaction.

Table 4: Comparison of Acidic Mucin Positivity in Various Studies

| Author | Benign Hyperplasia (%) | Carcinoma Prostate (%) |
|-----------------------|------------------------|------------------------|
| Arora HL | 33.30 | 60 |
| Pinder <i>et al.</i> | 0 | 38 |
| McMahon <i>et al.</i> | 5 | 50 |
| Agrawal <i>et al.</i> | 0 | 46.66 |
| Present study | 0 | 100 |

differentiated prostatic carcinoma (100%) correlate best with that of McMahon *et al.* Most of the authors observed that, acid mucin was present predominantly in CaP but rarely in normal gland. The intensity of positive reaction varies from deep blue to light from mucinous to non-mucinous area. Present study observed the same findings.

Conclusion

In mammalian tissue different types of mucins are present and they occur in the form of mixtures.

Normal Prostate showed presence of neutral mucin only. In neoplastic lesions acid mucin were predominately seen. In late stages of prostatic carcinoma neutral mucin may appear showing reshuffling to embryological state. Thus mucin histochemistry may act as a tool for early detection of prostate carcinoma and may help for early surgical procedure with good results. Also, this technique can be used in rural areas where limited facilities are available.

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