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COMPARATIVE ANALYSIS OF LEISHMAN GIEMSA COCKTAIL AND PAPANICOLAOU STAIN IN DIAGNOSING POTENTIALLY MALIGNANT AND MALIGNANT ORAL LESIONS

Pathology

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ABSTRACT

Background: Oral squamous cell carcinoma (SCC) is a common malignancy with high morbidity and mortality rates. Early detection of potentially malignant disorders (PMDs), such as oral epithelial dysplasia (OED), is crucial to improving prognosis. Exfoliative cytology is a non-invasive and cost-effective diagnostic tool traditionally utilizing the Papanicolaou (Pap) stain. However, newer techniques, such as the Leishman-Giemsa (LG) cocktail stain, offer promising alternatives with quicker staining times and enhanced cellular detail. **Aim:** This study aimed to compare the diagnostic efficacy of the Leishman-Giemsa cocktail with the conventional Papanicolaou stain in identifying potentially malignant and malignant oral lesions. **Methods:** A cross-sectional study was conducted from August 1, 2022, to January 31, 2024, at Gandhi Medical College, Bhopal, including 100 patients with potentially malignant or malignant oral lesions. Oral scrapings were taken and stained with either Pap stain or Leishman-Giemsa cocktail. Stains were evaluated based on cytoplasmic and nuclear details using a scoring system. Descriptive statistics, paired t-tests, and chi-square tests were employed to compare results, with significance set at $p < 0.05$. **Results:** Of the 100 patients, 82% were male, and 73% had leukoplakia, the most common lesion. Tobacco use was prevalent, with 90% of patients showing tobacco-stained teeth. Buccal mucosa was the most commonly affected site (60%). The Leishman-Giemsa cocktail demonstrated faster processing times and diagnostic accuracy comparable to the Pap stain. **Conclusion:** The Leishman-Giemsa cocktail is a viable alternative to the Papanicolaou stain for diagnosing potentially malignant and malignant oral lesions. It offers a practical, cost-effective, and efficient option for cytological screening, particularly in resource-limited settings. Further studies are warranted to validate these findings in larger populations.

KEYWORDS

Leishman Giemsa Cocktail, Papanicolaou Stain, Oral Cancer, Squamous cell carcinoma, Malignant lesion

INTRODUCTION

Oral squamous cell carcinoma (SCC) is the most common malignancy of the oral cavity, known for its aggressive behavior and significant morbidity and mortality rates.[1] The early detection of potentially malignant disorders (PMDs) like oral epithelial dysplasia (OED) is crucial, as timely intervention can improve prognosis and reduce the progression to SCC.[2] OED, characterized by distinct histological changes, serves as a precursor to malignancy, underscoring the importance of precise diagnostic tools in its early identification.[3] The economic burden of managing oral cancer is particularly severe in emerging nations, including India and other South-East Asian countries, where the incidence is rising.[4] This also shows the increasing importance of effective screening and diagnostic approaches.[5]

Exfoliative cytology has proven to be a practical, cost-effective, and non-invasive tool for detecting and evaluating potentially malignant and malignant oral lesions.[5] Among the traditional methods, the Papanicolaou (Pap) stain is widely utilized due to its ability to selectively stain cells from different layers, providing a detailed view of cellular morphology. However, the Pap stain procedure is labor-intensive and requires multiple sequential steps, making it costly and time-consuming.[6] These limitations have driven the search for alternative staining methods that can provide similar or better diagnostic accuracy with greater efficiency.

The Leishman-Giemsa cocktail, a novel cytological staining method, has emerged as a promising alternative. This technique combines the Leishman and Giemsa stains, leveraging the advantages of both to enhance cellular contrast and clarity. The Leishman-Giemsa cocktail allows for the precise visualization of cellular structures, including nuclei and cytoplasmic components, providing clinicians with valuable insights into the cellular composition of oral lesions. Its versatility extends to various sample types, including exfoliated oral epithelial cells and tissue biopsies, making it suitable for diverse clinical settings.[7]

One of the key benefits of the Leishman-Giemsa cocktail is its reduced staining time, which simplifies the diagnostic process and shortens turnaround times without compromising accuracy. Studies have indicated that this method offers superior sensitivity and specificity in detecting cancerous changes compared to traditional techniques. This increased diagnostic confidence is particularly valuable in settings with limited resources, where access to rapid and reliable diagnostic methods can significantly impact patient outcomes.

Given the rising incidence of oral cancer and the critical need for early detection, the Leishman-Giemsa cocktail represents a significant advancement in oral cytology and a feasible alternative for cytological screening. This study aims to compare the diagnostic efficacy of the Leishman-Giemsa cocktail with the conventional Papanicolaou stain in identifying potentially malignant and malignant oral lesions.[9,10] By evaluating the sensitivity, specificity, and overall utility of both staining techniques, this research seeks to determine whether the Leishman-Giemsa cocktail can serve as a viable alternative or complement to the Pap stain in oral cancer screening and diagnosis thereby contributing to improved management and outcomes for patients with oral lesions.

METHODOLOGY

Study Design and Setting

This cross-sectional study was conducted in the Department of Pathology, Gandhi Medical College, and associated hospitals (Hamidia Hospital), Bhopal, from August 1, 2022, to January 31, 2024.

Sample Size

The study included 100 patients with potentially malignant or malignant oral lesions.

Inclusion and Exclusion Criteria

Inclusion criteria comprised all patients with potentially malignant or malignant oral lesions. Patients who did not consent were excluded.

Ethical Approval

The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants.

Sample Collection and Staining

Oral scrapings were collected from lesions using wooden spatulas or brushes under aseptic conditions. Smears were prepared on clean glass slides. One smear was fixed in 95% ethanol and stained with Papanicolaou stain, while the other was air-dried and stained using the Leishman-Giemsa (LG) cocktail.

- Papanicolaou Stain: Standard staining procedure involving hydration, staining with Harris Hematoxylin, Orange G-6, and EA-50, followed by dehydration and clearing in xylene.
- Leishman-Giemsa Cocktail: Prepared by mixing Leishman stain and Giemsa working solution (1:1). Air-dried smears were stained with the LG cocktail, followed by a buffer rinse (pH 6.8), washing, and mounting.

Evaluation

Stained slides were evaluated for cytoplasmic and nuclear details using criteria adapted from Sujathan et al., with a scoring system ranging from 0 (poor) to 3+ (excellent). A single examiner assessed 50 well-stained cells per slide.

Data Collection and Statistical Analysis

Patient data, including demographics and clinical history, were recorded. Descriptive statistics, such as frequencies and means, were used to summarize categorical and continuous variables. Paired t-tests compared cytoplasmic, nuclear, and total scores between the stains, with Chi-square tests applied for categorical associations. Statistical significance was set at $p < 0.05$.

RESULTS

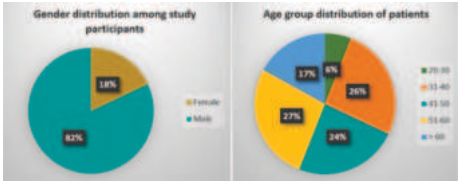


Figure 1: Age and Gender Distribution of Study Participants

The study was conducted among 100 patients. The majority of patients were male (82%), with females comprising 18% of the sample (Fig 1). Patients' ages ranged from 20 to over 60 years. The 51-60 age group had the highest frequency (27%), The predominance of older age groups reflects the cumulative effect of risk factors for oral lesions with advancing age.

Table 1: Risk Factors for Oral Lesions Among Patients

Medical illness		
DM	5	5.0
HTN	5	5.0
DM/HTN	1	1.0
NO	89	89.0
Tobacco packs/day		
1 per day	16	16.0
2-3 per day	51	51.0
≥4 per day	33	33.0
Tobacco chewing (years)		
<10 years	16	16.0
11-20 years	42	42.0
21-30 years	37	37.0
>30 years	5	5.0
No. of cigarette smoked per day		
0-1	32	32.0
2-3	60	60.0
≥4	8	8.0
Alcohol consumption		
No	48	48.0
Yes	52	52.0
Oral hygiene		
Normal	30	30.0
Poor	70	70.0

Table 1 provides an overview of lifestyle factors, medical history, and oral hygiene among patients with oral lesions. A majority (89%) had no medical illness, while diabetes mellitus (DM) and hypertension (HTN) each affected 5%. Tobacco consumption varied, with 51% of patients using 2-3 packs per day, and 42% reporting 11-20 years of tobacco chewing. Cigarette use was common, with 60% smoking 2-3 cigarettes daily. Alcohol consumption was reported by 52% of patients. Oral hygiene was generally poor, with 70% having suboptimal oral care practices.

Table 2 : Clinical Presentation of Patients

Clinical presentation of patients		
Leukoplakia	73	73.0
Erythroplakia	11	11.0
Tobacco Stained teeth	90	90.0
SMF	14	14.0
Lesions		
Leukoplakia	30	30
Ulcer	51	51
Ulcer proliferative	19	19
Mean duration of symptoms	2.89±0.56 months	
Site involved		
Base Of Tongue	1	1.0
Buccal Mucosa	60	60.0
Floor Of Mouth	1	1.0
Gingivobuccal Sulcus	4	4.0
Hard Palate	2	2.0
Lateral Border of Tongue	23	23.0
Lower Lip Mucosa	4	4.0
Soft Palate	3	3.0
Tip Of Tongue	1	1.0
Tonsillar Fossa	1	1.0
HPE findings		
WDSCC	41	41.0
MDSCC	21	21.0
PDSCC	3	3.0
NA	35	35.0

Table 2 summarizes the clinical presentation of patients with oral lesions. The most common clinical fpresentations were tobacco-stained teeth (90%), followed by leukoplakia (73%) and submucous fibrosis (SMF) (14%). Lesion types included ulcers (51%), leukoplakia (30%), and ulceroproliferative lesions (19%), with an average symptom duration of 2.89 months. The buccal mucosa was the most frequently affected site (60%), followed by the lateral border of the tongue (23%). Histopathological examination (HPE) revealed well-differentiated squamous cell carcinoma (WDSCC) in 41% of cases, moderately differentiated squamous cell carcinoma (MDSCC) in 21%, and poorly differentiated squamous cell carcinoma (PDSCC) in 3%, with 35% showing no abnormalities (NA)

Table 3: Distribution of Oral Lesions Across Various Patient Category

Characteristics		Lesion			Total	P value
		Leuko-plakia	Ulcer	Ulceropro-liferative		
Age of patient	20-30	3 (50%)	2 (33.3%)	1 (16.6%)	6 (100%)	0.968
	31-40	8 (30.7%)	14 (53.8%)	4 (15.4%)	26 (100%)	
	41-50	8 (33.3%)	11 (45.8%)	5 (20.8%)	24 (100%)	
	51-60	7 (25.9%)	15 (55.5%)	5 (18.5%)	27 (100%)	
	> 60	4 (23.5%)	9 (52.9%)	4 (57.1%)	17 (100%)	
Tobacco chewing years	<10 years	6 (37.5%)	8 (50%)	2 (12.5%)	16 (100%)	0.674
	11- 20 years	9 (21.4%)	23 (54.7%)	10 (23.8%)	42 (100%)	
	21-30 years	13 (35.1%)	17 (45.9%)	7 (18.9%)	37 (100%)	
	>30 years	2 (40%)	3 (60%)	0	5 (100%)	
Alcohol	No	17 (35.4%)	27 (56.2%)	4 (8.3%)	48 (100%)	0.031
	Yes	13 (25%)	24 (46.1%)	15 (28.8%)	52 (100%)	
Oral hygeine	Normal	24 (80%)	1 (3.3%)	5 (16.6%)	30 (100%)	<0.001
	Poor	6 (8.5%)	50 (71.4%)	14 (20%)	70 (100%)	

Table 3 presents the distribution of oral lesions (Leukoplakia, Ulcer, and Ulceroproliferative) across different patient demographics and lifestyle factors. Age distribution shows that patients aged 31-40 years had the highest prevalence of oral lesions, with ulcer cases being most common (53.8%) in this group. Tobacco chewing of 11-20 years was

associated with a higher prevalence of ulcers (54.7%). Alcohol consumption significantly influenced lesion type distribution, with higher rates of ulceroproliferative lesions (28.8%) among alcohol users (p=0.031). Oral hygiene had a strong association with lesion type; poor oral hygiene was associated with the majority of ulcers (71.4%) and a significant difference in lesion distribution (p<0.001).

Table 4: Comparing Cytoplasmic, Nuclear and Total Score Between Both Staining Methods

Staining method	Cytoplasmic score (Mean ± SD)	Nuclear score (Mean ± SD)	Total score (Mean ± SD)
Pap stain	2.06 ± 0.52	1.95 ± 0.57	4.01 ± 0.96
LG Cocktail stain	2.20 ± 0.58	2.26 ± 0.63	4.46 ± 0.98
p value	0.03	<0.001	<0.001

Table 4 shows the mean cytoplasmic score was higher for the LG Cocktail Stain (2.20) compared to the Pap Stain (2.06), with a statistically significant P value of 0.030. This suggests that the LG Cocktail Stain provided a slightly better cytoplasmic definition than the Pap Stain, potentially improving diagnostic accuracy.

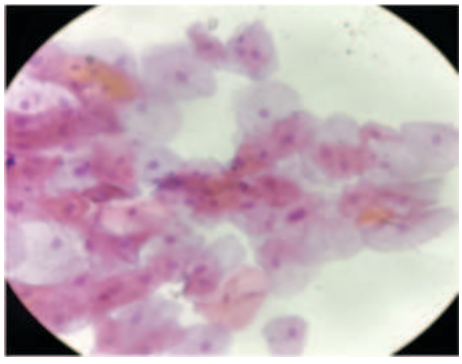
The mean nuclear score was higher for the LG Cocktail Stain (2.26) compared to the Pap Stain (1.95), with a significant P value of <0.001, which suggests that the LG Cocktail Stain offered better nuclear detail, which is crucial for accurate cytological diagnosis. The LG Cocktail Stain had a higher mean total score (4.46) compared to the Pap Stain (4.01), with a highly significant P value of <0.001, which indicates that the LG Cocktail Stain provided better overall staining quality than the Pap Stain.

Table 5: Comparison of Diagnostic Accuracy of Pap Stain and LG Cocktail Stain

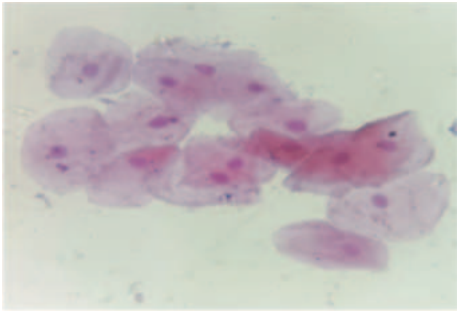
Stain	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Pap Stain	92.45	86.54	96.78	74.56
LG Cocktail Stain	96.82	82.65	97.56	77.65

The LG Cocktail Stain demonstrated higher sensitivity (96.82%) and PPV (97.56%) compared to the Pap Stain (92.45% sensitivity and 96.78% PPV).(Table 5) However, the Pap Stain had slightly higher specificity (86.54%) compared to the LG Cocktail Stain (82.65%). The higher diagnostic accuracy of the LG Cocktail Stain suggests it may be a more reliable method for detecting potentially malignant and malignant oral lesions.

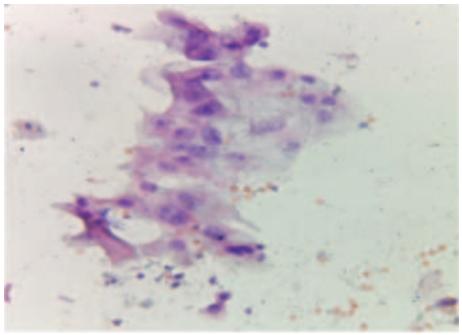
Microscopic Pictures



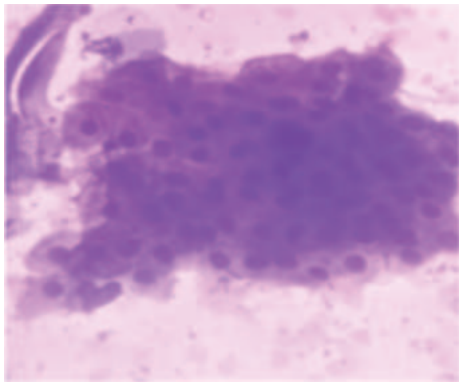
Exfoliated Buccal Mucosal Cells Stained With Papanicolaou Stain (40x)



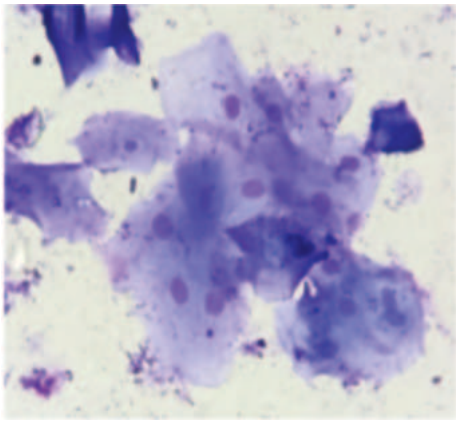
Exfoliated Buccal Mucosa Cells Showing Cytoplasmic And Nuclear Staining By Papanicolaou Stain (40x)



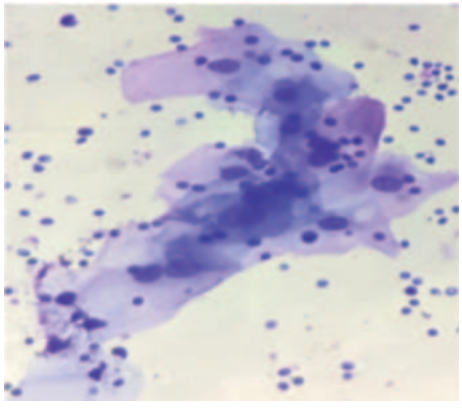
Photomicrograph From A Patient Of SCC Of Buccal Mucosa Showing Exfoliated Buccal Mucosa Cells Stained With Papanicolaou Stain Showing High Nucleo-cytoplasmic Ratio, Coarsely Granular Chromatin And Prominent Nucleoli (40x)



Photomicrograph From A Patient Of Squamous Cell Carcinoma Of Buccal Mucosa Showing Exfoliated Cells Stained With Leishman Geimsa Cocktail Stain Showing High Nucleo-cytoplasmic Ratio And Coarsely Granular Chromatin (40x).



Exfoliated Buccal Mucosa Cells Stained With Leishman Geimsa Cocktail Stain (40x)



Exfoliated Cells From Buccal Mucosa Showing Cytoplasmic And Nuclear Staining By Leishman Geimsa Cocktail Stain (40x)

DISCUSSION

The findings of this cross-sectional study involving 100 patients with potentially malignant and malignant oral lesions are consistent with existing literature, provide insight on critical epidemiological trends and staining techniques for diagnosing oral lesions. The study revealed a significant male predominance, with 82% of cases being male, in line with previous studies by Metgud et al.[11] and Doddagowda et al.[12]. This can be attributed to higher rates of tobacco and alcohol use among men, key risk factors for oral lesions. The age distribution showed that individuals aged 51-60 years had the highest occurrence of oral lesions (27%), closely followed by the 31-40 (26%) and 41-50 (24%) age groups. These findings mirror those of Rashmi Metgud et al.[11] and Garbyal et al.[13] who reported a similar age profile for oral lesions. Belgaumi and Shetty[7] and Mitra et al.[14] conducted studies that provide additional evidence supporting these findings.

Only a small proportion of patients (11%) had concurrent medical illnesses, such as diabetes mellitus (5%) and hypertension (5%), suggesting that systemic diseases were not major contributors to the development of oral lesions. This observation aligns with studies by Johnson et al.[15] and Gupta et al.[16] (2019), which highlighted the stronger association between oral lesions and lifestyle habits like tobacco use, rather than underlying systemic illnesses. In their screening investigation, Bose et al.[17] found that local etiological factors such as tobacco chewing and smoking were more significant than systemic disorders. Sidhu et al.[18] support this finding by demonstrating that patients with extensive tobacco and alcohol use, regardless of their systemic health state, had a higher prevalence of possibly malignant oral lesions. In addition, research conducted by Belgaumi and Shetty[7] and Metgud et al.[11] has specifically examined the diagnostic effectiveness of stains in identifying oral lesions, rather than investigating the influence of systemic disorders.

Clinically, leukoplakia was the most common finding (73%), followed by erythroplakia (11%), with tobacco-stained teeth observed in 90% of patients, indicating the strong influence of tobacco use on oral health. These findings correspond with studies by Warke and Khot[19], who reported similar lesion patterns among tobacco users. The most commonly affected site was the buccal mucosa (60%), followed by the lateral border of the tongue (23%), which is consistent with other studies, including those by Belgaumi and Shetty[7] and Metgud et al[11]. The frequent involvement of the buccal mucosa and tongue highlights the direct impact of carcinogens on specific anatomical sites exposed to tobacco use. Histopathologically, well-differentiated squamous cell carcinoma (WDSCC) was the most frequent diagnosis (41%), followed by moderately differentiated SCC (21%) and poorly differentiated SCC (3%). This distribution is similar to findings in studies by Belgaumi et al.[7] and Gupta et al[16], which also reported a higher frequency of WDSCC.

The study highlights the superior performance of the Leishman Giemsa (LG) Cocktail Stain compared to the Papanicolaou (Pap) Stain. The LG Cocktail demonstrated a higher mean cytoplasmic score (2.20) and nuclear score (2.26), compared to 2.06 and 1.95, respectively, for the Pap stain, suggesting better cytoplasmic and nuclear clarity. These findings are consistent with previous studies by Doddagowda et al.[20] and Gupta et al[16], which also reported enhanced diagnostic precision with LG Cocktail staining. The LG Cocktail stain's ability to provide higher cytoplasmic and nuclear detail makes it an excellent alternative to traditional stains, offering the advantage of rapid and accurate diagnosis in potentially malignant and malignant lesions.

The study found that the LG Cocktail Stain had a higher mean cytoplasmic score compared to other stains. This result, supported by previous studies by Doddagowda et al.[20], Belgaumi and Shetty (2013)[7], and Metgud et al.[11], highlights the stain's effectiveness in improving cytoplasmic detail and increasing diagnostic accuracy. The present study revealed that the average nuclear score was greater for the LG Cocktail Stain (2.26) in comparison to the Pap Stain (1.95), indicating improved nuclear clarity and enhanced precision in cytological diagnosis. This finding is consistent with the research conducted by Gupta et al.[16] which showed that the LG Cocktail Stain had better nuclear staining properties in comparison to other stains. Warke and Khot[21] conducted a study to examine the effectiveness of different staining techniques, such as the LG Cocktail, Giemsa, and Pap Stains, in the diagnosis of potentially cancerous oral lesions. According to their research, the LG Cocktail Stain showed better nuclear staining compared to the Giemsa and Pap Stains, making it the preferred option for cytological tests.

The higher total score and sensitivity of the LG Cocktail Stain (96.82%) and positive predictive value (97.56%) demonstrate its potential as a reliable diagnostic tool for detecting malignant oral lesions. Similar observations were made by Iqbal et al[22]and Bose et al, who emphasized its value in clinical practice, particularly in settings with limited resources. The LG Cocktail Stain's superior staining quality, ease of preparation, and cost-efficiency make it a practical option for mass screening programs and also highlights its important role in enhancing early diagnosis and improving patient outcomes in clinical practice.

Limitation

As this is a single-center study, the results may be influenced by regional factors and may not fully represent diverse patient demographics or oral lesion variations seen in other regions. The assessment relied on the expertise of a single examiner for slide evaluation, introducing potential observer bias. While the study highlights promising findings regarding the Leishman-Giemsa cocktail stain, the cross-sectional design restricts the ability to determine long-term diagnostic outcomes. Future multicentric studies with larger sample sizes and multiple examiners are recommended to validate these findings and assess the broader applicability of the Leishman-Giemsa cocktail in diverse clinical settings.

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