

Original Article

Comparative study of conventional exfoliative cytology and centrifuged liquid-based cytology in oral leukoplakia and oral squamous cell carcinoma patients

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ABSTRACT

Background: This study aims to compare the efficacy between conventional exfoliative cytology (EC) and centrifuged liquid-based cytology (CLBC) in control, leukoplakia, and oral squamous cell carcinoma (OSCC) patients. Oral leukoplakia and oral cancer require an early definitive diagnosis for better prognostic outcome. Oral EC, a minimally invasive technique that involves the examination of desquamated cells from the tissue surfaces used as a method of early diagnosis. CLBC is a modified technique that is used to achieve improved quality of the cytology findings.

Materials and Methods: A comparative study was done in 30 subjects, of which, 10 cases from control group, 10 oral leukoplakia, and 10 OSCC cases. These subjects were selected according to the appropriate inclusion and exclusion criteria. The cases in each group underwent conventional as well as CLBC. The comparison was carried out between these groups with respect to the cellular and background stromal factors. Appropriate qualitative evaluation of the samples was collected and statistical analysis was done using the Chi-squared test. The significance level of value was $P < 0.05$.

Results: Significant results were obtained for certain parameters such as cellular overlap clear background, uniform distribution in control, leukoplakia, and OSCC with a $P = 0.004^{**}$, $P = 0.001^{**}$, $P = 0.006^{**}$ using CLBC.

Conclusion: CLBC is better and give clearer vision as compared to conventional cytology and can be used in the early diagnosis.

Key Words: Cytology, early diagnosis, leukoplakia, oral cancer

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INTRODUCTION

Oral cancer is the sixth most common cancer worldwide.^[1] The incidence rate of oral cancer has increased since the last decade.^[2] Squamous cell carcinoma (SCC) is the most common type of oral cancer almost, accounting for 90% of cases. Increased fertility of the disease is chiefly due to late diagnosis

and inadequate treatment at appropriate time period. Furthermore, various molecular and biochemical changes are responsible for the tissue changes. Oral SCC (OSCC) cases mostly are preceded by cellular changes reflecting in the tissue to cause premalignant

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lesions in the oral cavity. Oral leukoplakia is the most common precursor lesion for causation of OSCC.^[2] Early detection and diagnosis of premalignant diseases prevent the commencement of OSCC and thus improves the prognosis of diseases. A conventional approach including histopathological examination of biopsied tissue still remains the diagnostic gold standard. However, less invasive techniques can be used for the same and needs to be explored more.^[2]

Exfoliative cytology (EC) is a simple, painless, and noninvasive technique. Also advantageous in cases contraindicated for biopsy where this less invasive or noninvasive techniques such can be used. The EC process includes scrapping of less cohesive cells from the lesional surface its examination under microscope to detect the cellular changes.^[3]

A modification of EC, liquid-based cytology (LBC) was introduced and exists since 1970s that was initially used only for gynecological purposes.^[4,5] Centrifugation of the liquid biopsy is done to make the smears from concentrated sediments of sample. The technique usually gives high cellular details.^[6] Studies conducted in normal mucosa and atrophic lesions by Garbar *et al.* in 2005 and Banerjee and Kamath in 2018 showed the use of centrifuged LBC (CLBC) technique which reduces inadequate samples, improves cellular details, and aids in the detection of molecular biology when compared with conventional cytology.^[4,6] Hence, this study aims to compare the efficacy of CLBC with conventional EC in normal oral mucosa, oral leukoplakia cases, and OSCC cases to know the possibility of the early detection of changes in oral mucosa.

MATERIALS AND METHODS

Sample

A comparative study was carried out in the Department of Oral and Maxillofacial Pathology and Oral Microbiology in Vinayaka Missions Sankarachariyar Dental College, Salem, India. The sample size was determined using the standard sample size formula. The sample for this comparative study includes 30 subjects – 10 controls from patients without any lesion or cancer, 10 oral leukoplakia, and 10 OSCC patients. The study was approved by our Institutions Ethical Committee Clearances (VMSDC/IEC/Approval No. 255). The following inclusion and exclusion criteria were applied.

The samples taken from patients were divided into three groups which are as follows: Group A: Smears

are taken from buccal mucosa of patients who reported oral prophylaxis without any signs of oral lesions whose age group between 35 and 50 years, Group B: Smears are taken from the lesional area from patients who are clinically diagnosed with oral leukoplakia and Group C: Smears are taken from the lesional area of patients who are provisionally diagnosed with ulcero-proliferative lesions suggestive of cancer and later confirmed with biopsy were included in this study. Smears from patients with OSCC who are undergoing radiation therapy are excluded from the study.

Methodology

The study was preceded after receiving consent from the patients. Two samples were taken with the help of a wooden spatula, one is used for a conventional method where smears were made in the glass slide and fixed immediately with 95% ethyl alcohol solution. The second sample was immersed in 74 mL of 95% alcohol and was subjected to centrifuging for 10 min at 2000 rpm. The obtained pellet of cells was then suspended in a glass slide and a smear was prepared. The smear is left for 2 h followed by H and E staining.

The slides were then evaluated under the microscope (Lawrence and Mayo, Chennai, Tamil Nadu, India) and qualitative analysis was done for both smears for each case. The efficacy of both techniques was assessed concerning the parameters/factors such as cell morphology, clear background, uniform distribution, cellular overlap, and inflammatory cells which are graded as follows.^[7]

Cell morphology - Good (<10% of cells deformed), fair (10%–20% of cells deformed), and poor (>20%–50%) of cells deformed.

Clear background - good (<10% debris in background), fair (10%–30% debris in background), and poor (>30% debris in background).

Uniform distribution - High (>50% of cells shows uniform distribution), moderate (10%–50% of cells shows uniform distribution), and low (<10% of cells shows uniform distribution).

Cell high (>50% of cells shows overlap), moderate (10%–50% of cells shows overlap), and low (<10% of cells shows overlap).

Inflammatory cells - high (>50% of inflammatory cells in background), moderate (10%–50% of inflammatory cells in background), and low (<10% of inflammatory cells in background).

Statistical analysis

The comparison of both techniques in each group was done using the Chi-squared test with consideration of $P < 0.05$ as significant.

RESULTS

H- and E-stained smears for conventional EC [Figure 1] and CLBC [Figure 2] in each group were evaluated for the parameters of cellular morphology, cellular background, uniform distribution of cells, cellular overlap, and inflammatory cells. Statistical analysis was done for the same using the Chi-squared test with the $P < 0.05$ as significant.

In the control group, clear background, uniform distribution, and cellular overlap showed significant results when compared to conventional cytology with the $P = 0.036^*$, $P = 0.004^{**}$ and $P = 0.014^*$.

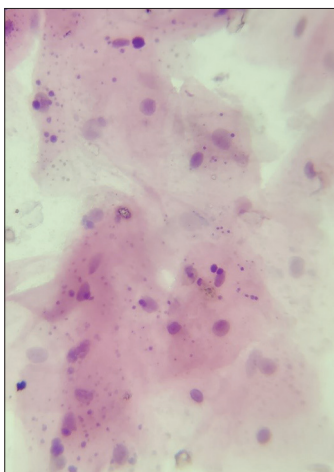


Figure 1: Conventional exfoliative cytology - overlapping of cells with a poor background in $\times 40$.

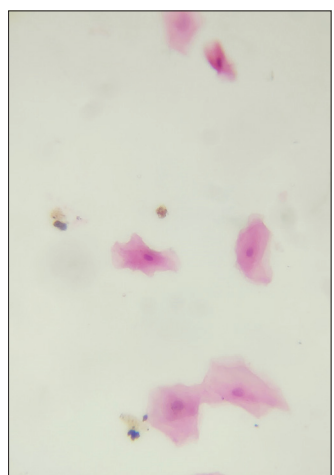


Figure 2: Centrifuged liquid-based cytology – single and separate cells with clear background in $\times 40$.

Other parameters in CLBC showed comparatively better cell morphology and low inflammatory cells in the background but the results were not statistically significant [Table 1].

In leukoplakia cases, statistically significant results were obtained for clear background, uniform distribution, and cellular overlap. The uniformity was 80% high in CLBC when compared to 30% and 60% of low and moderate grades observed in conventional cytology. Cellular overlap was around 90% low in CLBC smear when compared to 50% of high and moderate cases in conventional EC. Inflammatory cells showed same results with both techniques. Background clarity was good in 50% of cases, fair in 30%, and poor in the remaining 20% of cases in CLBC over 50% of cases with a $P = 0.034^*$ [Table 2]. When compared to conventional EC cases, only 50% of fair and poor grades were observed. Cellular morphology was 20% poor, 60% fair, and 20% good in CLBC smears compared to 30% poor and 70% fair with statistical nonsignificance.

Background clarity in OSCC smears was 60% good and 40% fair in CLBC with the $P < 0.006^{**}$. Uniform distribution of smear was 10% poor, 60% moderate, and 30% more in CLBC over 70% low and 30% moderate in EC with the $P = 0.014^*$. Other factors showed statistically nonsignificant better-quality smears prepared by CLBC over those of EC smears [Table 3].

Thus, overall, the results showed CLBC to be more effective with clear features of smear than those in EC smear slides.

DISCUSSION

The incidence rate of oral cancer cases keeps rising accounting for 3% of all malignancies worldwide. Multiple etiologic factors aid in causing oral potentially malignant disorders (OPMDs) and oral cancer.^[2] Oral leukoplakia is one of the common precancerous lesions with a prevalence rate of 0.1%–0.5%. The lesions are usually diagnosed based on the histopathological examination.^[2]

Oral cancer with 90% of cases of SCC is mostly preceded by OPMDs. Despite improved treatment protocols with the addition of novel modalities, the 5-year survival rate has not improved significantly. The predominant reason for this is late diagnosis of oral cancer cases.^[8] Histopathological examination of such

Table 1: Comparison between conventional exfoliative cytology and centrifuged liquid-based cytology parameters in control/normal mucosa

Control	Cytology		Total, <i>n</i> (%)	χ^2	<i>P</i>
	Conventional exfoliative, <i>n</i> (%)	Centrifuged liquid-based, <i>n</i> (%)			
Cell morphology					
Poor	2 (20)	2 (20)	4 (20)	0.000	1.000
Fair	5 (50)	5 (50)	10 (50)		
Good	3 (30)	3 (30)	6 (30)		
Clear background					
Poor	5 (50)	1 (10)	6 (30)	6.667	0.036*
Fair	5 (50)	5 (50)	10 (50)		
Good		4 (40)	4 (20)		
Uniform distribution					
Low	6 (60)		6 (30)	11.111	0.004**
Moderate	4 (40)	5 (50)	9 (45)		
High		5 (50)	5 (25)		
Cellular overlap					
High	6 (60)		6 (30)	8.571	0.014*
Moderate	2 (20)	5 (50)	7 (35)		
Low	2 (20)	5 (50)	7 (35)		
Inflammatory cells					
High	1 (10)		1 (5)	1.059	0.589
Moderate	1 (10)	1 (10)	2 (10)		
Low	8 (80)	9 (90)	17 (85)		
Total	10 (100)	10 (100)	20 (100)		

*Significant at 5%; **Significant at 1%

Table 2: Comparison between exfoliative cytology and centrifuged liquid-based cytology parameters in oral leukoplakia cases

Leukoplakia	Cytology		Total, <i>n</i> (%)	χ^2	<i>P</i>
	Conventional exfoliative, <i>n</i> (%)	Centrifuged liquid-based, <i>n</i> (%)			
Cell morphology					
Poor	3 (30)	2 (20)	5 (25)	2.277	0.320
Fair	7 (70)	6 (60)	13 (65)		
Good		2 (20)	2 (10)		
Clear background					
Poor	5 (50)	2 (20)	7 (35)	6.786	0.034*
Fair	5 (50)	3 (30)	8 (40)		
Good		5 (50)	5 (25)		
Uniform distribution					
Low	3 (30)	1 (10)	4 (20)	10.016	0.007**
Moderate	6 (60)	1 (10)	7 (35)		
High	1 (10)	8 (80)	9 (45)		
Cellular overlap					
High	5 (50)	1 (10)	6 (30)	16.667	0.001**
Moderate	5 (50)		5 (25)		
Low		9 (90)	9 (45)		
Inflammatory cells					
Low	10 (100)	10 (100)	20 (100)	-	-
Total	10 (100)	10 (100)	20 (100)		

*Significant at 5%; **Significant at 1%

lesions is the gold standard of diagnosis.^[8] However, biopsy techniques are invasive and time-consuming. Thus, economical and labor-saving techniques such as EC can be of assistance for the diagnosis.

EC involves the evaluation of less cohesive cells on epithelial surface. The procedure is advantageous in cases contraindicated for biopsy. The procedure involves spreading of the surface cells from the

Table 3: Comparison between exfoliative cytology and centrifuged liquid-based cytology parameters in oral squamous cell carcinoma cases

Squamous cell carcinoma	Cytology		Total, n (%)	χ^2	P
	Conventional exfoliative, n (%)	Centrifuged liquid-based, n (%)			
Cell morphology					
Poor	4 (40)		4 (20)	5.333	0.069
Fair	5 (50)	7 (70)	12 (60)		
Good	1 (10)	3 (30)	4 (20)		
Clear background					
Poor	4 (40)		4 (20)	10.400	0.006**
Fair	6 (60)	4 (40)	10 (50)		
Good		6 (60)	6 (30)		
Uniform distribution					
Low	7 (70)	1 (10)	8 (40)	8.500	0.014*
Moderate	3 (30)	6 (60)	9 (45)		
High		3 (30)	3 (15)		
Cellular overlap					
High		2 (20)	2 (10)	3.877	0.144
Moderate	6 (60)	7 (70)	13 (65)		
Low	4 (40)	1 (10)	5 (25)		
Inflammatory cells					
High	3 (30)		3 (15)	3.529	0.060
Moderate	7 (70)	10 (100)	17 (85)		
Total	10 (100)	10 (100)	20 (100)		

*Significant at 5%; **Significant at 1%

epithelium on slide followed by staining with Papanicolaou (PAP) or H and E stain and its microscopic examination. The stained smears are evaluated for cellular changes and background.^[3]

In 1970s, a technique of LBC was introduced to diagnose gynecological diseases. CLBC is the improved form of conventional EC. It involves centrifugation of scraped material in suspension to obtain the sediments and smear it on slides. The advantages involve concentrated material than the conventional EC smears.^[5] The technique is recently being experimented in oral lesions. Thus, this study was aimed to compare the efficacy of CLBC over EC in OPMD and OSCC cases.

Similar studies have been carried out before for evaluating the efficacy of CLBC over EC. A Banerjee and Kamath in 2018 compared brush cytology with CLBC in oral lesions using PAP stains. The comparison was done based in the parameters of cellularity, cellular overlapping, altered cytomorphology, and background. The study revealed more yield of cells in CLBC, cellular overlapping was significant higher in cases with CLBC smears, cytomorphological alteration was similar in both techniques and CLBC smears showed better background.^[6] Dwivedi *et al.* 2012 studied the utility of CLBC and conventional EC in normal mucosa, hyperkeratotic lesions,

ulcerated lesions, and atrophic lesions. The comparison was based on the criteria including cellularity, background clarity, uniformity in distribution, cellular overlapping, cellular elongation, blood, and microbial colonies. The conclusion declared was CLBC does not offer a significant advantage over conventional smears except for the clear background.^[8] Ahmed *et al.* in 2009 compared CLBC with conventional oral EC to evaluate the efficiency of CLBC in oral lesions using PAP and May Grunwal Giemsa method. The comparison was done on the basis of thickness, cellular distribution, leukocytes, and red blood cells as well as cell morphology. The study revealed equal reliability for both techniques while CLBC had an overall improvement on sample preservation, specimen adequacy, visualization of cell morphology, and reproducibility.^[9] Arunachalam *et al.*, 2021 compared CLBC with conventional brush cytology in normal mucosa, by using PAP-stained smears. The smears were evaluated based in cellularity, cell distribution, cellular overlapping, cell elongation, and cellular background and graded for the same. The results showed statistically nonsignificant better outcome in CLBC over conventional cytological smears in most of the criteria.^[10] Hegde *et al.*, 2018 evaluated the efficacy of CLBC over conventional EC. The study aimed at evaluating the CLBC efficacy over EC in normal mucosa and OSCC cases

with the help of PAP staining. The study found statistically significant difference in the parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, and cellular elongation. CLBC smear had less mucus, microbial colonies, and inflammatory cells than the conventional technique. Thus, concluding better efficacy of CLBC over the parameters of conventional cytology.^[7]

The present study revealed some statistically significant results in each group to favor CLBC technique over EC. The uniformity of distribution and clear background were definitely significant statistically for CLBC in normal mucosa, leukoplakia, and OSCC specimens, thus demonstrating better quality of smear over conventional EC. These results were similar to the study conducted by Hegde *et al.*^[7] Other parameters, including cell morphology, cellular overlap, and inflammatory components had better results for CLBC over EC but statistically nonsignificant. The results were similar to Dwivedi *et al.* regarding the clear background using CLBC,^[8] but the author also stated that conventional technique was significant on comparison. The conflicting results are possible because the study was done in reactive lesions and the present study was done in premalignant lesion and cancer.

Overall, the CLBC smears have been revealed to be of better quality for the diagnosis than conventional EC. The major shortcoming of conventional EC is observed to be inadequate cellular clarity which leads to false results. However, CLBC consists of concentrated sediments of cytology material and thus is easier to spread and provides more cellular information than the conventional EC. As mentioned by Ahmed *et al.*, CLBC is better than EC sample preservation, adequate specimen amount, reproducibility of the respective lesional cells, and its morphology are better viewed than those in EC.^[9] Similar features were observed in the present study. To overcome the shortcomings of EC, more CLBC studies are needed to be carried out in several lesions involving larger sample size and improved standardization of the technique.

CONCLUSION

Oral precancerous lesions and cancer need an early diagnosis for better treatment protocols. EC and CLBC are proven to be acceptable alternatives to routine histopathology diagnosis in cases where

invasive procedures are contraindicated. Since CLBC sediments are concentrated and help lower the chances of false diagnosis as can be seen in EC. Furthermore, the cellular morphology and uniformity are better appreciated in CLBC. About its obtained concentrated smears, standardized CLBC is definitely preferred over EC in the diagnosis of any oral lesions. However, studies need to be carried out on a larger sample size including various oral lesions to confirm its incorporation into routine clinical practice for the diagnosis.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Abati S, Bramati C, Bondi S, Lissoni A, Trimarchi M. Oral cancer and precancer: A narrative review on the relevance of early diagnosis. *Int J Environ Res Public Health* 2020;17:9160.
2. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. *Int J Oral Sci* 2013;5:59-65.
3. Verma R, Singh A, Badni M, Chandra A, Gupta S, Verma R. Evaluation of exfoliative cytology in the diagnosis of oral premalignant and malignant lesions: A cytometric analysis. *Dent Res J (Isfahan)* 2015;12:83-8.
4. Garbar C, Mascaux C, Fontaine V. Efficiency of an inexpensive liquid-based cytology performed by cytocentrifugations: A comparative study using the histology as reference standard. *Cytojournal* 2005;2:15.
5. Arul P, Smitha S, Masilamani S, Akshatha C. Micronucleus assay in exfoliated buccal epithelial cells using liquid based cytology preparations in building construction workers. *Iran J Pathol* 2018;13:30-7.
6. Banerjee A, Kamath VV. Evaluation and comparison of conventional brush-based and centrifugation liquid-based cytopathology. *Clin Cancer Investig J* 2018;7:104.
7. Hegde V, Nambiar S, Yadav N. Efficacy of centrifuged liquid-based cytology over conventional cytology: A comparative study. *J Cancer Res Ther* 2017;13:951-5.
8. Dwivedi N, Agarwal A, Raj V, Kashyap B, Chandra S. Comparison of centrifuged liquid-based cytology method with conventional brush cytology in oral lesions. *Eur Jf Gen Dent* 2012;1:192-6.
9. Ahmed HG, Edris AM, Mohamed EA, Hussein MO. Value of centrifuged liquid-based cytology by Papanicolaou and May-Grünwald in oral epithelial cells. *Rare Tumors* 2009;1:e12.
10. Arunachalam M, Gunasekaran N, Rajkumar K, Thayalan DK, Dinakaran J, Bharanidharan R. Comparative evaluation of centrifuged liquid-based cytology method over conventional brush cytology in normal oral mucosa. *SRM J Res Dent Sci* 2021;12:91-4.