Differentiating Benign and Malignant Prostatic Lesions using Mucin and DNA Histochemical Staining, Omdurman Teaching Hospital, Sudan

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Abstract

Background: Benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are the most common prostatic diseases in elderly men. Cost-effective methods are required to accurately differentiate benign hyperplasia from prostatic adenocarcinoma.

Methods: This is an analytical, cross-sectional study conducted on 109 archival paraffin-embedded prostate tissue samples obtained from Omdurman Teaching Hospital. Data collected included histopathological diagnosis, lesion type, patient age, and Gleason score for malignant cases. Three sections were prepared from each sample and stained using Periodic Acid–Schiff, Alcian blue (pH 2.5), and Feulgen reaction to assess mucin types and DNA intensity. The diagnostic value of these histochemical stains in differentiating benign and malignant prostatic lesions and their association with Gleason grading were evaluated.

Results: All 53 BPH cases showed positive staining for neutral mucins with complete absence of acidic mucins. In contrast, PCa samples demonstrated acidic mucin positivity in 22 cases (39.21%), neutral mucin positivity in 2 cases (3.5%), positivity for both mucin types in 17 cases (30.35%), and negativity for all mucins in 15 cases (26.78%). Statistically significant differences were observed between benign and malignant groups regarding mucin type and DNA staining intensity ($p < 0.001$). Additionally, Gleason score showed a significant association with both mucin type and DNA intensity in malignant cases ($p < 0.001$).

Conclusion: Mucin histochemical stain can be used as a useful biomarker in differentiating BPH from PCa. The intensity of DNA expression using Feulgen reaction may provide valuable prognostic information in prostate cancer.

Keywords: Prostate Adenocarcinoma, Benign Prostatic Hyperplasia, Mucin, DNA, Gleason

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Introduction:

Globally, prostate diseases are a major cause of morbidity and mortality in men. (1) Prostate cancer remains the second most frequent type of cancer among men globally (2), with 1.5 million new cases and 400,000 annual deaths in 2022, as reported by the latest global cancer statistics. (3) In many sub-Saharan African countries, prostate cancer represents a major health burden among men, with African American men showing significantly higher incidence and mortality rates compared to men of other ethnicities, particularly among younger age groups. (4)

Prostate cancer is the most common malignancy in Sudanese men and has an increasing incidence among the younger population. (5) Benign Prostatic Hyperplasia (BPH) is the most prevalent prostatic condition and typically poses a diagnostic dilemma due to its histological similarity with well-differentiated adenocarcinoma, (6,7) which could lead to misdiagnosis and negatively impact treatment options and prognostic evaluation. (8)

Even though numerous studies have described the histopathological features of prostatic adenocarcinoma (PCa) and BPH, very few attempts have been made, particularly in low-resource settings like Sudan, on the application of histochemical techniques for differentiation. Most available information is from developed countries, where advanced immunohistochemical and molecular facilities are readily provided. (9) Such techniques are, however, not available or unaffordable for utilization in the majority of pathology laboratories in most of the African region. (10) It is important to address this gap towards improving diagnostic accuracy, guiding appropriate management, and making the best use of available resources in such settings.

The expression of neutral and acidic mucins was found to show differences between nodular hyperplasia and adenocarcinoma, according to previous histochemical analyses. (11) This suggests that special stains similar to Periodic Acid Schiff (PAS) and Alcian blue may provide diagnostic support in situations where immunohistochemistry is limited.

This study aims to evaluate the diagnostic utility of histochemical demonstration, specifically mucin and Deoxyribonucleic Acid (DNA) staining, in distinguishing between benign and malignant prostatic lesions and to assess the potential of these markers in providing prognostic information. (8)

Materials and Methods:

Study Design and Area:

This is an analytical, cross-sectional study of prostatic lesions collected from the histopathology lab of Omdurman Teaching Hospital, a major public hospital in Omdurman, Sudan, serving as a key healthcare facility.

Sample Design:

The sample size included all archival paraffin-embedded tissue blocks of prostate gland lesions available at the study site during the study period. Samples were included from the stored Formalin -Fixed Paraffin -Embedded prostate tissue blocks from the Histopathology Lab of Omdurman Teaching Hospital. They included specimens with a definite histopathological diagnosis of either BPH or PCa. Those with insufficient tissue, which precludes reliable staining, and cases lacking essential clinicopathological details were excluded.

Data Collection Methods and Tools:

Data was collected using a data collection form that covered histopathological diagnosis, type of lesion, patient age, and Gleason score (for malignant cases).

paraffin-embedded tissue blocks were sectioned using a rotary microtome (Leica Ltd). With thickness adjusted to 5µm.

Three sections were prepared from each sample and dewaxed in xylene for four minutes, and hydrated through gradual concentrations of ethyl alcohol: 100%, 90%,70% taking two minutes in each concentration and then applied to distilled water for two minutes.

PAS and Alcian blue technique (2.5 pH) were applied to demonstrate neutral and acidic mucin, respectively, and for DNA demonstration, the Feulgen reaction was applied.

Alcian Blue staining technique for acid mucin:

Hydrated sections were treated with Alcian blue solution at room temperature for 30 minutes. Then they washed in running water for 2 minutes and rinsed in distilled water. Then stained with nuclear-fast red for 5 minutes, washed in tap water, then Dehydrated, cleared, and cover-slipped. (12,13)

Periodic Acid Schiff reaction (PAS) for neutral mucin:

Hydrated sections were treated with periodic acid for 5 minutes, rinsed in distilled water, and covered with Schiff's reagent for 15 minutes, then washed in running tap water for 10 minutes. Harris hematoxylin was used as a counterstain for 15 seconds and blued with ammoniacal water, then rinsed in tap water. The sections were then subjected to gradual concentration of alcohol for dehydration (70, 80, 95, and 100%), followed by clearing in xylene, and a mounted coverslip was applied

with Distyrene Plasticizer Xylene (DPX) (14,15)

Feulgen method for DNA:

5 M Hcl was used for hydrolysis at room temperature for one hour. Then Schiff reagent was applied for 45 minutes. Washed in running water for five minutes, rinsed in distilled water, and stained with one per cent Light green for two minutes, washed, dehydrated and cleared with xylene, and a coverslip was applied with DPX. (13,16)

Ethical consideration:

Ethical approval was granted by the Scientific Research Committee of Al-Neelain University, Medical Laboratory Sciences Faculty. Permission for using the archived, anonymized histopathology samples and associated clinical information was obtained from the Histopathology laboratory of Omdurman Teaching Hospital. The Committee waived informed consent in compliance with institutional and national guidelines for research using archived specimens because there was no direct patient contact and no identifiable personal information.

Results:

A total of 109 samples of prostatic lesion biopsies prepared as paraffin-embedded blocks were enrolled in this study. Fifty-three (48.7%) were diagnosed with BPH, and 56 (51.3%) were PCas.

Using the Gleason scoring, among the 56 samples diagnosed with prostatic adenocarcinoma, 24 (42.9%) were high-grade, 22 (39.3 %) moderate, and 10 (17.9%) were low-grade.

Histochemical demonstration of mucin showed acid mucin in 22 samples (20.2 %) and neutral mucin expressed in 55 samples (50.5 %), 17 samples (15.6 %) were positive for both acid and neutral mucin, and 15 (13.8 %) revealed absence of mucin expression.

DNA staining intensity for the 109 samples showed a weak reaction in 14 (12.8%), a moderate reaction in 48 (44.0%), and a strong reaction in 47 (43.1%).

All samples of benign hyperplasia were positive for neutral mucin with complete absence of acidic mucin expression, whereas the PCa group were positive for acidic mucin in 22 samples (39.2%) and neutral mucin in two samples (3.5 %),17 samples (30.3%) revealed positive expression for both types of mucins, and 15 samples (13.8%) were negative for mucin expression (Figure 1 and 2). Regarding the staining intensity of DNA. The group of benign prostatic hyperplasia showed weak intensity of DNA expression by Feulgen

Table 1: Distribution of Mucin Staining Pattern by Histochemical Methods

Diagnosis	Neutral mucin only	Acidic mucin only	Both types	Negative	Total
BPH (n = 53)	53 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	53
PCa (n = 56)	2 (3.5%)	22 (39.2%)	17 (30.3%)	15 (26.8%)	56
Total	55 (50.5%)	22 (20.2%)	17 (15.6%)	15 (13.8%)	109

The difference between BPH and PCa is significant at **($p < 0.001$)** (Chi-square test).

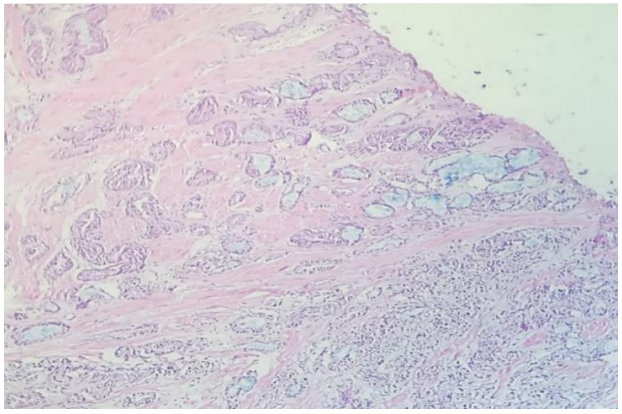

Figure 1: Positive Alcian Blue staining in prostate cancer

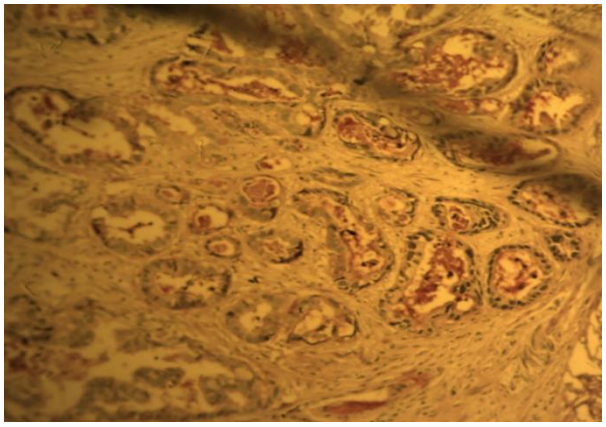

Figure 2: Positive PAS for neutral mucin in BPH

reaction in 11samples (10.09%), moderate in 42 samples (38.53%), no samples showed strong expression, while the PCa group revealed weak expression in 3 (2.75%), moderate in 6 samples (5.50%) and strong intensity reaction in 47 samples (43.11%) (Figure 3 and 4).

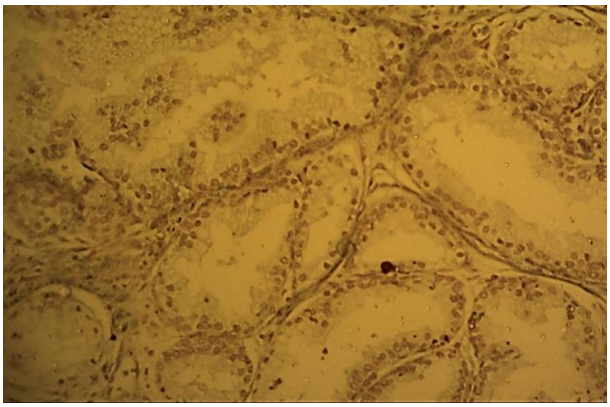

Figure 3: DNA staining in moderate grade (PCa).

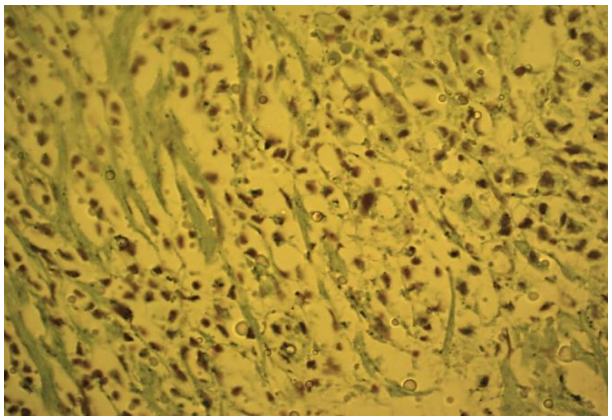

Figure 4: DNA staining in high-grade (PCa).

Table 2. DNA Staining Intensity by Feulgen Reaction.

Diagnosis	Weak (+)	Moderate (++)	Strong (+++)	Total
BPH (n = 53)	11 (20.8%)	42 (79.2%)	0 (0.0%)	53
PCa (n = 56)	3 (5.4%)	6 (10.7%)	47 (83.9%)	56
Total	14 (12.8%)	48 (44.0%)	47 (43.1%)	109

Discussion:

PCas exhibit a wide spectrum of appearances, ranging from anaplastic tumors to highly differentiated neoplasms that are distinguished from non-neoplastic glands with great difficulty due to their reliance on histomorphological appearance(18)

This study examined the mucin expression patterns and DNA staining intensity to compare between benign and malignant prostatic lesions, and to investigate the association between these markers and Gleason grading in adenocarcinomas.

The study finding demonstrates a significant difference in mucin expression within the type of lesions, with a complete absence of acid mucin among the prostatic hyperplasia group; the tendency towards the acidic type of mucin in malignant tissue proves the existing precepts that the mucin composition alteration indicates malignancy (8, 19, 20).

Acidic mucins, either sialomucins or sulfomucins, could be involved in the adhesion, invasion, or immune escape of tumor cells, potentially conferring aggressive tumor behavior (21). While neutral mucin is prevalent in benign lesions, supporting its role in mucin characterization as a method for malignancy assessment beyond conventional morphology.

The observation that there is a strong correlation between mucin subtype and Gleason grade in our population is in accordance with the finding that particular patterns of mucin expression are associated with the degree of tumor differentiation and aggressiveness, and underlines its potential use as a biomarker for both diagnosis and prognosis (22).

The Feulgen technique used for histochemical demonstration of DNA, characterized by its usefulness in archival formalin-fixed paraffin-embedded sections, giving a permanent result suitable for semiquantitative evaluation of DNA (23).

In our study, DNA intensity showed a significant relation with the type of prostatic lesion and with the Gleason score among the adenocarcinoma group. This finding supports the hypothesis that malignant cells typically exhibit increased DNA content due to genomic instability, such as aneuploidy or polyploidy, which is a hallmark of cancer. (24, 25)) And that is supported in previous studies that similarly reported that higher Feulgen reactivity correlates with more aggressive tumor behavior and higher histological grade. (23)

Taking all these factors into consideration, the changes in the expression of mucin and the intensity of DNA staining suggest the biological aggressiveness of PCa in the current study. This is also reflected in the clinicopathological features of the patients.

The prevalence of prostate cancer increases with age, with the highest rates being among men over 70 years of age. In Sudan, cancer patients come to hospitals at advanced stages of disease. (17) This may be attributed to the limited availability of specialized oncology and pathology services in rural regions, leading patients to seek care only at major referral centers in Khartoum, such as Omdurman Teaching Hospital. In the current study, there was a high proportion (42%) of adenocarcinomas that were initially presented as high grade, confirming that there is a high rate of late diagnosis in this setting.

Our findings confirm the value of the histochemical staining technique of mucin and DNA for differential diagnosis between prostatic hyperplasia and PCa, and that it may add value to the diagnosis.

Conclusions:

The present study confirms the utility of histochemical mucin staining and intensity of DNA staining as a tool for low-cost settings to differentiate between benign and malignant prostatic lesions, providing diagnostic and prognostic information about prostatic lesions.

Study Limitations:

The sample size was relatively small and restricted to a single institution, which may limit the generalizability of the findings.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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