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Assessing the efficacy of liquid-based cytology over conventional cytology in oral lesions: A comparative study.

[1]Dr. Khushali Shah, Govt. Dental College and hospital, Ahmedabad-380016.
[2]Dr. Sima Odedra, Govt. Dental College and hospital, Ahmedabad-380016.
[3]Dr. Vaishali Dodia, Govt. Dental College and hospital, Ahmedabad-380016.
[4]Dr. Pooja Monoara, Govt. Dental College and hospital, Ahmedabad-380016.
[5]Dr. Tarang Mehta, Govt. Dental College and hospital, Ahmedabad-380016.
[6]Dr. Jayasankar Pillai, Govt. Dental College and hospital, Ahmedabad-380016.
Corresponding Author: Dr. Khushali Shah, Govt. Dental College and hospital, Ahmedabad-380016.

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Abstract

Background: Exfoliative cytology is the study of cell that are shed off or desquamated cells from the epithelial surfaces. Centrifuged liquid-based cytology (CLBC) is a modified technique that is used in the current study.

Aim: To assess and compare the efficacy of CLBC with conventional cytology in apparently normal mucosa and in oral lesions after staining with Papanicolaou (PAP) stain.

Materials and Methods: The study sample for this comparative study was collected from 50 subjects with either normal oral mucosa or various oral lesions such as hyperkeratotic lesions, ulcerated lesions or atrophic lesions reported to the outpatient department of a government dental institute. Two smears were taken from the oral cavity using a sterile swab. One was spread on the slide using conventional technique and fixed immediately with 95% ethyl alcohol. For second sample the swab with scraped material was dipped and shaken in suspending solution composed of 20 ml of 95% ethanol +6 ml acetic acid +74 ml normal saline for 10 minutes and spun in centrifuge for 10 minutes at 2000 rpm. The obtained cell pellet was then re-suspended in 95% alcohol and the suspension was poured over a horizontally placed glass slide and left for two hours to allow sedimentation of cells. Both the smears were stained by PAP stain. The stained smears were then being compared for various morphological parameters. The Wilcoxon Signed rank test was applied and a p value less than 0.05 were considered statistically significant.

Results: There was a statistically significant difference ($p<0.001$) between centrifuged liquid-based cytology and conventional cytology when parameters like cellularity, cell distribution, cellular overlapping, cellular

background, the presence of RBCs and inflammatory infiltrate are evaluated. The only parameter which showed insignificant result was 'cellular elongation'.

Conclusion: CLBC would be useful for advanced procedures like immunocytochemistry especially in laboratories with limited access to expensive automated systems.

Keywords: Liquid based cytology, Conventional cytology, Oral lesions, PAP stain.

Introduction

Exfoliative cytology is the microscopic examination of a shed or desquamated cells from the epithelial surface. It is a cost effective and perhaps the best procedure for the initial evaluation and diagnosis of oral lesions. [1] It is simple, safe and reliable diagnostic procedure especially in population-based screening programs, where repeated samples might be required. [2]

In recent years, it is observed that the liquid-based cytology (LBC) technique is being used and preferred over the conventional exfoliative cytology technique. The LBC technique has also shown better efficacy over the conventional cytology method when the cytosmear parameters were observed in shedding oral mucosal cells [3]

Oral squamous cell carcinoma (OSCC) is one of the most common health problems in India. It arises from potentially malignant disorders (PMDs) such as leukoplakia, erythroplakia, and Oral Lichen Planus (OLP). [4] The LBC technique has its application in the diagnosis of oral cancers and also in PMDs. [5]

Studies in cervical cytology have shown that the LBC reduces the problems related to sampling and preparation of better smears and reduction in false-negative rates.[6] [7][8].

LBC technique has been shown to result in slides with clear background, with higher cellularity dispersed homogeneously and with reduced R.B. Cs, Inflammatory cells and mucous. The clear background thus obtained enhances sensitivity and quality. As compared to conventional smears, the use of liquid-based preparations greatly reduced the number of slides that are unsatisfactory, or satisfactory but limited by specimen artifacts, thus diminishing the false negative results.

Centrifuged LBC (CLBC) which is a modification of LBC is cost effective, simple technique with readily available equipment and it provides clear background by removing debris, blood and mucous cell. [9]

LBC gives better results, as it not only enhances both sensitivity and specificity, it also provides material for further investigations including immunocytochemistry, HPV testing, AgNORs, DNA ploidy or laser scanning cytometry in addition to sophisticated molecular methods. [10][11]

As there is a better scope and applicability of the LBC techniques in the diagnosis of pathologies, including the oral pathologies, the present study was undertaken with the objective of comparing the cellular parameters using both the conventional and the LBC techniques.

Materials and methods

This comparative study was conducted at Government Dental College and Hospital, Ahmedabad from December 2019 to February 2020. A total of 50 subjects of either normal oral mucosa or various oral lesions (n=10 Normal mucosa, n=17 Hyperkeratotic lesions, n=16 Ulcerative lesions, n=7 Atrophic lesions) reported to the out-patient department were included in the study. The ethical permission to carry out the present study was obtained from the Institutional ethical committee prior to the start of the study (IEC GDCH/ OP.5/2021) The subjects were informed with regard to research objectives, methods, possible benefits, and potential risks, and written consent was obtained from all patients. Two smears were obtained

from the lesion using cytological swab. One was spread on slide using conventional technique and fixed immediately in 95% ethyl alcohol. For the second sample the swab with scraped material was dipped and shaken in suspending solution composed of 20ml of 95% ethanol +6 ml acetic acid +74 ml normal saline for 10 minutes and spun in centrifuge for 10 minutes at 2000 rpm. The superficial fluid is poured off and the obtained cell pellet was re-suspended in 95% alcohol and the suspension was poured over a horizontally placed glass slide and it is evenly spread with the help of another glass slide and left for two hours to allow sedimentation of cells. The smears were then stained by PAP stain.

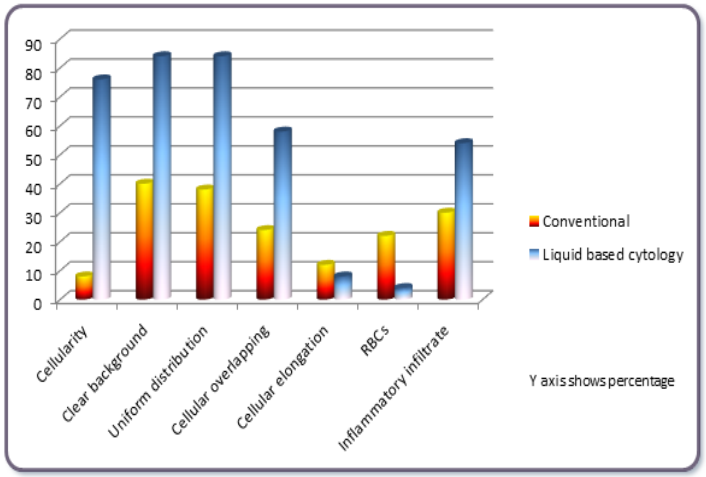
Evaluation of smear quality

Qualitative analysis of the smear obtained through conventional cytology and CLBC was made. Comparison between these two techniques was performed with respect to cellularity, cell distribution, cellular overlapping, cellular background, cellular elongation, the presence of RBCs and inflammatory infiltrate. All slides were evaluated under light microscope and given scoring according to the 'Adequate' (score 1) or 'Inadequate' (score 2) of the particular criteria and the information obtained was subjected to statistical evaluation by means of Wilcoxon Signed rank test. P value ≤0.05 was considered to be significant.

Table 1: Comparison of various criteria between conventional technique and CLBC technique

Criterias	Conventional method (%)	CLBC method (%)	P value*
Cellularity	4 (8)	38 (76)	.000
Clear background	20 (40)	42 (84)	.000
Uniform distribution	19 (38)	42 (84)	.000
Cellular overlapping	12 (24)	29 (58)	.001
Cellular elongation	6 (12)	4 (8)	.507
RBCs	11 (22)	2 (4)	.008
Inflammatory infiltrate	15 (30)	27 (54)	.016

Graph 1: The graph shows statistically significant difference between the CLBC and Conventional cytology.



Results

[Table 1]

Evaluation of smear quality: the stained smears were compared for quality of samples in terms of cellularity, clear background, uniform distribution, cellular overlapping, cellular elongation, presence of rbcs and inflammatory cells.

Assessment criteria

1. Cellularity: Adequate: - if there is presence of more than 40 cells per 10X field.

Inadequate: - if there is a presence of less than 40 cells per 10X field.

2. Cell Distribution: Adequate: -If there is uniform distribution of more than 70% of the cells in a given slide

Inadequate: - If there is no uniform distribution of more than 70% of the cells in a given slide

3. Cellular Overlapping: - Adequate: - If the clarity of the cell morphology and cell outline will be hampered in more than 70% cells

Inadequate: - If the clarity of the cell morphology and cell outline won't be hampered in more than 70% cells

4. Clear Background: - Adequate: - If cell morphology and cell outline will not hampered with background staining in more than 70% of the cells

Inadequate: - If cell morphology and cell outline will hampered with background staining in more than 70% of the cells

5. Cellular Elongation: -Adequate if elongation is seen in 70% of the cells in a given slide.

6. Presence Of RBCs: - Presence/Absence of RBCs

7. Inflammatory Infiltration: - Presence/Absence of inflammatory cells

Cellularity

Of 50 cases, adequate cellularity was seen in 38 cases (76%) using CLBC method in contrast to 4 cases (8%) with the conventional method. Highly significant difference was observed between two techniques. (p=0.000).

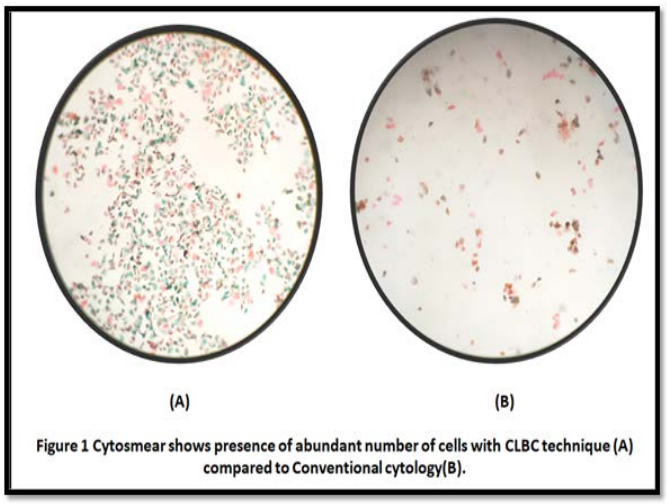


Figure 1 Cytosmear shows presence of abundant number of cells with CLBC technique (A) compared to Conventional cytology(B).

Clear Background

A clear background was seen in 42cases (84%) using CLBC method in comparison to only 20 cases (40%) showing a clear background in the conventional method. CLBC showing significantly higher scores compared to conventional methods. (p=0.000)

Uniform Distribution

The CLBC method gave us better results in 42 cases (84%) in comparison to conventional method 19 cases (38%) in terms of uniform distribution of the cells, which was showing highly significant results. (p=0.000).

Cellular Overlapping

Of 50 cases, CLBC shows 29 cases (58%) with the cellular clumping compared to conventional method showing 12 cases (24%) which shows significant results. (p=0.001)

Cellular elongation

Cellular elongation was seen in 4 cases (8%) with CLBC method as compared to 6 cases (12%) with the conventional methods. CLBC has slightly better results as compared to conventional method, yet they were not significant statistically. (p=0.507)

Presence of RBCs & Inflammatory infiltrates

Only 2cases (4%) of CLBC technique shows presence of abundant RBCs as compared to 11 cases (22%) with the conventional method which is showing statistically significant results. (p=0.008)

In CLBC method 27 cases (54%) shows inflammatory cells infiltration as compared to the conventional method which is showing only 15 cases (30%) so it shows statistically significant results. (p=0.016).

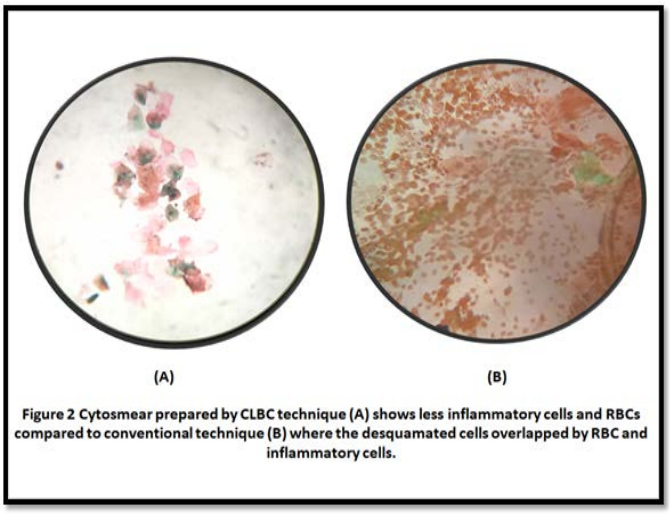


Figure 2 Cytosmear prepared by CLBC technique (A) shows less inflammatory cells and RBCs compared to conventional technique (B) where the desquamated cells overlapped by RBC and inflammatory cells.

Discussion

Exfoliative cytology is an advantageous diagnostic procedure because it is noninvasive, relatively painless, and inexpensive, and requires a minimum of technical skills. Despite its advantages, it has certain disadvantages such as false negative results because of inadequate sampling, inadequate cellularity, poor background or presence of debris and RBCs that hampers the diagnosis. [12] It is shown that a maximum of only 20% of the cells collected on a variety of collection devices can be mechanically transferred to the flat surface of a glass slide. [13] Since liquid-based cytology was developed in the 1990s various comparative studies have shown that it can offer significant advantages over the conventional exfoliative cytology.[14] LBC technology removes most mucus, protein, and red blood cells with the use of glacial acetic acid, distributes cells evenly, improves cell morphology, optimizes sample fixation, provides improved and unbiased sampling, controls cellular density, enhances nuclear detail, reduces scanty preparations, and eliminates air-drying artefacts in oral samples.[15] In a Brazilian study, the liquid-based preparations resulted in higher specimen resolution as well as presented a better cytological morphology for pemphigus vulgaris, squamous cell carcinoma, herpes simplex virus lesions, and fungal infections.[16] But LBC requires expensive automated devices and materials, and trained users for interpretations, which might not be affordable for many cytopathology laboratories in countries with poor resources.[17] In cervical uterine cancer screening, the liquid-based preparations have demonstrated a significant reduction in false-negative rates as compared with those of conventional smears.[18][19] In the present the centrifuged liquid-based cytology (CLBC) technique was applied using simple and readily available equipment to evaluate the efficiency of CLBC over conventional cytology using seven different criteria. The technique for processing of the specimen and preparation of smear was standardized by conducting several trials prior to scoring. The cells collected from the mucosal lesion or normal buccal mucosa with the help of the sterile swab were initially flushed in a liquid medium and then centrifuged. Each of the components of the reagent has a definite role. Isopropyl alcohol acts as a good fixative in cytological smears. This is important to preserve the morphology of the cells, as much as possible, in the condition in which they were present before being sampled.[9] Glacial acetic acid acts as a lysing agent and helps in the lysing of erythrocytes. Lysing of erythrocytes prior to slide preparation results in smears that are easier to interpret because of better visualization of epithelial cells and thus,

it enhances the clarity of the background. Physiological saline is iso-osmolar, which maintains the cells in a proper osmolarity condition in order to avoid any osmotic shock and prevent the destruction of epithelial cells.[20] Centrifugation at 2,500 rpm for 15 min with the sample dispersed in the reagent causes sedimentation of the cells at the bottom forming the cell button, whereas all the debris and mucus form the supernatant solution that can be discarded.[9] We found statistically significant difference with parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, presence or absence of RBCS and inflammatory infiltrate in our study in CLBC in comparison to the conventional technique. Only one parameter i.e., cellular elongation shows insignificant result. Dwivedi et al. performed a similar study on normal mucosa, hyperkeratotic lesions, ulcerated and atrophic lesions and found insignificant difference between the two techniques in terms of cellularity of smears. The authors attributed it to the inadequate scraping of the buccal mucosa in ulcerated areas as it caused discomfort to the patients. Additionally, in their study the cells were lost due to errors in sample processing.[9].In the present study, it was observed that CLBC technique (76%) offers better results than conventional technique (8%) in terms of cellularity. In CLBC method, as the sample collected was flushed out in a suspending solution, the number of cells lost due to adherence to the swab was minimized. Centrifugation technique, which was implemented in our study, helped us in getting a cell button with adequate concentration of cells. In a study conducted by Shaila et al., the slides prepared by the conventional wooden spatula method in normal oral mucosa were disregarded due to either excessive clumping or scarcity of cells.[21] Ogden et al. found less cell yield and cell dispersion,[22] whereas Ahmed et al. found a reduced amount of cells done on normal oral mucosa in the conventional method in comparison to the LBC method.[23]. In the present study the sterile swab instead of wooden stapula for the scraping of the cells from the mucosal surface. In a study conducted by Nambiar et al., on apparently normal mucosa from healthy subjects using LBC technique found adequate cellularity in 66% of the cases.[24] Kujan et al. in their study on apparently normal oral mucosa using LBC technique found adequate cellularity in 98% of the cases. However, as LBC is expensive the present method can be adopted as it provides better cellularity than the conventional smear technique using limited resources. [25] In terms of clear background, we have found that CLBC technique (84%) offers better results than conventional technique (40%). Ahmed et al and Hyama et al. also obtained similar results and reported that the scantiness of background staining in CLBC method enhances sensitivity and quality.[26][27]. In a study conducted by Nambiar et al., on apparently normal mucosa when clarity of the smear background was evaluated between the two techniques, most of the samples of CLBC (80%) showed clear background as compared to the conventional methods (48%) and this is concurrent with results obtained in the present study[3] Study done by Dwivedi et al. also showed similar results.[9] This was due to the use of glacial acetic acid in the suspending solution that lyses the red blood cells and centrifugation technique that removes mucin, debris, microbial colonies, and other artifacts present in the background. In terms of uniform distribution, the conventional method does not have a liquid medium for the uniform spreading of cells; scant cells were present in the center and most of the cells accumulated in the periphery. The CLBC method shows uniform distribution of cells compared to conventional technique and shows

similar results with the study done by Nambiar et al. [3] According to Dwivedi et al., the process of resuspending the cell pellet in alcohol and then pouring it over a horizontally placed glass slide led to sedimentation of cells and prevented the uniform distribution of cells in CLBC method, which they followed [9]. The present study method offered smears with uniform distribution compared to the conventional technique, which can be attributed to a small amount of sample taken per slide that was evenly spread with the help of a glass slide. Cellular overlapping was more seen in conventional techniques compared to CLBC technique. This is attributed to the mucus present in the conventional smears which led to more adherences of the cells, which were removed by cytocentrifugation in CLBC technique. Our study shows more cellular overlapping in CLBC technique (58%) compared to conventional technique (24%) this may be due to presence of more number of the cells may led to overlapping or it may occurred due to error in processing. Study conducted by Hedge et al., shows less cellular overlapping in CLBC technique (40%) compared to conventional technique (45%).[28] In terms of cellular elongation, Studies have shown that cell elongation to be a significant drawback with the LBC technique. However, our method revealed less cellular elongation (8%) as compared to the conventional technique (12%). Carefully performed centrifugation will not cause any significant distortion in cellular morphology of exfoliated cells and will not have any adverse effect on the diagnostic efficacy of the smear as evident with our smears. [9] In the study by Nambiar et al., the cellular elongation was observed only in 4% of cases with CLBC technique as compared to conventional technique which is 70%.[3] RBCs were present in a dense amount in conventional smear (22%) which were drastically reduced in CLBC technique (4%). This has been attributed to due to use of glacial acetic acid acts as a lysing agent and helps in the lysing of erythrocytes. Lysing of erythrocytes prior to slide preparation results in smears that are easier to interpret because of better visualization of epithelial cells and thus, it enhances the clarity of the background. Similar results obtained by Hedge, et al., showed that out of 90 cases, RBCs were seen only in 4% cases by conventional technique and in CLBC technique no RBCs were seen.[28] In terms of inflammatory cell infiltration, CLBC technique showed presence of more cells (54%) compared to conventional technique (30%). In the present study, CLBC technique shows more inflammatory infiltrates compared to conventional method as swab was collected from the oral lesions. Davey et al. and Dwivedi et al. conducted similar studies and reported that there was no evidence that LBC reduced the proportion of unsatisfactory slides in comparison to the conventional technique. [29][9]. But in the present study, it was found that there is a statistically significant difference between the two techniques and also proved CLBC shows better results than the conventional method.

Conclusion

Results shows that CLBC offer a significant advantage over conventional smear preparation as CLBC technique can offer better smears using materials already available in the laboratory setup; it is cost effective and hence can be implemented in laboratories with limited resources. CLBC may be useful for advanced procedures like immunocytochemistry especially in laboratories with limited access to expensive automated systems. Further studies with modifications and improvements may help in making this technique more useful.

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