Article published online: 2021-11-01

ORIGINAL ARTICLE

Comparison of centrifuged liquid based cytology method with conventional brush cytology in oral lesions

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ABSTRACT

Background: Exfoliative cytology is the study of cells that are shed or scrapped off from mucosal surfaces. Centrifuged Liquid based cytology is a modified technique employed in the present study. **Aims:** To compare the utility of centrifuged liquid based cytology with conventional cytology in oral lesions after staining with Papanicolaou (PAP) stain. **Materials and Methods**: 50 cases of oral lesions comprising of normal mucosa (n=14), hyperkeratotic lesions (n=17), ulcerated lesions (n=7) and atrophic lesions (n=12) were selected. Two smears were obtained from the lesion using a cytological brush. One was spread on the slide using conventional technique, fixed immediately in 95% ethyl alcohol. Second sample was suspended in suspending solution for 10 minutes then spun in centrifuge for 10 minutes. The supernatant was poured off and the obtained cell pellet was used to prepare a smear by sedimentation and left to dry overnight. Both the smears were stained by PAP. The stained smears were compared for seven morphological parameters such as adequacy of smear, clear background, cell distribution, smear thickness, cell morphology, and presence of blood, inflammatory cells, microbial colonies and artifacts. Wilcoxon Signed rank test was used and $P \leq 0.05$ was considered statistically significant. **Results:** There was a statistically significant difference ($P<0.001$) between centrifuged liquid based cytology and conventional cytology when clear background was evaluated while in all other parameters the difference was not significant. **Conclusion:** Centrifuged Liquid based cytology showed clearer background than conventional brush cytology in oral lesions.

Key words
Brush cytology, liquid based cytology, oral lesions, PAP

INTRODUCTION

Oral Exfoliative cytology 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, especially in population-based screening programs, where repeated samples might be required.[2] Early detection of a pre-malignant or cancerous oral lesion can improve the survival and the morbidity of patients suffering from these conditions.[3] Exfoliative cytology for screening of oral cancer and precancer has never achieved the same success as it has in cervical screening. Nevertheless, the cytologic smear has been used in the diagnosis of certain types of oral lesions, most of them related to viral and fungal diseases.[4]

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.105386

Liquid-based cytology (LBC), since its inception in the 1990s, has shown significant advantages over conventional exfoliative cytology. Studies on cervical cytology have shown that the LBC reduces the problems related to sampling, helps in preparation of better smears, and reduction in false-negative rates.[5,6] This technique has been shown to result in slides with a high cellularity dispersed in a homogeneous thin layer.[7]R.B.Cs, inflammatory cells and mucous are reduced and cells are distributed randomly throughout the slide. 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.[8]

LBC gives better results, as it is 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.[9,10] In most published series, LBC allows a good inter observer reproducibility.[11] However, LBC requires expensive automated devices and materials, which might not be affordable for many cytopathology laboratories with limited resources.

For many years, LBC has been developed for cervical cancer screening and not oral cancer, as it requires automated devices. The aim of this study was to compare the utility of a modified technique, centrifuged liquid based cytology (CLBC) with that of conventional brush cytology in oral lesions.

MATERIALS AND METHODS

The study sample for this comparative study was collected from 50 subjects with either normal oral mucosa (n=14), hyperkeratotic lesions, (n=17) ulcerated lesions (n=7), or atrophic lesions (n=12) reporting to outpatient department of Saraswati Dental College and Hospital, Lucknow. Two smears were obtained from the lesion using a cytological brush. One was spread on the slide using conventional technique and fixed immediately in 95% ethyl alcohol. For second sample the brush 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 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. The smears were then stained by PAP stain [Figure 1].

Evaluation of smear quality

The stained smears were compared for quality of sample in terms of cellularity, cell distribution, cellular clumping, cell morphology, and presence of blood and microbial colonies. Each criteria was graded and scored individually using the grading criteria suggested by Alves *et al.*[12] with some modifications as given below.

Assessment criteria

Cellularity

0. No cells
1. Scant cells
2. Adequate cells
3. Abundant cells (≥5,000 cells).

Clear background

0. Abundant debris present (inadequate for diagnosis).
1. Debris present but adequate for diagnosis.
2. Clear background.

Uniform distribution

0. Cells restricted to only one area of the slide

Figure 1: Photograph showing materials used for Centrifuged Liquid Based Cytology

1. Few areas with cells
2. Cells distributed evenly throughout slide.

Cellular overlapping

0. Cells present only in clumps
1. Few areas with clumping seen
2. Minimal overlapping of cells.

Cellular elongation

0. Marked change in morphology
1. Some change in morphology but adequate for diagnosis
2. No change in morphology.

Blood

0. Abundant blood obscuring the cells
1. Some blood present but adequate for diagnosis
2. No blood present.

Microbial colonies

0. Abundant colonies obscuring the cells
1. Some microbial colonies present but adequate for diagnosis
2. No microbial colony present.

All the slides were scored blindly by two observers and statistical evaluation was done using Wilcoxon Signed rank test and $P \leq 0.05$ was considered significant.

RESULTS

No adverse effect of fixation or staining, that could interfere with the final evaluation was observed. All the scores indicated categorical up gradation from lower to higher order.

The overall observations were as follows [Figure 2].

Cellularity

No significant difference was observed between two techniques either overall or for different types of specimen studied ($P>0.05$). Though in hyperkeratotic and ulcerative lesions, conventional technique had slightly better results yet they were not significant statistically. In normal oral mucosa specimen, CLBC had slightly better results as compared to conventional technique, but here again the difference was not significant statistically.

Background

Overall statistically significant difference between two techniques was seen ($P<0.001$) with CLBC showing significantly higher scores (1.68±0.47) as compared to brush cytology (1.26±0.44). It was seen that the percentage of smears with clear background was almost two and half times in CLBC (68%) as compared to brush cytology (26%). Among different types of specimen too this difference was evident. Amongst atrophic lesions liquid cytology had 7 specimen (58.3%) with clear background as against only 2 (16.7%) with brush cytology thereby liquid cytology showing a higher mean score of 1.58±0.52 as compared to brush cytology with 1.17±0.39, however, the difference was not statistically significant ($P=0.059$), similar trend was seen in ulcerative lesions but in case of hypertrophic lesions and normal tissue, the difference was not only proportionally higher but was also significant statistically too [Figure 3].

Cellular distribution

No statistically significant difference between two techniques was seen either overall or amongst different types of specimen. Though the scores of brush cytology were slightly better for atrophic and hyperkeratotic lesions, for ulcerative lesions these were poor as compared to CLBC.

Cellular overlapping

No statistically significant difference was seen between the two techniques in either overall or different types of lesions.

Cellular elongation

Statistically no significant difference between two techniques was seen overall, however in hyperkeratotic lesions, CLBC had less morphological change as compared to conventional smear, and this difference was statistically significant.

Presence of blood

No statistical difference between two techniques was seen either overall or in different types of specimen. However, in case of ulcerative lesions CLBC demonstrated some superiority over conventional technique yet the difference were not significant statistically.

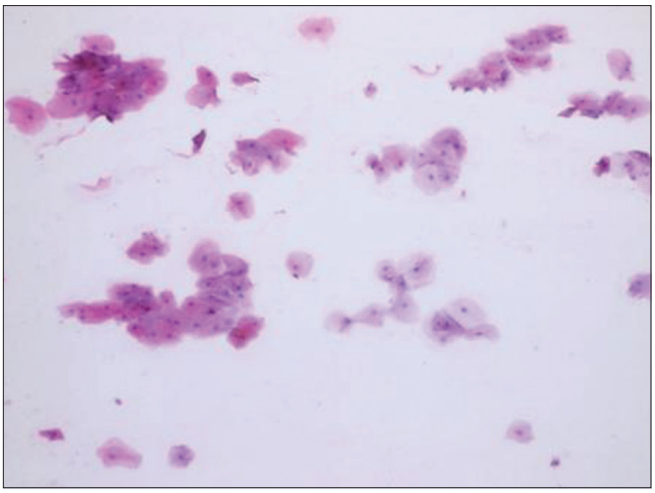

Figure 2: Photograph showing clear background with smears prepared by CLBC

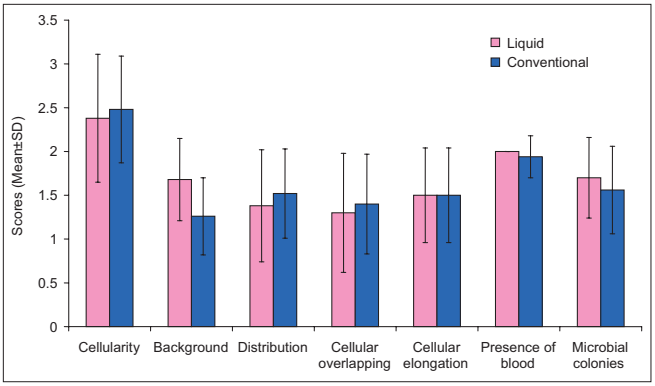

Figure 3: The graph shows a statistically significant difference between the CLBC and conventional cytology in clear background ($P\leq0.01$) while in all other parameters the difference was not statistically significant

Presence of microbial colonies

Both the techniques seemed to have similar efficacy regarding presence of microbial colonies with only slight differences in mean scores. No particular trends were seen either for any specific type of lesion or for overall diagnostic efficacy.

DISCUSSION

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.[5] The revolutionary modification of the cervical smear method by using liquid-based cytology (LBC) has been shown to reduce substantially the problems experienced with conventional smears such as presence of mucous, debris, blood and artifacts in the background and has resulted in significant improvement in cyto diagnostic accuracy.[13-15] 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.[16,17] Probably owing to the paucity

of studies on liquid-based cytology for examination of the oral mucosa, the conventional method for preparation of exfoliative cytology smear is still the standard practice as the automated system required for LBC is not readily available in many small laboratories.

In this study we aimed to obtain smears by a modified technique i.e. centrifuged liquid based cytology (CLBC) using simple and readily available equipments. The technique for processing of the specimen and preparation of smear was standardized by conducting several trials prior to scoring.

When various parameters were compared no statistically significant difference was found between the two techniques in terms of cellularity of the smear but overall we found conventional brush cytology smears comparatively having better cellularity than CLBC. Brush cytology resulted in only 6% smears with scant cells as compared to 14% in CLBC. Most of the smears with scant cells in either technique were those of ulcerated lesions (57.1%) probably because of lack of proper scraping due to discomfort experienced by the patient in taking the sample. Lower cellularity in CLBC may also be due to loss of cells during sample processing. But in general majority of CLBC (86%) smears yielded sufficient number of cells for making a diagnosis.

CLBC score better in terms of having a clear background with 68% of smears had a clean background with minimum debris as compared to only 26% in conventional smear and this difference was statistically significant. In all the slides of CLBC there was no blood present as compared to brush cytology with 6% slides with some blood. Lastly, both the techniques showed presence of microbial colonies but it was observed that in CLBC the microbial colonies were present in lesser numbers while in conventional brush cytology smear the number of colonies was higher though this difference was not statistically significant. We also observed that in liquid based preparations the microorganisms were usually those associated with the epithelial cells while in brush cytology smears the colonies were present in the background also. In one of the conventional smears the microbial colonies were so abundant that it completely obscured the cells precluding its use for diagnostic purpose. These results exhibit the superiority of CLBC in terms of providing a clean and clear background. The use of acetic acid in the suspending solution and cytocentrifugation clearly removes mucin, blood, debris, microbial colonies, and other artifacts present in the background as reported in earlier studies.[18,19]

In terms of uniform distribution of cells, we found no statistically significant difference between the two techniques. In fact in our results we found the scores of brush smears to be slightly higher than CLBC. This goes against the popular notion that liquid based cytology results in better distribution of cells. This difference could be because in most studies done on liquid based cytology automated systems for preparing thin smears were used which have been shown to result in a uniform distribution while we used a manual technique which might have resulted in less than desirable uniform distribution of cells.

It was observed that 12% of centrifuged liquid based smears showed so much of clumping that limited their diagnostic utility as compared to 4% of conventional smears. Though the reason for this finding is not clear but it could be possible that manual teasing of the cells during spreading of sample in conventional smear preparation helps in separating the cells and reducing the clumping while in our technique of CLBC the cells were allowed to sediment passively on the slide which might have caused the cells in clumps to remain as such. Again the use of automated instruments may result in formation of a uniform monolayer of cells but some cellular clumping is observed even in these automated systems.[12]

In terms of change in morphology no statistically significant difference was seen in both CLBC and brush cytology technique showing that a carefully performed centrifugation will not cause any significant distortion in cellular morphology of exfoliated cells and hence will not have any adverse effect on diagnostic efficacy of the smear. Although care has to be taken to avoid overzealous centrifugation to separate the debris, as it might lead to changes in cellular morphology.

Delavarian *et al.* evaluated the diagnostic value of a modified liquid-based cytology using OralCDx® Brush in oral potentially malignant lesions and oral cancer and found that modified technique was a useful tool for screening of oral premalignant and malignant lesions. In contrast, in our study we used simple cervical smear brush along with manual cytocentrifugation than automated liquid based cytology systems and the results suggested that except for clearer background there was no added advantage of CLBC as compared to brush cytology.[20]

This finding is concurrent with the findings of Davey *et al.*, they also reported that there was no evidence that liquid-based cytology reduced the proportion of unsatisfactory slides, or detected more high-grade lesions in high-quality studies, than conventional cytology.[21] Also in a randomized control trial conducted to compare liquid-based cytology and the Pap test with respect to testing positivity rates, histologically confirmed detection rates, and positive predictive values, it was found that liquid-based cytology is neither more sensitive nor more specific than the PAP test in detecting CIN or cancer.[22]

Thus overall in our study no technique was found to have statistically significant advantage over the other in overall assessment. Although CLBC scores better in

terms of clearer background but in all other parameters difference was not statistically significant.

CONCLUSION

To conclude with the results of our study show that although CLBC does not offer a significant advantage over conventional smear preparation to advocate its use for routine diagnostic and mass screening procedures but the clean, debris, blood and microbe free background achieved by this technique may be useful for advanced procedures like immunocytochemistry specially 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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How to cite this article: Dwivedi N, Agarwal A, Raj V, Kashyap B, Chandra S. Comparison of centrifuged liquid based cytology method with conventional brush cytology in oral lesions. Eur J Gen Dent 2012;1:192-6.

Source of Support: Nil, **Conflict of Interest:** None declared.