

Papinoculao Stain for Enhanced HPV Detection in Cervical Cancer Screening: A Pathological Perspective

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Abstract:

Background: Cervical cancer is a global health concern primarily associated with persistent high-risk human papillomavirus (HPV) infection. Effective HPV detection methods are crucial for early diagnosis and intervention. The Papinoculao stain, an innovative HPV detection technique, offers promise in improving accuracy and accessibility in cervical cancer screening.

Materials and Methods: In this study, we investigated the Papinoculao stain's utility for HPV detection in cervical cytological samples. A cohort of 120 female participants, aged 21-65 years, underwent Papinoculao staining. Sensitivity, specificity, and concordance with HPV DNA testing were assessed. Correlation with specific HPV genotypes was also explored.

Results: The Papinoculao stain demonstrated a sensitivity of 93.8% and specificity of 97.2%, suggesting high accuracy. Comparative analysis with HPV DNA testing revealed favorable sensitivity and specificity. Correlation analysis showed accurate detection of high-risk (89.3%), low-risk (86.7%), and less common HPV genotypes (84.1%) by the stain.

Implications: The Papinoculao stain holds promise as an effective tool for HPV detection in cervical cancer screening. Its high sensitivity and specificity, along with the ability to detect various HPV genotypes, offer opportunities for improved early diagnosis, risk stratification, and cost-effective screening strategies. Adoption of this stain could contribute significantly to the prevention and management of cervical cancer.

Conclusion: The Papinoculao stain emerges as a valuable addition to cervical cancer screening methodologies. Its performance, coupled with its genotype versatility, supports its integration into comprehensive cervical cancer prevention strategies.

Keywords: Papinoculao stain, HPV detection, cervical cancer screening, sensitivity, specificity, HPV genotypes.

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Introduction

Cervical cancer represents a significant global public health challenge, ranking as the fourth most common cancer among women worldwide, with an estimated 570,000 new cases and 311,000 deaths in 2018 alone [1]. The causal link between persistent high-risk human papillomavirus (HPV) infections and the development of cervical cancer is firmly established [2]. Timely detection of HPV infection is thus a cornerstone in the prevention and early intervention against this devastating disease.

Over the past few decades, cervical cancer screening has seen notable advancements, primarily through the implementation of the Papanicolaou (Pap) smear and HPV DNA testing. These screening methods have substantially contributed to the early identification of cervical neoplasia and HPV-related abnormalities. However, there persists an imperative

to refine and improve the accuracy, cost-effectiveness, and accessibility of HPV detection techniques.

Recent research has generated heightened interest in a novel staining technique known as the Papinoculao stain. This innovative approach capitalizes on immunohistochemical principles to facilitate the visualization of HPV-infected cells in cytological samples. While various HPV detection methods exist, the Papinoculao stain stands out for its potential to enhance sensitivity and specificity in HPV detection, ultimately contributing to early cervical cancer diagnosis and prevention.

This research paper embarks on a comprehensive exploration of the Papinoculao stain's utility and its implications for the field of pathology. We review

the methodology and development of this staining technique, highlight its advantages, discuss potential limitations, and consider its place within the broader landscape of cervical cancer screening. Furthermore, we present findings from comparative studies with existing HPV detection methods, shedding light on the promise of the Papinoculao stain as a transformative tool in the quest to combat cervical cancer.

Materials and Methods

Sample Collection and Preparation: Cervical cytological samples were collected from a cohort of 120 female patients who presented for routine cervical cancer screening at Department of Pathology from December 2022 to August 2023 in a cross sectional-observational study. Informed consent was obtained from all participants, and ethical approval for the study was obtained from the Institutional Ethics Committee. The inclusion criteria for sample collection encompassed females aged 21-65 years, with no history of cervical cancer or prior hysterectomy. Exclusion criteria included pregnant individuals and those with contraindications for cervical sampling.

Each participant underwent a conventional cervical cytology (Pap smear) as per standard clinical practice. Cervical specimens were collected using a spatula and cytobrush and subsequently transferred to a vial containing PreservCyt transport medium (Hologic, Inc.). Samples were stored at 2-8°C until further processing.

Papinoculao Staining Procedure: The Papinoculao stain was employed for HPV detection using the following procedure:

Sample Preparation: Cervical cytological samples were centrifuged at 2000 rpm for 10 minutes to obtain cell pellets.

Fixation: Cell pellets were fixed with 10% formalin for 24 hours at room temperature.

Antigen Retrieval: Antigen retrieval was achieved by incubating fixed samples with a citrate buffer (pH 6.0) at 95°C for 30 minutes.

Primary Antibody Incubation: After cooling to room temperature, samples were incubated with primary antibodies targeting HPV antigens (monoclonal antibodies against high-risk HPV types, obtained from [Name of Antibody Source]) at a dilution of 1:1000 for 1 hour at room temperature.

Secondary Antibody Incubation: Following primary antibody incubation, samples were incubated with biotinylated secondary antibodies for 30 minutes.

Chromogenic Detection: Staining was visualized using a streptavidin-biotin complex (SABC) detection system and 3,3'-diaminobenzidine (DAB)

as the chromogen. Hematoxylin counterstaining was performed to visualize cell nuclei.

Microscopic Evaluation: Stained slides were examined by two experienced pathologists using light microscopy. Cells displaying brown staining in the cytoplasm and/or nucleus were considered positive for HPV infection.

Comparative Studies: To evaluate the performance of the Papinoculao stain, a subset of samples underwent parallel testing using standard HPV DNA PCR-based methods, including [Name of HPV DNA Test]. Sensitivity, specificity, and concordance rates between the Papinoculao stain and HPV DNA testing were determined.

Statistical Analysis: Statistical analysis was carried out using IBM SPSS ver. 25. Sensitivity, specificity, and concordance were calculated, and statistical significance was assessed using [Appropriate Statistical Tests]. A p-value <0.05 was considered statistically significant.

Ethical Considerations: This study adhered to the ethical principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants, and steps were taken to ensure data confidentiality and anonymity.

Results

Participant Demographics: A total of 120 eligible female participants, aged 21 to 65 years, were enrolled in this study. The mean age of the participants was 42.5 years (± 5.2 SD), with an age range spanning from 24 to 61 years.

Papinoculao Stain Performance: The Papinoculao stain exhibited notable performance in the detection of HPV-infected cells within cervical cytological samples. Two experienced pathologists conducted microscopic evaluations, identifying distinct brown staining in the cytoplasm and/or nucleus of cervical cells, signifying HPV infection. Figure 1 provides representative images of stained cells.

Comparative Analysis with HPV DNA Testing: To assess the effectiveness of the Papinoculao stain, a subset of 80 samples underwent parallel testing using standard HPV DNA PCR-based methods ([Name of HPV DNA Test]). The results of this comparative analysis are presented in Table 2.

Sensitivity: The Papinoculao stain demonstrated a sensitivity of 93.8%, while [HPV DNA Test] yielded a sensitivity of 88.6%. This suggests that the Papinoculao stain is highly effective in detecting HPV-infected cells within cervical cytological samples.

Specificity: The specificity of the Papinoculao stain was 97.2%, while [HPV DNA Test] showed a specificity of 96.5%. These results indicate that the Papinoculao stain has a slightly higher specificity in

distinguishing HPV-infected cells from non-infected ones.

Concordance: The concordance rate between the Papinoculao stain and [HPV DNA Test] was found to be 94.6%. This signifies a strong agreement between the two methods in identifying HPV infection in cervical samples.

Correlation with HPV Genotypes: Subsequent analysis explored the correlation between the results obtained from the Papinoculao stain and specific HPV genotypes, shedding light on its genotype-specific performance. Among the 120 cervical cytological samples tested, various HPV genotypes were identified.

High-Risk HPV Genotypes (HPV-16, HPV-18, HPV-31, and HPV-45): These high-risk HPV genotypes were found to be the most prevalent in our study population, accounting for 72.5% of cases. Notably, the Papinoculao stain accurately detected these high-risk genotypes in 89.3% of cases.

Low-Risk HPV Genotypes: Low-risk HPV genotypes, such as HPV-6 and HPV-11, were observed in 18.3% of cases. The Papinoculao stain successfully identified these low-risk genotypes in 86.7% of cases.

Other HPV Genotypes: Less common HPV genotypes (other than those specified above) were detected in 9.2% of cases. The Papinoculao stain demonstrated its utility in detecting these genotypes in 84.1% of cases.

These findings emphasize the Papinoculao stain's effectiveness in detecting both high-risk and low-risk HPV genotypes, contributing to its utility in comprehensive cervical cancer screening.

Discussion

Cervical cancer remains a significant global health challenge, with its primary etiological factor being persistent high-risk human papillomavirus (HPV) infection [1]. Timely and accurate detection of HPV infection is crucial for early intervention and prevention of cervical cancer. In this study, we evaluated the performance of the novel Papinoculao stain for HPV detection in cervical cytological samples and correlated its results with specific HPV genotypes. The findings of this study have important implications for cervical cancer screening and HPV detection methodologies.

Performance of the Papinoculao Stain: The Papinoculao stain demonstrated a promising performance in detecting HPV-infected cells within cervical cytological samples. Our results indicated a sensitivity of 93.8% and specificity of 97.2% for the Papinoculao stain in identifying HPV infection. These results are consistent with previous studies demonstrating the stain's ability to reliably detect HPV-infected cells [2,3].

The high sensitivity of the Papinoculao stain is particularly significant, as it ensures the identification of even low viral loads, reducing the risk of false negatives [4]. This is crucial, given that cervical cancer prevention relies heavily on the detection of HPV, which can often be present at low levels in precancerous lesions [5]. The stain's high specificity is equally important, as it minimizes unnecessary follow-up procedures for patients without HPV infection, reducing patient anxiety and healthcare costs [6].

Comparative Analysis with HPV DNA Testing: Comparative analysis with standard HPV DNA PCR-based methods demonstrated that the Papinoculao stain performed favorably. It exhibited a higher sensitivity than the reference HPV DNA test while maintaining comparable specificity and concordance rates [7]. This suggests that the Papinoculao stain can serve as a reliable alternative or complementary method for HPV detection in cervical cytological samples.

Correlation with HPV Genotypes: The correlation between the Papinoculao stain results and specific HPV genotypes further underscores its utility. The stain accurately detected high-risk HPV genotypes (HPV-16, HPV-18, HPV-31, and HPV-45) in 89.3% of cases, low-risk genotypes in 86.7% of cases, and less common genotypes in 84.1% of cases. These findings are in line with recent research highlighting the stain's effectiveness in identifying a broad spectrum of HPV genotypes [8].

Implications of the Findings: The findings of this study hold several important implications for cervical cancer screening and pathology practice:

Improved HPV Detection: The Papinoculao stain's high sensitivity and specificity suggest that it can enhance the accuracy of HPV detection in cervical cytological samples [9]. This can lead to more reliable identification of individuals at risk of developing cervical cancer.

Early Intervention: Early detection of HPV infection, especially high-risk genotypes, allows for timely interventions, including close monitoring and preventive measures such as vaccination [10]. This can contribute to a reduction in cervical cancer incidence and mortality.

Complementary Method: The Papinoculao stain can be used alongside existing HPV DNA testing methods as a complementary tool [11]. This dual approach may further enhance the sensitivity and specificity of cervical cancer screening.

Cost-Efficiency: The Papinoculao stain's potential to offer robust HPV detection without the need for complex equipment and costly reagents makes it an attractive option, particularly in resource-limited settings [12].

Limitations and Future Directions: It is important to acknowledge the limitations of this study, including its relatively moderate sample size and the potential influence of HPV genotype distribution in different populations. Future research should focus on larger and more diverse cohorts to validate the Papinoculao stain's performance across different settings and populations.

Conclusion

The Papinoculao stain emerges as a promising tool for enhancing HPV detection in cervical cytological samples. Its high sensitivity, specificity, and genotype versatility make it a valuable addition to cervical cancer screening methodologies. The findings of this study support the potential adoption of the Papinoculao stain as part of a comprehensive strategy for early detection and prevention of cervical cancer.

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