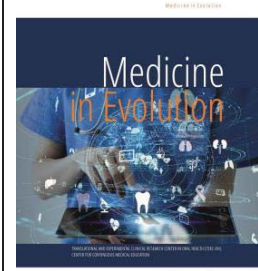


Assessing the Diagnostic Potential of Brush Biopsy for Oral Cancer Detection



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Abstract

1. Background/Objectives: This study aims to assess the effectiveness of brush biopsy in detecting malignancies in the oral cavity through clinical examinations of patients attending regular dental check-ups. Additionally, it evaluates the diagnostic value of brush biopsies by analysing the cytological results, risk factors, and lesion localization while discussing the role of oral cancer prevention and the method's advantages and limitations in routine dental practice. 2. Material and methods: After a detailed inspection and palpation of the soft tissue in the oral cavity, cells from a suspicious, potentially precancerous lesion were collected using a cytobrush, which was stroked multiple times over the lesion in a rotating motion under light pressure in order to obtain as many cells as possible. The present study included 60 patients who met the eligibility criteria, selected based on age distribution (18 to 90 years old) and assessed for key risk factors such as alcohol consumption and smoking habits. 3. Results: The obtained results confirmed that leukoplakia was the most frequently diagnosed condition, summing 25% of all cases, that represent 17 out of the lesions identified, while lichen planus represented 19% of diagnoses, with 13 cases detected, both being recognized as premalignant lesions requiring careful monitoring. 4. Conclusions: Brush biopsy is a non-invasive, cost-effective, and easily integrated diagnostic tool for early oral cancer detection, complementing traditional biopsy methods by enabling timely intervention, reducing unnecessary surgical procedures, and supporting preventive dental care, with further research needed to validate its clinical role.

Keywords: Oral cancer, brush biopsy, early detection, cytology, oral pathology

INTRODUCTION

Oral cancer is a significant public health concern, with a rising global incidence and substantial morbidity and mortality rates [1]. Early detection plays a central role in improving patient outcomes and survival rates [1]. Traditional diagnostic techniques, such as visual examination and tissue biopsy, have diagnostic limitations in terms of invasiveness, subjectivity, and potential sampling errors. Therefore, there is a growing need for reliable and non-invasive methods that can aid in the early detection of oral cancer [2, 3]. The brush biopsy technique has emerged as a potential tool for detecting oral cancer. This technique involves the use of a specially designed brush to collect cells from suspicious oral lesions. Brush biopsy offers several advantages over conventional biopsy methods, including its simplicity, cost-effectiveness, and ability to be performed in an outpatient setting [4].

A brush biopsy is a diagnostic procedure used to detect oral cancer. It is a minimally invasive technique, in which a small sample of cells from the oral cavity gets collected for an examination under a microscope [4,5]. The brush biopsy procedure involves using a small brush with fine bristles to gently collect cells from the surface of suspicious areas in the mouth, such as lesions, ulcers, or abnormal tissue [6]. Each lesion must be sampled separately to ensure accurate localization of results. During routine dental visits, the oral mucosa should be examined for abnormalities, and any unclear changes suspected of neoplasia should be documented along with a brush biopsy. The collected cells are preserved in a liquid medium and sent to a pathologist for evaluation of abnormalities or cancer signs [7, 8].

Clinically conspicuous lesions, particularly those with high-grade dysplasia or invasive squamous cell carcinoma, should undergo morphological assessment for early detection, as prognosis is significantly better in early stages [9]. Since oral mucosal carcinoma primarily affects the epithelium, brush biopsy is a useful diagnostic tool, reducing the need for overdiagnosis associated with frequent surgical biopsies. Compared to conventional surgical biopsies, brush biopsy offers several advantages, including lower costs, minimal material requirements, and a simple, rapid procedure [10]. Its non-invasive nature makes it suitable even for anxious patients. While surgical biopsies are typically reserved for highly suspicious lesions, brush biopsies have a broader range of indications. They are effective for exclusion diagnosis, early detection, and monitoring of oral mucosal carcinoma and potentially malignant oral disorders. Oral cancer, predominantly oral squamous cell carcinoma (OSCC), represents a significant public health issue worldwide [11].

The incidence of OSCC has been rising, and despite advancements in treatment, survival rates remain low due to late-stage diagnoses [12]. Common risk factors for oral cancer include tobacco use, alcohol consumption, human papillomavirus (HPV) infection, and poor oral hygiene [13]. Early detection plays a crucial role in improving prognosis, as it allows for timely intervention and reduces the likelihood of disease progression. Current diagnostic methods for oral cancer rely heavily on conventional biopsy techniques, which, while accurate, are invasive and can cause patient discomfort [14]. The brush biopsy has emerged as a non-invasive alternative that enables clinicians to collect epithelial cells from suspect lesions with minimal discomfort to the patient. This technique holds potential as a screening tool for the early detection of oral malignancies, particularly in dental settings where routine examinations are conducted [15]. Despite its advantages, the diagnostic accuracy of brush biopsy remains under continuous evaluation [15]. This study aims to investigate its effectiveness compared to standard histopathological methods, assess its feasibility in routine dental practice, and explore its role in identifying potentially malignant, premalignant and malignant lesions. By analyzing patient demographics, lesion

characteristics, and cytological findings, this research seeks to provide valuable insights into the potential implementation of brush biopsy in primary dental care settings.

Aim and objectives

The aim of this observational, prospective study is to evaluate the outcome of the clinical extra-oral and endo-oral examination of the patients that are referring on a regularly basis to the dental office for check-ups or planned treatments, and quantify the presence potentially malignant, premalignant or malignant lesions, as well as the use and efficiency of the brush biopsy technique for the detection of malignancy in the oral cavity.

In addition, a further evaluation of the cytological diagnosis, the disease spectrum, risk factors and localization on the oral mucosal will be performed in order to evaluate the diagnostic value of brush biopsies. Furthermore, the importance of oral cancer prevention, the advantages and disadvantages of the oral brush biopsy in general dental routine and the limits of this method are discussed.

MATERIAL AND METHODS

The Clinic of Oral Pathology of the “Victor Babes” University of Medicine and Pharmacy conducted a study in collaboration with the dental office “Zahnarztpraxis Dr. Sautré” in Düsseldorf, Germany, during the period between July 2023 – December 2023.

Patients that presented in the dental office for all kind of treatments and regularly check-ups were screened for oral lesions. Those patients with any kind of oral lesions (benignant lesions, fungal lesions, denture related lesions), were included in the data collection, as well as patients with potentially malignant lesions, including leukoplakia lesions, lichen planus and ulcerative lesions were quantified.

The inclusion criteria were represented by: >18 years of age, both genders, visible lesion in the oral cavity or need for oral cancer prevention, compliant patient

The exclusion criteria were represented by: <18 years of age, non-compliant patients

This examination protocol was divided into 3 parts: the medical history of the patient (questionnaire), the extraoral examination and the intraoral examination.

After a detailed inspection and palpation of the oral cavity, the cells from a suspicious, potentially precancerous lesion were collected, using a cytobrush (Figure 1, Figure 2). This type of brush was stroked over the lesion several times, performing a rotating motion in the same direction and under light pressure to obtain as many cells as possible (Figure 3, Figure 4).

After the oral inspection that focused as well on the oral mucosa, a cytobrush (Rovers Orcellex Brush RT, Oral Cell Sampler) was brushed over the clinically suspicious lesion by rotating it around 10 times to remove squamous epithelia from the epithelial lining (so-called forced exfoliation). The head of the brush was then loosened and stored a solution (BD SurePath™) to preserve the collected cells (Figure 5, Figure 6). An accompanying cytopathology form was filled out for each brush biopsy, containing at least the patient's name, date of birth and insurance details, but usually also information on localization, clinical picture and any previous illnesses and therapies.

The lesions were described in terms of their location, color, surface structure and size and in many cases a suspected diagnosis was made. Important risk factors, such as a previous squamous cell carcinoma diagnosis or a previously diagnosed lichen planus, were also noted on the accompanying form. The main concern of the question about malignancy was also made clear in all forms.



Figure 1. The Brush Biopsy Kit used (Celligence - die innovative Bürstenbiopsie - Mundwerk Deutschland GmbH & Co. KG)



Figure 2. The Brush Biopsy Kit used (Celligence - die innovative Bürstenbiopsie - Mundwerk Deutschland GmbH & Co. KG)



Figure 3. The Brush used for the Biopsy (Rovers Orcellex Brush RT, Oral Cell Sampler)



Figure 4. The Brush used for the Biopsy (Rovers Orcellex Brush RT, Oral Cell Sampler)



Figure 5. The Solution used: BD SurePath™



Figure 6. The Solution used: BD SurePath™

RESULTS

In a quarter, the dental clinic "Zahnarztpraxis Dr. Sautré" sees an average of 900 different patients. The study was conducted from July 2023 until December 2023, covering two quarters. This accounts for approximately 1800 different patients over the duration of the study. Among these 1800 patients, 60 met the criteria for inclusion in the study.

In the age groups of 18-30, 30-40, and 40-50, there were 6 patients each, accounting for 10% of the total patients each. Twelve patients fell within the 50-60 and 60-70 age brackets, representing 20% of the total patients each. The highest number of patients, 13, belonged to the 70-80 age group, accounting for approximately 22% of the total patients. Five patients were between 80-90 years of age, making up around 8% of the total patients. In total, the study included 60 patients.

The two most striking risk factors for oral cancer development are alcohol consumption and smoking. First, the risk factors from the whole patient collective were evaluated. From all patients, 28% claimed regular alcohol consumption and 10% tobacco consumption only. The combination of both risk factors significantly increases the risk of development of oral cancer and was stated by 17% of patients. Of the patient collective 45% did not mention any risk factors (Table 1). It has to be noted that some patients may feel prejudged by their doctor and fear to claim any risk factors.

Table 1. The risk factors of the patient collective

Risk Factors Mentioned	Percentage
No risk factors mentioned	45%
Alcohol consumption	28%
Smoking + Alcohol consumption	17%
Smoking	10%

Twenty-seven out of the total 60 patients denied any risk factors, including alcohol consumption or use of tobacco. Alcohol was consumed by 17 patients a regular basis. The combination of smoking and alcohol use was seen in 10 patients. Purely smoker were 6 patients. It can be seen that, if you are smoker, it is more likely that you also consume alcohol, than vice versa. In a subsequent analysis, the study examined the risk factors associated with patients who underwent brush biopsy. Among the patients included in the study, it was found that 11 individuals (42%) did not report any specific risk factors. However, a significant proportion, constituting 27% of the patients, reported a history of both smoking and alcohol consumption (Table 2). Additionally, four patients reported smoking only, while another four patients reported alcohol consumption only.

Table 2. The risk factors of the patient a Brush Biopsy was performed

Risk Factors Mentioned	Percentage
No risk factors mentioned	42%
Alcohol consumption	27%
Smoking + Alcohol consumption	16%
Smoking	15%

Most of the 69 lesions were located on the vestibular mucosa, the jugal mucosa, or tongue. A number of 16 lesions (24%) were localised in the vestibule (left and right combined) and a total of 14 lesions (20%) were found on the cheek (jugal mucosa, right and left combined). The tongue mucosa was involved in 12 cases (16%). Lesions were also found on the labial mucosa (7), the floor of the mouth (6), the edentulous ridge (5), the hard palate (4), the gingiva (3) and the retromolar area (2) (Table 3).

Table 3. Location of the Lesion

Area	Percentage
Vestibule	23%
Jugal Mucosa	20%
Tongue	18%
Labial Mucosa	10%
Floor of the mouth	9%
Edentulous Ridge	7%
Hard Palate	6%
Gingiva	4%
Retromolar Area	3%

In the clinical evaluation, the most frequently diagnosed condition was leukoplakia, accounting for 25% of all cases, with a total of 17 lesions identified. Following closely behind was lichen planus, comprising 19% of diagnoses with 13 lesions detected. Both leukoplakia and lichen planus are recognized as premalignant lesions, necessitating careful monitoring and management. In addition to leukoplakia and lichen planus, various other lesions were observed within the group of patients included. These cases represented by 8 cases of ulcerations (12%), 6 instances of hyperkeratinisation (9%), and 6 aphthous lesions (9%). Furthermore, 4 cases of hyperkeratinisation referring to bite marks, 3 instances of pressure point ulceration due to poorly fitting dentures, and 3 cases of candida were identified (Table 4). Less frequently observed were 2 cases of erythroplakia, along with individual instances of a burning lesion, prevention, petechiae, fibroma, and persisting irritation ulcer. Each of these conditions calls for specific attention and, where necessary, appropriate treatment to ensure the patient's oral health and well-being.

Table 4. Clinical diagnosed lesions

Condition/Lesion	Percentage
Leukoplakia	25%
Lichen Planus	19%
Ulcer	12%
Hyperkeratinization	9%
Aphthous lesion/Inflammation	9%
Bite marks	6%
Pressure Point Ulcer	4%
Candida	4%
Prevention of rezidive of OSCC	3%
Erythroplakia	2%
Burning lesion	2%
Prevention	1%
Petechiae	1%

From the 69 lesions which were included in the study, in case of 32 lesions a brush biopsy was performed. Out of these 32 brush biopsy samples, the most frequent diagnosis was leukoplakia, with 19 results in total (60%). The 2nd frequent cytological diagnosis was “no malignant cells” which refers to clinical diagnosis like ulcerations. Three cases of lichen planus were detected and each one case of mycosis, erythroplakia and candida (Table 5).

Table 5. Biopsy Results

Condition/Lesion	Percentage
Leukoplakia	60%
No malignant cells	22%
Lichen planus	9%
Mykosis	3%
Erythroplakia	3%
Candida	3%

Out of the 32 biopsies performed, 10 were taken from the vestibule, accounting for 31% of the total. Eight lesions were biopsied from the tongue, while seven were taken from the jugal mucosa. In three cases, biopsies were taken from the floor of the mouth, two from the hard palate, and one each from the retromolar area and the edentulous ridge. Interestingly, although seven lesions were found on the labial mucosa, no biopsies were taken from them. Similarly, no biopsies were taken from the three lesions located on the gingiva (Table 6).

Table 6. Location of the Brush Biopsy performed

Area	Percentage
Vestibule	31%
Tongue	25%
Jugal Mucosa	22%
Floor of the mouth	10%
Hard Palate	6%
Retromolar area	3%
Edentulous ridge	3%

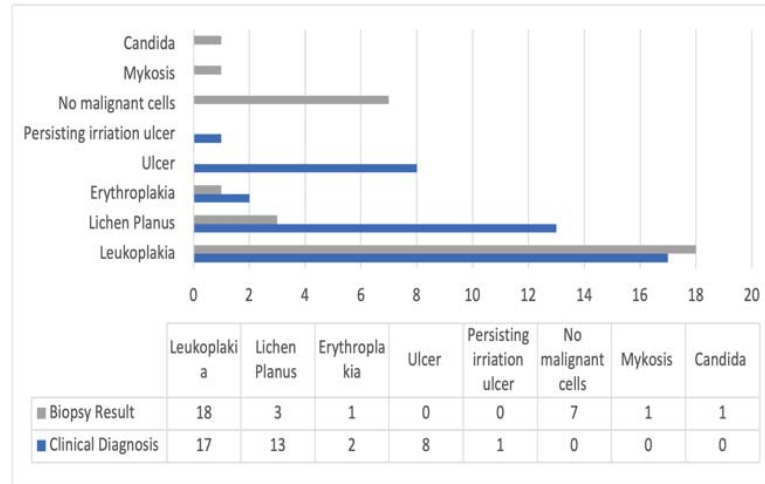


Figure 1. The Clinical Diagnosis of the Lesions vs. The Cytological Diagnosis

A comparison of clinical and cytological diagnoses revealed notable findings. Leukoplakia was clinically diagnosed in 18 lesions, with 17 confirmed by biopsy, indicating high diagnostic accuracy. However, lichen planus showed a significant discrepancy – while it was clinically diagnosed in 13 cases, only 3 were confirmed cytologically, suggesting challenges in its clinical identification (Figure 7). Erythroplakia was clinically identified in two cases, but only one was cytologically confirmed. The diagnosis of "no malignant cells" was mainly associated with biopsies of ulcerative lesions or those performed as a preventive measure, such as monitoring potential recurrence of oral squamous cell carcinoma (OSCC). Additionally, one case of *Candida albicans* infection and another of mycosis were identified, emphasizing the importance of cytological evaluation in accurately distinguishing various oral lesions.

DISCUSSIONS

The findings from this study align with the existing literature on the effectiveness of oral brush biopsy (OBB) in the early detection of oral cancer. The results of this study, which included 60 patients from July 2023 to December 2023, demonstrate that OBB is a reliable method for identifying precancerous and cancerous lesions in the oral cavity.

The patient cohort consisted of 29 females and 31 males, with a median age of 60 years. A total of 69 lesions were found, of which 32 were subjected to brush biopsy. The most common lesion identified was leukoplakia, with 18 clinically diagnosed cases and 17 confirmed by biopsy. This high prevalence underscores the importance of routine screening in detecting such lesions early.

Compared to conventional scalpel biopsy, brush biopsy provides a non-invasive alternative with high patient acceptance. Prior studies, including Neumann et al. (2022) and Deuerling et al. (2019), report high sensitivity and specificity for brush biopsy. The study highlights its potential as a routine screening tool in general dental practice, particularly in detecting leukoplakia and early-stage OSCC. However, discrepancies in diagnosing lichen planus underscore the need for combined diagnostic approaches. The findings of this study align with existing literature on the effectiveness of oral brush biopsy (OBB) for early oral cancer detection. Conducted between July and December 2023 with 60 patients, the study confirmed OBB as a reliable method for identifying precancerous and cancerous oral lesions. The patient cohort included 29 females and 31 males, with a median age of 60 years. Among

69 detected lesions, 32 underwent brush biopsy, with leukoplakia being the most common diagnosis.

Comparison with previous studies, such as Neumann et al., highlights similar findings, with leukoplakia being the most frequently detected lesion. The study by Neumann et al., conducted in Germany between 2014 and 2016, involved 670 patients and 814 brush biopsies analyzed using liquid-based cytology (LBC). The sensitivity for cancer detection was 100%, with a specificity of 86.5%, reinforcing the reliability of OBB in general dental practice.

A study by Deuerling et al. (2019) at the University of Leipzig further supported LBC's efficacy, analyzing 1,352 samples between 2012 and 2018. The findings showed a sensitivity of 95.6% and specificity of 84.9%, demonstrating that LBC is highly sensitive, minimally invasive, and an efficient alternative to traditional scalpel biopsy.

Gupta et al. [] in India compared exfoliative cytology, modified brush biopsy, and scalpel biopsy in 225 cases of oral precancerous lesions. The modified brush biopsy showed higher sensitivity (81.69%) than routine exfoliative cytology (48.57%), confirming its usefulness as a screening tool, particularly in resource-limited settings.

A 2023 study by Kokubun et al. in Japan analyzed 653 patients undergoing cytological and histological examinations. The findings indicated that while OBB had a sensitivity of 69% and specificity of 75%, some cases required histological confirmation, especially for deep-margin lesions.

Despite its advantages, oral brush biopsy presents certain limitations that must be considered. One of the main concerns is the possibility of false-negative results, particularly in cases of squamous cell carcinoma (SCC), where tumoral cells may be located in deeper layers, making them difficult to detect [36]. Additionally, the specificity of OBB is lower than that of scalpel biopsy, which means it may not always accurately distinguish between dysplastic and non-dysplastic lesions [28]. Some conditions, such as lichen planus and erythroplakia, also pose diagnostic challenges, often requiring additional histological confirmation to ensure an accurate diagnosis [24]. Another important factor influencing the reliability of the method is its dependence on sample quality. The accuracy of liquid-based cytology and brush cytology largely depends on proper sample collection and analysis, and any deficiencies in this process may lead to inadequate or inconclusive results [36]. Furthermore, while OBB is highly effective for early detection, its diagnostic value in advanced lesions remains limited, as it may not be sufficient for identifying deeply invasive or complex lesions, which necessitate histological follow-up [23].

Overall, these studies affirm that oral brush biopsy, particularly when combined with liquid-based cytology, is a highly sensitive, non-invasive, and practical tool for early oral cancer detection. It enhances diagnostic accuracy in general dental practice, supports early intervention, and reduces oral cancer mortality rates.

CONCLUSIONS

Brush biopsy proves to be a valuable diagnostic tool for early oral cancer detection. Its non-invasiveness, affordability, and integration into routine dental check-ups support its widespread adoption. While it does not replace traditional biopsy methods, it serves as an effective preliminary screening technique, prompting timely intervention. Further research and larger cohort studies are recommended to validate its role in clinical practice.

The daily use of brush biopsy in dental offices for cancer detection holds great potential. It is a time-efficient procedure that can be easily integrated into routine dental check-ups. Additionally, it is cost-effective for both patients and dentists, providing an affordable alternative for early cancer detection compared to invasive biopsy techniques.

While brush biopsy does not replace surgical biopsy, which remains essential for an accurate diagnosis, it helps reduce the need for unnecessary surgical interventions. In conclusion, liquid-based brush cytology is a highly sensitive and reliable method for diagnosing oral neoplasia. Its advantages over traditional invasive biopsy methods could facilitate early detection and treatment, ultimately improving patient outcomes.

Conflicts of Interest

The author declares no conflict of interest.

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