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Cell Block Method: An Imperative Tool for Cytological Diagnosis of Oral Potentially Malignant Lesions

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ABSTRACT

Globally, oral squamous cell carcinoma (OSCC) is one of the commonest reported malignancies usually arising from oral potentially malignant lesions (OPMLs) such as leukoplakia, erythroplakia, oral submucous fibrosis (OSMF) and oral lichen planus (OLP). Hence timely and early diagnosis of these disorders is of prime importance to halt their malignant transformation. A search of published works was done using the online databases of PubMed, Scopus, Web of Science, and Cochrane Library". Data from resource-constrained laboratory settings worldwide shows limited documentation of the efficacy of advanced cytological techniques, including LBC and cell block preparations. The oral mucosa can be a suitable area for regular cytological screening due to its easy accessibility. Liquid-based cytology (LBC) can preserve the cellular details and reduce the overlapping of cells, enabling precise interpretation, reducing false-negative results and aiding in the diagnosis of premalignant and malignant lesions of the oral cavity with more accuracy compared to exfoliative cytology. The remaining sample in LBC can be used in cell block formation and various ancillary tests like immunocytochemistry, immunofluorescence, and molecular studies. Literature showed a scarcity of data available regarding Pakistan. Therefore, the review is aimed to explore the cell block method as a minimally invasive technique for reducing morbidity and mortality associated with OSCC.

Keywords: Cell Biology; Oral Squamous Cell Carcinoma; Malignancy.

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INTRODUCTION

Oral carcinoma is the sixth most frequent malignancy globally, accounting for about 500 000 new cases every year and 3.6% of cancer deaths, creating significant health problems and burden worldwide[1]. In Pakistan and India, oral carcinoma represents a major health concern accounting for up to 40% of all malignancies and is the most prevalent carcinoma in males while the third most prevalent cancer in females[2]. Globally, a 5-year survival rate of 50% is seen among patients with oral carcinoma even though the oral cavity is easily reachable for routine examination and follow-ups. However, unfortunately, individuals report in the

terminal stages of malignancy (mostly at stage III or IV); thus, no improvement has been noted in the survival rate for oral cancer over the years[3]. If oral cancer is diagnosed at early stages and timely treatment is given for localized lesions, morbidity and mortality can both be minimized, and survival rates are reported to reach up to 82% [4].

Oral carcinoma is mainly associated with malignant transformation of oral potentially malignant lesions (OPMLs) [5]. OPMLs if remain untreated can progress to invasive tumors of the oral cavity in which affected epithelium shows epithelial dysplasia histologically and include oral leukoplakia (most common), erythroplakia, erythro-leukoplakia, oral submucous fibrosis (OSMF), oral lichen planus and oral lupus erythematosus. Leukoplakia is the commonest OPML while erythroplakia being less frequent but more serious shows the malignant potential of almost 85% [6]. The prevalence of OPMLs is between 1% and 5% globally depending mostly on the place of origin, the nature of the population under study, pattern of tobacco and alcohol use and areca quid chewing[5,7]. These lesions are usually asymptomatic in the initial stages but may be diagnosed by dental physicians on routine examination of the oral cavity due to their characteristic clinical appearance. If an appropriate and conclusive diagnostic approach is adopted for the detection of these lesions in the early stages, morbidity and mortality of the patients can be reduced. Thus, OPMLs are potentially high-risk lesions that transform into malignancy based on their indiscernible course in most cases. Hence, early diagnosis through regular screening of patients presenting with recurrent oral infectious or inflammatory lesions remains indispensable. Therefore, the review is aimed to explore the cell block method as a minimally invasive technique for reducing morbidity and mortality associated with OSCC.

A narrative review methodology and analysis of published works were planned, carried out, and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. In this review, a search of published works was done using the online databases of PubMed, Scopus, Web of Science, and Cochrane Library for relevant publications up to October 2020. The following medical subject headings (MeSH) were used in the search strategy: ''oral potentially malignant lesions'', "exfoliative cytology", "liquid-based cytology", ''oral squamous cell carcinoma'' and "cell block". The reference lists of the articles were also searched to identify missed studies. No restriction was applied on time of publication or language. To facilitate the screening process of studies from online databases, all search results were downloaded into an EndNote library (version X8).

Only hospital and clinic-based studies were included where the cytological analysis was carried out by pathologists up to October 2020. Studies considering individuals of age group 18-60 years irrespective of gender who underwent brush biopsies of intraoral leukoplakia, erythroplakia, erythroleukoplakia, proliferative verrucous leukoplakia, oral lichen planus, or oral submucous fibrosis were included. Population-based studies and studies referring only to clinical features were excluded. Pregnant women were also excluded. Different information was extracted from the shortlisted studies such as first author name, year of publication, the geographic region in which the study was carried out, duration of the study, sample size, gender and age of the studied sample, the prevalence rate of oral lesions, OPMLs if observed or not, study setting (urban, rural or both), study design, sampling method, laboratory techniques used, any statistically significant results found and conclusions made by the authors regarding the efficacy of different laboratory techniques in the diagnosis of OPMLs.

DISCUSSION

Incidence of oral potentially malignant lesions and oral carcinoma is very high in South Asian countries that may be attributed to specific eating habits. Though histopathology is thought to be a gold standard method in detecting these lesions, it may not be possible to perform a biopsy in all suspected cases as it is a costly, time-consuming, invasive technique having surgical as well as psychological implications on the patient[8].

Role of Cytology in the Diagnosis of OPMLS

Diagnostic cytology plays an imperative role in the detection of epithelial and cellular abnormalities and infectious diseases. It is a very simple, rapid, cheap and reliable method for diagnosing cutaneous premalignant and malignant tumors, immunobullous lesions, infectious diseases and genodermatosis[9]. Cytology is an accepted, widely employed diagnostic modality for the timely diagnosis of oral cancers but its role in detecting OPMLs is still debatable[10]. Oral exfoliative cytology is a well-established and more sensitive technique that can detect the oral cavity's primary cancerous lesions, even when the lesions seem to be innocuous, and there is no suspicion of cancer, and when the prognosis is excellent[11].

Recent developments in the discipline of cytology have converted diagnostic cytopathology to an advanced diagnostic tool with limited false-positive and false-negative results. Liquid-based cytology (LBC) gives improved and higher quality results compared to conventional cytology, as it increases the sensitivity and specificity of the diagnosis and provides residual material for additional investiga-

tions[12]. Thus, this review will emphasize the importance of liquid-based cytology and early detection of OPMLs to prevent malignant transformation. In Pakistan, very few reports have been documented regarding the cytological diagnosis of these lesions however, to the authors' knowledge; none of the studies has yet been reported on the diagnostic efficacy of advanced cytological techniques, including LBC and cell block preparations.

Different Cytological Techniques for OPMLs Detection

1. Exfoliative Cytology

Exfoliative cytology (EC)is a screening and diagnostic test used for early detection of oral diseases, such as squamous cell carcinoma, pemphigus, potentially malignant disorders, candidiasis, and salivary gland lesions[13]. This technique is relatively simple, cost-effective, non-invasive, and rapid, well-received by the patients, enabling the professionals to monitor the follow-up after providing the necessary treatment. EC usually consumes the specimens of exfoliating cells compared to histopathology, in which entire tissue is submitted for processing. Conventional cytology has played a significant role in the detection of uterine cervical cancer during a gynecological examination since the beginning of the Papanicolaoutechnique in the 40s. In the past, disputes occurred in the use of EC because of a large number of false-negative results and subjective interpretation of atypical oral mucosa cells. Liquid-based cytology (LBC) tests have substituted conventional cytology, SurePath or ThinPrep being the primary screening tests in most of the laboratories[14].

2. Liquid-based Cytology

Liquid-based cytology (LBC)has proven to be superior to conventional cytology by reducing the difficulties associated with sampling thus helping in the formation of improved smears and reducing the false-negative rates. The clear background thus obtained enhances not only the quality of the smear but also increases the diagnosticsensitivity[15]. In this technique, the cells, after sampling, are first suspended in a suitable fixative (preservative) medium followed by centrifugation, and a smear is then prepared[16,] (Figure 1).

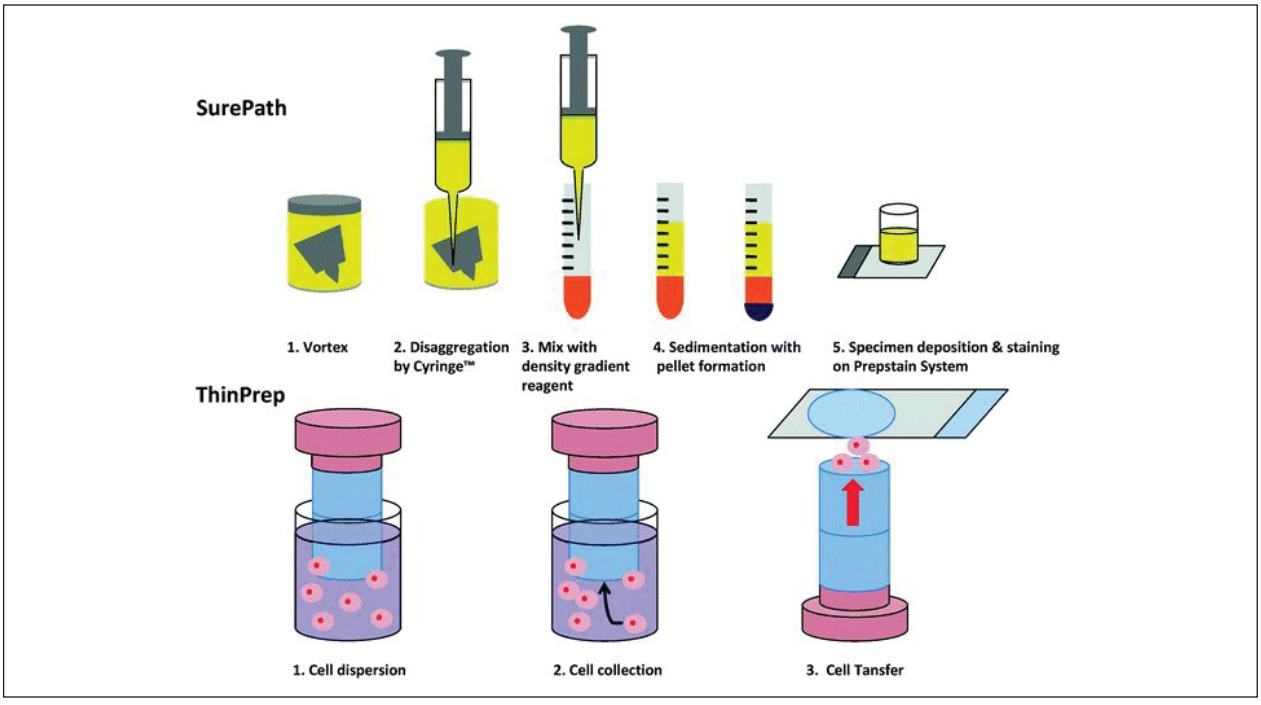

Figure 1: Liquid-based cytology techniques: SurePath and ThinPrep processing techniques[17].

3. Manual Liquid Based Cytology

Manual liquid-based cytology (MLBC) is a technique that enables cells to be suspended in a monolayer and thus improves detection of precursor lesions and improvement of specimen adequacy. The residual sample can be utilized for additional tests like detection of HPV-DNA and immunocytochemistry, enhancing the utility of MLBC just like ThinPrep and SurePath techniques[18,19].

As in LBC, a filtration process is used and there is computer-assisted thin layer deposition of cells resulting in better cell retrieval abilities and improved cell preservation, therefore, it is an expensive technique that might not be economical for the majority of cytopathology laboratories.

Therefore, to achieve the accuracy of LBC and to limit the expenses of an automated method, smears obtained from modified manual liquid-based cytology (MLBC) method can be used in which centrifugation is performed at higher speeds (approx. 3000 rpm for 10 minutes)[20]. Various studies have indicated that LBC has advantages over conventional cytology in terms of background, cellularity and nuclear details thus helping in an accurate diagnosis (Table 1).

Table1: Comparison between exfoliative cytology and liquid-based cytology in squamous epithelial lesions by different authors over the years (2014 – 2019).

Author(s)	Place of Study	Year	Patients	Sampled Lesions	EC vs. LBC % (Different Parameters)	*p*-Value	Conclusion
Waris et al. [21]	Pakistan	2019	300	Oral mucosal lesions	Detection rate of cytology, 57.7%(Epithelial Dysplasia), 54.3% (Keratosis), 74.7% (Inflammation)	N/A	Cytology detected dysplasia, keratosis, inflammation, bacterial and candida growths more accurately than naked eye examination. Therefore, it can be used as a diagnostic tool for detection of these lesions on routine basis.
Kondo et al.[22]	Japan	2019	241	Oral intraepithelial lesions	Sp= 80% (LBC) 73% (EC) PPV= 92%(LBC) 86%(EC) NPV= 41%(EC) 29%(LBC)	0.024	LBC showed significant specificity, positive predictive value, and low rate of inadequate specimen, so it was suitable for oral cytology.
Remmerbach et al. [23]	Germany	2017	113	OSCC	Sn = 98%(LBC) 96%(EC) Sp = 69%(LBC) 90%(EC) PPV= 89%(LBC) 96% (EC) NPV= 91%(LBC) 90%(EC)	N/A	Both techniques (EC and LBC) show high sensitivity. Therefore, they provide a quick and reliable screening tool for dentists to identify oral lesions at an early stage.

Hegde et al.[24]	India	2017	90	OSCC	Clear background= 85%(LBC) 30%(EC) Cellularity =67% (LBC) 34% (EC)	≤0.05	LBC has better efficacy as compared to EC in all the parameters accessed.
Qadir et al.[25]	Pakistan	2015	35	Oral mucosal changes in HIV/AIDS patients	Inflammation =65% Smears 65% smears 48.5% smears 51.4% smears fungi= 48.5% Smears micronuclei=51.4% smears	0.001	Oral mucosal changes in HIV/AIDS patients like inflammation, fungi, dysplasia, and micronuclei formation were found on cytology smears which can easily be ignored on routine clinical examination of these patients. Thus, cytology markedly increases the diagnostic accuracy.
Mulki et al. [8]	India	2015	108	OLP OSCC	Sample adequacy=282 (LBC) 269 (EC) Cellular clarity =291 (LBC) 261 (EC)	<0.05	LBC can be considered as an alternative to EC whenever a surgical biopsy is not possible.
Singh et al. [26]	India	2015	1000	ASCUS CIN Cervical carcinoma	U/S smears =1.7% (LBC) 4.3% (EC)	<0.05	The detection rate of LBC and EC was similar whereas reduction in U/S rate and availability of residual sample to perform HPV DNA testing has made LBC better than EC.

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Moosa et al.[27]	Pakistan	2014	80	Pre-cancerous lesions of the cervix	**Quality Index** =0.65 (MLBC) 0.56 (EC) **Average score** clear background =1.87 (MLBC) 1.36 (EC) Cellularoverlapping = 1.68 (MLBC) 1.29 (EC)	<0.01	MLBC can be used for cervical screening in low resource areas instead of LBC or EC as this technique is superior to EC in terms of clear background, increased cellularity and decreased cellular overlapping thus increasing diagnostic accuracy.

Sn=sensitivity, Sp= specificity, EC=Exfoliative cytology, LBC=Liquid Based Cytology, OSCC= Oral Squamous Cell Carcinoma, OLP= Oral Leukoplakia, ASCUS= Atypical Squamous Cells of Undetermined Significance, CIN= Cervical Intraepithelial Neoplasia, U/S= Unsatisfactory, HPV= Human Papillomavirus, N/A= Not Applicable.

Literature Highlighting the Role of LBC and MLBC

Studies show that specimens prepared using both ThinPrep and SurePath showed higher nuclear details and better-defined cytoplasm than those prepared using conventional smear[28]. Moreover, LBC along with immunocytochemistry and cell block formation with immunohistochemistry leads to enhanced morphological details thus resulting in a correct diagnosis. Waris et al. conducted a cross-sectional study showing the importance of cytology as compared to the clinical examination of oral mucosal lesions in terms of detection of epithelial dysplasia (57.7%), keratosis (54.3%), inflammation (74.7%), bacterial (48.7%) and candidal (7.7%) loads[21]. Qadir et al. performed a study on oral mucosal changes in patients with HIV/AIDS also concluded the same results that inflammation (65%), certain fungi (48.5%), and micronuclei (51.4%) are more easily detected on cytological smears. These findings show significant results (*p*-value 0.001)[25]. A study by Moosa et al. compared MLBC with EC, concluding that MLBC is relatively superior to EC in terms of certain parameters like clear background (1.87 vs. 1.36) and cellular overlapping (1.68 vs. 1.29). MLBC showed higher quality index (0.65 vs. 0.56) and a significant *p*-value of <0.01, thus concluding that MLBC can be used as a screening method in low resource settings[27].

A study by Mulki et al. compared the exfoliative cytology with liquid-based cytology, which showed that LBC is better than EC in terms of cellular clarity and sample adequacy (*p*-value <0.0001 for both parameters)[8]. The same results were shown by a research conducted by Kondo et al. which showed significantly better specificity (80% vs. 73%), positive predictive value (92% vs. 86%), and low rate of an inadequate specimen of LBC as compared to EC[22]. A case-control study by Vidal et al. evaluated the sensitivity, specificity, and concordance between EC and LBC, which included 182 patients with primary OSCC (case group) and 179 individuals with normal buccal mucosa (control group). LBC showed improved specificity of 95% as compared with conventional cytology, which was 75%[29]. A comparative study by Dwivedi et al. reported a statistically significant difference (*p*<0.001) between LBC and EC when various parameters (cellularity, background, cellular overlapping, and presence of microbial colonies) were compared. The percentage of the smears with a clear background was 68% in the case of LBC as compared to 26% for EC[15].

Pankaj et al. in the study showed that the unsatisfactory rate of conventional cytology in their study was 7.1% as compared to 1.61% for LBC, and this difference is statistically significant[30]. Similar results were shown by Singh et al. they concluded that 4.3% of smears were reported as unsatisfactory by EC while only 1.7% of smears were unsatisfactory by the LBC technique[26]. A comparative study performed by Hegde et al. showed similar results in terms of adequate cellularity (67% in case of LBC as

compared to 34% in case of EC) and clarity of the background (80% in case of LBC in comparison with 30% in case of EC)[24].

Cell Blocks: Merits and Demerits

Cell blocks (CBs) made from residual LBC samples, aspirates, and fluid samples may also have implementations of practice in the discipline of cytopathology. Obtaining sufficient cell block material allows for further ancillary studies like immunocytochemical staining, fluorescence in situ hybridization, and other molecular studies to be performed on the cytology specimen, thus helping in the definitive classification of the lesion and modifying the treatment options. Several methods are used for cell block preparation, but the Plasma Thrombin method is most widely used as it is a simple and cost-effective technique in which liquid fixative is centrifuged, the supernatant formed is then decanted. After that plasma and reconstituted thrombin are added and the solution is quickly agitated thus forming a clot will within 30–60 seconds which is then placed into a labeled cassette containing formalin. The specimen is then processed routinely in the histopathology laboratory[31]. Disadvantages regarding CB technique include loss of cytological material during tissue processing or sectioning, suboptimal CBs with thick smears having clot and tissue fragments thus hindering the cellular details, suboptimal cellularity and expensive as well as skill-based technique requiring extra staff therefore, their quality may be compromised in low-setting areas[32,33].

Literature Highlighting the Role of Cell Block Method

Several studies have proved that cell blocks are an integral part of cytology preparations and ancillary testing; however, the authors could not find any literature on oral potentially malignant lesions through careful and selective searching of recommended databases. Sale et al. and Pallavi et al. conducted studies on benign oral solid and cystic lesions showing that CBs provide a better microscopic evaluation than conventional cytology in terms of specific parameters like cellular morphology, nuclear details, and staining quality[34,35]. CBs also show a better detection rate for different oral cavity cystic lesions, thus diagnosing them more accurately. A study by Woo et al. on pleural effusion cytology concluded that CBs show better sensitivity (94.3%) as compared to LBC (81.3%) and diagnose potentially malignant as well as malignant lesions more efficiently thus can be used along with LBC[36]. Kulkarni et al. and George et al. conducted studies on cervical smears which showed higher sensitivity (75%) and specificity (93%) of CBs as compared to LBC (66% and 84% respectively) and CS (50% and 70%, respectively) concluding that CBs have higher detection rate for malignant lesions[37,38]. The same results were obtained by Sadullahoğlu et al., Zhang et al. and Qin et al. who made a common conclusion that CBs have higher diagnostic accuracy (91.7%), sensitivity (90%), and specificity (98.3%) for detecting malignant lesions[39,40,41]. Thus, the CB technique and LBC increase cytological diagnosis and can be used as an integral part of cytopathology (Table 2).

Table 2: Comparison between cell block and liquid-based cytology techniques by different authors from 2014-2020.

Authors	Year/ Type/ Place of Study	Site of sample	Total No. of Samples	CS/LBC vs. CB % (Different Parameters)	*p*-Value	Conclusion
Sale et al. [34]	2020 Original article India	Oral lesions i.e., odontogenic tumors, calcifying epithelial odontogenic tumors, Epidermoid cysts and radicular cyst	30	Sn= 93.7% (CB)71.1% (CS) Sp=89.5% (CB), 42.2% (FNAC) PPV=90.9% (CB) Acc=88.9%(CB)	≤ 0.01	CBs provide better microscopic evaluation as compared to smears because they enable improved cellular morphology, nuclear details and staining quality when compared with the results of FNAC as well as conventional cytology.

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Pallavi et al.[35]	2019 Original article India	odontogenic cystic lesions	17	CB DR =71% for OKC 66.7% for DC 66.7% for RC50% for AMB CB detects keratin in OKC, epithelial cells in DC, inflammatory cells in RC and tumor epithelial cells in AMB which were not detected by FNAC	N/A	CB can be used as a preoperative diagnostic technique for jaw bone lesions as it is a simple, rapid and economical method in differentiating OKC from other lesions by the presence of keratin flakes, epithelial cells, mixed inflammatory cells, erythrocytes and hemorrhagic areas as compared to FNAC.
Woo et al.[36]	2018 Original article Korea	Pleural Effusion	1014	Sn= 94.3%(CB), 81.3% (LBC) Sp=98.7%(CB), 99.4%(LBC) (LBC) DR. for malignant lesions 78.9% (CB), 68.3% (LBC)	<0.05	CB should be used along with LBC in routine clinical practice to improve diagnostic accuracy esp. in lesions with malignant potential or frankly malignant lesions.
George et al.[38]	2017 Original article Dominican Republic	Cervical smears	325	CB Inflammation = 58% (ASCUS),65%(AGC) Atrophy= 10%, Reactive changes= 47% Detection rate for LSIL= 71%	0.228	Inflammatory and atrophic changes are easier to diagnose in CB as compared to LBC. CBs can be useful in the detection of initial diagnosis of ASCUS and AGC.
Kulkarni et al. [37]	2017 Original article India	Cervico-vaginal smears	50	Sn= 75% (CB) 66% (LBC), 50% (CS) Sp=93%(CB), 84% (LBC) 70% (CS) CB/Hp=74% CPS/Hp=54%	≤0.05	CB showed increased sensitivity and specificity in the diagnosis of neoplastic conditions of the cervix. It also helps to distinguish b/w HSIL and SCC.
Sadullahoglu et al. [39]	2017 Original article Turkey	Bronchial aspiration & bronchial brushings	240	Sn= 54.8%(CB) 45%.6%(LBC) DR= 55.1% (CB), 43.8% (LBC)		CB resulted in a 10.1% increase in diagnostic sensitivity. Thus, adding CB to LBC contributes to the improvement in the cytological diagnosis of BA as well as BB.

Zhang et al. [40]	2016 Original article China	Endometrial samples	184	Sn=82.8%(CB), 79.3%(LBC) Sp=98.3%(CB), 97.4%(LBC) PPV=92.3%(CB), 88.5%(LBC) NPV=95.8%(CB), 94.9(LBC)	<0.01	CB and LBC together increase the diagnostic accuracy of EC to 95.8%.
Qin et al. [41]	2014 Original article China	FNA specimen of pancreatic lesions	72	Sn=90%(CB) 73%(LBC) 70%(CS) NPV=66.7% (CB), 31.6% (LBC), 30% (CS)	<0.05	CB immunohistochemistry provides higher diagnostic efficacy as compared to CS and LBC.

CB= Cell Block, LBC= Liquid based cytology, CS= Cytology smear, Sn= Sensitivity, Sp= Specificity, NPV= Negative predictive value, PPV= Positive predictive value, Acc= Accuracy, CR= Concordance rate, Hp= Histopathology, DR= Detection rate, React. Ch.= reactive changes, DR.= Detection rate, LSIL, Low-grade squamous intraepithelial lesion, ASCUS=Atypical squamous cells of undetermined significance, AGC= Atypical glandular cells, BA= Bronchial aspiration, BB= Bronchial brushings, FNAc= Fine needle aspiration cytology, OKC= Odontogenic keratocysts, DC= Dentigerous cyst, RC= Radicular cyst, AMB= Ameloblastoma.

LBC can be a better substitute for conventional smears because of the reduced rate of unsatisfactory smears. However, as the detection rate of epithelial lesions is similar using conventional and liquid-based techniques, conventional cytology has proven to be the best screening technique in a low-resource setting considering its cost-effectiveness over LBC. To achieve better results, MLBC can be used in these cases as a good alternative.

CONCLUSION

Timely and accurate evaluation of oral potentially malignant lesions is essential to prevent their malignant transformation. Therefore, to get an accurate diagnosis, the latest techniques with better and validated sensitivity and specificity may be used. LBC and CB techniques are strongly advocated in the greatest interest of public health as they improve the sample quality and reduce the probability of false-negative results compared with the conventional technique, hence recommended for routine diagnostic purposes. Moreover, as in Pakistan, minimal data is available describing these techniques for the detection of oral lesions. Therefore, studies on these lesions with skilled professionals and focusing on diagnostic efficacy must overcome the pitfalls and get a better yield for timely diagnosis to reduce consequent morbidity and mortality.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

MJ did substantial contributions to the conception, design and drafting of the manuscript. RA contributed to the conception of the manuscript, analysis and interpretation of data. GZ did the analysis and interpretation of data. NN revised the manuscript critically for important intellectual content, final approval of the version to be published.

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