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Alternate Special Stains for the Detection of Candidal Organisms in Oral Cyto-Smears among Geriatric Patients

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Abstract

Background: Oral candidiasis is frequently encountered in geriatric patients due to age-related systemic and oral changes. Special stains aid in improving the detection of candidal organisms in oral cytosmears.

Aim: To compare the efficacy of Periodic Acid–Schiff (PAS) and Alcian Blue stains in the detection of candidal organisms in oral cytosmears among geriatric patients.

Materials and Methods: A total of 40 oral cytosmears were obtained from geriatric patients, of which 20 smears were stained with PAS and 20 with Alcian Blue. The smears were evaluated microscopically for the presence and staining intensity of candidal organisms, and the findings were statistically analyzed.

Results: Both PAS and Alcian Blue stains successfully demonstrated candidal organisms. PAS stain showed superior staining intensity and clearer visualization of fungal hyphae and spores compared to Alcian Blue.

Conclusion: PAS and Alcian Blue are effective special stains for detecting candidal organisms in oral cytosmears; however, PAS demonstrated higher diagnostic efficacy and better staining characteristics.

Keywords: Oral Candidiasis, PAS stain, Alcian Blue

1. Introduction

The geriatric population is steadily increasing worldwide as a result of improved healthcare and life expectancy. Aging is associated with progressive physiological, immunological, and functional changes that predispose elderly individuals to a wide range of systemic and oral diseases[1]. Oral health in geriatric patients is often compromised due to factors such as reduced salivary flow, nutritional deficiencies, systemic illnesses, polypharmacy, and diminished manual dexterity, all of which contribute to an increased susceptibility to opportunistic infections[1].

Among oral opportunistic infections, candidal infections represent one of the most common conditions affecting elderly individuals. Candida species, particularly Candida albicans, are normal commensals of the oral cavity; however, under favorable conditions such as immunosenescence, altered oral microflora, xerostomia, denture use, and poor oral hygiene, these organisms can transform from a benign colonizing

state to a pathogenic form[1]. This transition results in clinical manifestations ranging from pseudomembranous and erythematous candidiasis to angular cheilitis and denture-associated stomatitis, which significantly impair oral function and quality of life in geriatric patients[1].

The diagnosis of oral candidiasis in the elderly can be challenging, as clinical presentations may be atypical or masked by underlying mucosal changes related to aging. Laboratory confirmation plays a crucial role in accurate diagnosis and timely intervention[2].

Oral exfoliative cytology is a simple, non-invasive, and cost-effective diagnostic technique that allows for the detection of fungal organisms at the cellular level,particularly in geriatric patients[6].The diagnostic yield of cytosmears can be significantly enhanced by the use of special stains that selectively demonstrate fungal cell wall components. Among these, Periodic Acid–Schiff (PAS) and Alcian Blue stains are widely employed for the identification of candidial organisms[3].This current study aimed to detect and evaluate the staining efficacy of PAS and Alcian blue of candidal organisms among geriatric patients.

2. Materials and Methods

2.1. Sample collection

This study was conducted by the Department of Oral and Maxillofacial Pathology,Adhiprasakthi dental college and hospital,Chengalpattu.The study sample comprised 20 geriatric outpatients who reported to the Department of Oral Medicine and Radiology (OMR) . After obtaining informed consent, oral cytosmears were collected from the mucosal sites using a sterile wooden spatula/cytobrush. From each patient, two cytosmears were prepared on clean, labeled glass slides to ensure adequate sample representation. The smears were immediately fixed in 95% ethyl alcohol to preserve cellular morphology and prevent air-drying artifacts. All collected samples were subsequently subjected to routine and special staining procedures for cytological evaluation.

2.2 PAS staining

The smear was oxidized in 0.5% periodic acid solution for 5 mins.The slide was then rinsed using distilled water and placed in Schiff reagent for 15mins. The smear was washed with lukewarm tap water for 5 mins and counter-stained with Mayer's hematoxylin solution for 1 min , following this the smear was washed under tap water for 5 mins and left to dry.

2.3 Alcian blue staining

The slide was stained with Alcian blue stain for 15 mins and washed under running water for 15 mins. The slide was then rinsed with distilled water and counter stained with eosin stain for 1 min. Absolute alcohol was then used to dehydrate the smear.

3. Results

The present study evaluated and compared the staining efficacy of Periodic Acid–Schiff (PAS) and Alcian Blue stains in the detection of fungal organisms in oral cytosmears obtained from geriatric patients.The intensity of fungal staining was evaluated using a semi-quantitative scoring system, where a score of 0 indicated absence of staining, 1 indicated mild staining, 2 indicated moderate staining, and 3 indicated intense staining.

The results were analyzed to determine the distribution of staining intensity scores for each stain, thereby enabling a comparative assessment of their effectiveness in demonstrating fungal organisms in oral cytological smears. This comparative analysis highlights the relative diagnostic utility of PAS and Alcian Blue stains in the detection of mycotic organisms among the geriatric population.

Figure 1: Intensely Stained Fungal Organism demonstrated by PAS(Score-3)

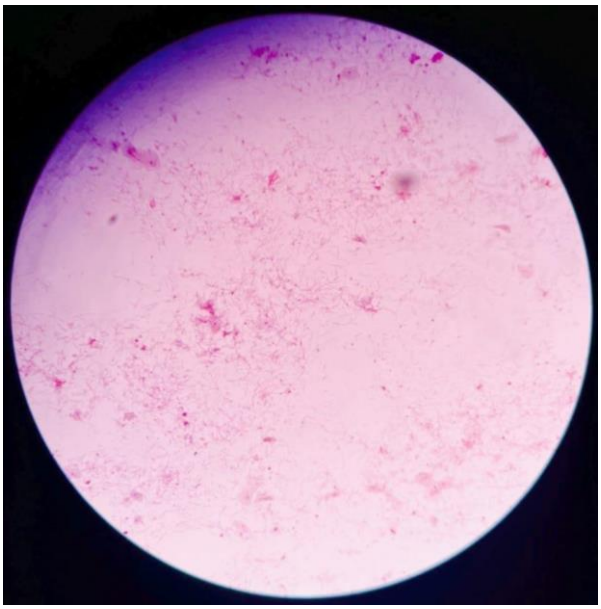

Figure 2: Moderately Stained Fungal Organism demonstrated by Alcian blue(Score-2)

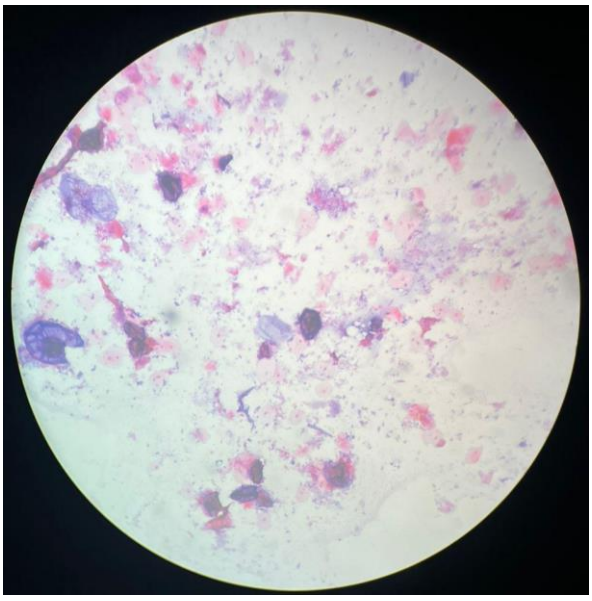

Table 1: Comparison of Mean Staining Intensity Scores and Percentage Distribution of PAS and Alcian Blue for Fungal Organisms (n = 20)

Stain	Staining Intensity Score	Number of Samples (n)	Percentage (%)	Mean Staining Intensity (Mean ± SD)	Statistical Comparison
PAS	0 – Not stained	1	5%		
	1 – Mildly stained	3	15%	2.20 ± 0.89	
	2-Moderately stained	7	35%		

	3 – Intensely stained	9	45%		p = 0.167*
Alcian Blue	0 – Not stained	3	15%		
	1 – Mildly stained	6	30%	1.55 ± 0.94	
	2-Moderately stained	8	40%		
	3 – Intensely stained	3	15%		

- Comparison of mean staining intensity scores between PAS and Alcian Blue was performed using the Chi-square test,$p < 0.05$ considered statistically significant.
- PAS demonstrated a significantly higher mean staining intensity compared to Alcian Blue ($p < 0.05$).
- Among the 20 oral cytosmear samples evaluated, PAS staining demonstrated a higher proportion of intensely stained fungal organisms (45%) compared to Alcian Blue (15%), with a significantly greater mean staining intensity score (2.20 ± 0.89 vs1.55 ± 0.94; p =0.167).

4. Discussion

Oral candidiasis is a prevalent opportunistic infection among geriatric patients, primarily due to immunosenescence, systemic illnesses, xerostomia, denture use, and compromised oral hygiene[1]. Early and accurate diagnosis is essential to prevent disease progression and improve oral health–related quality of life in this vulnerable population[1]. Oral exfoliative cytology provides a simple and minimally invasive diagnostic approach; however, its effectiveness largely depends on the staining technique employed for fungal visualization[5].

In the present study, both Periodic Acid–Schiff (PAS) and Alcian Blue stains successfully demonstrated the presence of candidial organisms in oral cytosmears obtained from geriatric patients. The detection of yeast cells and hyphal forms using both special stains confirms their diagnostic utility as adjuncts to routine cytological evaluation. These findings emphasize the role of special stains in enhancing fungal detection, particularly in cases where routine stains may fail to clearly identify candidial elements[6].Comparatively, PAS stains exhibited higher efficacy than Alcian Blue in the detection of candidial organisms[3]. PAS consistently produced intense magenta staining of fungal cell walls[13], offering superior contrast against the epithelial background. This enhanced staining quality facilitated clearer visualization of candidial morphology, including budding yeast forms and pseudohyphae, thereby improving diagnostic confidence[3]. Figure 1 illustrates an intensely stained fungal organism using Periodic acid–Schiff stain (Score 3), highlighting the strong affinity for polysaccharides such as chitin and glucan, which are abundantly present in the candidial cell wall.

In contrast, Figure 2 shows a moderately stained fungal organism with Alcian blue stain (Score 2).Although Alcian Blue was effective in detecting candidial organisms, the staining intensity and contrast were comparatively lower than PAS. Alcian Blue primarily binds to acidic mucopolysaccharides and glycoproteins, which may account for the relatively subtle staining of fungal elements in some smears[8]. Nevertheless, Alcian Blue proved useful as a supportive stain by aiding in the differentiation of fungal structures from background cellular debris and mucin, thereby contributing to overall diagnostic accuracy. The findings of the present study are in agreement with previous cytological and histopathological studies that have reported superior sensitivity of PAS stain in identifying fungal organisms when compared to

other special stains. The combined use of PAS and Alcian Blue enhances the likelihood of detecting candidal infections, especially in geriatric patients where fungal load may be minimal or clinical presentation atypical[7].

Despite the encouraging results, the present study is limited by a relatively small sample size and the absence of fungal culture or molecular confirmation. Future studies incorporating larger sample populations and correlating cytosmear findings with culture or advanced diagnostic techniques may further validate the comparative efficacy of these stains.

5. Conclusions

Both PAS and Alcian Blue stains were effective in detecting candidal organisms in oral cytosmears of geriatric patients. However, PAS stain demonstrated higher efficacy with superior clarity and sensitivity in identifying fungal elements compared to Alcian Blue. Owing to its ability to distinctly highlight fungal cell walls, PAS can be considered the stain of choice for routine screening and diagnosis of oral candidiasis in the geriatric population, with Alcian Blue serving as a useful adjunct[12].

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