Study of Mucin Histochemistry in Benign hyperplasia and Malignant Lesions of Human Prostate Gland

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Abstract

Aim: Prostatic enlargement occurs due to nodular hyperplasia, prostatitis and neoplasm of prostate gland. Incidence of prostate cancer increases by 1% yearly which has been reported in the last three years. Early detection of prostate cancer is important. Also, the differentiation between benign hyperplasia and malignant lesions of prostate is very important for the treatment of patient. Aim of the present study is to evaluate the usefulness of Mucin stains in differentiating between benign hyperplasia and malignant lesions of prostate.

Material and Methods: The study was done on ninety-five specimens of benign hyperplasia (n=73) and malignant (n=22) prostates which were collected from postmortem and surgically resected specimens in KIMSU and KHMRC hospital. Routine Hematoxylin & Eosin and special stains such 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: We tabulated our results according to color intensity into different grades ranging from -ve to +++. Acid mucins were present predominantly in prostate carcinoma.

Conclusion: Hence, mucin histochemistry may be a valuable and cost-effective tool for the differentiation between benign hyperplasia and carcinoma of prostate.

Key words: Prostate, Carcinoma of prostate, Mucin, Histochemistry, Benign hyperplasia, Acid mucin, PAS.

Introduction

Benign prostate hyperplasia (BPH) is a histological diagnosis describing a hyper-proliferative process of epithelial and stromal cells in the transition zone of the prostate. [1] Age itself is the major risk factor for BPH. The prevalence of BPH rises markedly with aging. [2]Carcinoma of the prostate (CaP) is responsible for 10% of internal malignancies death in males. [3]As the age increases the incidence of CaP rises progressively especially after the age of 50 years with a peak incidence in the age group of 75 years and above.[4] However, most of the patients of CaP die of other unrelated causes because they never have symptoms or very late diagnosis. [5]BPH and neoplasm are the two major causes of prostate hypertrophy and to differentiate between both, the prostate needle biopsy is commonly used. However, in this procedure limited amount of tissue is available for diagnosis. Sometimes BPH may mimic adenocarcinoma of prostate. The diagnosis of CaP is one of the most challenging areas of surgical pathology. [6]For precise differential diagnosis of BPH and CaP there is a need of a marker which is specific, cost effective and can be easily used in remote areas.

Various types of mucins are present in mammalian tissue. Mucosubstances are tissue components, other than glycogen which are rich in carbohydrates and present in connective tissue, or secreted by certain epithelial structures. [7]. Muco-substances secreted by epithelia are known as "mucins"[8]. Mucins perform different types of functions like lubrication, protection against acids etc. Two types of mucosubstances are present: A) Neutral mucins and B) Acidic mucins

Neutral mucins are slightly alkaline which help for reducing the pH and toxicity of substances. Acidic mucins are sub classified into weakly acidic and strongly acidic.[8, 9, 10]

Histochemistry is a 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

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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.[11, 12]

Specific chemical composition of mucosubstances is documented by various workers with the help of new histochemical methods with special stains but there have been few studies of human prostate mucosubstances. [13].We studied the Mucin Histochemistry in BPH and Malignant Lesions of Human Prostate Gland with the help of H&E and combination of special stains such as P.A.S., PAS-Diastase, P.A.S.-Phenyl hydrazine, Alcian Blue pH 2.5 and 1, Aldehyde fuchsin, combined AB (2.5) –PAS and combined AF-AB (2.5)

Materials and Methods

This study was conducted in the Department of Anatomy, Krishna Institute of Medical Sciences, "Deemed to be University", Karad from Dec 2013 to Dec 2017 after taking permission from Ethical Committee of KIMS, Karad. We used ninety-five specimens of benign hyperplasia (n=73) and malignant (n=22) prostates which were collected from surgically removed specimen from Krishna Hospital and Medical Research centre, Karad.

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 wax and blocks were prepared by histopathological technique and cut at 5-6 microns. Sections were stained with Hematoxylin and Eosin, 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 1and 2.5, Aldehyde fuchsin(AF), combined AB(2.5)-PAS and combined AF-AB(2.5).

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.

   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 andsulphomucins.
5. Aldehyde Fuchsin – This stain only stains sulphomucins and confirms their presence.
6. Combined AB-PAS – This staining procedure will stain all different types of mucin. Neutral –Magenta, Carboxylated –Blue, Sulphated —Purple.
7. Combined AF-AB — This staining procedure helps for differentiation and confirmation of carboxylated and sulphatedmucins. Carboxylated –Blue, Sulphated—Purple

All the results were tabulated according to color intensity into different grades ranging from -ve to +++. [13, 14]

Colour Index[13, 14]:

1) +++: Strong positive reaction.

2) ++: Moderate reaction

3) +: Weak reaction

4) ±: Trace

5) -ve: Negative reaction

Benign Prostatic Hypertrophy (BPH) -

When stained with H and E there is hyperplasia of glandular and stromal tissue with papillary buds and in folding. Glandular component is made up of nodules of small and large acini lined by basal and secretory cells. Some glands show papillary in folding and projections containing central fibrovascular core and others are dilated and cystic. The stromal component often shows both fibrous and smooth muscle elements[7,8].

When the section of Benign Prostatic Hypertrophy are stained with (Table no1,Fig.No.1 & 2)

PAS stain, the glandular acinar cell showed magenta colour indicating presence of PAS positive substances like carbohydrate and neutral mucin**.**

PAS-D- stain the intensity of magenta was same indicating the absence of non-mucinous carbohydrate like glycogen.

PAS- PH -total absence of staining indicates the presence of neutral mucin only.

AB 2.5 pH- the glandular acini are not staining showing absence of acid mucin.

AB 1.0 pH- no glandular acini are stained showing absence of sulphomucin.

AF stain- no glandular acini stained, confirming absence of sulphomucin.

AB-PAS -staining many glandular acini are intensely stained with magenta colour with no blue colour glandular acini. It shows the presence of neutralmucin**.**

AF-AB- staining no glandular aciniare stained, showing absence of acid mucin.

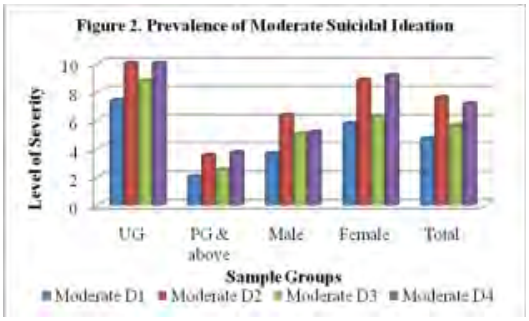


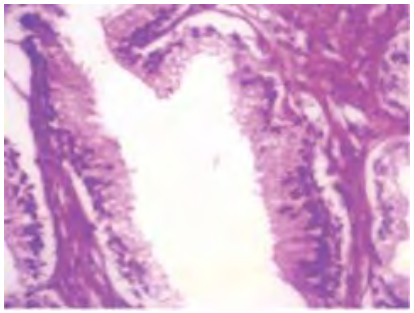
Table No 1:Mucin Histo-chemistry of Benign hyperplasia of Prostate

Sr. No	Stain	Glandular Acini Colour	Intensity	Inference
1	H& E	NucleusBlueTissue-pink	—	Identified & Confirmed
2	PAS	Magenta	+++	PAS positive substance
3	PAS D	Magenta	+++	No glycogen. Mucosubstances are present.
4	PAS PH	Magenta	-ve	Neutral mucin
5	AB pH 2.5	Blue	-ve	No Acid mucin
6	AB pH 1.0	Blue	-ve	No Sulphomucin
7	AF	Violet/Purple	-ve	No Sulphomucin
8	AB -PAS	Blue &Mangeta	M +++B -ve	Predomintaly Neutral mucinAcid mucin-Nil
9	AF -AB	Violet/purple & Blue	B -veP -ve	Sulpho&Sialomucin- Nil

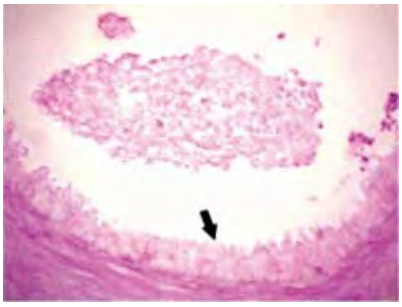
From the above table it is observed that though there is glandular hypertrophy the acini show presence of neutral mucins only. No acid mucins are seen.

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: Mangeta; B: Blue; P: Purple. +++: Strong reaction; ++: Moderate reaction; +: Weak reaction; ±: trace; – ve: Negative reaction.

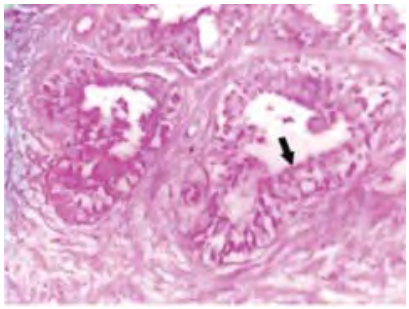
Fig. 1: Mucin Histochemistry of Benign Prostatic Hypertrophy Using Different Stains



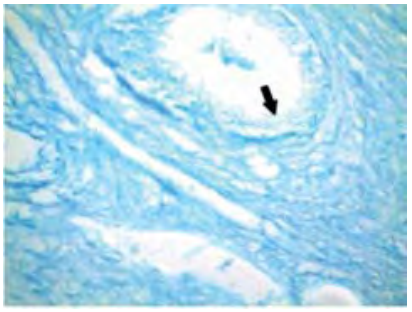
Benign Prostatic Hypertrophy H and E40x



Benign Prostatic Hypertrophy PAS PH 40x

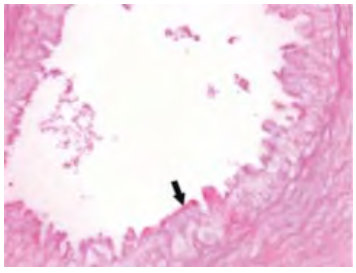


Benign Prostatic Hypertrophy PAS 40x

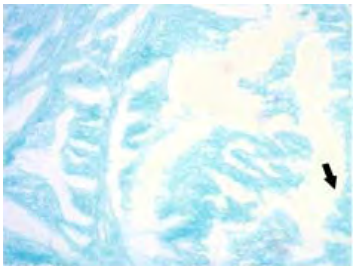


Benign Prostatic Hypertrophy AB 2.5 40x

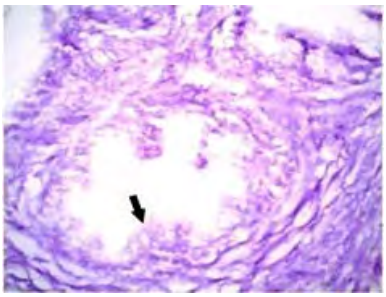
Fig. 2: Mucin Histochemistry of Benign Prostatic Hypertrophy Using Different Stains



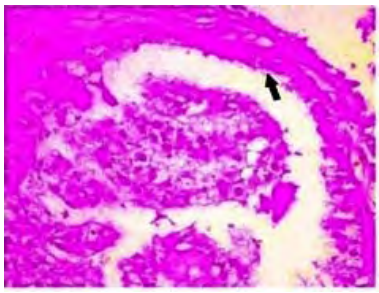
Benign Prostatic Hypertrophy PAS D 40x



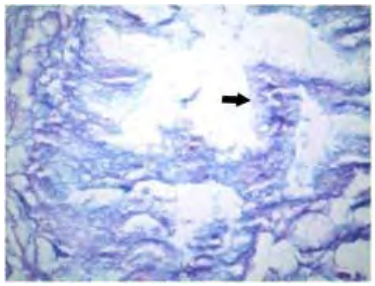
Benign Prostatic Hypertrophy AB 1 40 x



Benign Prostatic Hypertrophy AF 40 x



Benign Prostatic Hypertrophy AB PAS 40 x



Benign Prostatic Hypertrophy AF AB 40 x

Carcinoma of Prostate-

In **H and E** staining of CaP there are small glands, sometimes medium to large glands seen. Papillary/cribiform glands or solid growth, single cells or necrosis. Cytoplasm is usually finely granular, may be clear, foamy due to intracellular lipids. Nuclear enlargement is seen with hyperchromasia and prominent nucleoli. Malignant transformation is accompanied by loss of basal cells[7,8].

When the section of Carcinoma of Prostate gland are stained with – (Table no 2 & Fig No. 3 & 4)

PAS stain-the glandular acinar cell showed magenta colour indicating presence of PAS positive substances like carbohydrate and neutral mucin**.**

PAS-D-stain the intensity of magenta was same indicating the absence of non-mucinous carbohydrate like glycogen.

PAS-PH - Less intensity of staining indicates the presence of combination of acidic and neutral mucin.

AB 2.5 pH - many acinar cell showed blue colour indicating presence of acid mucin.

AB1.0 pH- few acinar cell stained bluecolour showing presence of little amount of sulphomucin.

AF- few acinar cell were stained purple showing presence of little amount of sulphomucin.

AB –PAS- few acinar cells were stained magenta while the other showed blue colour showing presence of neutral and acid mucin.

AF-AB- majority of acinar cell showed blue colour while few were stained purple showing presence of both sialomucin and sulphomucin with predominately sialomucin.

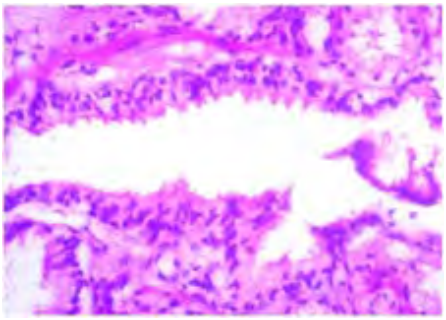
Table No 2 Mucin Histochemistry of Carcinoma of Prostate

Sr. No	Stain	Glandular Acini Colour	Intensity	Inference
1	H& E	Nucleus:Blue		
	Tissue-pink	__	Identified & Confirmed	
2	PAS	Magenta	+++	PAS positive substance
3	PAS D	Magenta	+++	No glycogen. Mucosubstances are present.
4	PAS PH	Magenta	++	Neutral mucin and acid mucin present
5	AB pH 2.5	Blue	++	Acid mucin present
6	AB pH 1	Blue	+	Few Sulphomucin
7	AF	Violet/Purple	+	Few Sulphomucin
8	AB -PAS	Blue &Mangeta	M ++	B ++ Neutral mucin and Acid mucin are present
9	AF - AB	Violet/purple & Blue	B ++ P +	Predominately Sialomucin with few Sulphomucin

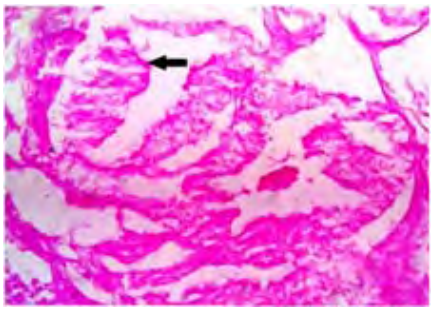
From the above table it is observed that in prostate carcinoma both neutral and acid mucins are seen. In acidic mucinssialomucins are more than sulphomucins.

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: Mangeta; B: Blue; P: Purple. +++: Strong reaction; ++: Moderate reaction; +: Weak reaction; ±: trace; – ve: Negative reaction.

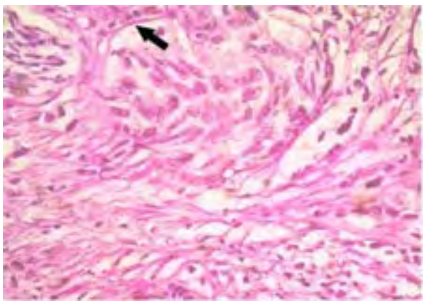
Fig. 3: Mucin Histochemistry of Prostatic Carcinoma Using Different Stains



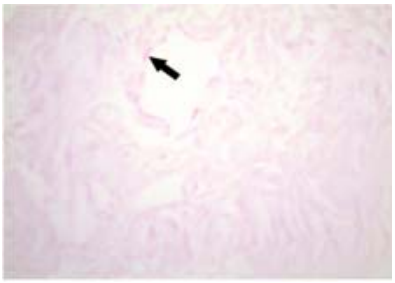
Prostatic Carcinoma H and E 40 x



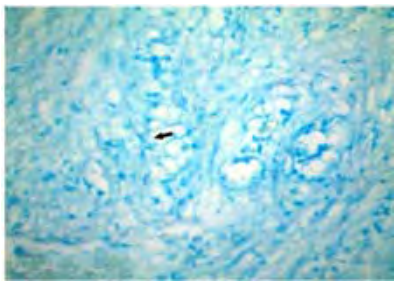
Prostatic Carcinoma PAS 40 x



Prostatic Carcinoma PAS D 40 x

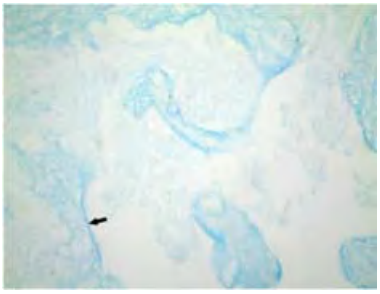


Prostatic Carcinoma PAS PH 40 x

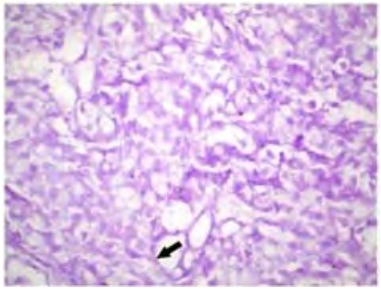


Prostatic Carcinoma AB 2.5 40 x

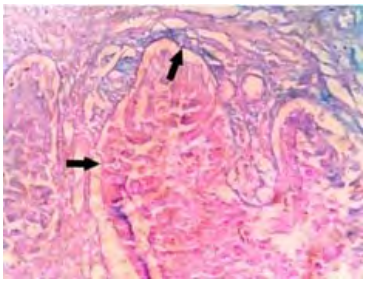
Fig. 4: Mucin Histochemistry of Prostatic Carcinoma Using Different Stains



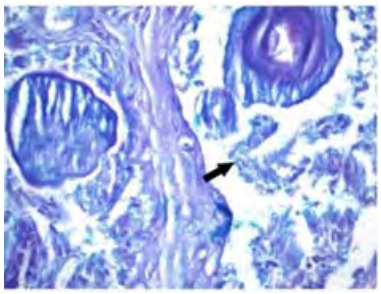
Prostatic Carcinoma AB 1 40 x



Prostatic Carcinoma AF 40 x



Prostatic Carcinoma AB PAS 40 x



Prostatic Carcinoma AF AB 40 x

Discussion

The enlargement of prostate gland is very common and generally starts by 5th decade. If it is more than normal it may cause urinary obstruction leading to hypertrophy of urinary bladder, diverticulosis of urinary bladder and so on. Prostatic enlargement may be due to benign hyperplasia or CaP. Carcinoma of Prostate is common in males after lung carcinoma. An early diagnosis of CaP may be lifesaving.

In the present study 95 prostatic specimens which included BPH and histologically identified prostate carcinoma sections were studied for mucin histochemistry. In any pathological condition,

before the nuclear changes are obvious the cells will show changes in their function earlier and help in early diagnosis. In BPH there is only increase in the mass of the glandular and fibromuscular part of the gland, but the mucin remains normal i.e. neutral mucins were observed. In carcinoma acid mucin presence was clearly seen but in late stages of carcinoma neutral mucin may start reappearing [(19)].

Frank et al. observed that in BPH there was only presence of neutral mucin and acid mucin was present predominantly in CaP.[15]Our findings also go along with them. Our study observed the presence of neutral mucins only in BPH (Table no 1).In cases of prostatic carcinoma; we noticed positivity for acidic mucin in 100%. When compared with other authors their reported values were on the lower side (Table no.3).

Table no.3- Showing comparison of acidic mucin positivity in various studies

Author	Benign hyperplasia	Carcinoma prostate
Arora H L[13]	33.30%	60%
Pinder et al.(16)	0%	38%
McMahon et al.(18)	5%	50%
Agrawal et al.(17)	0%	46.66%
Present study	0%	100%

In our study positivity for acidic mucin in well 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 benign hyperplasia of prostate. The intensity of positive reaction varies from deep blue to light from mucinous to non-mucinous area. Present study observed the same findings.

Present study concludes that mucin histochemistry stains can be used as tools to distinguish the well differentiated adenocarcinoma from the prostatic hyperplasia.

Conclusion

Benign hyperplasia of prostate gland gives the histo-chemical reaction for neutral mucins. Various stains like Periodic Acid Schiff (PAS), Periodic Acid Schiff-Diastase(PAS-D), Periodic Acid Schiff-Phenyl hydrazine(PAS-PH), Alcian blue at pH 1.0 (AB-1) **,** Alcian blue at pH 2.5 (AB-2.5), Aldehyde fuchsin(AF) , Combined Alcian Blue (2.5)- Periodic Acid Schiff (AB2.5-PAS) , Combined Aldehyde Fuchsion - Alcian blue (2.5)(AF-AB 2.5) were used to identify and confirm mucin in BHP and CaP. Present study concludes that mucin histochemistry stains can be used as an adjunct tool to distinguish the BPH from the CaP.

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