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Comparative Study of Conventional Staining Techniques in Cytology

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Abstract: ***Background: Exfoliative cytology is the histopathologic examination of cells that have been obtained by their physical removal, followed by their placement on a glass slide, and then appropriately stained [1].The aim of this study was to compare the staining of cytologic Papanicolaou stain, hematoxylin andeosin stain, and may-grunwald-giemsa stain in oral mucosa smear. Materials and Methods: A total of 150 Cytological oral smears were taken from 50 volunteers using a sterile wooden tongue depressor. In each case, the surface epithelium of the buccal mucosa was scraped and applied to a clean frosted glass slide. The scraped cells were placed onto pre-cleaned slides. three slides were made from each subject. One was air-dried and stained with the (MGG) stain, while the other was wet fixed and stained with PAP and H&E. Results: The nuclear structure was excellent stained with Pap and H&E and was poor with MMG representing 88%, 80, and 5% respectively while the cytoplasm and background were excellent stained with Giemsa representing 80% and 60% and respectively. Conclusion: Pap-stained smears were better than H&E and Grunwald- Giemsa stain for oral cytology.***

Keywords: Comparative, Staining Techniques, Cytology

1. Introduction

Diagnostic cytology is an art and science of the interpretation of cells from the human body that either exfoliate (desquamate) freely from the epithelial surfaces or is removed from various tissue sources by various clinical procedures. [2].Oral exfoliative cytology is particularly valuable for mass screening purposes; with a sensitivity of 94%, and specificity of 100% [3]. Recent advances in technology facilitate the use of reliable quantitative techniques such as cytomorphometry, histometric, and computer-assisted image analyzer. The evaluation of parameters such as nuclear area (NA), cytoplasmic area (CA), and ratio of NA/CA (N/C), may increase the sensitivity of exfoliative cytology for early diagnosis since these are precise, objective, and reproducible [4].

Application of diagnostic cytology in this part of the human body can both either differentiate between benign and malignant conditions or sometimes like other body cytology specimens though it helps in the subclassification of inflammatory diseases, such as Herpes simplex, bacterial infections, and sometimes in other metaplastic and hyperplastic conditions.

The purpose of biologic staining is to ease the perception of structures by increasing the contrast between them. Two fundamentally different methods are used for routine fixation and staining of cytologic specimens. romanowsley-type stains (e.g., Wright's, May-Granwald-Giemsa, Diff-Quik) are based on air-drying, and are widely used in veterinary medicine [5, 6}. The trichrome Papanicolaou (Pap) and bichrome hematoxylin and eosin (H&E) stains, which are based on wet-fixation, have not gained popularity in veterinary cytopathology, although a few authors advocate their use. Some cytopathologists prefer the non-permanent new methylene blue as a complementary nuclear stain in addition to romanowsley stains [7, 8].

2. Materials and Methods

The study was launched after the Ethical Committee in Al- Rayan Medical College, had approved the proposal. Each participant involved in the studywas informed and asked to sign a written consent form.

A total of 150 Cytological oral smears were taken from 50 volunteers using a sterile wooden tongue depressor. In each case, the surface epithelium of the buccal mucosa was scraped and applied to a clean frosted glass slide. The scraped cells were placed onto pre-cleaned slides. Three slides were made from each subject. One was air-dried and stained with the (MGG) stain, while the other was wet fixed and stained with PAP and H&E [8].

Table 1: Papanicolaou stain procedure

Step	Staining Reagent	Time	Step	Staining Reagent	Time
1	tap water	Rinse	6	95% Ethanol	10 dips
2	Harris Hematoxylin	1-3min	7	EA-50	2.5 min
3	tap water	Rinse	8	95% ethanol	10 dips
4	95% Ethanol	10 dips	9	100% Ethanol	1 min
5	orange G-6 stain	1.5 min	10	2 changes of xylene	2 min

Table 2: Haematoxylin and Eosinprocedure

Step	Staining Reagent	Time
1	water	Rinse
2	Harris Hematoxylin	1-3min
3	In 1% acid alcohol	2sec
4	ammoniated water	10 dips
5	water	1.5 min
6	Eosin	20sec
Wash in water, dehydrate, clear, and mount		

Table 3: May-Grunwald-Giemsa procedure

Step	Staining Reagent	Time
1	May-Grunwald	10 min
2	pH 6.8 buffer	Rinse
3	Giemsa solution	30 min
4	pH 6.8 buffer	5-20 min
Mount in DPX		

The data analysis protocol was according to the density of color and the clearance of nuclei, cytoplasm and background.

Statistical analysis: Statistical analysis was carried out on all samples using the Frequencies, cross tabulation and chi-square were calculated, to determine statistical significance ($P<0.05$) with 95% confidence level.

3. Results

This is a descriptive study to compare cytological stain (Pap, H&E, and MMG), the nuclear structure excellent stained with Pap and H&E and poor with MMG representing 88%, 80, and 5% respectively as shown in table (4). The cytoplasm and background were excellent stained with May-Granwald-Giems are presenting 80% and 60% and respectively as shown in tables (5 and 6).

Table 4: Comparison of staining quality for three types of cytologic stains in nuclear structure

Cell Feature	Excellent	Good	Poor
Pap	88%	20%	10%
H&E	80%	24%	16%
Giemsa	5%	25%	80%

P. value (0.034)

Table 5: Comparison of staining quality for three types of cytologic stains in cytoplasm

Cell Feature	Excellent	Good	Poor
Pap	88%	20%	10%
H&E	20%	80%	10%
Giemsa	50%	30%	20%

P. value (0.023)

Table 6: Comparison of staining quality for three types of cytologic stains

Cell Feature	Papanicolaou	Hematoxylin and Eosin	May-Grunwald-Giemsa
Nucleus	Excellent	Excellent	poor
Cytoplasm	Good	Good	Excellent
background	poor	Poor	Excellent

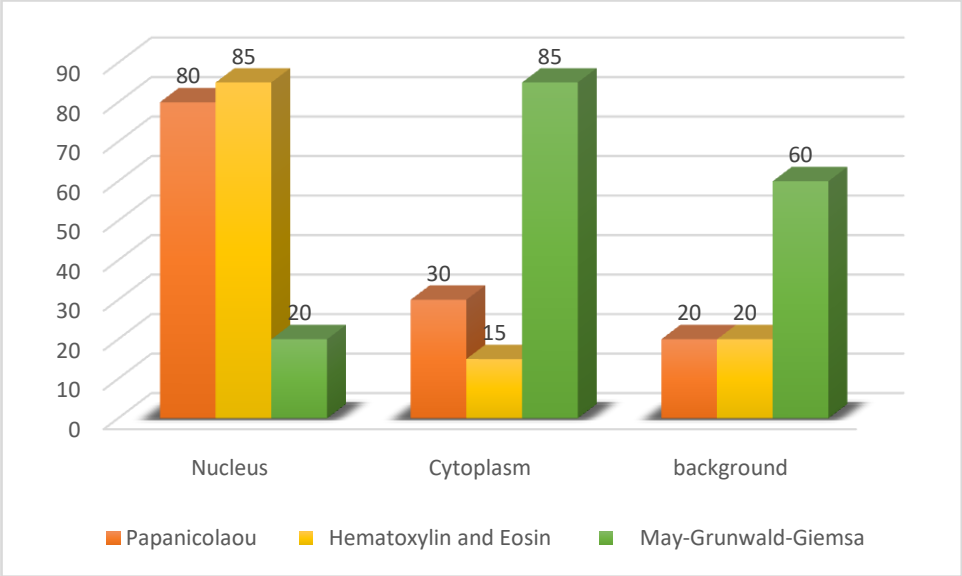

Figure 1: Comparison of staining quality for three types of cytologic stains

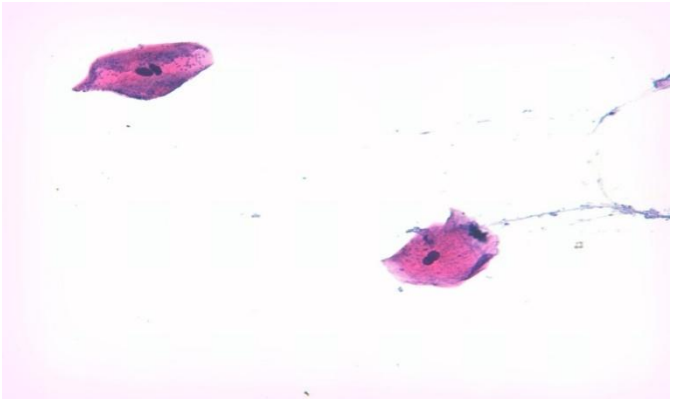

Figure 1: Photomicrograph of a buccal cellby Pap Stain 400x)

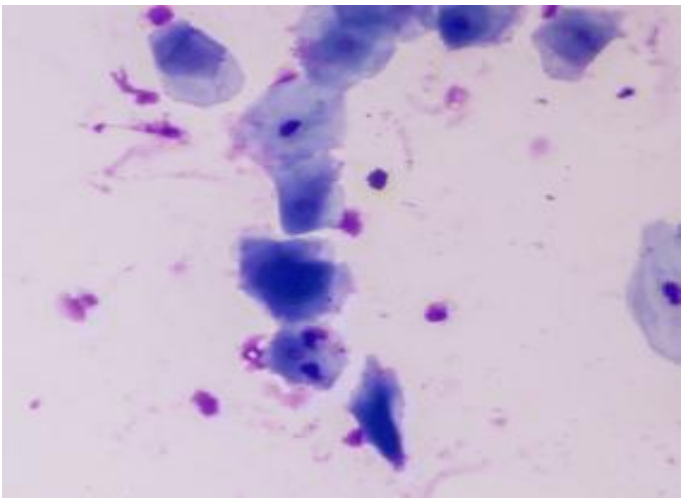

Figure 2: Photomicrograph of a buccal cell by May-Grunwald-Giemsa Stain (400x)

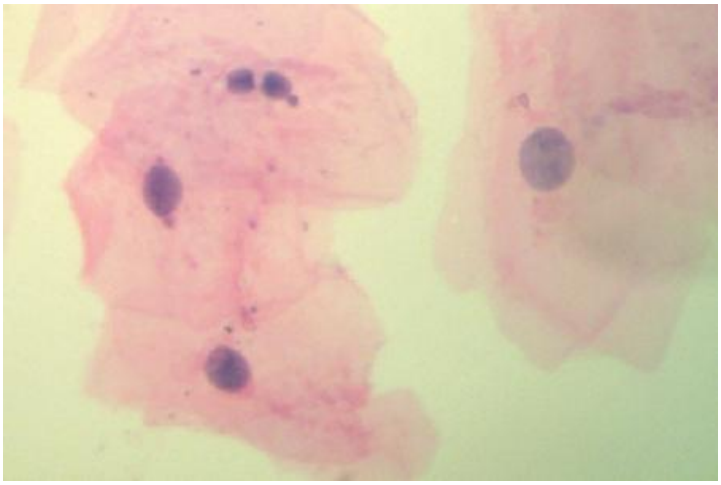

Figure 3: Photomicrograph of a buccal cell (H&E Stain 400x)

4. Discussion

Oral exfoliation cytology is a simple and non-invasive method. Diagnostic techniques that can be used for early detection potentially malignant lesions [9]. Cytological assesses parameters such as, nuclear shape, nuclear-cytoplasmic ratio, color density, and vacuolated cytoplasm. These quantitative techniques may increase the sensitivity of exfoliative cytology for the early diagnosis of oral cancer [10]. The present results, In a comparative study of May-Grunwald-Giemsa and Pap stains, May-Grunwald-Giemsa stains were considered the stain of choice for veterinary cytopathology, with Pap stains recommended for special diagnostic situations. [11] However, because of the transparency of the cytoplasmic counter stains in methods based on wet-fixation or rehydration, H&E and Pap stains were superior to May-Grunwald-Giemsa for the examination of diagnostically important tissue fragments. May-Grunwald-Giemsa stains applied to wet-fixed or rehydrated specimens may increase the transparency of cellular components. Nuclear and nucleolar details were better assessed in H&E- or Pap-stained smears because of the delicate staining of chromatin by hematoxylin, and hematoxylin's lower affinity for euchromatin background material including colloid, mucus, chondroid matrix, myxoid matrix and other secretory products of diagnostic importance are better demonstrated by May-Grunwald-Giemsa stains than Pap or H&E. In cytopathology, particularly of tumors, nuclear features are important in assessing malignancy. Thus, stains that increase nuclear detail are desirable, especially when used in conjunction with stains that emphasize cytoplasmic and extracellular details for differentiating cell origin. Use of H&E or Pap stain in addition to Romanowsky stain facilitates assessment of nuclear detail and tissue fragments and bridges the gap between cytologic and histologic interpretations [11].

References

[1] Leopold G. Koss, Diagnostic Cytology and its Histologic Bases, Volume I&II, Third Edition, Philadelphia, JB Lippincott, 1979

[2] Pisinger, C. and M. Døssing, *A systematic review of health effects of electronic cigarettes.* Preventive medicine, 2014. **69**: p. 248-260.

[3] Manyanga, J., et al., *Electronic cigarette aerosols alter the expression of cisplatin transporters and increase drug resistance in oral cancer cells.* Scientific reports, 2021. **11**(1): p. 1-14.

[4] Freeman KP, Carroll BS: Wet-fixed, trichrome-stained cytologic specimens in private equine practice. Comp Cont Educ Pract Vet 11 :485-492, 1989

[5] Roszel JF, MacVean DW, Monlux AW: Use of cytology for tumor diagnosis in private veterinary practice. J Am Vet Med Assoc 173:1011 -1014, 1978

[6] Perman V, Alsaker RD, Riis RC: Cytology of the Dog and Cat, American Animal Hospital Assoc., South Bend, IN 1979

[7] Tvedten H: Cytology of neoplastic and inflammatory masses. In: Small Animal Clinical Diagnosis by Laboratory Methods, ed. Willard MD, Tvedten H, Turnwald GH, 2nd ed., pp. 321-341, W. B. Saunders, Tokyo, 1994

[8] Stanley Lawrence Lamberg, Robert Robert Rothistein: Laboratory Manual of Histology and Cytology, AVI Publishing Company, INC Westport, Connecticut, 1978

[9] Einstein, T. and B. Sivapathasundharam, *Cytomorphometric analysis of the buccal mucosa of tobacco users.* Indian journal of dental research: official publication of Indian Society for Dental Research, 2005. **16**(2): p. 42-46

[10] Boon GD, Rebar AH, DeNicola DB: A cytologic comparison of Romanowsky stains and Papanicolaou-type stains. I. Introduction, methodology and cytology of normal tissues. Vet Clin Pathol 11 :22-30, 1982

[11] Rebar AH, Boon GD, DeNicola DB: A cytologic comparison of Romanowsky stains and Papanicolaou-type stains. II. Cytology of inflammatory and neoplastic lesions. Vet Clin Pathol11 :16-25, 1982