



ORIGINAL RESEARCH PAPER

Oral Pathology

A COMPARATIVE STUDY TO EVALUATE THE EFFICACY OF REHYDRATED AIR DRIED BUCCAL CYTOLOGICAL SMEARS USING EDIBLE OILS WITH CONVENTIONAL WET FIXATION

KEY WORDS: Cytofization, Edible Oil, Air-dried Smears, Rehydration.

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ABSTRACT	Objectives: 1) To evaluate the rehydrating and fixation efficacy of two edible oils in air dried buccal smears. 2) To compare it with conventional wet fixation method. Methods: 45 Buccal smears taken from the healthy individuals. Control group was labeled as wet fixation with 95% Ethanol and other two study groups I & II were labeled as air dried fixation and rehydrated with coconut oil and olive oil respectively. Single blinded study was done. The staining quality was assessed with respect to nuclear details, cytoplasmic details and background staining was done. Results: Air dried smears rehydrated with coconut oil and olive oil were in par with the ethanol and can be used as an excellent alternative for fixation of the cytological smears.
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INTRODUCTION
 Cervical PAP smear is a useful method in early cancer detection and it is routinely carried out throughout the world. This approach was then carried out in buccal smears to detect the cancerous and precancerous lesions from the exfoliating cells.¹

Cytology provides rapid diagnosis by minimally invasive technique. Exfoliative cells from oral epithelium have been widely used in cytology to detect abnormal nuclear and cellular morphological changes. Accuracy of cytopathological diagnosis depends on multiple steps among which fixation plays an important role in fixing the cells and making them amenable to evaluation and reach an appropriate diagnosis.

Smears collected by the oral pathologists are routinely fixed immediately in 95% ethanol by dipping the freshly spreaded smears (wet fixation). Drying artefacts and alteration of the cellular morphology are some of the limitations of wet fixation that cause difficulties in smear interpretation. To overcome this, alternative method like rehydration of air-dried smears were carried out using sequential tap-water and acetic acid and 50% aqueous glycerine etc.² Literature search revealed that there were experimental studies in rehydrating air dried buccal smears using different oils. Hence, the present study was undertaken to assess the fixing ability of coconut oil and olive oil by rehydrating the air dried smears compared with that of conventional wet fixation using 95% ethanol.

MATERIALS AND METHODS
 The study was carried out in 15 healthy volunteers after taking written informed consent. Inclusion criteria consists of subjects with normal, healthy buccal mucosa free of any pathology. Initially, the subjects were asked to rinse their mouth with water and are then subjected for the oral smear collection. Three buccal smears were collected from each volunteer using wooden spatula. One of the smears was immediately fixed in 95% ethanol for 10 mins. Other two smears were allowed to air-dry in room temperature for 2 hours, followed by rehydration using coconut oil (group I) and olive oil (group II) respectively. All the fixed slides were then stained by Pap stain.

Slides Evaluation
 All the slides were labeled and evaluated by two separate

oral pathologists. In each slide, a minimum of 20 epithelial cells without overlap and shrinkage were considered for evaluation. The pathologists evaluated the smears based on the six parameters (Nuclear staining, cytoplasmic staining, cell morphology, clarity of staining, uniformity of staining and background clearance).

The statistical analysis was done using Chi-square test and Cohen's kappa test in IBM SPSS software version 20.

Scoring Criteria

Criteria	Score=1 acceptable	Score=0 Unacceptable
Nuclear staining	Round , smooth and clear nuclear membrane	Granular , disintegrated and out of focus
Cytoplasmic staining	Intact cytoplasmic membrane and transparent cytoplasm	Disintegrated cytoplasmic membrane, granular cytoplasm and out of focus
Cell morphology	Absence of folds, no overlap and maintained nuclear cytoplasmic ratio	Overlapping cells, folded and disintegrated cells
Clarity of staining	Crispness in staining and transparency	Obliterate the nucleus and cytoplasm
Uniformity of staining	Uniformly stained throughout the individual cell	Stained in different shades of color in an individual cell
Background clearance	Clear background with no debris present	Debris present

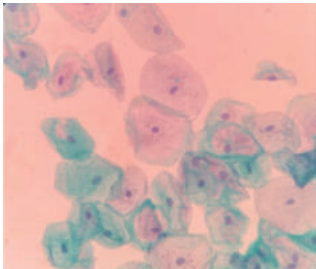


Fig 1: Buccal smears fixed using conventional wet fixation using 95% ethanol (PAP 40X magnification)

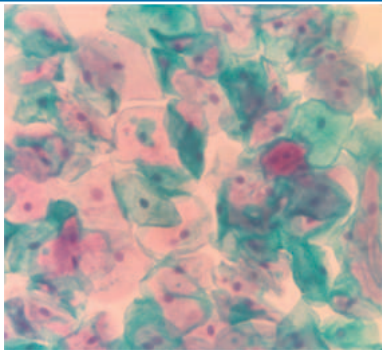


Fig 2: Air dried buccal smears rehydrated with coconut oil (PAP 40X magnification)

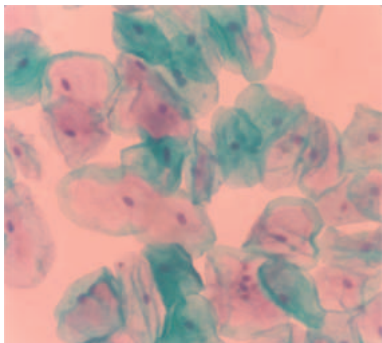


Fig 3: Air dried buccal smears rehydrated with olive oil (PAP 40X magnification)

Observer 1

Criteria	Coconut Oil		Olive Oil		95% Ethanol		p Value
	N	%	N	%	N	%	
Nuclear staining	15	100	15	100	15	100	0.290
Cytoplasmic staining	15	100	14	93	15	100	0.360
Cell morphology	12	80	10	67	10	67	0.649
Clarity of staining	13	87	14	93	13	87	0.799
Uniformity of staining	12	80	11	73	12	80	0.659
Background clearance	11	73	11	73	10	67	0.897

Observer 2

Criteria	Coconut Oil		Olive Oil		95% Ethanol		p Value
	N	%	N	%	N	%	
Nuclear staining	15	100	15	100	15	100	0.290
Cytoplasmic staining	15	100	15	100	15	100	0.290
Cell morphology	11	73	11	73	10	67	0.897
Clarity of staining	14	93	15	100	13	87	0.343
Uniformity of staining	13	87	11	73	12	73	0.598
Background clearance	10	67	12	80	9	60	0.484

RESULTS

The observation by both the reviewers showed that the nuclear staining was excellent in wet and air dried rehydrated smears (100%) with perfect agreement. (Kappa value = 1)(Fig 1-3)

The analysis of cytoplasmic staining revealed that coconut oil and ethanol provides 100% quality staining with both the reviewers with perfect agreement. (Kappa value = 1),

whereas only 93% air dried smears rehydrated with olive oil showed better staining by first reviewer.

On analyzing cell morphology, smears fixed with coconut oil showed better preservation of cellular morphology (80%) under the observation of the first reviewer (Kappa value = 0.74) whereas according to the second observer, the smears air dried and rehydrated with both the oils had an equal morphology preservation property (73%) than the ethanol. (Kappa value = 0.62)(Fig 2 and Fig 3)

According to both the reviewers, it was a perfect agreement that the clarity of staining was better in olive oil than the other two cytofixatives. (Kappa value = 1)

Both the reviewers agreed that the air dried smears fixed and rehydrated with the coconut oil provided better uniformity in staining over the other two cytofixatives. (Kappa value = 0.86)(Fig 2)

Lesser debris was seen in the slides fixed with olive oil, hence olive oil had a better background clearance property over coconut oil and ethanol. (Kappa value = 0.74)(Fig 3)

The evaluation of all the slides by two reviewers showed that there was no statistical significance seen between the air dried smears rehydrated (fixed) with edible oils and smears fixed with ethanol. (Table 2, Table 3)

DISCUSSION

Exfoliative cytology helps in the study of physiologically exfoliated cells and pathologically collected malignant cells usually from the mucous membrane. Conventional wet fixation with ethanol requires immediate fixation to prevent air-drying artefacts. To overcome such flaws, studies were carried out on rehydration of air dried smears with various aqueous and non-aqueous solutions for better visualization of the epithelial cells.³

Gill G W et al (1998) suggested that air dried smears exhibit more flatter epithelial cells that would help in examining the cytoplasmic details over wet fixation.⁴

Rehydration of vaginal exfoliative cytological smears using tap water was attempted in 1954 by Lencioni et al with favourable results. Later Sivaraman and Iyengar (2002) suggested that the rehydrated air-dried PAP stained cervical smears are a satisfactory alternative to wet fixed cervical smears.

In recent times, rehydration of air dried buccal smears were carried out with various alternatives like normal saline, corn syrup, honey, liquid paraffin etc., with promising results.

Sherin Singnarpi et al (2017) concluded that the procedure of air drying and rehydration with olive oil was inexpensive, easy, convenient and almost equally effective to routine fixation.⁵

Antony JVM et al (2021) compared wet fixed smears with air dried smears rehydrated with coconut oil and showed that the rehydration of air dried buccal smears had good overall staining properties and concluded that air dried smears rehydrated with coconut oil can be used as an alternative to wet fixed smears.⁶

In this study, comparison of rehydrated air dried smears was carried out using both coconut oil and olive oil with 95% ethanol. The results revealed that all the cells in the PAP stained smears were in par with each other in respect to microscopical evaluation. Among the three groups, rehydrated smears using coconut oil showed excellent cytomorphological details with good background clearance, whereas rehydrated smears using olive oil provided good

cellular features with clear background devoid of debris.

CONCLUSION

From this study, it is evident that the smears from all the three groups showed better fixation and staining of the cells by maintaining the nuclear and cytoplasmic details with acceptable background clearance. Hereby we conclude that the edible oils like coconut oil and olive oil can be used as potential rehydrating agents to fix the air dried smears and can also be used as an better and efficient alternative for conventional wet fixation. In addition, they are readily available, biocompatible, cheap and can be used as a satisfactory alternative source, particularly in poor laboratory settings and remote areas especially during mass screening programs.

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