

# Efficacy and Diagnostic Accuracy of Centrifuged Liquid-Based Cytology over Conventional Oral Exfoliative Cytology in Normal Oral Mucosa and Oral Squamous Cell Carcinoma: Comparative Study

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## ABSTRACT

**Background:** The five-year survival rate of oral cancer remains 50% or less despite the treatment advances. An effective screening test is necessary for early detection of oral cancer.

**Aim:** To assess the efficacy and diagnostic accuracy of centrifuged liquid-based cytology in comparison to conventional exfoliative cytology in normal oral mucosa and oral squamous cell carcinoma.

**Materials & Methods:** The study population comprised of 40 individuals, equally divided into two groups: healthy individuals and previous histopathologically proven oral squamous cell carcinoma cases. Exfoliative cytology was done and the samples were processed by conventional cytology as well as by centrifuged liquid-based cytology and stained with Papanicolaou stain. Seven cytological parameters were analysed to assess the quality of slides prepared. A cytological diagnosis of the case group was also made and was compared with histopathological diagnosis to evaluate the diagnostic accuracy.

**Results:** The present study revealed statistically significant superior results in all the cytological parameters evaluated by centrifuged liquid-based cytology compared to conventional exfoliative cytology ( $P \leq 0.05$ ). There was an 'almost perfect agreement' found between centrifuged liquid-based cytology diagnosis and histopathology ( $k=0.89$ ) while only a 'substantial agreement' was found between conventional exfoliative cytology with histopathology ( $k=0.79$ ).

**Conclusion:** The present study summarizes that centrifuged liquid based cytology is more effective than conventional oral exfoliative cytology in screening oral cancer. Further studies with larger sample size in different oral lesions are necessary to validate the use of this cytology technique.

**Key-words:** Centrifuged liquid-based cytology; Cytodiagnosis; Oral squamous cell carcinoma.

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## INTRODUCTION

Oral squamous cell carcinoma (OSCC) commonly known as oral cancer is one among the three major cancers in India.<sup>1</sup> The presence of easily identifiable oral potentially malignant disorders (OPMDs) along with well-known risk factors, makes OSCC a preventable disease.<sup>2</sup> However, the five year survival rate of OSCC remains 50% or less which indicate voids in early detection.<sup>2</sup>

Biopsy followed by histopathological examination is the gold standard technique for detecting oral cancer.<sup>3</sup> Being an invasive procedure, it can only be carried out at a clinical setting with trained operators and with expensive armamentarium.<sup>3</sup> Compared to biopsy, exfoliative cytology is an easy procedure, which can be carried out at oral cancer screening camps with minimal investment.<sup>4</sup> However, the slides prepared by conventional exfoliative cytology are of inferior quality with inadequate cells and more false negative results, which may hinder a proper diagnosis.<sup>5</sup>

Liquid based cytology (LBC) is a comparatively newer cytological technique, widely used for screening cervical cancer, introduced in 1990's.<sup>6</sup> In LBC, the collected material from the mu-

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cosal surfaces is suspended in a preservative liquid followed by processed in cytospin, an automated device resulting in excellent

quality slides. Only a portion of the sample is required for preparing the slides and the rest can be used for other investigations like immunocytochemistry, human papilloma virus (HPV) testing and DNA ploidy analysis.<sup>6,7</sup> Sukegawa S et al found that automated LBC was superior to conventional cytological methods for the primary screening of oral cancer and precancerous lesions of oral cavity.<sup>8</sup> However, this excellent cytological technique remains less popular due to its higher cost. Maskem et al in 2001 introduced a cost-effective modification of liquid based cytology known as, "manual liquid-based cytology" for cervical cancer screening.<sup>9</sup>

A few other cost-effective modifications of liquid based cytology were reported in the literature by Banerjee A et al, Hegde V et al, Shukla S et al, Delavarian Z et al, and Perez Sayans et al.<sup>10-14</sup> Centrifuged liquid based cytology (CLBC) is one such modification, which can be carried out with easily available equipment in a basic medical laboratory without much compromise in the final results.<sup>10</sup>

The literature search revealed paucity of studies assessing the quality as well as diagnostic accuracy of CLBC in oral cancer compared with the gold standard scalpel biopsy.<sup>6,9,10</sup> Hence, the present study was undertaken to assess the efficacy and diagnostic accuracy of CLBC in comparison to conventional oral exfoliative cytology (COEC) in OSCC and in normal oral mucosa by keeping histopathology as benchmark. If found effective, CLBC can create revolution in oral cancer screening.

## SUBJECTS AND METHODS

### Study population:

The present study was carried out in patients availing treatment at Azeezia College of Dental Sciences and Research, Kollam, Kerala after obtaining approval from the institutional ethical committee. The sample size was calculated from Hegde V et al.<sup>11</sup>

$$\text{Sample size of one group, } n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (p_1q_1 + p_2q_2)}{(p_1 - p_2)^2}$$

$$n = 20$$

The study population was equally divided into two groups:

Group 1: Oral squamous cell carcinoma patients and Group 2: Healthy controls.

The patients with histopathologically proven squamous cell carcinoma were included in the study group. Age and sex matched healthy individuals without habits of smoking, paan chewing and alcohol consumption were selected as controls.

### Cytology technique:

All the subjects were asked to rinse their mouth with distilled water before sample collection. For CLBC a commercially available LBC kit was used (Eziprep Liquid based cytology kit, VDNA Laboratories, Chennai) (Figure 1). The kit contains a preservative liquid, a separator solution, centrifugation tubes, Pasteur pipette, slide carrier to stabilize glass slide and two funnels to pour the sample into the slides. An oral cytobrush (Astra Biosciences, Thrissur) was used for sample collection (Figure 1). The samples were collected from oral cancer lesions in case group and from buccal mucosa in healthy subjects by rotating the cytobrush 360° ten times. The collected sample was transferred to a preservative liquid provided with the Eziprep LBC kit. After 30 minutes of fixation 7 ml from the total 14 ml sample was collected on a Pasteur pipette. It was layered over 4 ml of separator solution already taken in a centrifuge tube. The sample was then centrifuged at 1000 rpm for five minutes in a swinging type centrifuge (Rotek, B&D Laboratories, Ernakulam)

(Figure 1). After centrifugation, the supernatant was poured off and to the cell pellet at bottom, one drop of distilled water was added and mixed to make it homogenous. One slide was placed inside the slide carrier and two funnels were locked in their position. Five drops of normal saline added to each funnel for wetting the slides. Then using a micropipette, 50 µl of the cell pellet was collected and loaded to each funnel (Figure 1). One drop of fixative was added to each funnel and were kept for five minutes. After five minutes the remaining excess fluid was thrown out and the funnels were removed. The slides were washed gently in running tap water.

In COEC also the samples were collected from oral cancer lesions from patients and normal buccal mucosa in healthy subjects by rotating the cytobrush 360° ten times. The collected sample was directly spread on a glass slide and the cytological smear was fixed with alcohol.

All the slides were stained with Papanicolaou stain (Rapid PAP, Bio Lab diagnostics, Mumbai) following the manufactures protocol and were mounted with DPX.

### Evaluation of the slides:

All the slides were evaluated by two experienced cytopathologists. The quality of the slides was evaluated by assessing seven parameters: adequate cellularity, clear background, uniform cellularity, cellular clarity, cellular overlapping, nuclear hyperchromatism and presence of blood, mucin and microbial colonies. The first four parameters were scored as good, average and poor. Cellular overlapping was scored as high, moderate and low. Nuclear hyperchromatism was graded as marked, medium and minimum. Blood mucin and microbial colonies were scored as present or absent.<sup>15</sup>

Cytological diagnoses were performed based on the diagnostic guideline for oral cytology, proposed by the Diagnostic Guideline Committee for Oral Cytology of the Japanese Society of Clinical Cytology. The cytological diagnoses are classified into NILM (negative for intraepithelial lesion or malignancy), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), SCC (squamous cell carcinoma), and IFN (indefinite for neoplasia or non-neoplasia).<sup>16</sup> The diagnoses NILM, LSIL, HSIL were categorized as 'negative for OSCC', IFN was categorized as 'indeterminate' and SCC was categorized as 'positive for OSCC'. The histopathological grading of OSCC was done following the Broder's criteria.<sup>17</sup> Finally, the cytological diagnoses were correlated with the histopathology to evaluate diagnostic accuracy.

### Statistical data analysis:

Data analysis was performed using SPSS software (Version 20.0). Chi-square test, Cohen kappa, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), negative likelihood ratio (NLR), accuracy, false negative rates and false positive rates were evaluated.

## RESULTS

The study population comprised of two groups, one consisting of 20 subjects histopathologically diagnosed as OSCC and the other consisting of 20 healthy individuals. The mean age of the OSCC patients and control group were 64.4 and 64.3 years respectively. Among the OSCC group 75% of patients were having habits of smoking, paan chewing and alcoholism. Tongue was the most common site for OSCC (40%), followed by buccal mucosa (35%), gingiva and lip (10% each) and alveolar mucosa 5%. The histopathological grading showed 75% of the cases to be well-differentiated, 15% of cases to be moderately differentiated and 10% of cases to be poorly

differentiated squamous cell carcinoma.

All the evaluated cytological parameters showed statistically significant difference between slides prepared by CLBC and COEC in total subjects. The slides prepared by CLBC showed adequate and uniform cellularity, clear background, evident nuclear hyperchromatism and minimal cellular overlapping. Also, the slides made by CLBC were devoid of blood, mucin and microorganisms. In all the parameters used for assessing the quality of slides, CLBC showed excellent results compared to COEC (Figure 2,3,4,5 and Table 1).

Among the forty samples, one case was diagnosed as 'indefinite' by both the techniques, and was excluded from the final statistical analysis for diagnostic accuracy. The CLBC showed 89.47% sensitivity, 100% specificity, 10.53% false negative rate, no false positive cases, 100% PPV, 90.91% NPV, 0.11% NLR, 94.87% accuracy. The COEC technique showed 78.9% sensitivity, 100% specificity, 21.1% false negative rate, no false positive cases, 100% PPV, 83.3% NPV, 0.2% NLR, 89.7% accuracy. So CLBC showed better sensitivity, NPV, NLR and accuracy than COEC and less false negative cases (Table 2). The diagnostic accuracy of CLBC technique with histopathology was compared using Cohen Kappa statistics. The kappa value was 0.89 which indicated an 'almost perfect agreement' between CLBC and histopathology. Similarly, the diagnostic accuracy of COEC was evaluated against the histopathology and kappa value was found to be 0.79 which indicated 'substantial agreement'.

**Table 1:** Comparison of cytological parameters between COEC and CLBC

PARAMETER		CLBC (%)	COEC (%)	P value
Adequate cellularity	Good	73.75	45.00	P<0.01
	Average	13.75	16.25	
	Poor	12.50	38.75	
Clear background	Good	71.25	27.50	P<0.01
	Average	15.00	50.00	
	Poor	13.75	22.50	
Uniform cellularity	Good	75.00	32.50	P<0.01
	Average	12.50	45.00	
	Poor	12.50	22.50	
Cellular clarity	Good	71.25	37.50	P<0.01
	Average	15.00	37.50	
	Poor	13.75	25.00	
Cellular overlapping	High	13.75	46.25	P<0.01
	Medium	15.00	38.75	
	Low	71.25	15.00	
Nuclear hyperchromatism	Marked	50.00	31.25	P<0.05
	Medium	25.00	38.75	
	Minimal	25.00	30.00	
Presence of blood, mucin and microbial colonies	Present	15.00	43.75	P<0.01
	Absent	85.00	56.25	

CLBC showed superior diagnostic agreement with histopathology compared to COEC (Table 3,4).

The CLBC and COEC showed kappa values of 1 and 0.95 respectively in Cohen kappa analysis for inter-observer variability (Table 3,4). Both the techniques showed an 'almost perfect agreement' in inter-observer variability which indicates great agreement between the cytopathologists in diagnosing the cases.

## DISCUSSION

The present study revealed the average age of patients with OSCC was 64.4 years which was in accordance with the studies conducted by Pires FR et al<sup>18</sup> (mean age: 62 years), Rikardsen OG et al<sup>19</sup> (mean age: 66 years) and Troeltzsch M et al<sup>20</sup> (mean age: 60 years).

Smoking, paan chewing and alcoholism are well known risk factors of oral cancer. About 75% of the OSCC patients in the present study were practising at least one of these habits. Pires et al<sup>18</sup> reported 80% and T Smitha et al<sup>21</sup> reported 78% prevalence of these habits in their studies.<sup>12</sup>

The commonest clinical type of oral cancer was ulcero-proliferative lesions (70%) followed by exophytic masses, ulcers and white plaques (10% of cases per each type). Razavi SM et al in their study reported, 42.5 % of exophytic lesions, 41.3% of ulcers, 9% of red and

**Table 2:** Diagnostic accuracy of CLBC and COEC

	CLBC	COEC
Sensitivity	98.47	78.9
Specificity	100	100
False negative	10.53	21.1
False positive	0.0	0.0
PPV	100	100
NPV	90.91	83.3
NLR	0.11	0.2
Accuracy	94.87	89.7

**Table 3:** Comparison of diagnostic agreement of CLBC with histopathology

CLBC	Histopathology diagnosis		
	Positive	Negative	Total
Positive	17	0	17
Negative	2	20	22
Total	19	20	39

26. Kappa = 0.89\*\*, P = 0, Almost perfect agreement

**Table 4:** Comparison of diagnostic agreement of COEC with histopathology

COEC	Histopathology diagnosis		
	Positive	Negative	Total
Positive	15	0	15
Negative	4	20	24
Total	19	20	39

Kappa = 0.79\*\*, P = 0, Substantial agreement

white plaque and 5% of white plaques.<sup>22</sup>

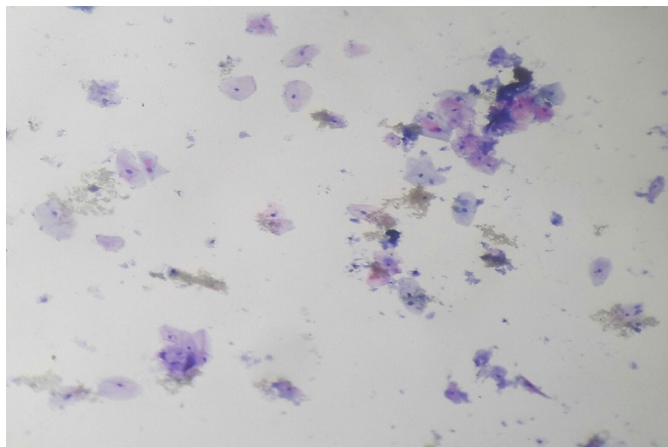
The histopathological grading of oral cancer patients comprises predominantly of well differentiated OSCC (75%) followed by moderately differentiated OSCC (15%) and poorly differentiated OSCC (10%). In a study done by Razavi SM et al also reported more cases as well differentiated squamous cell carcinomas (46.3%) followed

by moderately differentiated (33.8%), poorly differentiated (16.3%) and anaplastic (3.8%) OSCCs.<sup>22</sup>

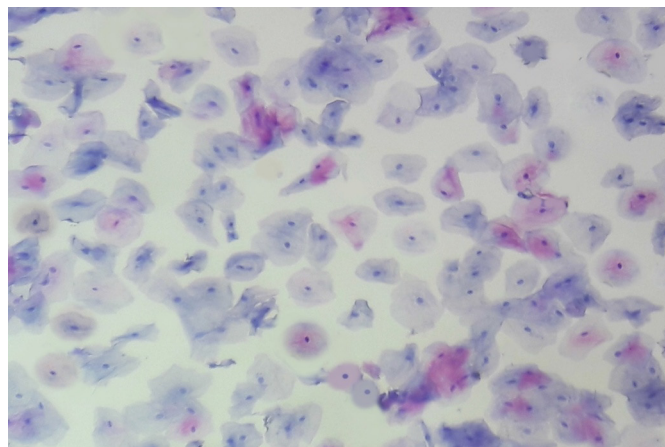
Most of the slides prepared by CLBC showed adequate cellularity compared to the slides prepared by COEC and the differences were statistically significant (Table 1). Nambiar S et al in a comparative study between CLBC and COEC in normal oral mucosa and Hegde V et al in a comparative study in normal oral mucosa and OSCC subjects also reported similar results.<sup>10,11</sup> The parameter clear background also showed statistically significant differences between CLBC and COEC (Table 1). Nambiar S et al and Dwivedi N et al also reported better results by CLBC in normal mucosa and oral mucosal lesions in their studies respectively.<sup>6,10</sup> About 75% of slides prepared by CLBC showed uniform cellular distribution and 71.25% showed cellular clarity and the differences were statistically significant compared to COEC (Table 1). Nambiar S et al reported uniform distribution in 36% of slides prepared by CLBC method while only 12% of slides prepared by COEC method showed uniform cellular distribution.<sup>10</sup> Hegde V et al also reported better results in CLBC compared to COEC in OSCCs as well as in healthy controls.<sup>11</sup> Majority of the slides prepared by CLBC showed 'low cellular overlapping' while the slides prepared by COEC showed 'high cellular overlapping' (Table 1). Nambiar S et al reported that 80% of slides prepared by COEC showed cellular overlapping while only 38% of slides prepared by CLBC showed cellular overlapping and the results were



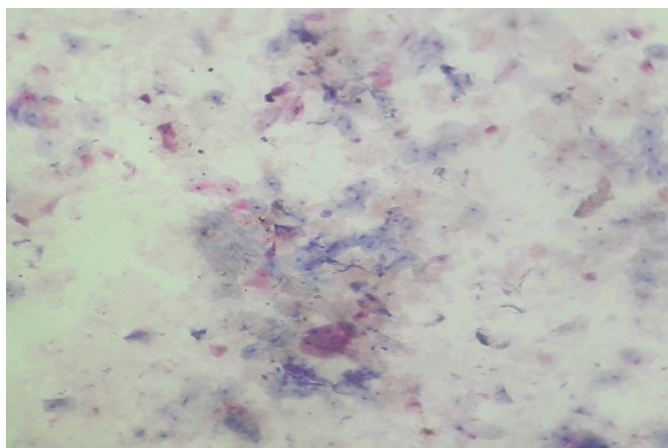
**Fig. 1:** Armamentarium for CLBC. **A)** Eziprep LBC Kit **B)** Cytobrush **C)** Micropipette **D)** Centrifuge



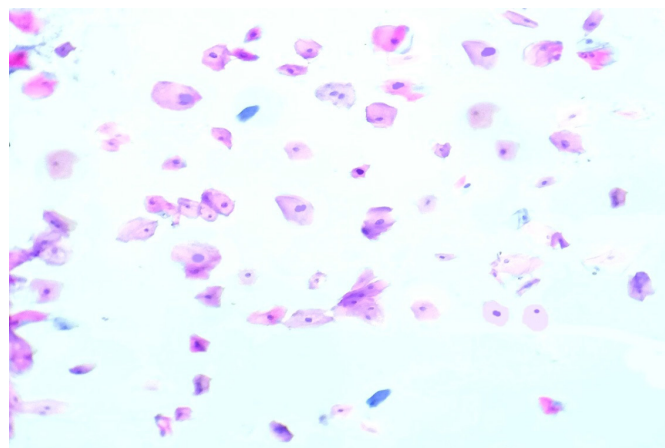
**Fig. 2:** COEC prepared slide from healthy controls (Pap, x40)



**Fig. 3:** CLBC prepared slide from healthy controls (Pap, x40)



**Fig. 4:** COEC prepared slide from OSCC patients (Pap, x40)



**Fig. 5:** CLBC prepared slide from OSCC patient (Pap, x40)

statistically significant.<sup>10</sup>

The parameter nuclear hyperchromatism was graded as marked, moderate and minimum. A statistically significant difference observed between the two techniques in total subjects ( $P < 0.01$ ) (Table 1). Bhandari PA and Ghadkari conducted a comparative study in OPMDs and malignancies using wooden spatula and tooth brush. However, no statistically significant difference in nuclear hyperchromatism was found.<sup>23</sup>

Majority of the slides prepared by CLBC are devoid of blood, mucin or microbial colonies compared to the slides prepared by COEC and the differences were statistically significant (Table 1). Hegde V et al reported that CLBC showed less mucin and inflammatory cell content compared to COEC and the difference was statistically significant.<sup>11</sup>

There is great inter-observer agreement in present study. Delavarian Z et al reported “substantial agreement” for Oral CDX brush used modified liquid based cytology in comparison to scalpel biopsy ( $Kappa = 0.806$ ) in OPMDs and malignancies.<sup>13</sup> The CLBC showed good sensitivity, specificity, PPV, NPV, less false positive and false negative cases and better accuracy than COEC (Table 2). Delavarian Z et al reported 88.8% sensitivity, 100% specificity, 100% PPV, 80% NPV, and 0.11 NLR.<sup>13</sup> Vidal AKL conducted a comparative study between OSCC patients and healthy controls by LBC and COEC and reported 96.9% sensitivity, 75% specificity and 96.3% accuracy in cases and 91% sensitivity, 57.5% specificity and 82.7% accuracy in controls.<sup>23</sup> The diagnostic concordance between LBC and histopathology was reported as above 90%. Perez-Sayans M et al conducted a non-computer assisted LBC in OSCC patients and reported 69% sensitivity, 100% specificity, 100% PPV and 71% NPV.<sup>14</sup> Bhatia PV et al compared the quality as well as the diagnostic accuracy of a modified brush biopsy and liquid based cytology in oral sub-mucous fibrosis and reported satisfactory results with liquid based cytology.<sup>25</sup>

## CONCLUSION

To conclude, the present study inferred that CLBC is a superior cytological technique than COEC in screening oral cancer. CLBC technique provided slides with better diagnostic quality and accuracy than COEC which is almost comparable with that of the slides prepared by LBC. Further studies with larger sample size, in different oral lesions are necessary to validate the use of this cytology technique.

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