

## MUCIN PROFILE OF PROSTATIC LESIONS: A HISTOCHEMICAL APPROACH FOR DIFFERENTIATION IN A TERTIARY CARE CENTER.

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### Abstract

#### Background

Accurate microscopic differentiation between benign and low-grade malignant prostatic lesions poses a diagnostic challenge, particularly in core biopsies where limited tissue can hinder definitive interpretation. Immunohistochemistry (IHC) markers like AMACR and p63 are commonly used but are expensive and technically demanding, limiting their use in resource-constrained settings. Hence, there is a need for alternative diagnostic methods that are cost-effective, reproducible, and easy to perform.

#### Aim

To evaluate the diagnostic utility of histochemical mucin stains, Periodic Acid Schiff (PAS), Alcian Blue, and Mucicarmine in distinguishing benign from malignant prostatic lesions.

#### Materials and Methods

This study included 100 prostatic biopsies received over 18 months in a tertiary care hospital. All specimens were stained with PAS, Alcian Blue, and Mucicarmine. The staining patterns were analyzed and correlated with histopathological diagnoses made using routine Hematoxylin and Eosin (H&E) staining.

#### Results

Neutral mucins (PAS positivity) were predominantly found in benign lesions (90.7%). In contrast, acidic mucins stained by Alcian Blue and Mucicarmine were more commonly seen in malignant lesions (66.7% and 53.3%, respectively), particularly in well-differentiated adenocarcinomas. These differences were found to be statistically significant ( $p < 0.001$ ).

#### Conclusion

Mucin histochemistry offers an effective, low-cost, and accessible diagnostic adjunct for differentiating benign from malignant prostatic lesions, making it particularly valuable in settings with limited access to immunohistochemistry.

#### Recommendations

We recommend incorporating Alcian Blue and Mucicarmine into routine histopathological evaluation when IHC is not feasible.

**Keywords:** Prostate, Mucin, PAS, Alcian Blue, Mucicarmine, Histochemistry, Benign Prostatic Hyperplasia, Adenocarcinoma.

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#### Background

Prostatic carcinoma ranks among the most prevalent malignancies in men and represents the second leading

cause of cancer-related mortality in Western countries. Its incidence in India is steadily rising due to urbanization and lifestyle modifications. While serum

PSA testing and histopathological evaluation remain the mainstays of diagnosis, differentiating low-grade adenocarcinoma from benign prostatic hyperplasia (BPH) can be particularly challenging, especially on limited biopsy material.

Immunohistochemical markers are helpful in these scenarios but are often cost-prohibitive in resource-poor settings. Mucin histochemistry offers a simpler alternative by highlighting biochemical differences in glandular secretions. Neutral mucins are typically present in benign glands, whereas acidic mucins may be expressed in malignant epithelium. This study aims to evaluate the diagnostic significance of PAS, Alcian Blue, and Mucicarmine stains in differentiating benign and malignant prostatic lesions.

## Materials and Methods

This retrospective cross-sectional observational study was conducted on 100 prostatic biopsies received in the Department of Pathology over 18 months (1 November 2016 to 31 May 2018). Formalin-fixed, paraffin-embedded (FFPE) sections were stained with Hematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS), Alcian Blue, and Mucicarmine.

- PAS: Demonstrates neutral mucins (magenta staining).

- Alcian Blue (pH 2.5): Detects acidic mucins (blue staining).

- Mucicarmine: Highlights epithelial acidic mucins (deep red staining).

Histological categorization into benign, premalignant, and malignant lesions was performed using H&E-stained slides. Malignant lesions were further graded using Gleason scoring. All mucin staining results were recorded and statistically analyzed using the Chi-square test, with  $p < 0.05$  considered significant. No missing data was reported.

The study was carried out at Sri Manakula Vinayagar Medical College, a multi-specialty tertiary care hospital with medical research facilities.

PAS (Periodic Acid-Schiff) Procedure: Sections were deparaffinized, hydrated, and oxidized in periodic acid for 5 minutes, followed by Schiff's reagent for 15 minutes. After thorough washing in running tap water,

counterstaining with hematoxylin was done. Neutral mucins appear magenta.

Alcian Blue Staining Procedure: The tissue sections are first deparaffinized and brought to distilled water through descending grades of alcohol. The slides are then treated with 3% acetic acid for 3 minutes to adjust the pH, followed by staining in 1% Alcian Blue solution prepared at pH 2.5 for 30 minutes. After staining, the slides are thoroughly rinsed in running tap water for 10 minutes. Nuclear counterstaining is performed using Nuclear Fast Red for 5 minutes. The slides are then rinsed briefly in distilled water, dehydrated in graded alcohols, cleared in xylene, and mounted using a suitable mounting medium. Acidic mucins take up the Alcian Blue stain and appear blue, whereas the nuclei appear red.

Mucicarmine Staining Procedure: Deparaffinized and hydrated sections are first stained with Weigert's Iron Hematoxylin for 10 minutes to highlight the nuclei. After rinsing in running tap water for 5 minutes, the sections are stained in mucicarmine solution for 30 minutes. The mucicarmine solution typically contains aluminum hydroxide and carmine, which selectively stain epithelial mucins. Following staining, the sections are rinsed in distilled water, dehydrated through graded alcohols, cleared in xylene, and mounted with a coverslip. In the stained slides, epithelial mucins appear deep rose to red, nuclei appear black, and other tissue elements take on a yellowish background.

## Results

Out of 100 cases studied, majority (75%) of the cases were of BPH including pure BPH and BPH associated with other lesions. 15 cases were diagnosed to be malignant, among which 9 (60%) cases were well differentiated, 2 (13.3%) were moderately differentiated and 4 (26.7%) were poorly differentiated.

To detect neutral mucin, all the cases were stained with Periodic acid Schiff. Among benign cases, 68/75 (90.7%) were found to be positive, 8/10 (80%) cases of pre-malignant were positive, and 8/15 (53.3%) cases of malignant were positive, with a p-value of 0.001 (Table 1)

**Table 1: Association between diagnosis and PAS staining**

Sl. No.	PAS	Diagnosis			p value#
		Benign N (%)	Premalignant N (%)	Malignant n (%)	
1	Negative	7 (9.3)	2 (20)	7 (46.7)	0.001*
2	Positive	68 (90.7)	8 (80)	8 (53.3)	

*Note: # p value based on Chi-square test, \* statistically significant ( $p < 0.05$ )*

All the sections were subjected to an Alcian blue stain to detect the presence of acidic mucins. It was positive in 10/15 (66.7%) cases of malignancy, 2/10 (20%) premalignant cases and 1/75(1.3%) benign case with with p-value <0.01 (Table 2)

**Table 2: Association between diagnosis and Alcian blue staining**

Sl.No.	Alcian blue	Diagnosis			p value#
		Benign n(%)	Premalignant n(%)	Malignant n (%)	
1	Negative	74 (98.7)	8 (80)	5 (33.3)	<0.001*
2	Positive	1 (1.3)	2 (20)	10 (66.7)	

*Note: # p value based on Chi-square test, \* statistically significant (p<0.05).*

The sections were also subjected to Mucicarmine to detect the presence of acid mucins. It was positive in 8/15 (53.3%) malignant cases, 2/10 (20%) pre-malignant cases, and 1/75 (1.3%) benign cases, with p-value <0.001 (Table 3)

**Table 3: Association between diagnosis and Mucicarmine staining**

Sl.No.	Mucicarmine	Diagnosis			p value#
		Benign N (%)	Premalignant N (%)	Malignant n (%)	
1	Negative	74 (98.7)	8 (80)	7 (46.7)	<0.001*
2	Positive	1 (1.3)	2 (20)	8 (53.3)	

*Note: # p value based on Chi-square test, \* statistically significant (p<0.05).*

## Discussion

Differentiating well-differentiated prostatic adenocarcinomas from benign conditions such as benign prostatic hyperplasia (BPH) remains one of the more nuanced challenges in surgical pathology, especially when dealing with limited core biopsy specimens. In this context, the use of cost-effective histochemical stains such as PAS, Alcian Blue, and Mucicarmine offers a pragmatic approach, particularly in resource-constrained settings.

The results of our study align with findings from previous literature, where acidic mucins—demonstrated by Alcian Blue and Mucicarmine—were predominantly observed in malignant lesions, especially in well-differentiated carcinomas, and were virtually absent in benign lesions. On the contrary, neutral mucins, as detected by PAS staining, were significantly present in benign and some premalignant cases, highlighting their potential to exclude malignancy.

## Generalizability

While the findings are consistent with prior studies and offer a reproducible pattern of mucin expression, the generalizability is currently limited to histologically confirmed prostatic lesions from a

single tertiary care center. Further multicentric studies involving varied population demographics and a larger sample size would enhance external validity.

## Limitations

- Sample Size: Although 100 cases were evaluated, subgroup sizes (e.g., premalignant and poorly differentiated carcinoma) were relatively small.
- Lack of IHC Correlation: No direct comparison with IHC markers (e.g., AMACR, p63, or 34βE12) was performed, which could have strengthened diagnostic confidence.
- Retrospective Nature: The study design did not allow for real-time decision-making or longitudinal follow-up.

## Recommendations

We recommend incorporating Alcian Blue and Mucicarmine into routine histopathological evaluation when IHC is not feasible. Future studies should:

- Combine mucin staining with selected IHC panels for enhanced diagnostic accuracy.
- Assess the utility of mucin profiles in needle biopsies, TURP chips, and radical prostatectomy specimens.

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### List of Abbreviations

- BPH: Benign Prostatic Hyperplasia
- PAS: Periodic Acid Schiff
- IHC: Immunohistochemistry
- FFPE: Formalin-Fixed Paraffin-Embedded
- H&E: Hematoxylin and Eosin

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### Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

### Author Contribution

- Dr. Srikanth. K, Dr. Mourouguessine Vimal: Study conception, data analysis, manuscript writing
- Dr. Vijay David Raj: Histological review, statistical analysis, literature review

- Dr. NatarajanSuresh: Data collection, staining, and documentation

All authors have read and approved the final manuscript.

### Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

### Personalization

As a practicing pathologist and educator, this study stems from the everyday diagnostic dilemmas faced in routine histopathology. The intent was to develop a diagnostic strategy that is both practical and resource-sensitive, bridging the gap between affordability and diagnostic accuracy in peripheral and teaching hospitals.

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