ORIGINAL ARTICLE

Comparison of specimen adequacy and smear quality in oral smears prepared by manual liquid-based cytology and conventional methods

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ABSTRACT

Background: Liquid-based cytology (LBC), recommended in the mass screening of potentially malignant cervical and oral lesions, suffers from high cost owing to the use of expensive automated devices and materials. Considering the need for cost-effective LBC techniques, we evaluated the efficacy of an inexpensive manual LBC (MLBC) technique against conventional cytological technique in terms of specimen adequacy and smear quality of oral smears. **Materials and Methods:** Cytological samples were collected from 21 patients using a cytobrush device. After preparation of a conventional smear, the brush containing the remaining sample was immersed in the preservative vial. The preserved material was processed by an MLBC technique and subsequently, direct smears were made from the prepared cell button. Both conventional and MLBC smears were stained by routine Papanicolaou technique and evaluated by an independent observer for the thickness of the smear, cellular distribution, resolution/clarity of cells, cellular staining characteristics and the presence of unsatisfactory background/artifacts. Each parameter was graded as satisfactory; or satisfactory, but limited; or unsatisfactory. Chi-square test was used to compare the values obtained (significance set at $P \leq 0.05$). **Results:** MLBC technique produced a significant number of satisfactory smears with regard to cell distribution, clarity/resolution, staining characteristics and background/artifacts compared to conventional methods. **Conclusions:** MLBC is a cost-effective cytological technique that may produce oral smears with excellent cytomorphology and longer storage life.

Key words: Early diagnosis, manual liquid-based cytology, oral cytology

INTRODUCTION

The high incidence of oral cancer, associated with widespread use of smoking and smokeless tobacco in the Indian subcontinent, mandates the implementation of simple and cost-effective methods to screen the population at risk. The commonly employed method involves scraping of exfoliated cells from high-risk users and subsequently preparing cytosmears by either conventional or liquid-based cytology (LBC) techniques for early detection of potentially malignant oral disorders, thereby improving the survival and the morbidity of the patient.[1]

LBC is a technique in which cells are scattered in a fixative liquid, to produce a thin layer of cells on slides. Compared to conventional cytosmear preparation, LBC methods have been reported to produce smears with better fixation of cells, good nuclear details[2] and adequate cells for detection of infectious

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agents such as human papillomavirus through molecular biology techniques.[3] Further, the number of inadequate smears is lesser with LBC technique and the incidence of abnormal cells being obscured by overlapping of epithelial cells (unsatisfactory smears/satisfactory but limited smears) is also reduced.[4-6] Considering the higher sensitivity and specificity thus obtained and the reported good interobserver reproducibility, automated LBC techniques are widely used and preferred over conventional smear preparations in cervical screening. Their use has further been recently extended to non-gynecologic cytology such as breast cytology and oral cytology.

Though highly effective compared to conventional smear preparations, automated LBC techniques suffer from high costs involved and thus may not be affordable for the routine mass screening of oral potentially malignant disorders, mandating the need for cost-effective manual LBC (MLBC) methods.[1,4-7]

MATERIALS AND METHODS

Cytological samples were obtained from 21 patients who reported to the outpatient department, after obtaining informed consent from all the subjects before the study. Patients with any systemic illness or on any medication were not included in the study. Biopsy was performed after smear collection to confirm the histological diagnosis.

Specimens were collected using a cytobrush device. A conventional smear was prepared from the brush, fixed in 95% ethanol and subsequently stained by routine papanicolaou (PAP) technique.

The brush containing the remaining sample was immersed in the preservative vial (SurePath preservative fluid). The preserved material was processed by the MLBC technique described by Maksem *et al*.[5] which is as follows:

- Vortex mixing of the specimen
- Centrifuging at 3000 rpm for 10 min
- Decanting and blotting the excess fixative
- Adding 1–2 ml of polymer solution to the tube
- Vortex mixing
- Applying 3–6 drops of suspension to glass slide
- Allowing to dry
- Staining with PAP stain.

The smears prepared by both the techniques were evaluated by an independent observer for the thickness of the smear, cellular distribution, resolution/clarity of cells, cellular staining characteristics and the presence of unsatisfactory background/artifacts. Each parameter was graded as satisfactory; or satisfactory but limited; or unsatisfactory. Chi-square test was used to compare the values obtained (significance set at $P \leq 0.05$).

RESULTS

Satisfactory smears

MLBC technique produced a significant number of satisfactory smears [Figure 1, Table 1] with regard to cell distribution, clarity/resolution, staining characteristics and background/artifacts, compared to conventional methods [Figure 2]. The number of satisfactory thin smears was higher with MLBC, though not significant.

Satisfactory smears with limitations

Both the techniques produced smears that were satisfactory but limited by various factors such as cell overlapping [Table 1].

Unsatisfactory smears

There were no unsatisfactory smears with MLBC technique, whereas unsatisfactory smears were observed for every parameter with conventional cytology [Table 1].

Overall satisfaction

MLBC produced a higher percentage of satisfactory smears as compared to conventional cytology [Table 1].

DISCUSSION

LBC, developed in the 1990s, showed a considerable advantage over conventional cytology. Cervical smears made by LBC showed a reduction in sampling error, better transfer, better fixation of the sample and a reduction in false-negative results.[2-6]

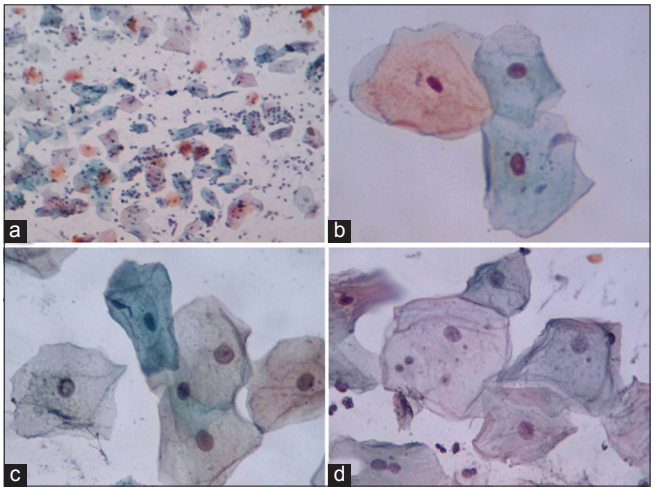

Figure 1: Significant number of satisfactory smears produced by manual liquid-based cytology method, with regard to cell distribution, clarity, staining characteristics and background artifacts. (a) Low power view (H&E stain, ×100), (b-d) high power view (H&E stain, ×400)

Table 1: Frequency distribution of specimen adequacy and smear quality in both smears

Parameters	Technique	Satisfactory		Satisfactory but limited		Unsatisfactory		χ^2 value (DF-2)	*P*
		Smears	Frequency %	Smears	Frequency %	Smears	Frequency %		
Thickness	Conventional	1	4.8	19	90.5	1	4.8	5.04	0.08
	MLBC	6	28.6	15	71.4	0	0		
Cell distribution	Conventional	0	0	19	90.5	2	9.5	28.78	<0.001
	MLBC	17	81	4	19	0	0		
Clarity/resolution	Conventional	6	28.6	13	61.9	2	9.5	8.44	0.015
	MLBC	15	71.4	6	28.6	0	0		
Staining characteristics	Conventional	8	38.1	11	52.4	2	9.5	8.51	0.014
	MLBC	17	81	4	19	0	0		
Background/artifacts	Conventional	4	19	9	42.9	8	38.1	11.32	0.004
	MLBC	11	52.4	10	47.6	0	0		
Total	Conventional	19	18.1	71	67.6	15	14.3	50.3	<0.001
	MLBC	66	62.9	39	37.1	0	0		

MLBC: Manual liquid-based cytology

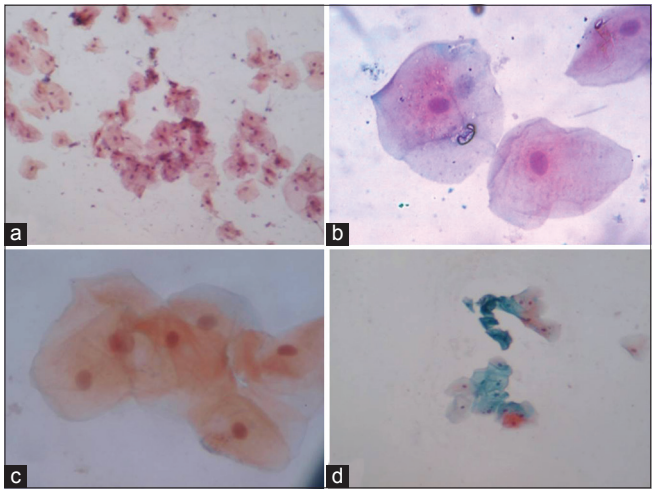

Figure 2: Significant number of satisfactory smears produced by manual liquid-based cytology method, with regard to cell distribution, clarity, staining characteristics and background artifacts. (a) Low power view (H&E stain, ×100), (b-d) high power view (H&E stain, ×400)

MLBC technique refers to a modification of routine LBC techniques wherein slides are prepared using a polymer solution and allowed to dry, forming a membrane. Studies on cervical smears have reported satisfactory results with MLBC comparable to conventional smears, highlighting the cost-effectiveness of MLBC compared to automated LBC techniques.[4,5]

Kavatkar *et al*.[4] prepared cervical cytology smears by MLBC method and compared the morphology with direct scrape smears and further correlated with histopathology wherever possible. They found the MLBC method to be comparable to the conventional scrape smear. Maksem *et al*.[5] applied MLBC technique on 100 gynecological specimens and found unclumped, monolayered, uniform and random cell-spreads, with satisfactory crispiness of cells.

Previous studies to compare the efficacy of LBC versus conventional cytology in oral lesions have applied automated LBC methods, reporting thinner smears with uniform distribution of cellular material, which along with a clear background due to the reduction of background artifacts, resulted in specimens with enhanced cytomorphology.[1,7,8] Enhanced cytomorphology enables better details of cytopathic effects in oral lesions such as nuclear hyperchromatism, binucleation and multinucleation (acantholytic cells of pemphigus vulgaris) and cytological alterations and increased nuclear-cytoplasmic ratio (oral squamous cell carcinoma).

Hayama *et al*.[1] compared specimen adequacy and diagnostic agreement between liquid-based preparations and conventional smears in various oral lesions. Though they found both techniques to be diagnostically reliable, they observed that the liquid-based method showed an overall improvement on sample preservation, specimen adequacy, visualization of cell morphology and reproducibility.

Navone *et al*.[7] reviewed the literature for the efficacy and efficiency of LBC and conventional cytology in the early diagnosis of oral squamous cell carcinoma and potentially malignant oral lesions. They concluded that though conventional cytology helps in screening, LBC gave better results, enhancing both sensitivity and specificity and provided material for further investigation.

Delavarian *et al*.[8] concluded that a modified liquid-based brush biopsy technique is very useful in the diagnosis of potentially premalignant and malignant oral lesions with very high sensitivity, specificity and predictive values.

Considering the inexpensive nature of the manual method and the satisfactory results reported earlier with cervical smears,[4,5] we compared the efficacy of oral smears prepared by MLBC method with conventional smears.

Similar to the findings of authors who employed automated LBC techniques in oral lesions,[1,7,8] our oral smears, prepared

by MLBC technique, showed more regular and homogenous distribution of cells compared to conventional smears. Considerable reduction in background artifacts was also noted in our study, similar to the findings of these earlier investigators.

Similar to the findings in cervical smears prepared by MLBC technique,[4,5] our smears produced cells with sharper outlines consistently and the cellular staining was of good quality.

Thus, employing a manual method of LBC on oral smears, we observed thin smears, with homogenous cell distribution and a clear background. The cells also had crispier outlines and good staining quality.

CONCLUSION

Our study, reiterates the significance of employing MLBC as an ideal technique for preparing oral smears. Besides producing cytosmears with excellent cytomorphology, MLBC also provides the pathologist with enough cellular material to make additional slides for techniques such as PAS or silver staining. Further, better preservation methods employed in MLBC ensures longer storage life for the cytological specimens.

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Conflicts of interest

There are no conflicts of interest.

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