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Original Article

Efficacy of centrifuged liquid-based cytology over conventional cytology: A comparative study

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ABSTRACT

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

Aims: To compare the efficacy of CLBC with conventional cytology in apparently normal mucosa and histologically proven cases of oral squamous cell carcinoma after staining with Papanicolaou stain.

Materials and Methods: The study sample was collected from fifty individuals with no habits and apparently normal oral mucosa (Group 1) and forty cases of histologically proven cases of oral squamous cell carcinoma (Group 2). One smear was taken and spread on the slide by a conventional technique. The second sample was flushed out in a suspending solution, centrifuged, and the cell pellet obtained was used to make the smear. The stained smears were compared for nine parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, cellular elongation, mucus, inflammatory blood, and microbial colonies. Chi-square test was used for statistical analysis and $P \leq 0.05$ was considered statistically significant.

Results: There was a statistically significant result with parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, and cellular elongation in CLBC technique, in comparison with the conventional technique. The presence of mucus, microbial colonies, and inflammatory cells were also less in CLBC technique in comparison with the conventional technique.

Conclusion: CLBC has better efficacy over conventional method in all the parameters analyzed.

KEY WORDS: Centrifuged liquid-based cytology, conventional technique, exfoliative cytology, oral squamous cell carcinoma, Papanicolaou stain

INTRODUCTION

Oral cancers are a major health problem in India. Early diagnosis greatly increases the probability of cure with minimum impairment and deformity.[1,2]

Exfoliative cytology is one of the early diagnostic modes of detection, where the microscopic examination of a shed or desquamated cells from the epithelial surface is done.[3-5]

Liquid-based cytology (LBC) has been designed to improve the quality of conventional cytology.[6-8] LBC requires expensive automated devices which might not be affordable for many cytopathology laboratories.[4]

Centrifuged LBC (CLBC) which is a modification of LBC. It is cost-effective, yet efficient technique and uses simple and readily available equipment, provides debris, blood, and microbes free background.[4]

MATERIALS AND METHODS

In this prospective comparative study, the efficacy of CLBC over conventional cytology was studied, so that this technique can be applied in premalignant and malignant lesions as well.

The study sample was collected from fifty individuals with no habits and apparently normal oral mucosa (Group 1) and forty cases of histologically proven cases of oral squamous cell carcinoma (Group 2). The subjects were informed

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with regard to research objectives, methods, possible benefits, and potential risks, and written consent was obtained from all patients.

Two cytological smears were obtained, from normal buccal mucosa (Group 1) and the lesional area (Group 2) using a soft toothbrush. One smear was made using the conventional technique and fixed immediately in 95% ethyl alcohol. The second sample was flushed out in a suspending solution composed of 20 ml of 95% ethanol, 6 ml acetic acid, and 74 ml of normal saline. This was centrifuged for 10 min at 2000 rpm. The obtained cell pellet was resuspended in 95% alcohol, and smear is prepared with the help another slide and left for 2 h following which both smears were stained by Papanicolaou method.

Evaluation of smears

Qualitative analysis of the smear obtained through conventional brush cytology and CLBC were made. Comparison of smears obtained from the normal oral mucosa and patients with oral squamous cell carcinoma in both the techniques was carried out with respect to cellularity, cell distribution, cellular clumping, cellular morphology, the presence of blood, mucous, inflammatory cells, and microbial colonies. All the slides were evaluated blindly by two independent observers and information obtained was subjected to statistical evaluation by means of Chi-square test. $P < 0.005$ was considered significant.

RESULTS

Statistically significant results were obtained in terms of cellularity, clarity of the background, uniform distribution, cellular overlap, cellular elongation, and mucus content.

However, the presence of inflammatory cells, blood, and microbial colonies did not show any statistically significant difference between the two techniques, in control as well as in patients with oral squamous cell carcinoma.

DISCUSSION

Incidence of oral premalignant lesions and oral cancers is very high in India as compared with Western population. Though histopathology is considered as gold standard in diagnosing these lesions, it may not be feasible to perform a biopsy in all suspected cases (the patient may be medically compromised).[9]

The seminal work by Papanicolaou and Traut in studying the cells from precancerous and cancerous lesions of the cervical mucosa paved the way for oral cytology.[6,10] Exfoliative cytology is an advantageous diagnostic procedure because it is noninvasive, relatively painless, and inexpensive and requires a minimum of technical skills.[9] However, over a period, as the field of oral cytology started to grow, they experienced certain limitations and, therefore, felt the need for improvements.[6]

LBC offers significant advantages over the conventional exfoliative cytology.[4] LBC technology removes most mucus, protein and red blood cells with 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.[11] In a study in Brazil, 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.[6] However, 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.[11]

The revolutionary modification of LBC with a significant improvement in cytodiagnostic accuracy with increased sensitivity is CLBC. The efficiency of the inexpensive CLBC method relies on cytocentrifugation.[4]

Here, we have compared the conventional technique with CLBC technique in both groups. (Group 1 - apparently normal mucosa and Group 2 - histologically diagnosed cases of oral squamous cell carcinoma). The cells collected from the buccal mucosa with the help of a brush was initially flushed in a liquid media 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 they were present before being sampled.[4] Glacial acetic acid acts as a lysing agent and helps in 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 to avoid any osmotic shock and prevent the destruction of epithelial cells.[12] Centrifugation at 2500 rpm for 15 min with the sample dispersed in the reagent causes sedimentation of the cells at the bottom forming cell button, whereas all the debris and mucus form the supernatant solution that can be discarded.[4]

We found statistically significant difference with various parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, and cellular elongation in our study with CLBC in comparison with the conventional technique.

Kujan *et al*. in his 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.[13] Ahmed *et al*. in his study on oral lesions using CLBC technique found optimal cellularity and stated that this method gave better diagnostic accuracy when compared to the conventional method.[11] Shah and Deshmukh conducted a study on exfoliative cytology and cytocentrifugation on the oral premalignant lesion and malignant lesion, where 80% of smears obtained with cytocentrifugation showed high cellularity.[14]

CLBC technique (67%) offered smears with adequate cellularity than the conventional technique (34%). This is attributed to the CLBC technique where sample collected through the brush was flushed out, followed by centrifugation of the sample which resulted in pellet with a better concentration of cells in comparison with the conventional method. Less cell yield obtained in conventional technique may be due to loss of cells to the brush [Figures 1 and 2].[13]

Most of the samples of CLBC (85%) showed clear background with minimum mucus, inflammatory cells, and microbial colonies as compared to the conventional method (30%) [Figures 3 and 4]. Optimum results obtained in CLBC are due to the reagent used and centrifugation of the sample. There was no evidence of erythrocytes in any of the slides present in CLBC as compared to conventional technique. This result is similar to the study done by Dwivedi *et al*. and is attributed to glacial acetic acid used in the suspending reagent which will lyse all the erythrocytes.[4] CLBC also showed complete removal of mucus from the smears than conventional technique because of the reagent used in CLBC technique helps in removal of mucus from the smears and enhances clarity and brings about less cohesiveness of cells. Mucin, debris, and microbial colonies formed a supernatant solution in the cytocentrifugation technique which were eliminated and hence increased the background clarity and cellular details [Figures 5 and 6]. Shah and Deshmukh also

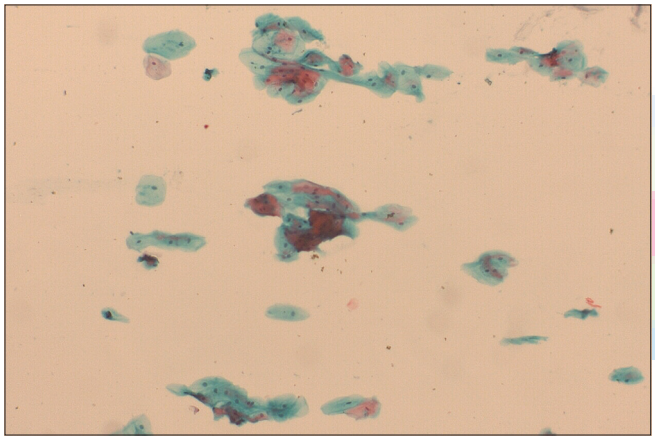

Figure 1: Photomicrograph showing cellular clumping, elongation, and overlapping with less uniform distribution of cells in conventional technique (Group 1 - normal mucosa)

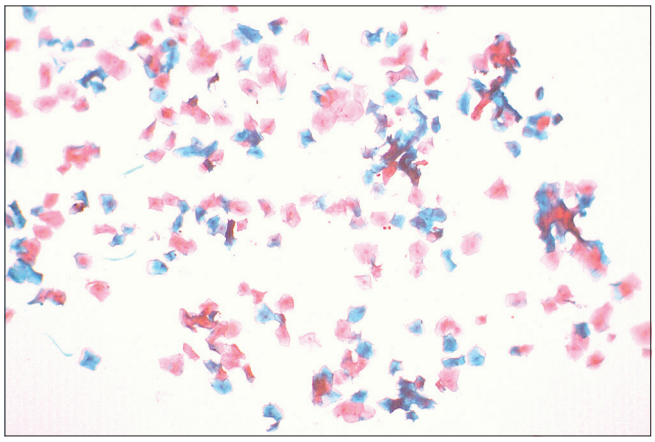

Figure 3: Photomicrograph showing less cellular clumping, elongation, and overlapping with uniform distribution of cells (Group 1 - normal mucosa)

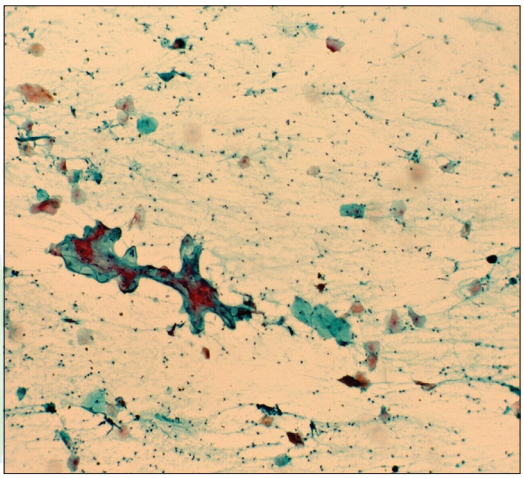

Figure 2: Photomicrograph of smear obtained with conventional technique showing less cellularity, unclear background, clumped cells, cellular elongation, and dense inflammatory infiltrate (Group 2 - oral squamous cell carcinoma cases)

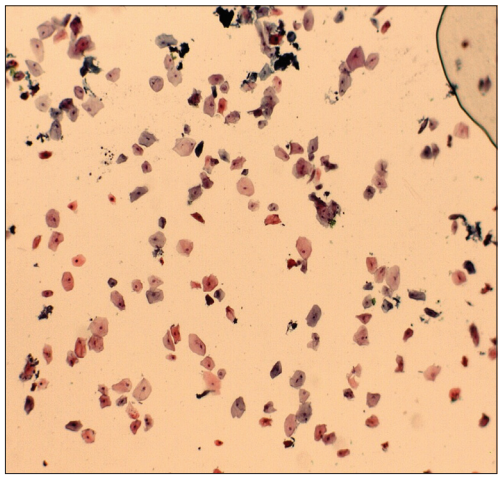

Figure 4: Photomicrograph of smear showing obtained with centrifuged liquid based cytology technique showing adequate cellularity, clear background, less cellular clumping, and less inflammatory infiltrate (Group 2 - oral squamous cell carcinoma cases)

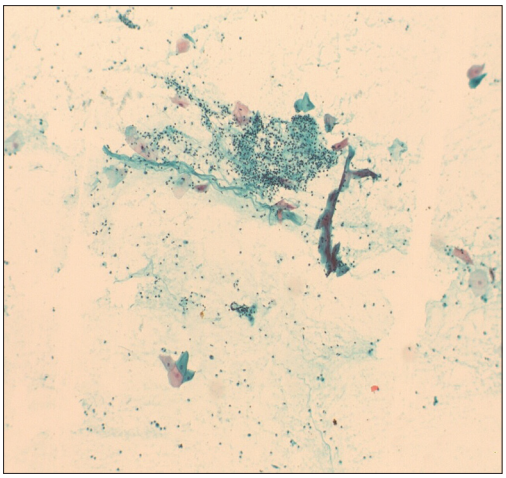

Figure 5: Photomicrograph of smear obtained with conventional technique showing less cellular areas of epithelial cells with unclear background with dense localized collection of inflammatory cells obscuring the details of the epithelial cells, cellular clumping can also be seen (Group 2 - oral squamous cell carcinoma cases)

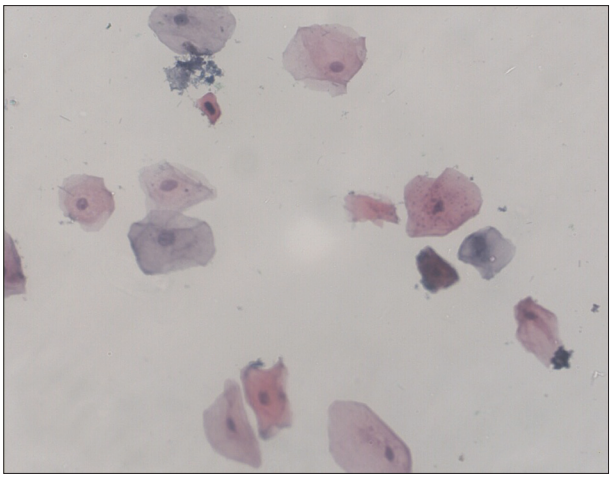

Figure 6: Photomicrograph of smear obtained with centrifuged liquid based cytology technique showing uniformly distributed epithelial cells in clear background. Less of cellular clumping and mild inflammatory cells can also be seen (Group 2 - oral squamous cell carcinoma cases)

concluded that cytocentrifugation reduces the amount of debris which is an integral part of exfoliative cytology.[14]

Conventional method does not have a liquid media for uniform spreading of cells; hence, scant cells were present in the center and most of the cells accumulated in the periphery. 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.[4] In our method, CLBC technique (40%) offered smears with uniform distribution than the conventional technique (8%) which can be attributed to a small amount of sample taken per slide which was evenly spread with the help of a glass slide.

Studies have shown that cell elongation to be a significant drawback with the LBC technique. However, our method revealed much less cellular elongation as compared to the conventional technique. 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.[4] Cellular overlapping and cellular elongation were more seen in conventional technique (45%) of the cases, but it was less seen in CLBC technique (40%). This is attributed to the mucus present in conventional smears which led to more adherences of the cells, which were removed by cytocentrifugation in CLBC technique.

Microbial colonies and inflammatory cells were present in a dense amount in conventional smears which were drastically reduced in CLBC technique. This has been attributed to discarding the supernatant fluid which contained microbial colonies and debris. However, statistically no significant difference was observed between both the techniques.

Table 1: Comparison of parameters between CLBC and conventional technique

Criteria	Normal mucosa and OSCC (*n*=90) (%)		*P*
	Conventional technique	CLBC	
Cellularity	31 (34)	61 (67)	0
Clear background	27 (30)	77 (85)	0
Uniform distribution	8 (8)	38 (40)	0
Cellular overlap	59 (45)	36 (40)	0
Cellular elongation	41 (45)	6 (6)	0
Mucus	38 (42)	0	0
Inflammatory cells	40 (44)	37 (41)	0.651
Blood	4 (4)	0	0.043
Microbial colonies	13 (14)	12 (13)	0.829

OSCC=Oral squamous cell carcinoma, CLBC=Centrifuged liquid based cytology

Davey *et al.* and Dwivedi *et al*. with similar studies done reported that there was no evidence that LBC reduced the proportion of unsatisfactory slides in comparison with the conventional technique.[4,15] However, in our study, we found statistically significant difference between the two techniques and it proves that CLBC technique is better than the conventional method. A modification in the CLBC method which we incorporated rendered better results than the previous studies.

CONCLUSION

CLBC method is strongly advocated in the best interest of public health as it improves the sample quality and reduces the likelihood of false negative results in comparison with the conventional technique, hence recommended for routine diagnostic purposes. Good clarity of background with adequate cellularity with evenly dispersed cells can be useful for diagnostic augmentation and advance procedures such as immunochemistry. Further implementation of this study on larger sample size with skilled professionals in cytology may help in overcoming the drawbacks obtained in this technique. As this method is relatively technique sensitive, improvement on this front can yield better results.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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