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LEISHMAN GIEMSA COCKTAIL, A CYTOLOGICAL STAINING COMPARABLE TO PAPANICOLAOU STAIN FOR ORAL CANCER DIAGNOSIS – A PROSPECTIVE OBSERVATIONAL STUDY.

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Abstract

Background:
Oral squamous cell carcinoma (OSCC) is the most common tumor in the head and neck region. Exfoliative cytology in the form of scrape cytology is a valuable aid for screening oral lesions.

Aim:
The study aims to evaluate and compare Pap, MGG, and LG cocktail staining in exfoliated cells of oral malignancy.

Material & methods:
This prospective study was conducted in the department of Pathology at Hitech Medical College, Rourkela, over a period of two years. According to conventional staining protocols, one air-dried smear was stained with MGG, and the other with LG cocktail, and the third with Papanicolaou stain. The stained slides were examined independently by two pathologists, and the results were recorded. Biopsy results were also compared with the cyst diagnosis. Statistical analysis was done.

Result:
The study evaluated the diagnostic efficacy of Pap, MGG, and LG cocktail stains in exfoliated cells of oral malignancy, specifically squamous cell carcinoma (SCC). Among the 103 suspected cases, 92 were diagnosed as SCC, with a 100% correlation between cyst diagnosis and histological results for SCC cases. The LG cocktail stain demonstrated the highest specificity at 95%, followed by Pap (93.7%) and MGG (93.2%). Sensitivity was similar across all stains, with LG cocktail slightly outperforming Pap and MGG. MGG showed the highest negative predictive value at 84.7%, indicating its superior ability to rule out malignancy.

Conclusion:
LG Cocktail staining is a simple, cost-effective, less time-consuming, one-step process and can be used for an oral cancer screening program. Most importantly, the LG cocktail staining technique was found to give results comparable to the Pap stain.

Recommendation:
It is recommended to use the LG cocktail stain for improved sensitivity and comparable specificity in diagnosing oral squamous cell carcinoma from exfoliated cells.

Keywords: *Leishman stain, MGG stain, LG cocktail stain, Papanicolaou stain, efficacy.*
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Introduction:

Oral squamous cell carcinoma (OSCC) is a frequent head and neck cancer that arises in the oral mucosa. 1 The Global Cancer Observatory (GCO) reported that 377,713 instances of OSCC were reported globally in 2020, with Asia accounting for the majority of these occurrences. 2 Middle-aged to elderly men are the most vulnerable, and OSCC affects more men than women.3. There is conclusive evidence that tobacco use is associated with oral cancer. Other risk factors are alcohol use, herpes virus infection, candidiasis, syphilis, nutritional deficiency, poor dental hygiene, immune deficiency, and genetic factors. 4

Exfoliative cytology is a valuable aid for screening malignant and benign oral lesions. It is an easy, economical, noninvasive, and feasible method for the detection of malignancies. PAP (Papanicolaou Stain), a polychromatic stain developed by George N. Papanicolaou, the father of cytopathology, in 1942 and subsequently modified by him in 1954 & 1960, is used in exfoliative cytology. The procedure of staining is time-consuming, has multiple steps, is expensive, and is associated with drying artifacts. 4-5 Subsequently, Allegra et al. 6 emerged as one of the pioneers in advocating the utility of scrape cytology of buccal mucosa as a screening device in populations with a high risk for oral cancer. In addition to Pap stain, many laboratories are using MGG stain or Leishman's stain.

To overcome these limitations, the combination of Leishman's and Giemsa stains (LG cocktail) was used in the present study. This combination of stains is basic, easy to use, and cost-effective. The present study was done for the evaluation and comparison of Pap, MGG, and LG cocktail staining in exfoliated cells of oral malignancy. Giemsa is a good cytoplasmic stain, whereas Leishman is a good nuclear stain. When mixed, the LG cocktail provides a moderate metachromasia to the ground substance and brilliantly stained cellular components. The study aims to evaluate and compare Pap, MGG, and LG cocktail staining in exfoliated cells of oral malignancy.

MATERIAL AND METHODS:

Study Design:

This was a prospective observational study designed to evaluate the cytological diagnosis of clinically suspected squamous cell carcinoma (SCC) using scrape cytology. The study was conducted to assess the correlation between cytological features and biopsy results.

Study Setting:

The study was conducted in the Department of Pathology at Hitech Medical College, Rourkela, from January 2022 to January 2024. The participants were referred for scrape cytology for suspected SCC, and the study was carried out within this designated period.

Participants:

A total of 103 patients who were clinically suspected of having squamous cell carcinoma and referred for scrape cytology to the Department of Pathology were included. Inclusion criteria involved patients with lesions suggestive of SCC based on clinical examination. Patients with incomplete data or those who did not consent to participate were excluded.

Variables:

- **Primary Variables:** Cytological features of SCC, including nuclear features (chromatin type, vesicularity, membrane integrity) and cytoplasmic features (transparency, cell membrane type).
- **Secondary Variables:** Clinical features of lesions (number, site, size, and types of lesions including ulcerative, whitish patch, submucosal fibrosis, growth).

Data Sources/Measurement:

The data were collected through detailed clinical history and local examination of the lesion. Cytological samples were collected by scraping the lesion with a scalpel blade, and three smears were prepared on clean, dry glass slides. One slide was fixed with 95% ethanol, the second air-dried slide was stained with Giemsa (MGG), and the third was stained using an LG cocktail (Leishman stain mixed with Giemsa). After staining, cytological features were assessed, focusing on the nuclear and cytoplasmic characteristics.

Statistical Methods:

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study participants. Cytological findings were evaluated using a scoring system for nuclear (1-3) and cytoplasmic (1-3) features. The diagnostic accuracy of cytological findings was compared with biopsy results, and statistical analyses (e.g., sensitivity, specificity, and diagnostic accuracy) were performed to assess the correlation between cytological diagnoses and biopsy results.

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Results:

Out of 103 clinically suspected cases of SCC that were subjected to scrape cytology, the maximum number of cases were seen in the 5th decade, followed by the 7th decade. 57 males and 46 females gave a history of tobacco consumption. In most cases, the presentation was as an ulcer or as a growth. 92 cases were reported as squamous cell carcinoma and 11 cases as dysplasia, out of which 4 cases were low-grade and 7 cases were high-grade dysplasia.

While correlating between cyst diagnosis and histological interpretation, there is a 100% correlation in the case of SCC cases. Out of 4 cases of low-grade dysplasia, 1 case was found to be an inflammatory lesion in histopathology. Out of 7 cases of high-grade dysplasias, 2 cases were found to be inflammatory lesions in histopathology.

While coming to histopathologic diagnosis, 70 cases (68%) were well-differentiated S.C.C., 19 cases (19%) were moderately differentiated S.C.C., 3 cases (2%) were poorly differentiated S.C.C., 2 cases (2%) were low-grade dysplasia, and 8 cases (9%) were high-grade dysplasia.

The comparison of diagnostic efficacy between the three stains—PAP, MGG, and LG cocktail—shows that the specificity of the LG cocktail stain was the highest at 95%, followed closely by PAP and MGG with specificity rates of 93.7% and 93.2%, respectively. However, when considering sensitivity, the LG cocktail stain showed slightly better performance (88.27%) compared to MGG (88%) and PAP (88.76%), though the differences were minimal. The negative predictive value of MGG (84.7%) was notably higher than that of the PAP (76.2%) and LG cocktail (76%) stains, indicating that MGG may be more effective in ruling out malignancy. Despite this, the overall diagnostic performance of the LG cocktail was comparable to MGG, with a slight edge in sensitivity (Table 1).

Table 1: COMPARISON OF DIAGNOSTIC EFFICACY OF PAP, MGG, AND LG Cocktail stains

Stain	Specificity (%)	Sensitivity (%)	Negative predictive value
PAP	93.7	88.76	76.2
MGG	93.2	88	84.7
LG cocktail	95	88.27	76

It is observed that the sensitivity of LG Cocktail is higher than Pap, while specificity was comparable, and both were better than Pap while the specificity was comparable.

Discussion:

The sensitivity of the LG Cocktail stain in this study was found to be 95%, which was higher than that of the Pap stain. The specificity of the LG Cocktail stain was 88.27%. These results indicate that while LG Cocktail provided superior sensitivity in detecting squamous cell carcinoma (SCC), its specificity was slightly lower compared to Pap. When compared to MGG, the cytoplasmic staining in the test group was observed more effectively using Pap and LG Cocktail stains. Although Pap outperformed LG Cocktail in cytoplasmic staining, the difference was not statistically significant. This aligns with Sujathan et al.7, who recommended using both stains together to enhance diagnostic efficacy, as MGG is superior for cytoplasmic staining and Pap for nuclear staining.

Regarding nuclear staining, the LG Cocktail stain produced relatively better results than both Pap and MGG in this study. This is consistent with findings by Gabryal et al. 8 and Shetty et al. 9, who reported that LG Cocktail provides clearer chromatin granularity and vesicularity, especially in air-dried smears. Although the LG Cocktail stain lacked the nuclear transparency seen with Pap, its ability to highlight nuclear features in SCC was more pronounced. This suggests that LG Cocktail is a valuable tool for nuclear assessment in cytology, as it offers enhanced chromatin detail compared to MGG.

Comparing the diagnostic performance of the stains, this study found no statistically significant difference between Pap and LG Cocktail in diagnosing SCC cases. Out of the 92 SCC cases, both Pap and LG Cocktail stains diagnosed nearly identical numbers of cases ($p = 0.158$). In contrast, both Pap vs. MGG ($p = 0.001$) and MGG vs. LG Cocktail ($p = 0.001$) showed statistically significant differences, with MGG performing inferiorly. These findings corroborate the results of previous studies, where Pap and LG Cocktail were found to have comparable diagnostic accuracy in SCC detection, while MGG underperformed in comparison to both 10.

Cytomorphologically, this study revealed that nuclear enlargement and variation in nuclear size were more exaggerated in air-dried smears, and intense background staining sometimes obscured the cellular details. This finding is consistent with the results observed in MGG-stained smears, where excessive metachromasia occasionally hindered the visualization of cell clusters. However, in LG Cocktail-stained smears, the nuclear

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metachromasia of malignant cells was better detected, allowing for clearer identification of cellular details and improving the assessment of cytoplasmic granules, intracellular, and extracellular mucin. This enhanced visibility of malignant features aligns with the findings of Gabryal et al.9 and Shetty et al.10, who similarly observed that LG Cocktail offered better chromatin clarity and cellular feature identification.

Finally, in terms of practicality and cost-effectiveness, the MGG Cocktail was found to be more expensive and time-consuming than the LG Cocktail. The MGG staining process takes approximately 45 minutes, while LG Cocktail staining can be completed in under 10 minutes without the need for additional fixation. This makes the LG Cocktail stain a more efficient and affordable option for rapid cytological evaluation. In comparison, although the Rapid Pap kit offers a faster turnaround time (around 5 minutes), it requires multiple steps and is considerably more expensive than both MGG and LG Cocktail staining methods.

CONCLUSION:

LG Cocktail staining is a simple, cost-effective, less time-consuming, one-step process and can be used for an oral cancer screening program. LG Cocktail needs no prior fixation of air-dried smears and can be used with tap water in place of buffer. Most importantly, the LG cocktail staining technique was found to give results comparable to the Pap stain.

Generalizability:

The study results are relevant for pathology departments using scrape cytology and stains like Pap, MGG, and LG Cocktail for SCC diagnosis, particularly in settings with limited resources. However, the findings may have limited generalizability to other populations with different SCC prevalence or socio-demographics. Further studies in diverse populations are necessary for broader applicability.

Source of Funding:

This study was self-funded by the researchers and did not receive any external financial support.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this study.

Author Contributions:

All authors contributed to the study conception, data collection, analysis, and manuscript preparation.

Ethical Approval:

The study was approved by the Ethical Review Committee of Hitech Medical College, Rourkela, India, under the reference number [insert reference number]. The study adhered to the ethical principles outlined in the Declaration of Helsinki.

Informed Consent:

Informed consent was obtained from all participants included in the study. Participants were fully informed about the study's objectives, the procedures involved, and their right to withdraw at any time without consequence.

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