Original Article

# Establishing the accuracy of a new and cheaper sample collection tool: Oral cytology versus oral histopathology

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## Abstract

**Objective:** High cost of tools used for the collection of oral cytology sample; deters their use as mass screening tool in the developing countries. Hence, this study aims to assess the diagnostic accuracy of a new, cheaper sample collection tool in the field of oral exfoliative cytology through comparison with histopathology.

**Materials and Methods:** Fifty-seven patients out of 394 cases were selected for the study in whom the cytological examination was performed using the wooden end of a sterile cotton swab followed by biopsy. The cytological smear classified in accordance with the Pap classification was compared with the histopathological diagnosis.

**Results:** The study recruited 11 cases of oral potentially malignant disorders, 33 cases of oral carcinomas, and 13 cases of other lesions. The sensitivity and specificity of the cytology test using the wooden end of a sterile cotton swab was 75.0% and 61.5%, respectively. The Spearman's correlation coefficient was 0.618, with the $P < 0.01$.

**Conclusion:** Our study proves that the use of wooden end of a sterile cotton swab stick offers a low cost and fairly effective solution which is not only easily available but can be potentially applied as a mass screening tool at primary health-care centers.

**Keywords:** Oral carcinomas, oral exfoliative cytology, oral potentially malignant disorders, Pap classification

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**Submitted:** 12-Sep-2019, **Revised:** 23-Sep-2019, **Accepted:** 26-Sep-2019, **Published:** 08-May-2020

## INTRODUCTION

Exfoliative cytology refers to the microscopic examination of cells obtained from the body for diagnostic purposes. It has gained its popularity in the field of gynecology as a diagnostic tool for cervical cancer. Since its advent and generous usage in cervix cytology along with the Papanicolaou technique in the 1940s, the number of deaths from cervical cancer has decreased.[1] However, the role of exfoliative cytology in screening for oral cancer still is not as well established as it is in the diagnostic process of the uterine cervix cancer.[2]

Cytological study of oral cells was first attempted by Montgomery and von Haam.[3] It is now a well-known noninvasive technique that is well accepted by the patient. It remains an attractive option for early diagnosis of varied

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_273_19



**How to cite this article:** Sood A, Mishra D, Yadav R, Bhatt K, Priya H, Kaur H. Establishing the accuracy of a new and cheaper sample collection tool: Oral cytology versus oral histopathology. J Oral Maxillofac Pathol 2020;24:52-6.

oral lesions such as potentially malignant disorders, oral carcinomas, vesiculobullous disorders, fungal infections and viral infections.[4,5]

Oral cells can be obtained by a wide variety of techniques which may require a scraping of the surface of the mucosa, rinsing of the oral cavity or taking a sample of saliva from the patients.[2] The use of cytology tool yields cells from deeper layers of the epithelium which when appropriately stained and subjected to microscopic evaluation can provide critical data with regard to dysplastic changes and cellular abnormality. It has also been asserted that oral cytology holds a higher diagnostic value than specialist's oral examination. However, its diagnostic accuracy still needs to be assessed.[6]

There have been a number of tools in English literature used for collection of sample from the oral cavity [Table 1].[2] Even though the sensitivity and specificity of such tools for the diagnosis of oral lesions have been reasonably high; their high cost deters their use a mass screening tool in the developing countries. Hence, this study aims to assess the diagnostic accuracy of a new, cheaper sample collection tool in the field of oral exfoliative cytology through comparison with histopathology.

## MATERIALS AND METHODS

This cross-sectional pilot study was conducted at the Centre for Dental Education and Research, All Institute of Medical Sciences from August 2017 to July 2018. We retrospectively included patients presenting with oral lesions to the center, in whom a scrape cytological examination using the wooden end of a sterile cotton swab stick [Figure 1] had been performed along with a biopsy. A total of 394 cases were subjected to cytological diagnosis during this period, among whom 57 cases who simultaneously underwent a biopsy procedure fulfilled the selection criteria.

The sample for the cytological smear was taken from the most representative site of the oral lesion using the wooden end of a sterile cotton swab stick. The round wooden end


**Figure 1:** Wooden end of a sterile cotton swab used for oral scrape cytological examination

**Table 1: Comparison of oral smear collection tools reported in literature**

| Collection device | Disadvantages | Advantages | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| Cotton tip applicator[24] | Difficult to use in lingual, mandibular regions<br>Long inflexible handles<br>Few epithelial cells collected | Economical<br>Easy availability | 93.8-96 | Not stated |
| Metal spatula[23,24] | Prone to cellular and nuclear distortion<br>Difficult to use in lingual, mandibular regions<br>Long inflexible handles<br>Can be excessively invasive | Easy availability | 55-60 | 100 |
| Wooden spatula/ tongue depressors[24] | Delivers thick cellular aggregates due to mechanical damage<br>Difficult to use in lingual, mandibular regions<br>Long inflexible handles<br>Difficulty in penetrating keratotic lesions | Economical<br>Easy availability | 86.5-97.5 | 88.9-100 |
| Curette[17,24] | May cause patient discomfort | Adequate sampling from thick keratotic lesions | 87-100 | 99 |
| Tooth brush[19] | Easily available<br>Economical | | 77 | 100 |
| Nylon flocked swab[28] | Use of preservatives may affect sample | Designed to collect more cells and pathogens | 81 | 73 |
| Cytobrush[17,22] | Expensive | Collects more cells<br>Less distortion | 90-99 | |
| Cytobrush Plus GT | Expensive | Superior cell collection from lateral border of the tongue | | |
| Oral CDx™ brush[17,26,27] | Expensive | Aided by computer assisted reporting | 95-96.3 | 90-95 |
| Orcellex[21] | Expensive | Purported to collect cells from all layers of epithelium<br>Useful in thick keratin layers | 60 | 99 |

was rolled over the chosen site for 20–30 s keeping constant contact during the mentioned duration, and a smear was prepared from the sample acquired. The smear was then subjected to conventional Pap staining procedure which has been previously standardized.[7] The cytological smears were classified in accordance with the Pap classification and subsequently compared with the histopathological diagnosis. On the basis of histopathological diagnosis, the cases were classified into three groups – malignancies, oral potentially malignant disorders (OPMDs) and others which consisted of epithelial hyperplasia and verrucous hyperplasia without dysplasia.

The statistical analysis was done using IBM SPSS Statistics Version 21 (North Castle Drive, Armonk, NY, U.S.A) and sensitivity, specificity was calculated. Spearman correlation test and bivariate random-effects ordered regression model were also performed to evaluate the correlation with histopathology and to ascertain the odd's risk, respectively. For the purpose of statistical analysis and comparison with histopathological diagnosis, lesions classified as Class I and Class II (Pap classification) were considered to be negative for dysplasia/malignancy while lesions classified as Class III, Class IV and Class V were considered to be positive for dysplasia/malignancy.

## RESULTS

The study consisted of 11 cases of OPMDs (included histopathologically diagnosed oral epithelial dysplasia [9 cases], oral submucous fibrosis [1 case] and oral lichen planus [1 case]), 33 cases of oral carcinomas and 13 cases under the category of "Others" (included clinically suspicious lesions with no dysplasia/malignancy). Out of the subjects selected for the study, 86.2% were males and 12.1% were females with most of them reporting around 35 years of age. About 63.8% cases reported with a smokeless tobacco habit while 19% did not report with any habit. The case distribution in accordance with Pap classification is shown in Table 2. Histopathological evaluation revealed 75.9% of cases reporting with dysplasia/malignancy. Histopathological and cytological correlation of cases is given in Table 3. Thirty-three cases which were diagnosed histopathologically as oral carcinomas were classified as Papanicolaou Class V (11), Class IV (9), Class III (9) and Class II (4) in cytology. Eleven cases of OPMDs were cytologically classified as Class II (6), Class III (4) and Class I (1). Others group cytologically showed 8 cases in cytological Class I and II, 5 cases in Class III and IV. The sensitivity and specificity of the cytology test using the wooden end of a sterile cotton swab was 75% and 61.5%, respectively. The Spearman's correlation coefficient was 0.618 with the $P < 0.01$. Logistic regression revealed odds risk to be 2.9 with $P < 0.05$.

**Table 2: Case division on basis of papanicolaou classification**

| Category | Frequency (%) |
|---|---|
| Class I | 4 (6.9) |
| Class II | 15 (25.9) |
| Class III | 17 (29.3) |
| Class IV | 10 (17.2) |
| Class V | 11 (19.0) |
| Total | 57 (100.0) |

**Table 3: Histopathological and cytological correlation of cases**

| Cytological category | Histological division | | |
|---|---|---|---|
| | Malignancy | OPMDs | Others |
| Class I | - | 1 | 3 |
| Class II | 4 | 6 | 5 |
| Class III | 9 | 4 | 4 |
| Class IV | 9 | - | 1 |
| Class V | 11 | - | - |
| Total | 33 | 11 | 13 |

OPMDs: Oral potentially malignant disorders

## DISCUSSION

Oral and oropharyngeal cancer is a well-known health burden. Delayed diagnosis accounts for major morbidity and mortality as most of the oral cancer cases are diagnosed at Stage III or Stage IV. Early detection and treatment can improve survival rates to 82%; however, this can decrease to 32% if metastasis has occurred.[8] Thus, early detection and management can substantially improve prognosis and survival rate of patients. Cytology including fine-needle aspiration technique is recognized as cost-effective, simple, accurate and a safe procedure for making a specific diagnosis that dictates management decisions by the treating clinicians.[9] Although scalpel biopsy and histological assessment have been considered as the gold standard for the diagnosis of oral cancer and potentially malignant disorders, the high sensitivity and specificity for cytology offer the most potential in comparison with other adjunctive techniques namely vital staining, light-based detection, oral spectroscopy and blood or saliva analysis.[10] In addition, when compared to histopathology, the cytopathology technique is dramatically swifter, office-based and the results can be known in as little as an hour. Apart from aiding in diagnosis of oral cancer and potentially malignant disorders, oral cytology has also been used for the diagnosis of anemia, diabetes mellitus, infectious diseases in particularly oral hairy leukoplakia and others such as Behcet's disease[11-15] as well as identification of potential biomarkers for oral cancer progression.

Through this study, we intended to evaluate the diagnostic efficacy of exfoliative cytology, collected by a novel

sampling tool, against the currently used gold standard of surgical biopsy coupled with the histopathological assessment. The importance of this comparative analysis is to contribute to the development of safe, minimally invasive and inexpensive tool for screening/early diagnosis and surveillance of oral squamous cell carcinoma and OPMDs.

The cytological diagnosis of the lesions is highly dependent on the adequacy and representativeness of the test sample, which in turn is dependent on the sample collection tool. This issue has been raised by the recent attempts of the development of an oropharyngeal Pap-test equivalent by Fakhry *et al*. which was found to be unsuitable due to insufficient sampling of the relevant cells in the tonsillar epithelium.[16] Over the years, several oral smear collection tools have been introduced, such as spatulas, dermatological curette, Fisherbrand sterile polyester swab, interproximal brush and toothbrush are to name a few [Table 1].[6,17-23] Variable results have been published with regard to the superiority of any one sampling tool hence the search for the optimum sample collection tool in the field of cytology continues till date.

The use of a sterile cotton swab or a cotton tip applicator, wooden/metal spatula for the collection of oral smears has been proposed long back. The cotton tip end of the sterile swab was reported to collect fewer cells on account of its nonadhesive surface and cellular entrapment within the cotton mesh. The wooden or metal spatula, on the other hand, presented with thick cellular aggregates hence cellular distortion.[22-24] Hence, in the current study, an attempt was made to evaluate the wooden end of a sterile cotton swab stick. The wooden end of the stick not only reduces cellular distortion but also allows for the collection of cells from posterior regions of the oral cavity especially in patients with impaired mouth opening.

In this study, four cases of malignancy were classified cytologically as Class II (negative for dysplasia/malignancy), that may be explained by nonrepresentative sampling or subjectivity as cytology was not performed by the same examiner. However, majority of malignant cases (29 [87%]) were graded under cytological Grade III, IV and V (positive for dysplasia/malignancy), indicating that this tool used in exfoliative cytology may be beneficial as a supplementary tool for diagnosing oral cancer. In the current study, the sensitivity and specificity of the wooden end of a sterile cotton swab was 75.0% and 61.5%, respectively. Both the specificity and the sensitivity of the oral CDx™ brush is higher than that of the current tool as reported by Scheifele *et al*.[20] The Spearman's coefficient was found to be significant with a value of 0.618 which means that there is a moderate correlation between cytological and histopathological diagnosis. The odds risk was 2.9 indicating that a lesion categorized as Class V (Pap classification) is nearly three times more likely than a Class I lesion to show dysplasia/malignancy on histopathological examination.

In our study, 86.2% of the case subjects were males ($n = 50$) and 12.1% of the cases were females ($n = 7$) which is similar to the distribution reported by Mehrotra *et al*.[25] Tobacco habit was reported in nearly 64% of the case subjects, while 19% did not report with any habit in our study. The remaining subjects abstained from providing information regarding tobacco usage. Mehrotra *et al*. reported 54% tobacco users in his study while 38.3% cases had tobacco habit in the study by Babshet *et al*.[19]

Tools used for collecting oral scrape smears should be commonly available in clinics, should not need special transportation or preservation method, nonirritating, inexpensive and provide an adequate number of epithelial cells following exfoliation. The current tool appears to meet all these criteria. This is of special significance for oral cancer screening in India, where the incidence of oral cancers and oral premalignant lesions is quite high in comparison to the Western population. Oral CDx™ brush, despite its high sensitivity and specificity is difficult to apply at a mass screening level due to the expense involved. Oral CDx brush is a computer-aided analysis of the epithelium of the oral mucosa.[26,27]

Others, including Cytobrush™ and Orcellex™ are also plagued by similar issues of poor availability and costly procurement. The use of wooden end of a sterile cotton swab offers an inexpensive and fairly effective solution which is abundantly available and can be easily applied as a mass screening tool at primary health-care centers.

## CONCLUSION

There are relatively few studies in the English literature based on the validation of oral cytology sample collection tools. Our experience with the currently purposed sample collection tool is promising but needs to be evaluated on a larger scale taking into account the complexities associated with inter-observer variation, to pave the way for its application on a large scale in the field of oral exfoliative cytology.

### Financial support and sponsorship
Nil.

### Conflicts of interest
There are no conflicts of interest.

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