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THE DIAGNOSTIC UTILITY OF PAPANICOLAOU (PAP) AND PERIODIC ACID-SCHIFF (PAS) STAINING IN PRIMARY DIAGNOSIS OF ORAL CARCINOMA: A COMPREHENSIVE ANALYSIS

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ABSTRACT Oral squamous cell carcinoma (OSCC) remains a global health burden with poor survival rates when diagnosed late. Exfoliative cytology and histopathology using specialized stains offer non-invasive or minimally invasive diagnostic avenues. This paper evaluates the clinical applications, technical nuances, and diagnostic efficacy of Papanicolaou (PAP) and Periodic Acid-Schiff (PAS) stains in the primary diagnosis of oral carcinoma. Evidence synthesized from contemporary literature indicates that PAP stain excels in detecting cytomorphological alterations and nuclear atypia in exfoliative cytology, while PAS stain reliably identifies glycogen content and keratinization patterns in tissue sections. Used synergistically, these stains enhance diagnostic accuracy, facilitate early detection, and provide prognostic insights. Technical modifications, limitations, and future directions integrating ancillary techniques are also discussed.

KEYWORDS : Papanicolaou Stain, Periodic Acid-schiff Stain, Oral Squamous Cell Carcinoma, Exfoliative Cytology, Cytomorphology, Glycogen, Keratin, Early Diagnosis.

1) INTRODUCTION

Oral carcinoma, predominantly oral squamous cell carcinoma (OSCC), ranks as the sixth most common malignancy globally, with over 377,000 new cases annually (3). Despite advances in treatment, 5-year survival rates remain dismal (□50–60%) due to late-stage diagnosis, underscoring the need for early detection strategies (3),(7). Histopathological examination remains the diagnostic gold standard, but scalpel biopsies are invasive and unsuitable for screening. Exfoliative cytology, augmented by specialized stains, offers a non-invasive alternative for preliminary diagnosis and monitoring of high-risk lesions (1),(3). Among these, Papanicolaou (PAP) and Periodic Acid-Schiff (PAS) stains have emerged as critical tools. PAP stain highlights nuclear and cytoplasmic details in cytological specimens, while PAS detects glycogen and glycoproteins in tissues, aiding in the identification of dysplastic and malignant changes (1),(2),(4). This paper comprehensively reviews their applications, technical protocols, diagnostic efficacy, and integrative roles in OSCC diagnosis.

2) PAP Stain: Principles and Applications in Oral Carcinoma

2.1) Technical Principles

The PAP stain is a polychromatic technique incorporating multiple dyes to differentiate cellular components. Key steps include:

- Nuclear staining with Harris's hematoxylin (blue).
- Cytoplasmic counterstaining with Orange G (keratin) and Eosin Azure (EA-50) (superficial cells: pink; intermediate cells: blue-green) (5),(2).
- Ethanol-based fixation preserves cellular architecture and prevents artefactual changes. Recent modifications for stored oral swabs involve extended fixation (10 minutes in 95% ethanol) and prolonged staining (hematoxylin: 10 min; OG-6: 5 min) to optimize results in archival samples (5).

2.2) Diagnostic Applications

- Cytomorphological Alterations:

PAP stain detects diabetes-associated changes in oral epithelium, including karyomegaly (enlarged nuclei), binucleation, vacuolated cytoplasm, and dyskaryosis (nuclear atypia). These changes reflect metabolic disturbances impacting cellular maturation and are prevalent in high-risk cohorts (1).

- Oral Cancer Screening:

In brush biopsies, PAP-stained smears classify lesions using the Papanicolaou system (Class I–V). Studies report 91.2% sensitivity and 100% specificity for detecting OSCC, with high positive predictive value (PPV: 100%) (3). Nuclear hyperchromasia, irregular membranes, and increased nuclear-cytoplasmic ratios are hallmark features of malignancy (Class IV/V).

- Fungal Detection:

A PAP-Calcofluor White (CFW) modification enhances visualization of Candida hyphae (apple-green fluorescence), prevalent in immunocompromised OSCC patients (4).

Table 1: Diagnostic Efficacy of PAP Stain in Oral Lesions

Application	Key Findings	Study
OSCC detection	Sensitivity: 91.2%; Specificity: 100%; PPV: 100%	3
Diabetic Cytomorphology	Significant karyomegaly, binucleation, and cytoplasmic vacuolation vs. controls	1
Candida infection	60% detection in OSCC smears (PAP-CFW)	4
Long term sample storage	Modified protocol restores nuclear/cytoplasmic detail in frozen swabs	5

3) PAS Stain: Principles and Applications in Oral Carcinoma

3.1) Technical Principles

PAS staining targets glycogen and glycoproteins via:

- Periodic acid oxidation of glycol groups to aldehydes.
- Schiff reagent binding, forming **magenta complexes (1),(2).

Variants Include:

- AB-PAS: Alcian blue highlights acidic mucins before PAS, differentiating glycogen from mucins.
- PAS-diastase: Diastase digestion confirms glycogen (diastase-sensitive) vs. glycoproteins.

3.2) Diagnostic Applications

- Glycogen Content Analysis:

Reduced epithelial glycogen is a marker of dysplasia and OSCC. Diabetic patients show PAS positivity correlating with glycemic control, while OSCC exhibits patchy or absent staining due to altered glucose metabolism (1),(4).

- Keratin Visualization:

PAS intensely stains keratin pearls (insoluble proteins) in well-differentiated OSCC. Comparatively, modified PAP stain (phloxine-B-enhanced) shows superior specificity for keratin, staining pearls magenta while preserving nuclear detail (2).

- Fungal Identification:

In histology, PAS stains Candida hyphae magenta, aiding diagnosis in precancer/cancer tissues (sensitivity: 55.6% in OSCC) (4).

Table 2: Diagnostic Efficacy of PAS Stain in Oral Lesions

Application	Key Findings	Study
Glycogen in OSCC	Patchy loss in malignant epithelium vs. uniform positivity in normal mucosa	1

Keratin detection	Staining intensity: Ayoub-Shklar > Modified PAP > AB-PAS	2
Candida in histology	Sensitivity: 55.6% in OSCC sections	4

4) Comparative Analysis: PAP vs. PAS in Diagnostic Workflows

4.1) Complementary Roles

- PAP for Cytological Triage:

Optimized for brush biopsies, PAP identifies nuclear atypia and infectious agents, guiding the need for scalpel biopsies. Its non-invasive nature suits screening in high-prevalence regions (3).

- PAS for Histological Grading:

Detects keratinization patterns critical for OSCC grading. Well-differentiated tumors (keratin-rich) show better prognosis, while PAS-negative/poorly keratinized tumors indicate aggressiveness (2),(8).

4.2 Diagnostic Accuracy

- PAP-AgNOR Synergy:

Adding silver-stained nucleolar organizer regions (AgNOR) to PAP increases sensitivity to 100% by quantifying proliferation-associated nuclear dots (OSCC: 5.38±0.34 dots/nucleus vs. normal: 2.57±0.32) (3).

- Keratin Detection:

Modified PAP surpasses PAS in specificity for keratin pearls (excellent in 33–40% of OSCCs) due to polychromasia, while Ayoub-Shklar (PAS variant) offers higher intensity (2).

Table 3: PAP vs. PAS: Technical and Diagnostic Comparison

Parameter	PAP Stain	PAS Stain
Primary target	Nuclear chromatin, cytoplasmic keratin	Glycogen, glycoproteins, keratin
Optimal sample	Exfoliative cytology (smears)	Tissue sections
Key strengths	Nuclear detail, screening suitability	Glycogen quantification, keratin intensity
Limitations	Variable glycogen staining; requires expertise	Limited nuclear detail; diastase control needed
Innovations	PAP-CFW for fungi; AgNOR adjunct	AB-PAS for mucins; modified protocols

5) Technical Considerations and Limitations

5.1) Preanalytic Variables

- Fixation Artifacts:

Delayed ethanol fixation in PAP causes cellular shrinkage, while overfixation in PAS obscures glycogen. Modified PAP protocols for frozen samples address this (5).

- Staining Consistency:

PAP requires rigorous differentiation in alcohols to prevent cytoplasmic overstaining. PAS needs diastase controls to confirm glycogen specificity (1),(5) .

5.2) Diagnostic Pitfalls

- False Negatives:

PAP may miss hypoglycogenated dysplasia (requires PAS confirmation). PAS can overlook early nuclear atypia (requires PAP/hematoxylin) (1),(2).

- Keratin Variability:

Not all keratin pearls stain with PAS (20–30% false negatives), implying biochemical heterogeneity in OSCC (2).

6) Future Directions

- Automated Image Analysis:

Integrating PAP-stained cytology with AI algorithms could standardize dysplasia grading. Similarly, PAS glycogen quantitation software may predict metabolic dysregulation (4),(6).

- Molecular Adjuncts:

Coupling PAP/PAS with p16 immunohistochemistry or HPV in situ hybridization would enhance screening for HPV-associated OSCC (7),(8).

- Point-of-Care Staining:

Rapid PAP kits (e.g., RAPID-PAP) enable same-day diagnosis in resource-limited settings (3).

7) CONCLUSION

PAP and PAS stains are indispensable tools for the primary diagnosis of oral carcinoma, each offering unique advantages. PAP stain is the cornerstone of cytological screening, detecting nuclear and cytoplasmic abnormalities with high sensitivity, particularly when combined with AgNOR. PAS stain provides critical insights into glycogen metabolism and keratinization patterns, aiding histological grading and prognostication. Their synergistic use mitigates individual limitations, offering a comprehensive diagnostic approach. Future innovations, including automated analysis and molecular integration, will further solidify their role in early OSCC detection. For optimal outcomes, laboratories should adopt modified protocols for challenging samples and prioritize integrative reporting of cytological and histological findings.

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