Original Article

Evaluation and Comparison of Conventional Brush-based and Centrifugation Liquid-based Cytopathology

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Abstract

Background: Brush cytopathology is a traditional method of collecting shed cells by scrapping off the mucosal surface of the oral cavity. Liquid-based cytopathology by centrifugation is a technique that causes the cells to be suspended in a monolayer enabling better morphological assessment. **Aims:** The aim of this study is to compare the efficacy of conventional brush cytopathology and centrifugation-based liquid cytology (CBLC) in oral lesions after staining with rapid Papanicolaou (PAP) stain. **Materials and Methods:** Forty cases of oral lesions comprising normal mucosa (n = 10), hyperkeratotic lesions (n = 11), ulcerated lesions (n = 15), and inflammatory lesions (n = 14) 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. The second sample was suspended in prepared fixative solution for 10 min and then spun in centrifuge for 10 min. 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 rapid PAP (Biolabs Pvt. Ltd.). The stained smears were compared statistically for cellular yield, cell distribution, cell morphology, and background noise (presence of blood, inflammatory cells, microbial colonies, and artifacts). Results and **Conclusion:** The efficacy and quality of the smears based on the liquid-based preparations indicated its superiority in obtaining a higher yield of cells and clearer background. The increased cellular yield in liquid-based preparations also showed increased clumping and overlapping of cells which proved as a drawback for CBLC.

Keywords: *Brush cytology, centrifugation, manual liquid-based cytology, Papanicolaou*

Introduction

The high mortality rate from oral cancer has been known to be due to several factors. Undoubtedly, the most significant out of them is delayed diagnosis. Studies have demonstrated that the survival and cure rate dramatically increase when oral cancer is detected in its precancerous stage or as an early-stage disease. Given the significant morbidity and mortality associated with advanced oral cancer and its treatment, there has been a compelling need to provide clinicians with an accurate diagnostic technique that will increase the detection of early-stage oral cancer. Advances in the early detection of oral cancer are unfolding.

The usefulness of cytology in the oral cavity was first examined by Montgomery and Von Haam.[1] Oral cytology is considered one of the best ways for the initial evaluation of the oral lesions microscopically due to its simplicity and reliability.[2] Exfoliative brush cytology and centrifugation liquid-based cytopathology (CLBC) are some of the examples of oral cytology, which have the potential to assist the diagnostic portion of the "screening gap" that currently challenges the early detection of many epithelial cancers.

Exfoliative brush cytology is the microscopic evaluation of the desquamated epithelial cells from the mucosal surface. Clumping, overlapping of cells, and smears masked by mucus and debris may yield false-negative observations, resulting in reduction in reliability of these tests.[3]

The advent of liquid-based cytology (LBC) has given a new dimension to the existing cytology techniques. It has shown an array of possibilities to improve the quality of conventional cytology[4] and deliver a better diagnosis. In liquid-based preparations, the sample is collected and transported in a vial containing preservative fluid which allows immediate fixation of the cells.[5] Smears made from the sediment are then stained and diagnosed. The technique generally

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yields a high cellular detail in a homogenously dispersed background and hence increases the sensitivity.[6] LBC has been performed in fine needle aspirates from various organs such as salivary glands, thyroid, lymph nodes, and bones, which are the prime focus of interest for oral and maxillofacial pathologists.[7] Later, it has also been used in investigations such as immunohistochemical analysis of lymphomas, analysis of proliferating cell nuclear antigen, and cell block preparation from the mucosal scrapings.

Previous studies done on smears obtained from the cervical region by the use of LBC have shown a significant ease in sampling and helped in preparing better-quality smears with consequent reduction of diagnostic errors. The present study has been conducted to compare the efficacy and performance of the centrifugation-based liquid cytology (CBLC) to conventional brush cytology (CBC) in various oral lesions.

Materials and Methods

The study group consisted of 44 patients reported to the Department of Oral Medicine and diagnosis of the institution comprised normal mucosa – 11, hyperkeratotic lesions – 10, ulcerative lesions – 15, and inflammatory lesions – 4. The study protocol was approved by the Institutional Ethical Committee. Informed consent was obtained from the patients who participated in this study. Two passages of cytology smears were obtained from the oral site of interest using cytobrush.

Conventional brush cytology

The first passage of smear was spread on the glass slide using conventional technique, followed by air drying for 5 min and then fixation by 95% ethyl alcohol for 8 min. It was then stained by rapid Papanicolaou (PAP) stain.

Centrifugation-based liquid cytology

The second passage of the scrapping was flushed out in the vial containing the preservative solution by suspending the brush head into it. The preservative solution was composed of 20 ml of 95% alcohol, 6 ml of acetic acid, and 74 ml of normal saline. The brush head containing the sample was suspended in the vial for 10 min in a stable platform before proceeding with the processing. The vial was subjected to rotation at 2000 rpm for 10 min in a centrifuge. The supernatant liquid was then decanted, and the cell pellet was obtained as a whole and resuspended in 95% alcohol. The suspension was dropped on the clear glass slide with the help of a measuring dropper and allowed to sediment for 15 min. After this, the smear was prepared and stained with rapid PAP stain (Biolabs PVT. Ltd.).

Evaluation of smears

Qualitative and quantitative analyses of the smears obtained through conventional technique and CBLC were done. The assessment of the parameters was done by observing five randomly selected fields (×40). The efficacy of these two techniques was compared and evaluated based on cellularity, cellular overlapping, altered cytomorphology, and background. Cellularity depicts the yield of the cells in a slide for observation. Cellular overlapping was determined by the ratio (cellular overlapping ratio [COR]) of number of overlapping cells seen to the total number of cells seen per field. Altered cytomorphology was determined by the ratio (altered cytomorphology ratio [ACR]) of number of cells showing altered cytomorphology to the total number of cells seen per field. The criteria for assessing the cellular yield were 12–54 cells (sufficient), 55–98 cells (adequate), and 99–139 cells (abundant), and the background characteristics have been depicted as intense, adequate, and clear.

All the slides were scored twice after evaluation by a single observer. Statistical evaluation was done using Mann–Whitney U-test for assessing the COR and ACR and Chi-square test for assessing the cellular yield and the background of the two techniques in SPSS 13 (IBM).

The evaluation and comparison of the two techniques have been done based on the assessment of the cellular yield/cellularity, COR, ACR, and background characteristics. CLBC showed a statistically significant improvement in the yield of cells as well as a tendency toward achieving a better background. The observations based on analysis of the various parameters are discussed below.

Cellularity

The yield of the cells was found to be more in case of CLBC. There was a statistically significant difference in the sample adequacy between these two techniques found in normal mucosa, ulcerative lesions, and inflammatory lesions. Hyperkeratotic lesions did show a higher yield of cells by CLBC, but the observations were not statistically significant [Figure 1a and Table 1].

Cellular overlapping

The overlapping of cells was found to be consistent in all the slides. There was a significant higher overlapping of cells observed in cases of normal mucosa and hyperkeratotic lesions using liquid-based preparations. There were no significant differences seen in the cases of normal mucosa and inflammatory lesions [Figure 1b and Table 2].

Altered cytomorphology

Change in the shape or cellular distortion is a common finding seen in smears. In almost all cases, it showed equal occurrence in both the techniques. The CLBC showed greater CORs among the ulcerative lesions [Figure 2a and Table 3].

Background

On overall observations from all the different groups, it is observed that CLBC showed a better background in most of the cases. The probability of getting a clearer

background is above 65% in all the groups using LBC. Among the different groups, the difference in obtaining a better background was too evident [Figure 2b]. There was a statistically significant difference noted in cases of normal mucosa and ulcerative lesions.

In ulcerated and inflammatory lesions, the conventional smear showed red blood cells (RBCs), microbial colonies, and inflammatory cells in the background, which obscured the view of the squamous cells [Figure 3A1 and B1], but on using the liquid-based preparations, the RBCs and inflammatory cells were totally absent in the background [Figure 3A2, B2 and Table 4].

Discussion

The prevalence and incidence of oral premalignant lesions and oral cancers in India are higher compared to the west. Scalpel biopsy is always considered as the best method to render diagnosis, but due to the lack of feasibility in few situations, cytology act as an adjunct procedure to biopsy.[8]

Since 1990, a lot of research has been done on the LBC, and various comparative analyses have been done

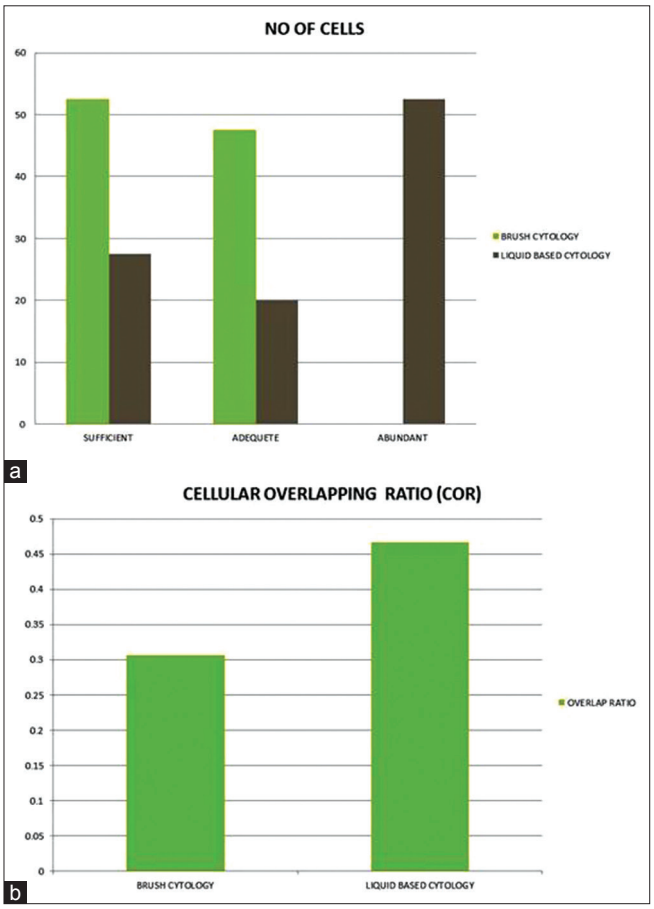

Figure 1: (a) The increased cellularity in centrifugation liquid-based cytology cytosmears. (b) The increased cellular overlapping in centrifugation liquid-based cytology cytosmears

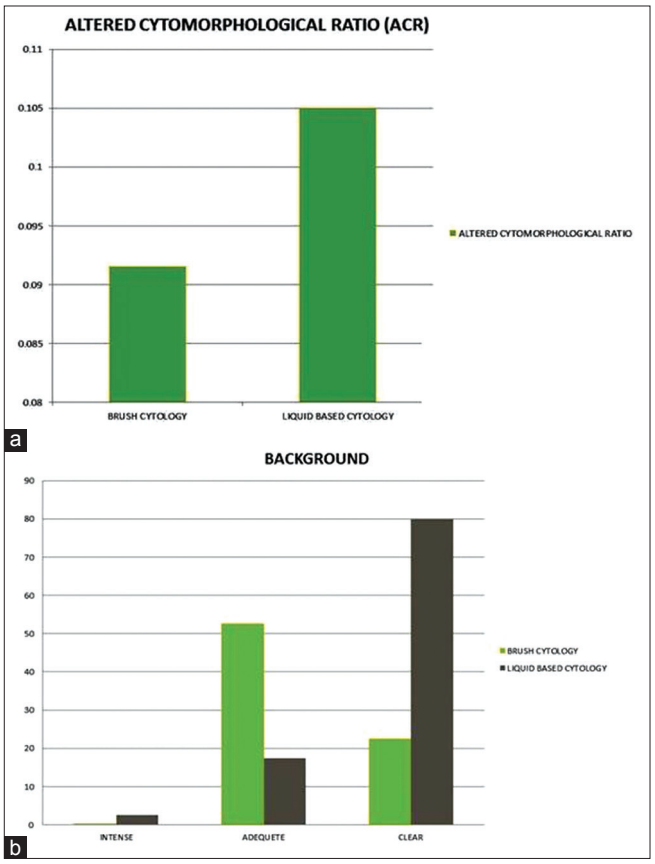

Figure 2: (a) The increased alteration of cell morphology in centrifugation liquid-based cytology cytosmears. (b) The increased tendency of obtaining clearer background in centrifugation liquid-based cytology cytosmears

Table 1: Cellularity - Comparative analysis of the harvest of viable diagnostic cells by the conventional brush and liquid cytology techniques

Cases	Technique	Sufficient (%)	Adequate (%)	Abundant (%)	*P*
Normal mucosa	MBC	18.20	81.80	0	0.03
	CLBC	18.20	36.40	45.50	
Hyperkeratotic lesions	MBC	70	30	0	0.179
	CLBC	30	70	0	
Ulcerated lesions	MBC	80	20	0	0.005
	CLBC	53.30	0	46.7	
Inflammatory lesions	MBC	100	0	0	0.02
	CLBC	0	100	0	

*Chi-square test. CLBC: Centrifugation liquid-based cytopathology, MBC: Manual based cytology

indicating its advantage over the conventional cytology. Most of the studies were done in the gynecological cases where swabs were obtained from the uterine cervix, vulva, etc. and it was observed that the LBC reduced sampling error, yield of cells, and fixation.[2,9,10]

LBC was approved by the Food and Drug Administration (FDA) in 1996, realizing its advantages over conventional technique in evaluating the gynecological specimens.[11] A study showed an increased chance of diagnostic accuracy with LBC compared to conventional cytology (86% vs. 77%). It was also found that manual LBC was more sensitive in diagnosing precursor lesions.[12]

In another study, manual LBC was compared to direct scrape smears in terms of preservation of the morphology of cells where the diagnostic accuracy was found to be 88%. The distribution of the cells showed uniformity along with the presence of cellular overlapping in few areas, which is also in concordance with our study. Polymorphs were seen along with the squamous cells, and it did not obscure the cellular morphology. In this study, the inflammatory component was almost absent in the group of inflammatory lesions. This proves that LBC is better for diagnosing cases based on its cellular features (epithelial lesions).[13] There was an agreement in diagnosis on SurePath preparations.[14]

In a study done with fine needle aspiration (FNA) cytology samples, the diagnostic agreement seen in between the two techniques was similar to the above-mentioned studies. Cellularity was low in manual LBC when compared to

Table 2: Cellular overlapping ratio - Comparative analysis of ratio of overlapping cells seen in the two techniques

Cases	Technique	Mean (COR)	*P*
Normal mucosa	MBC	0.307	0.005
	CLBC	0.481	
Hyperkeratotic lesions	MBC	0.192	<0.001
	CLBC	0.541	
Ulcerated lesions	MBC	0.377	0.917
	CLBC	0.411	
Inflammatory lesions	MBC	0.325	0.386
	CLBC	0.442	

*Mann–Whitney U-test. COR: Cellular overlapping ratio, CLBC: Centrifugation liquid-based cytopathology, MBC: Manual based cytology

Table 3: Altered cytomorphology ratio - Comparative analysis of cells with altered morphology (potentially dysplastic) harvested in the two techniques

Cases	Technique	Mean (ACR)	*P*
Normal mucosa	MBC	0.048	0.171
	CLBC	0.059	
Hyperkeratotic lesions	MBC	0.172	0.561
	CLBC	0.116	
Ulcerated lesions	MBC	0.039	0.001
	CLBC	0.151	
Inflammatory lesions	MBC	0.047	0.245
	CLBC	0.086	

*Mann–Whitney U-test. ACR: Altered cytomorphology ratio, CLBC: Centrifugation liquid-based cytopathology, MBC: Manual based cytology

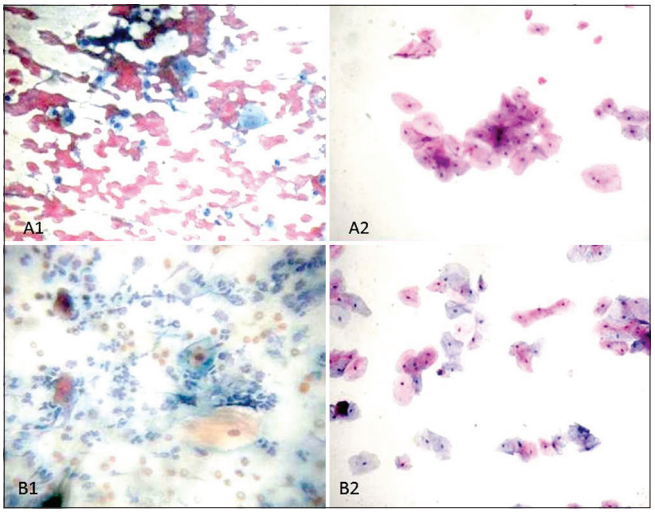

Figure 3: (Top) - Cytosmear of an inflammatory lesion showing the clarity in the background with centrifugation liquid-based cytology which is devoid of mucus, microbial colonies, and inflammatory cells (×40). (A1) Conventional brush smear. (A2) Centrifugation liquid-based cytology. (Bottom) - Cytosmear obtained through liquid-based preparations in ulcerated lesion showing clearer background compared to conventional technique which is devoid of red blood cells and inflammatory cells (×40). (B1) Conventional brush smear. (B2) Centrifugation liquid-based cytology

Table 4: Background - Comparative analysis of background noise (clutter, debris, stain problems, etc.) in smears from the two techniques

Cases	Technique	Intense (%)	Adequate (%)	Clear (%)	*P*
Normal mucosa	MBC	9.1	72.7	18.2	0.01
	CLBC	0	18.2	81.8	
Hyperkeratotic lesions	MBC	10	40	50	0.139
	CLBC	0	10	90	
Ulcerated lesions	MBC	46.7	46.7	6.70	0.002
	CLBC	6.7	26.7	66.70	
Inflammatory lesions	MBC	25	50	25	0.009
	CLBC	0	0	100	

*Chi-square test. CLBC: Centrifugation liquid-based cytopathology, MBC: Manual based cytology

conventional technique, but the reverse was observed in our study.

In all the groups taken into consideration in our study, the yield of cells was seen more upon using LBC. Nuclear overlapping was lesser in the FNA samples when processed with LBC which aided in better diagnosis.[15] A similar study was done in different lesions associated with breast, lymph nodes, salivary glands, bones, and thyroid gland where the parameters such as cellularity, background, preservation of cytoplasm, and nucleus showed a significant improvement upon using LBC, which is in concordance with our findings. The stromal components were preserved in cases of salivary glands neoplasms. LBC was not useful in diagnosing infectious cases of goiter as it yielded a clearer background. Similar results were seen in our study on observing the inflammatory lesions.[7]

The cellular features are preserved in manual LBC, and it gives a better picture of cellularity which is required for diagnosis. There were marked reductions in artifacts, cellular overlapping, mucous background, etc.[16] In our study, similar results were obtained except that more cellular overlapping was seen in LBC. This may be due to the manual technique or due to the increased yield of cells. The background obtained was comparatively clearer in LBC. Similar findings were also observed in other studies.[17] The use of acetic acid in the fixative solution also aids in rendering a clear background by removing blood, mucinous debris, and microbial colonies.[16]

Owing to the improvements in the application of LBC in cervical pathology, researchers have observed the similar findings related to oral lesions as well. A comparison of the diagnostic agreement and the efficacies of the two techniques was done where a high level of significance was found tilted toward liquid-based preparations.[18]

It was also found that the slides processed by liquid-based preparations showed thin, uniform distribution of cells, which is accordance with our study; however, due to the high cellular yield, there was an increased propensity for cellular overlapping which was found to be reduced in other studies.[18,19] A few studies were done on oral squamous cell carcinomas to evaluate the efficacies of the two techniques, where certainly the mucus content and the inflammatory component were decreased in the slides smeared using liquid-based preparations. In our study, we found similar cellular elongations or altered cytomorphology in both the techniques, which is probably due to method of smearing. Previous studies have shown that cellular elongations are seen less using LBC but was found to be statistically insignificant with manual preparation, which is in accordance to our studies, except in the cases of ulcerative lesions.[19] LBC study done on normal mucosa also showed a high yield of cells compared to conventional smear technique as there may be a loss incurred due to the adherence of the cells to the bristles; similar observations were found in our study too.[20,21] In our study, they were an increased chance to observe cellular overlapping probably due to increased pouring of sample of the slides for smearing; similar features were also seen in other studies.[17]

The background seen in cases prepared by liquid cytology was clear, mucus-free, and devoid of RBCs. This is due to the centrifugation of the collected sample and also the glacial acetic acid present in the preservative which leads to the lysis of the RBCs.[22] Artifacts in the cytology slides are common due to several reasons such as improper fixation, improper smearing, and associated debris. In our study, alteration in the cytomorphology (elongation, curling of cells) was noticed in both the techniques which is similar to other studies.[19,23]

Centrifugation-based LBC is an inexpensive, cost-effective, and technique ease method. In many published reports, it is proved to be more sensitive than conventional technique.[18,24] There are patents based on LBC techniques such as thin PREP (approved by FDA), SurePath, and Cytospin Oral CDX, which demonstrated the advantages of LBC over the conventional technique.[13,22,25-27] A major benefit of LBC is the preservation of sample for a prolonged period, which can be used for various purposes such as immunohistochemical assay by preparing cell blocks,[28] human papillomavirus detection, p16 positivity,[29-31] and also DNA testing.

Conclusion

LBC shows better results than CBC in many aspects that are already discussed, but there are pitfalls in liquid-based preparations too. In cases where the stromal components are an essential component, conventional cytology proves better. Although centrifuge-based LBC yielded better results, it requires more of an infrastructure and technical expertise compared to CBC.

Liquid-based preparations by centrifugation are superior to conventional smears with regard to clear background, yield of cells, and cell preservation. It is easier and convenient to interpret LBC smears by virtue of its background and it offers better visualization of the individual cells and understanding the dysplastic nature of them. The use of LBC should be limited to specific lesions where the study of the epithelial cells is more envisaged and enhancing cellular characteristics. Background characteristics such as the microbial colonies and inflammatory cells were lost in the LBC, hence making diagnosis of inflammatory group of lesions difficult.

In Indian scenario, we need to consider the techniques which have the potential to cater the need of the masses as well as are financially feasible to procure. Our study is based on the usage of simple equipment and materials which are readily available and cost-effective. It should be strongly advocated in the interest of diagnosing precancerous as well as cancerous lesions of oral cavity

as a screening tool which can help the clinicians to render better health-care services.

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Conflicts of interest

There are no conflicts of interest.

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