

Comparative Analysis Study of Efficacy of Papanicolaou smear & Leishman-Giemsa Cytological Technique in the Diagnosis of Oral Neoplastic Lesions

Agrawal Apurva¹, Kumar Kaushal², Singh Hari Chandra³, Agrawal Kailash Chandra⁴

¹Assistant Professor, Department of Pathology, Pt. JNM Medical College, Raipur, Chhattisgarh, India

²Ex-Senior Resident, Department of Pathology, AIIMS Patna, Bihar, India

³Associate Professor, Department of Pathology, VSSMC, Burla, Samablpur, Odisha, India

⁴Professor and HOD, Department of Pathology, VSSMC, Burla, Samablpur, Odisha, India

Running Title: Cytological evaluation of oral neoplastic lesions- Is it the time to abandon Papanicolaou smear as the preferred modality of exfoliative cytology?

Abstract: ***Introduction:** Papanicolaou (Pap) is the most commonly used staining procedure in exfoliative cytology. It provides excellent results but is expensive and time consuming as it requires multistep procedure. Leishman-Giemsa (LG) stain, a relatively new staining technique, is cost effective, simple and less time consuming with good staining characteristics. **Objectives:** To compare the efficacy and reliability of Leishman-Giemsa stain over Papanicolaou stains in cytological diagnosis of oral lesions. **Method:** 109 clinically suspected cases of oral squamous cell carcinoma were enrolled into the study. In all patients, two smears were taken and stained with Papanicolaou and Leishman-Giemsa stains. The diagnostic efficacy of each stain was evaluated by comparing with the histopathological diagnosis. **Results:** Among 62 confirmed cases of squamous cell carcinoma, the number of cases diagnosed by Papanicolaou and Leishman-Giemsa stain was 56 and 57 respectively. The P value obtained for the confirmed cases of squamous cell carcinoma in comparison for Leishman-Giemsa vs. Papanicolaou was 0.11. Hence, no statistical significant difference was observed between the diagnostic ability of Papanicolaou and Leishman-Giemsa stains. **Conclusion:** Leishman-Giemsa stain is a simple, cheap and less time consuming alternative to Papanicolaou stain without compromising the quality of staining for diagnosis of oral malignancy.*

Keywords: Exfoliative cytology, Leishman-Giemsa stain, Oral neoplastic lesions, Papanicolaou stain

1. Introduction

Cytological examination is an important screening procedure to detect malignancy in oral lesions.^[1] Oral carcinoma is the most common malignant neoplasm all over the world. Due to increase in tobacco chewing and smoking habits, its incidence is feared to rise further. Delay in the diagnosis is very common with these tumours, subsequently leading to high mortality and morbidity.^[2] Due to heavy burden on already scarce healthcare resources, especially in developing countries like India and the other South-East-Asian countries, the role of screening has become more vital to facilitate the early diagnosis of these difficult to detect malignant lesions.^[2]

Exfoliative cytology is a simple, economical and non-invasive procedure. It is the preliminary procedure of choice for detection of malignancy in oral lesions.^[3-5] Material for this study can be obtained by aspiration as well as by scraping. Most commonly used stain now-a-days for this procedure is Papanicolaou (Pap). It is being used universally with good results and the main advantage is that different stages of keratinization are stained with different colours. However, it is expensive and time consuming, and results in loss of cellular details and drying artefacts.^[6,7]

Romanowsky stains are universally used for staining peripheral smears. They have the remarkable property of making subtle distinctions in shades of staining, and staining of granules differentially. Leishman-Giemsa (LG) is a relatively new type of Romanowsky stain. This staining technique is a one-step procedure which is easy, cost-effective and has good staining properties. Despite these advantages it has only been rarely used in exfoliative cytology.

This study was conducted to compare the efficacy and reliability of Leishman-Giemsa stain over Papanicolaou stains in cytological examination of oral neoplastic lesion, so further define the role of Leishman-Giemsa staining technique in the field of exfoliative cytology.

2. Materials and Methods

This study was conducted between August 2011 and July 2013 in the department of Pathology, VSSMC, Burla, Samablpur, Odisha. The study includes 109 clinically suspected cases of oral squamous cell carcinoma within an age group of 22 to 85 years. All the patients were screened for oral neoplastic lesions using both Papanicolaou smear and Leishman-Giemsa staining technique. The diagnosis was confirmed with the help of histo-pathological examination.

The cases in which smears were inadequate (less than 50 cells) and patients who left follow up for histopathological examination were excluded. The patients were asked to rinse mouth, scrape or aspiration was done as per the requirement and two smears were prepared from each patient. First one was ether alcohol-fixed; stained with Papanicolaou and other air-dried smear; stained with Leishman-Geimsa. Papanicolaou (Pap) Staining Procedure: ^[8]

- 1) Fixation of the smear with 95% ethyl alcohol : 30 minutes
- 2) Tap water cleaning : 2 to 5 minutes.
- 3) Stained with Harris haematoxylin stain : 1 to 2 minutes.
- 4) Washed with running tap water : 2 to 5 minutes.
- 5) 0.05% aqueous Hydrochloric acid : 1-dip
- 6) Running tap water : 2 to 5 minutes
- 7) 95% alcohol : 10 dips
- 8) 95% alcohol : 2 minutes
- 9) Stained with Orange G-6 stain : 2 minutes
- 10) Rinsed in 95% alcohol : 2 times
- 11) Stained with Eosin Azure-36 stain : 2 minutes
- 12) Rinsed in 95% alcohol : 1 minute
- 13) Rinsed in 95% alcohol : 1 minute
- 14) Dehydrated in Absolute alcohol : 2 minutes
- 15) Cleared in Xylene : 1 minute
- 16) Cleared in Xylene : 1 minute
- 17) Mount in DPX

Leishman-Geimsa Staining Procedure:

- 1) Covered the slide with Leishman stain : 1 minute.
- 2) Equal volume of 1:1 (diluted) Giemsa stain : 5-7 minutes
- 3) Washed in running water : 1 minutes
- 4) Cleared in xylene : 30 Seconds
- 5) Mounted with coverslip

All slides were examined for nuclear and cytoplasmic detail to know about the staining characteristics. Each stained slide was evaluated for 50 well-stained cells and scored according to the scoring criteria of Sujathan et al. ^[9]

Cytoplasmic details were evaluated based on transparency and nature of cell membrane ^[9] and scored as:

- 0 - not preserved,
 1+ - non-transparent with intact cell membrane
 2+ - non transparent masking nuclear details
 3+ - transparent, intact cell membrane without masking nuclear details

Nuclear detail was assessed based on the nature of the chromatin, vesicularity, membrane integrity ^[9] and scored as:

- 0 - poor preservation,
 1+ - smudgy,
 2+ - fair preservation but chromatin granularity not appreciable,
 3+ - excellent preservation with crisp chromatin.

The cytological evaluation of the stained slides was performed by the single examiner. The diagnostic reliability was evaluated by comparing the two stains with each other and the histo-pathologic examination. The data was statistically evaluated using Student t-test, p value < 0.05 was considered significant by using SPSS for windows 20.

Results

When the smears were compared with the histopathology reports, it was found that 62 of the 109 clinically suspected

cases were diagnosed as squamous cell carcinoma, 44 as benign and in 3 patients, the tissue received did not show features suggestive of any groups and were excluded from data analysis. Of the 62 confirmed cases of squamous cell carcinoma, the number of cases diagnosed by Papanicolaou and Leishman-Geimsa were 56 cases and 57 cases with sensitivity of 90.32% & 91.94% respectively & specificity of 84.09% and 86.36% respectively.

The P value obtained on comparison of Leishman-Geimsa vs. Papanicolaou in nuclear and cytoplasm staining were 0.17 and 0.24 respectively. The P value obtained for the confirmed cases of squamous cell carcinoma in comparison for Leishman-Geimsa vs. Papanicolaou was 0.11. Hence, no statistically significant difference was observed between the diagnostic ability of Papanicolaou and Leishman-Geimsa stains.

The time required for staining with Papanicolaou stain is about 45-60 minutes whereas time required for Leishman-Geimsa stain is only 7-8 minutes. Leishman-Geimsa stain is also cost effective with 8-9 rupees per case while Papanicolaou stain cost 24-28 rupees per case.

3. Discussion

Papanicolaou is the most commonly used staining technique in exfoliative cytology since 1928 discovered by Dr. Georgios Nikolaou Papanicolaou. Papanicolaou stain contains haematoxylin, a nuclear stain; Orange G-6 & Eosin Azure - 36, two cytoplasmic stain. ^[10] It is a reliable procedure and has the benefit of staining cells from various layers differentially, like superficial cells are orange to pink, and intermediate and parabasal cells are green to blue. The chromatin patterns are well visible; the cells from borderline lesions are easier to interpret. But during fixation and staining there is significant loss of cells from smears along with cellular architecture; the procedure is time consuming and is also associated with drying artefacts. ^[11]

Leishman and Geimsa both being a differential stain but Leishman is a good nuclear stain, when used alone and gives an intense extracellular ground substance staining while Giemsa is a good cytoplasmic stain. When both are mixed, they provide a moderate metachromasy to the ground substance and brilliantly stained cellular components. ^[12] It was observed that the cytoplasmic staining was better appreciated in Leishman-Geimsa stain when compared to Papanicolaou stain and nuclear stain was better in Papanicolaou stain, but the difference was statistically insignificant which coincides with the study done by Gabryal et al., ^[12]

According to the study done by Sujathan et al., ^[9] Giemsa was a better cytoplasmic stain and Papanicolaou was a better nuclear stain, and combined use of both increases the efficacy of diagnosis. Though the nuclear transparency of Papanicolaou was absent in Leishman-Geimsa, the chromatin granularity and vesicularity was better appreciated in air-dried Leishman-Geimsa stained smears. This is in accordance with Gabryal et al. ^[12] Additionally, the nuclear enlargement and variation in nuclear size is exaggerated in air-dried smears which is helpful in cytological diagnosis. If

the background staining is too intense, it may also prevent adequate visualisation of cell clusters.^[9]

Finally, the cytological diagnosis of Papanicolaou and Leishman-Geimsa stained smears was compared with the histopathology reports. It was observed that no statistically significant difference was found between the diagnostic ability of Papanicolaou and Leishman-Geimsa stains. The overall observations of the present study was that Leishman-Geimsa stain is comparable to Papanicolaou stain, which is in accordance with the study by Gabryal et al.^[12] and Mitra et al.^[13] The sensitivity of Leishman-Geimsa stain to diagnose malignant tumours was 91.9%, which was higher than Papanicolaou and the specificity was 86.36%.

Ideal stain used in a mass screening programme must be easy, rapid and economical in spite of the good staining characteristics. The time required for staining with Papanicolaou stain, i.e., for fixation and staining is about 45 minutes. Papanicolaou stain requires multiple steps and large volumes of alcohol.^[8] The cost is also higher than the Leishman-Geimsa stain. On the other hand, Leishman-Geimsa staining procedure of air-dried smears requires no additional fixation as in Papanicolaou stain and can be completed in less than 10 minutes, with the least expenditure. One more advantage of Leishman-Geimsa is that it can stain smears for long intervals. Though Rapid Papanicolaou kit is available for faster turnaround time of approximately 5 minutes, it still requires multiple steps and is very expensive when compared to the Leishman-Geimsa staining.^[14] Therefore, Leishman-Geimsa Stain offers good staining characteristics, easy single-step procedure with significantly reduced procedure time and low costs.

Benefits of the Leishman-Geimsa over Papanicolaou stain

Leishman-Geimsa Stain	Papanicolaou stain
1. Cheap	1. Relatively expensive
2. One-step procedure	2. Multistep procedure
3. Single solution	3. Multiple solutions
4. Effective time, < 10 minutes	4. Effective time, 45 minutes
5. No need for prior fixation	5. Needs prior fixation

4. Conclusion

In this study, Leishman-Geimsa staining technique was found to give results comparable to the Papanicolaou stain and with advantages of a single step procedure, cost effectiveness and time saving. The positive findings in this study support the idea of utilising Leishman-Geimsa method for early detection of oral cancer, especially in mass screening programmes.

References

- [1] Das BK, Mallick NC. The diagnostic perspective of oral exfoliative cytology: An overview. *J Indian Dent Assoc.* 2000;71:7-9.
- [2] Sugeran PB, Savage NW. Exfoliative cytology in clinical oral pathology. *Aust Dent J.* 1996;41:71-4. [PubMed: 8670036]

- [3] Bernstein ML, Miller RL. Oral exfoliative cytology. *Journal of American Dental Association* 1978; 96:625-9.
- [4] Diniz FM, Garcia GA, Crespo AA, Martins CL, Gandara JM. Applications of exfoliative cytology in the diagnosis of oral cancer. *Med Oral.* 2004;9:355-61. [PubMed: 15292877]
- [5] Mehrotra R, Hullmann M, Smeets R, Reichert E, Daniel O. Oral cytology revisited. *J Oral Pathol Med.* 2009;38:161-6. [PubMed: 19213102]
- [6] Culling CA, Allison RT, Barr WT. Cellular pathology technique. 4th ed. London: Butterworth; 1985. Fine needle aspiration cytology; pp. 442-92.
- [7] Orell SR, Sherett GF, Barr WT. 4th ed. New York: Churchill Livingstone; 2005. Fine needle aspiration cytology; pp. 179-82.
- [8] Bancroft J, Gamble M. 5th ed. Edinburgh: Churchill Livingstone; 2002. Theory and practice of histological techniques; pp. 105-20.
- [9] Sujathan K, Pillai RK, Kannan S, Chandralekha B, Mathew A, Nair KM. Cyodiagnosis of serous effusions: A combined approach to morphological features in papanicolaou and May-Grünwald
- [10] Saxe A, Phillips E, Orfanou P, Husain M. Role of sample adequacy in fine needle aspiration biopsy of adequacy in fine needle aspiration biopsy of palpable lesions. *Am J Surg* 2001;182(4):369-71.
- [11] Culling CFA, Allison RT, Barr WT. Cellular pathology technique. 4th edn. London: Butterworth, 1985 :491.
- [12] Garbyal RS, Agarwal N, Kumar P. Leishman-Geimsa cocktail: An effective Romanowsky stain for air-dried cytologic smears. *Acta Cytol.* 2006;50:403-6. [PubMed: 16901003]
- [13] Mitra S, Bose S, Mukherjee G. Comparative studies on the Leishman Giemsa stains and papanicolaou stains for cytological diagnosis of oral lesion. *Sci Cult.* 2011;77:139-40.
- [14] Dighe S, Ajith D, Pathuthara S, Chinoy R. Papanicolaou stain: Is it economical to switch to rapid, economical, acetic acid, and papanicolaou stain? *Acta Cytol.* 2006;643-6. [PubMed: 17152276]

Illustrations (Figures)

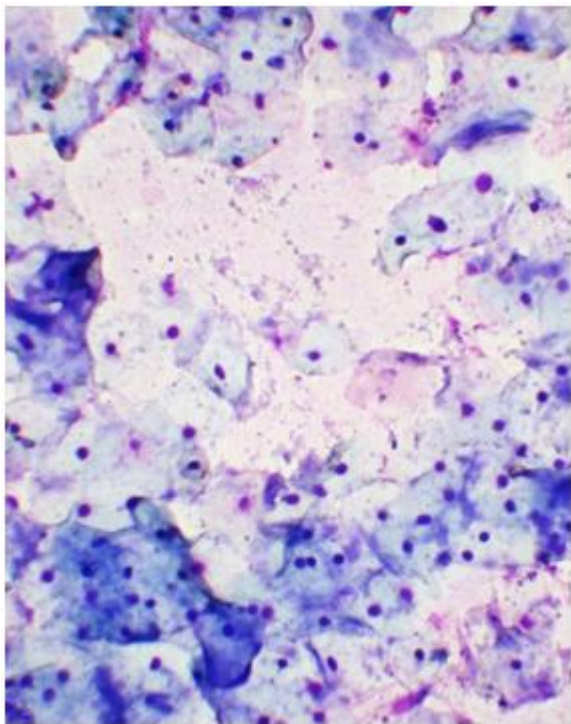


Figure 1: Smear shows mature looking squamous cells with folded cytoplasmic membrane. (Leishman-Giemsa, X100)

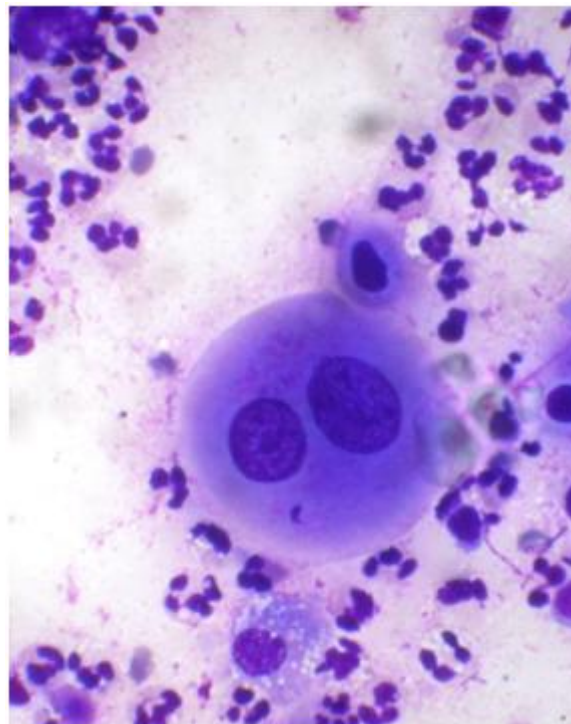


Figure 3: Smear shows binucleate cell with clear cellular details and enlarged nucleus with granular chromatin and prominent nucleoli (Leishman-Giemsa, X400)

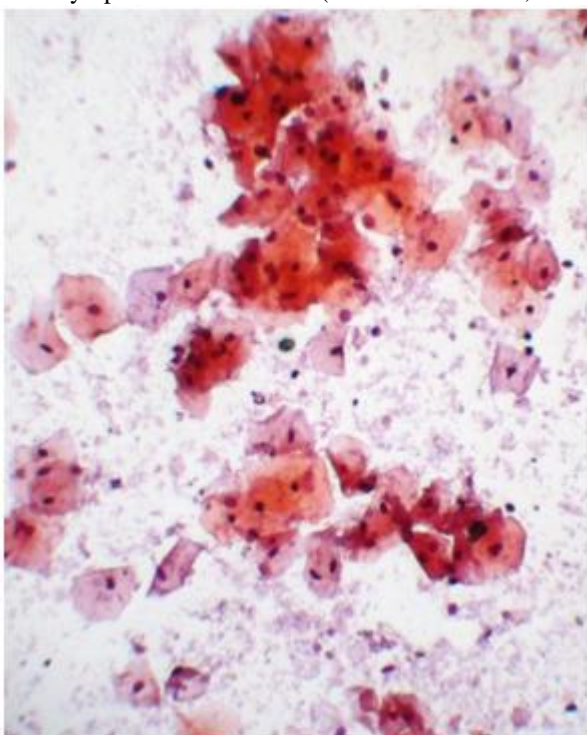


Figure 2: Smear shows orangeophilic squamous cells. (Papanicolaou, X100)

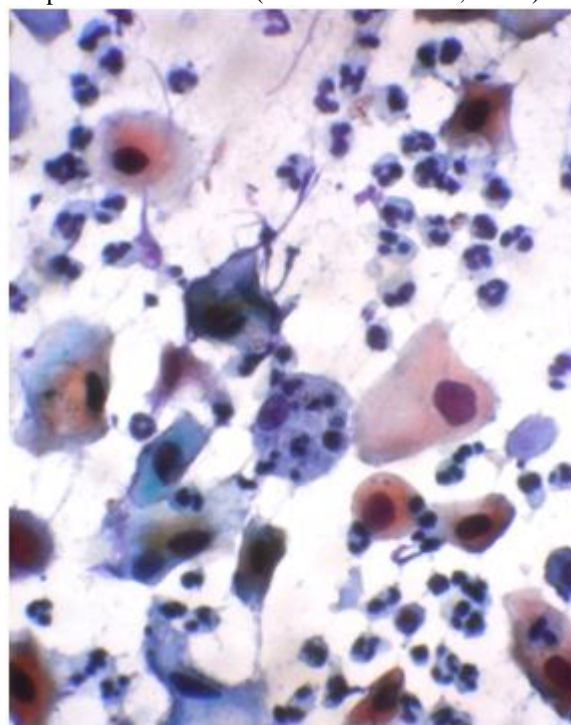


Figure 4: Smear shows differential staining of cytoplasm (Papanicolaou, X400)