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Feulgen Staining: Unveiling Distinctions in Prostatic Hyperplasia and Prostate Cancer Identification

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Abstract

Introduction: The escalating incidence of prostate cancer in sub-Saharan Africa, particularly in Sudan where it ranks third in cancer prevalence after breast cancer, necessitates comprehensive diagnostic approaches. Nuclear deoxyribonucleic acid (DNA) content has emerged as a pivotal prognostic factor in prostate tumors. This study delves into the semi-quantification of nuclear DNA in prostate cancer compared to prostatic hyperplasia, employing the Feulgen reaction technique.

Methods: Paraffin wax sections from patients previously diagnosed with prostatic hyperplasia and prostate cancer were selected for analysis. Clinicopathological data were retrieved from the archives of the National Health Laboratory in Khartoum, Sudan. Each case contributed two sections—one stained with Hematoxylin and eosin for diagnosis confirmation, and the other utilized for DNA demonstration through the Feulgen reaction.

Results: A cohort of 46 patients with clinically and pathologically diagnosed prostatic tumors participated in the study. Of these, 23 (50.1%) exhibited high-grade prostatic adenocarcinoma, 11 (23.9%) had moderate-grade prostatic adenocarcinoma, two (4.3%) presented with low-grade prostatic adenocarcinoma, and 10 (21.7%) manifested benign hyperplasia. Significant disparities in DNA staining intensities were observed in high-grade prostatic adenocarcinomas. The DNA staining intensities of moderate and high-grade prostatic adenocarcinomas were markedly higher than those of benign hyperplasia ($P<0.000$).

Conclusion: Feulgen reaction for DNA detection proves to be a promising avenue for providing valuable prognostic information in prostate cancer. The discernible differences in DNA staining intensities between various grades of prostatic adenocarcinomas and benign hyperplasia highlight the potential clinical implications of this technique in patient management. Active research in this domain should be prioritized to explore its clinical utility further.

Keywords: Prostatic hyperplasia; Prostate cancer; Feulgen reaction.

Introduction

Cancer has evolved into a formidable global health challenge, with the incidence of prostate cancer on the rise in sub-Saharan Africa, particularly in Sudan where it ranks as the third most prevalent cancer after breast, leukemia, and lymphoma. This disease stands as a prominent cause of mortality in the United States. Recognizable alterations in nuclear shape and size offer valuable biomarkers, including nuclear enlargement, irregularity, hyperchromatic, and nucleolar prominence, crucial for distinguishing high-grade prostatic cancer from prostatic hyperplasia [1]. While computer-assisted nuclear morphometry holds significant untapped potential for uncovering biomarkers in prostate cancer research, the Feulgen reaction, introduced by

Robert Feulgen and Heinrich Rossenbeck for DNA identification, emerges as an objective method with high interobserver reproducibility. It proves to be a valuable diagnostic and prognostic tool, offering advantages over overflow cytometry, such as the potential for long-term storage of biological material and the determination of minute DNA amounts. The Feulgen reaction allows the identification of nuclear phenotypes that discriminate between regions corresponding to heterochromatin and euchromatin domains. This staining technique, based on the reaction of Schiff reagents with aldehyde groups in deoxyribose molecules via hydrochloric acid hydrolysis, provides proportional staining intensity indicative of DNA concentration. The highly subjective nature of morphologic grading in prostate cancer underscores the need for an accurate and preferably objective method for malignancy grading. Nuclear DNA content has demonstrated prognostic value in prostate tumors and various other tumor types. The Feulgen DNA reaction, applicable to Hematoxylin and Eosin-destined cytological specimens, enables detailed retrospective studies based on Feulgen-stained archival slides. In this study, our focus is on semi-quantifying nuclear DNA in prostate cancer compared to prostatic hyperplasia using the Feulgen reaction technique [2-5].

Materials and Methods

This study was conducted at the Faculty of Medical Laboratory Sciences, Histopathology Department of Sudan University for Science and Technology, and the National Health Laboratory (NHL) in Khartoum state, Republic of Sudan, spanning from May 2015 to June 2016. Paraffin wax sections were meticulously chosen from patients previously diagnosed with prostatic hyperplasia and prostatic cancer at the National Health Laboratory. Comprehensive clinicopathologic data were retrieved from the archives of the National Health Laboratory, encompassing patients aged between 40 to 90 years. Exclusion criteria involved tiny biopsies and focal nodular prostate cancer. A total of forty-six paraffin blocks were enrolled, comprising thirty-six prostate cancer specimens and ten prostatic hyperplasia specimens. The tissues were meticulously sliced into 4 µm thick sections and mounted on microscope slides [6-8].

Standardized procedures were employed, involving incubation of all sections in an oven at 60ºC for one hour. Subsequently, three changes of xylene for two minutes each were performed to remove the wax, followed by treatment with three changes of absolute ethyl alcohol for two minutes each. The sections were then gradually rehydrated through ethyl alcohol and placed in water. For each case, two sections were prepared—one stained with Hematoxylin and Eosin as per Fischer et al.'s method for diagnosis confirmation, and the other paraffin section utilized for DNA demonstration via the Feulgen reaction.

In the Feulgen reaction, sections underwent sequential steps, commencing with placement in normal hydrochloric acid (N-HCl) at room temperature for one minute, followed by treatment with preheated (NHC_l) at 60°C for ten minutes. Subsequently, sections were rinsed in (NHC_l) at room temperature for one minute. They were then transferred to Schiff's reagent for 45 minutes, followed by rinsing in three changes of bisulfite solution for two minutes each. After rinsing in water, counterstaining was performed in 1% light green SF for one minute. Finally, sections were dehydrated in alcohol, cleared in xylene, and mounted in D.P.X. All sections were stained in the same batch to eliminate inter-batch variation. The slides were evaluated by a pathologist who remained blind to the clinical characteristics and histopathology of the study arms.

Statistical analysis involved data collection using SPSS version 16.0. Qualitative and quantitative variable differences were assessed using the Student t-test and Fisher exact test, respectively. The correlation between prostatic tumor and DNA content status was determined through Pearson correlation analysis. All tests were two-sided, with a p-value <0.05 considered statistically significant [8,9].

Results

The mean age of the study group was 64 years, with a median of 65, ranging from 40 to 90 years. The main histological grades of the 46 patients studied, within the age range of 40 to 90 years, are presented in Figure 1.

The staining intensity of the Feulgen reaction for DNA demonstration in paraffin sections was compared between prostatic adenocarcinoma and benign prostatic hyperplasia. Of the 46 patients with clinical and pathological diagnoses of prostatic tumors, 23 (50.1%) had high-grade prostatic adenocarcinoma, 11 (23.9%) had moderate-grade prostatic adenocarcinoma, two (4.3%) had low-grade prostatic adenocarcinoma, and 10 (21.7%) had benign hyperplasia [10].

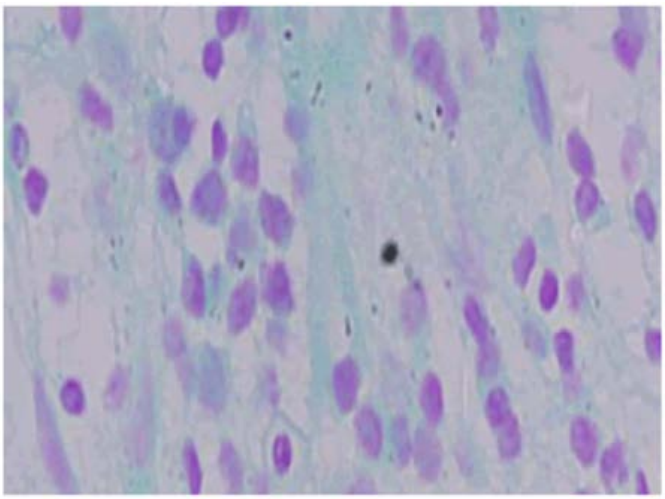

Figure 1: DNA in high-grade prostatic adenocarcinoma using Feulgen reaction technique, X40.

Table 1: Feulgen reaction and prostatic tumor grade.

Prostatic tumor grade	Hyperplasia	Low grade	Moderate grade	High grade	Total
Feulgen reaction for DNA	No (%)	No (%)	No (%)	No (%)	No (%)
Negative	1 (2.2 %)	0 (0.0%)	3 (6.5 %)	3 (6.5 %)	7 (15.2%)
Weakly positive (+)	5 (10.9%)	2 (4.3 %)	1 (2.2 %)	1 (2.2%)	9 (19.6 %)
Moderately positive (++)	3 (6.5 %)	0 (0.0%)	5 (10.9%)	2 (4.3 %)	10 (21.7 %)
Strong positive (+++)	1 (2.2 %)	0 (0.0 %)	2 (4.3 %)	10 (21.7%)	13 (28.3 %)
Strongly positive (++++)	0 (0.0 %)	0 (0.0 %)	0 (0.0)	7 (15.2 %)	7 (15.2 %)
Total	10 (21.7 %)	2 (4.3 %)	11 (23.9 %)	23 (50.1 %)	46 (100 %)
p: Value with chi-square test	p=0.000	p=0.000	p=0.000	p=0.000	

A detailed comparison and the staining results of the Feulgen reaction for DNA in different grades of prostatic adenocarcinoma and benign prostatic hyperplasia among the study group are presented in Table 1.

Most of the benign hyperplasia and low-grade prostatic adenocarcinoma cases showed weak positivity (Figures 2 and 3). The staining intensity of DNA in moderate and high-grade prostatic adenocarcinoma was significantly higher than in benign hyperplasia (all $p<0.000$) [11-15].

Discussion and Conclusion

In this study, the relationship between DNA positivity using the Feulgen reaction and the degree of malignancy was investigated in prostatic hyperplasia and carcinoma. The Feulgen reaction technique allows for convenient retrospective studies, correlating DNA data directly with the known clinical course of tumor diseases, eliminating the need for long-term prospective investigations. The mean age of the study group, 64 years, aligns with previous similar findings [16].

The focus on the semi-quantification of nuclear DNA patterns as a biomarker for the early stage of pre-neoplastic changes in benign prostatic epithelium is associated with precancerous alterations. The study suggests that DNA demonstration with the Feulgen reaction could serve as a marker for this change in biological behavior. The histological grades showed a statistically significant increase in staining intensity ($p<0.005$) as the disease progressed from benign hyperplasia to prostatic cancer, consistent with previous studies.

Accurate preoperative prediction of progression in localized tumors and recurrence is crucial for selecting the right patients for curative therapy, providing appropriate counseling, and selecting patients for adjuvant therapy. The study indicates that DNA positivity can be considered an independent predictor of

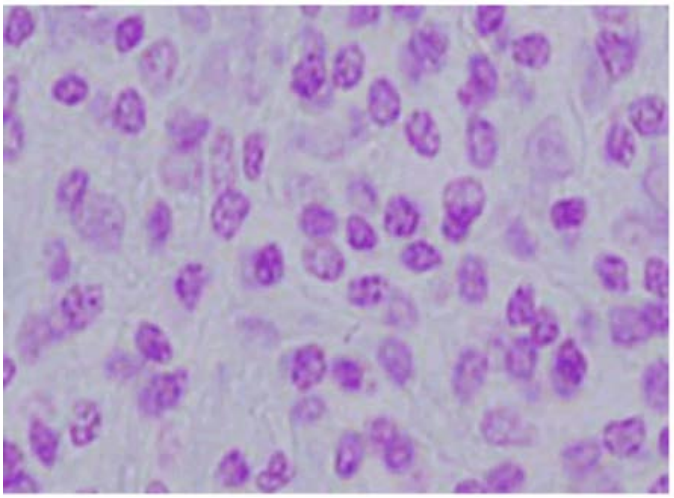

Figure 2: DNA in moderate grade prostatic adenocarcinoma using Feulgen reaction technique, X40.

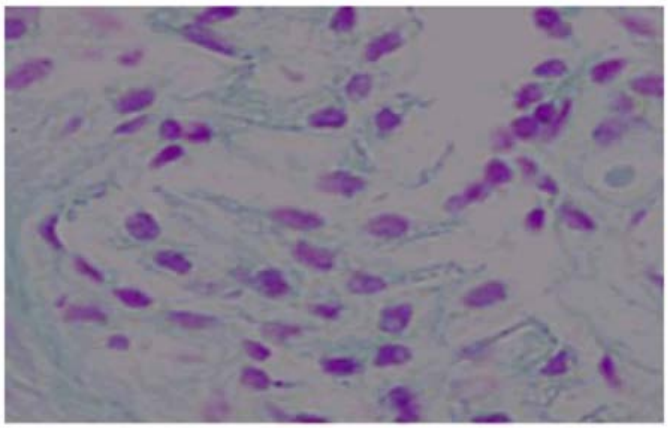

Figure 3: DNA in Benign nodular hyperplasia using the Feulgen reaction technique, X40.

progression and recurrence in prostate cancer. The incorporation of information from the anatomic stage, histologic grade, PSA level, age, and comorbidity into a manageable prognostic score remains uncertain, emphasizing the need for further research in this area.

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