

Efficacy of Manual Liquid Based Cytology over Conventional Cytology in Oral Squamous Cell Carcinoma

Sudhir Mishra¹, Jagadish Hosmani¹, Nandini Manoli², Ramakant Nayak¹, Manjunath G. V.²

¹Department of Oral Pathology and Microbiology, Rajiv Gandhi University, Belgaum, karnataka, India

²Department of Pathology, JSS University, Mysore, karnataka, India

Email address:

mishrasudhir5050@gmail.com (S. Mishra), jhosmani@gmail.com (J. Hosmani), nanda-dr05@yahoo.com (N. Manoli), ramakantnayak30@hotmail.com (R. Nayak), manjugatti@yahoo.com (Manjunath G. V.)

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Abstract: Context/Background: Oral cytology has come long way from its primitive Papanicolaou days. Liquid Based cytology has shown significant advantages over conventional exfoliative cytology. However, LBC requires expensive automated devices and materials which might not be affordable for many cytopathological laboratories in countries with limited resources. Manual liquid Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer and thus improves detection of lesions and improvement of adequacy. Aim: To study and evaluate the diagnostic efficiency and reliability of MLBC in comparison with conventional PAP smear (CPS) of oral squamous cell carcinoma. Materials and Methods: Two smear were prepared from 50 patients, clinically diagnosed with Oral Squamous Cell Carcinoma. Each smear was subjected to MLBC and CPS methods. The slides were evaluated by two pathologists for the staining characteristics of nucleus and cytoplasm. The diagnostic efficiency of each smear was evaluated by comparing the cytological diagnosis of each method with histopathological diagnosis. Results: Increased detection rate with MLBC was 29.41%. Identifying cellular atypia by MLBC was more sensitive (44%) compared to CPS (34%) with similar specificity (100%). The percentage agreement by the two methods was 77.28%. Conclusion: MLBC is an easy, cost effective technique comparable to CPS; however, it warrants further study in its potential application in screening of oral precancer and cancer.

Keywords: Conventional Pap Smear, Manual Liquid Based Cytology, Oral Cytology, Oral Squamous Cell Carcinoma

1. Introduction

At the world level, head and neck cancer is the sixth most common cancer.¹ Oral squamous cell carcinoma (OSCC) accounts for about 40% of head and neck and 90-95% of oral malignancies.^{2,3} Detecting oral malignant and potentially malignant lesions in early stages dramatically affects survival rates. Unfortunately, 50% of patients have regional or distant metastases at the time of diagnosis, which reflects a significant diagnostic delay.^{4,5} Five-year survival is about 76% to 80% if diagnosis is performed in stage 1 and 2. Late diagnosis in stage 3 and 4 can decrease this value to 41% and 9% respectively.^{2,3,6,7} Despite recent advances in treatment modalities, the survival rate of patients with oral cancer has not significantly improved. Therefore, new avenues are being explored in the era of evolving personalized patient management by early detection. Oral cytology has come a

long way from its primitive Papanicolaou days.^{8,9} It has made major strides in its eventful development. Conventional Pap Smears (CPS) sensitivity reduces to less than 50% when there is presence of obscuring blood, inflammation or thick areas of overlapping epithelial cells.^{10,11} These problems with the CPS, gave rise to the advanced technologies, Liquid based cytology. Liquid-based cytology (LBC), since its inception in the 1990s, has shown significant advantages over conventional exfoliative cytology. Although conventional cytology is useful when diagnosing oral precancer & cancer, LBC gives better results, as it not only enhances both sensitivity and specificity, but also provides material for further investigation (AgNORs, DNA, immunohistochemistry, etc.).^{8,12} In most published series, LBC allows a good inter-observer reproducibility.⁸ However, LBC requires expensive automated devices and materials, which might not be affordable for many cytopathology laboratories in countries with poor resources.^{8,9}

On the other hand, Manual Liquid Based Cytology (MLBC) introduced by Maskem et al in 2001 is a technique that enables cells to be suspended in a monolayer and thus improves detection of precursor lesions and improvement of specimen adequacy. Many studies have shown that with proper training, MLBC results in a higher diagnostic yield than traditional cervical smears.^{8,13}

Therefore the overall aim of our study was to compare efficacy of manual liquid based cytology over conventional cytology in screening of oral squamous cell carcinoma. Specific objectives of the study were: a) Compare the morphological view (nuclear and cellular parameter) in oral squamous cell carcinoma according to CPS and MLBC. b) Compare the validity of two methods in terms of sensitivity.

2. Materials and Methods

The study included 50 patients in the age range of 20 to 70 years with clinical diagnosis of oral cancer. A plastic spatula and wooden ice cream stick was used to collect the samples. Spatula was rotated against the lesion. Wooden stick material from one side of the spatula was spread onto a clean glass slide and fixed by bio-spray for conventional method. The material from plastic spatula was dipped into a bottle with fixative prepared in our laboratory. The specimens were subjected to two methods for morphological diagnosis namely Conventional Pap Smear (CPS) and Manual Liquid Based Cytology (MLBC).

2.1. CPS Method

This method included the standard procedure of usual staining of the glass slides with the spread smear. Rapid pap method of staining was used.

2.2. MLBC Method

We report here an indigenous method which is specific to our laboratory using chemicals available in the laboratory, a simple equipment, fixative and polymer solution prepared by us, thus making it a low cost manual method of oral cancer Pap smear screening. The method was accomplished in the following steps of processing.

The material collected in the liquid fixative (containing sodium chloride, sodium citrate, 10% formalin and isopropyl

alcohol) was further processed after a minimum duration of 24 hours. The procedure involved first the mixing of the sample properly before transferring it to a clean test tube and centrifuging it at 1,000 rounds per minute (rpm) for 5 minutes. The supernatant was then decanted. Two millilitre of polymer solution containing agarose, polyethylene glycol, poly-l-lysine and alcohol was added to the deposit. This was further centrifuged at 2,000 rpm (600-800g) for 5-10 minutes. The supernatant was discarded. Two millilitre of phosphate buffer solution is added to the deposit. The supernatant was discarded and from the deposit smear was made on a clean glass slide using a Pasteur pipette. The prepared slides were fixed by drying it room temperature for 2-3 hrs. The slides were further fixed by dipping it in 95% alcohol for 15 minutes and stained with rapid pap stain.

3. Observation

The smears were studied by two independent observers and cytological smear are categorized into one of five classes.

*Table 1. Cytology Categories into 5 Classes.*¹⁴

CATERGORIES	RESULTS
CLASS I	NORMAL
CLASS II	ATYPICAL
CLASS III	INDETERMINATE
CLASS IV	SUGGESTIVE FOR CANCER
CLASS V	POSITIVE OF CANCER

4. Statistical Analysis

The frequency distribution of leading morphological features was worked out to compare the same according to the two methods under study. Increased detection rate (IDR) was calculated as following, $IDR = ((Pm-Pc)/ Pc)*100$, where, Pm is the number of positive cases through MLBC and Pc is the same through CPS.

Subsequently, in order to compare the validity of CPS and MLBC in the diagnosis of oral cancer, sensitivity of the same were estimated considering the histopathological examination (HPE) as the gold standard method.

5. Results

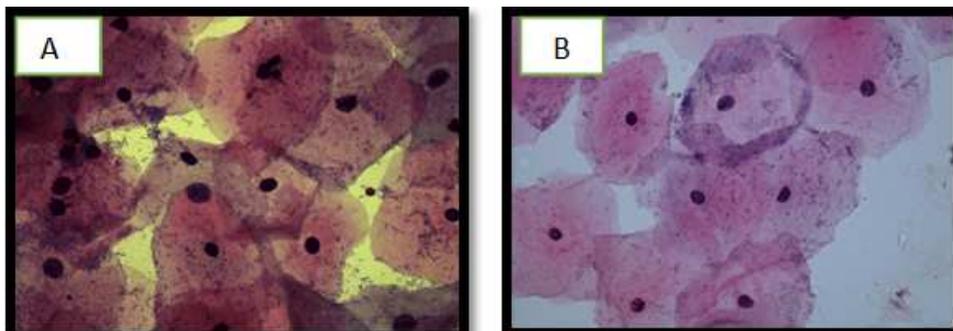


Figure 1. Normal Smear by both CPS and MLBC Methods. A) CPS (40x) B) MLBC (40x).

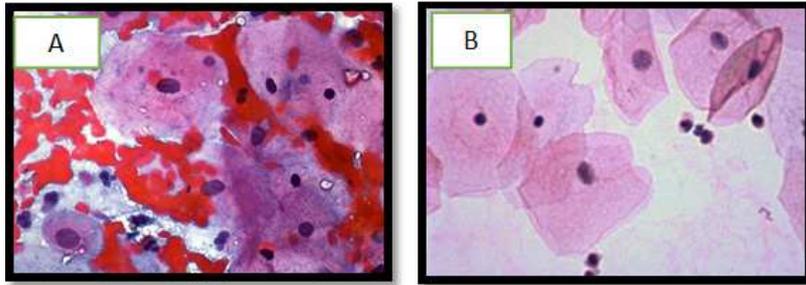


Figure 2. Normal Smear by both CPS and MLBC Methods. A) CPS (40x) B) MLBC (40x).

Class I - CPS showed superficial squamous cells with overlapping, bacterial colonies and cells are obscured by RBCs with normal morphology whereas MLBC showed increase in the number of cells with minimal overlapping, bacterial colonies and absent of RBCs thus increasing the adequacy of cells for the study which was confirmed by HPE. (figure 1. 2)

Class II - CPS showed superficial squamous cells with minor atypia, overlapping and bacterial colonies whereas MLBC showed increase in the number of cells with minimal overlapping, bacterial colonies and minor atypia with no evidence of malignant change with clear background thus increasing the adequacy of cells for the study which was confirmed by HPE. (Figure 3)

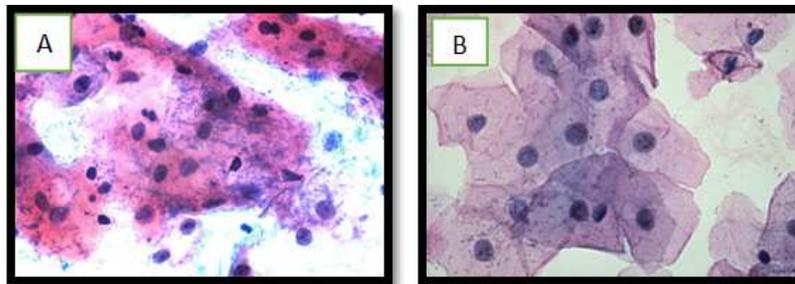


Figure 3. Atypical Smear by both CPS and MLBC Methods. A) CPS (40x) B) MLBC (40x).

Class III - CPS showed superficial squamous cells with malignant characteristics (hyper chromatic nucleus, altered N:C ratio) whereas MLBC showed malignant characteristics

with more clear cellular and nuclear detail thus increasing the adequacy of cells for the study which was confirmed by HPE. (Figure 4)

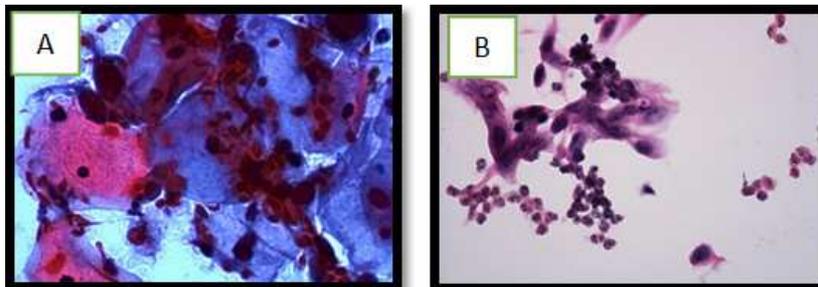


Figure 4. Indeterminate Smear by both CPS and MLBC Methods. A) CPS (40x) B) MLBC (40x).

Class V - CPS showed superficial squamous cells with obvious malignant characteristics (hyper chromatic nucleus, altered N:C ratio) whereas MLBC showed malignant

characteristics with more clear cellular and nuclear detail, clear background thus increasing the adequacy of cells for the study which was confirmed by HPE. (Figure 5)

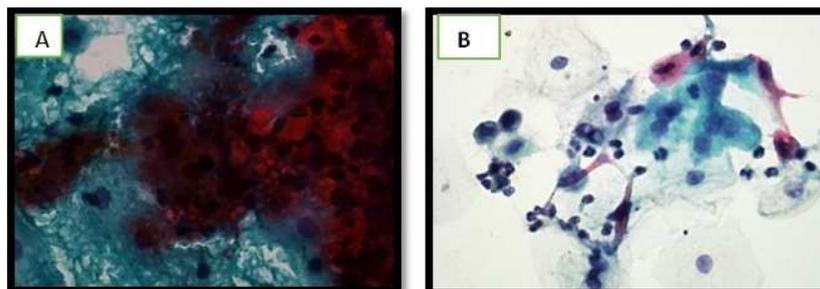


Figure 5. Positive Smear by both CPS and MLBC Methods. A) CPS (40x) B) MLBC (40x).

Cellularity was adequate in all of the MLBC cases whereas it was unsatisfactory in many CPS cases. The background was observed to be clean in all cases of MLBC which was not the case in majority of CPS. There were artefacts were present in most CPS samples. Architectural and cellular morphologic changes were present in most of CPS samples. Inflammatory infiltrate were prominently present in CPS but decreased in MLBC cases. Nuclear changes were very clear by MLBC, but not so clear by CPS. Diagnostic features of 50 cases according to both CPS and MLBC were divided (table 2).

Table 2. Diagnostic features of 50 cases according to both CPS and MLBC

SL NO	CATERGORIES	CPS	MLBC
1.	CLASS I	32	27
2.	CLASS II	6	6
3.	CLASS III	8	12
4.	CLASS V	3	4
5.	UNSTATISFACTORY	1	1

Validity of the two methods

To compare the validity of the two methods, we estimated sensitivity of the two methods considering HPE as the gold standard. In the diagnosis of atypical cell, MLBC was more sensitive than CPS (44% vs. 34%). (Table 3)

Table 3. Sensitivity of MLBC and CPS.

CATERGORIES	SENSITIVITY (%)
Conventional pap smear	34
Manual liquid based cytology	44

6. Discussion

Oral cytology appeared to be a promising diagnostic tool as it was thought to have potential for early detection of malignant lesions. The issue of whether oral cytology could be applicable for mass population screening is somewhat unsettled, although the majority opinion seems to be that it was not practical at that time.¹⁵ Over a period of time, as the field of oral cytology started to grow, many investigators including Montgomery and von Haam experienced the limitations of oral cytology and therefore felt the need for improvements. The conventional pap smear has been utilized for oral cancer screening for many years. Despite being credited for early detection, conventional papanicolaou smear (CPS) has its limitation. False negatives in CPS may be related to inadequate sampling, inadequate transfer of the sample onto the glass slide or deficiencies in the microscopic assessment of the slide.^{8,13} Liquid based cytology has been developed to address the sampling problems of conventional Pap smear.¹⁶ This technique commonly used in developed countries, but may not be affordable in the developing countries due to paucity of resources. To overcome these problems, a new slide preparation method namely the Manual Liquid Based Cytology (MLBC) was introduced by (Maksem *et al.*, 2001).⁸ The present work was done to evaluate the Manual liquid based cytology and to compare

the sensitivity of the same with conventional Pap smear.

Manual liquid based cytology (MLBC) are cost effective, improves detection of suspicious lesions and specimen adequacy. Although a clinician may have excellent collection and sampling technique, only approximately 20% of the cells collected are smeared on the glass slide in CPS.^{16,17,18} In our study the MLBC method was found to be superior to the conventional pap smear.

In most published series liquid based cytology was found to be more sensitive than conventional pap smear.^{8,9,19}

The present study showed that MLBC is more sensitive than conventional pap smear (44 vs 34) for atypical cell. Our study found increased detection rate with MLBC as compare to CPS.

There were several studies carried out to compare specimen adequacy and diagnostic agreement between liquid – based preparations and conventional smears in oral lesions. Study conducted by Hayama *et al.* show LBC demonstrated 41% overall improvement in smear thickness and 66% in cell distribution, and a reduction in cell overlying and presence of blood than CPS. It showed an overall improvement on sample preservation, specimen adequacy, visualization of cell morphology and reproducibility.⁹ Similar study conducted by Navon *et al* show LBC has higher sensitivity than conventional cytology (95.1 vs 85.7) % and higher specificity (95.9 vs 99.0)% respectively.²⁰

A study by Nandini *et al* found MLBC was more sensitive in diagnosis LSIL (low grade squamous intraepithelial lesion) and more specific in the diagnosis of inflammation than CPS.⁸ Result shown by Nandini *et al.* is similar to our study.

Manual method of liquid based cytology which we are following is an inexpensive, cost effective method of LBC which we have adapted and are comparing it with conventional pap smears (CPS) for its adequacy and utility. The other advantages of MLBC method is that the residual specimens can be used for ancillary testing like imunocytochemistry by cell block.

In conclusion, the low cost manual liquid based cytology method of oral screening was found to be better than the standard commercial method. It also over comes the limitations of CPS. However, it warrants further study in its potential application in screening of oral cancer.

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