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Comparison of Transepithelial Cytology and Histopathology in the Diagnosis of Potentially Malignant and Malignant Lesions of the Oral Mucosa

SUMMARY

Background/Aim: *The objective of the study was to compare cytological diagnoses using transepithelial cytology (oral brush biopsy) with histopathological diagnoses obtained by incisional biopsy in patients with benign lesions, oral potentially malignant disorders and oral squamous cell carcinoma.* ***Material and Methods:*** *The study included 57 patients. Brush biopsy was performed after local anaesthesia administration using the cervical brush. It was immediately followed by an incisional biopsy. Modified Bethesda System was used for cytological analysis and correlated with histopathological diagnoses according to intraepithelial neoplasia.* ***Results:*** *Good agreement was shown between cytological and histopathological diagnosis (kappa = 0.791). The sensitivity of the study was 92.85%, specificity 100%, positive predictive value (PPV) was 100% and negative predictive value (NPV) 93.54%.* ***Conclusions:*** *Oral brush biopsy, which allows the collection of epithelial cells of all layers can provide fast, precise and efficient cytological results which are in good agreement with the 'gold standard' – incisional biopsy followed by histopathology.*

Key words*: Incisional Biopsy, Oral Brush Biopsy, Oral Carcinoma, Oral Potentially Malignant Disorder, Transepithelial Cytology*

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Introduction

Oral cancer represents a global health burden with a 5-year survival rate lower than 50% [1]. Oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy (excluding non-melanoma skin cancer)[2] and represents 80-90% of all malignant neoplasms of the oral cavity[3]. OSCC is often preceded by a group of lesions named 'Oral potentially malignant disorders' (OPMDs). OPMDs are disorders that have a statistically increased risk of progressing to OSCC and include leukoplakia, erythroplakia, leuko-erythroplakia, oral lichen planus (erosive and atrophic form), actinic cheilitis, palatal lesions associated with cigar-smoking, discoid lupus erythematosus and other inherited disorders[4-6]. Early detection of OSCC and OPMD is a key factor in the improvement of a long term prognosis, quality of patients' life and survival rate[7, 8]. Since early detection has a vital role in survival rate, it would be significant to standardize and introduce a simple, inexpensive and noninvasive method for early cell atypia detection within oral epithelium/mucosa.

Incisional biopsy with histopathological assessment remains the golden standard in the diagnosis of OPMDs and malignant lesions[9], but even this method shows some limitations[10]. Transepithelial cytology is a quick, easy to use and simple procedure, based on an atraumatic brush technique, allowing physicians to acquire complete transepithelial specimens over a wider area[11]. Recent progress in cytological procedures has led to the promotion of liquid-based cytology to improve the sensitivity of cytological smears[11]. This improvement from conventional cytology is beneficial, especially for molecular studies, but in the case of source-limited countries, the high cost of technology is a limiting factor for health care systems[11].

This prospective study aimed to compare transepithelial oral brush cytology with the golden standard in the diagnosis of oral lesions – incisional biopsy in terms of cytological and histopathological diagnosis and their consequent correlation.

Material and Methods

This cross-sectional study was conducted between December 2019 and September 2021. Patients were referred for an examination, diagnosis and possible treatment of suspicious lesions to the Department of Oral Medicine and Periodontology and Department of Maxillofacial Surgery, School of Dental Medicine, University of Belgrade.

The study was approved by the Ethical Committee (No 36/7) of the School of Dental Medicine, University of Belgrade and complies with the ethical and scientific principles as set out in the Declaration of Helsinki. All patients were informed about the study details and signed the informed consent before entering the study. The study included 59 patients with the following clinical diagnosis: benign lesions, OPMDs or OSCC. Inclusion criteria for the study were patients above 18 years and the presence of the aforementioned clinical diagnosis. Exclusion criteria were history of radio or chemotherapy of head and neck region.

Anamnestic data and clinical examination

During the clinical examination, a visual inspection of the oral cavity was performed under the appropriate overhead light as well as a complete head and neck examination – the inspection and palpation based on the World Health Organization oral cancer diagnosis protocol[12].

Brush biopsy

At the second visit, the patients underwent a brush biopsy procedure after local anaesthesia (Septanest, Septodont, UK) administration. A conical, sterile, cervical brush (Gima, Gessate, Milan, Italy) was pressed against the lesion and rotated 10 times clockwise to provoke pinpoint bleeding and to secure an adequate transepithelial cell collection. The cervical brush was rotated only in the area of the lesion, to avoid potential dissemination of malignant cells into the surrounding healthy tissue. Collected cells were smeared on glass slides (Citotest Labware Manufacturing CO., Haimen, China), spray fixed using fixation solution (Citospray, Biognost, Zagreb, Croatia) and hematoxylin and eosin (H&E) stained by H&E stainer (Myr, Tarragona, Spain) using manufacturer protocol.

Surgical biopsy

A surgical biopsy was performed immediately after the brush biopsy. A scalpel blade 11 was used to collect a tissue sample. All lesions were sampled by the incisional biopsy, according to the clinical experience of two clinicians: a specialist in oral medicine and a specialist in maxillofacial surgery. The samples were immediately immersed in a 10% formalin solution (Merck, Darmstadt, Germany). Resorbable sutures were placed and removed after 7 days.

Tissue samples were moulded in paraffin blocks and cut on a microtome (Leica RM2245, Nussloch, Germany) at 4μm thickness, placed on slides and dried at 60°C for 60 minutes in an incubator (Binder, Tuttlingen, Germany). Dried glass slides were stained in an H&E stainer (Myr, Tarragona, Spain).

Samples evaluation

All samples were evaluated by an experienced pathologist subspecialized for oral and ear, nose and throat (ENT) pathology. The slides with an insufficient number of cells, as well as the slides contaminated with blood, necrotic material or exudate were discarded[13]. Cytological diagnoses were performed based on Bethesda System modified in 2001 [14] and were classified into NILM (negative for intraepithelial lesion or malignancy), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion) and OSCC (oral squamous cell carcinoma). Histopathological diagnoses were classified according to squamous intraepithelial neoplasia (SIN) system as SIN 0 (absence of dysplasia), SIN I (mild epithelial dysplasia), SIN II (moderate epithelial dysplasia), SIN III (severe epithelial dysplasia/carcinoma in situ) and OSCC (oral squamous cell carcinoma)[15].

Data and statistical analysis

SPSS 22.0 software package for Windows (SPSS Inc. Chicago, USA) was used for the statistical analysis. Agreement between diagnostic procedures was presented as a proportion of agreement and also as kappa coefficient – which corrects for the agreement that would be expected by chance. Coefficient between 0.81 and 1.00 was considered as 'very good agreement' (almost perfect agreement) between 0.61 and 0.80 as a 'good agreement' (substantial); between 0.41 and 0.60 a 'moderate agreement'; between 0.21 and 0.40 a 'fair agreement' and less than 0.20 a 'poor agreement (slight)[16]. Histopathological and cytological results were compared as ordered in Table 1. Additionally, the specificity and sensitivity of brush biopsy results were compared to histopathological results. To obtain these results, cytological and histopathological data were divided into two categories: benign (NILM, LSIL, SIN 0, SIN I and SIN II) and malignant (HSIL, SIN III and OSCC).

Table 1. Cytological and histopathological results paired for a proportion of agreement and kappa coefficient

	Cytological diagnosis	Histopathological diagnosis
1	NILM	SIN 0
2	LSIL	SIN I, SIN II
3	HSIL	SIN III
4	OSCC	OSCC

NILM – negative for intraepithelial lesion or malignancy, LSIL - low-grade squamous intraepithelial lesion, HSIL - high-grade squamous intraepithelial lesion, OSCC - oral squamous cell carcinoma, SIN 0 - the absence of dysplasia, SIN I - mild epithelial dysplasia, SIN II – moderate epithelial dysplasia, SIN III- severe epithelial dysplasia/carcinoma in situ

Results

Two samples out of 59 (3.39%) were discarded from the analysis. One discarded cytological slide (sampled from leukoplakia) was discarded due to insufficient cellularity, while one OSCC sample was contaminated by necrotic masses.

A good level of agreement (Table 2) was shown between the cytological and the histopathological diagnosis (49/57, 85.96%, kappa = 0.791, p<0.001). Absolute agreement for the OSCC diagnosis and between NILM and SIN 0 was observed.

Table 2. Agreement between cytological and histopathological diagnosis

Cytological diagnosis	Histopathological diagnosis			
	SIN 0	SIN I, SIN II	SIN III	OSCC
NILM	**13 (100%)**	0	0	0
LSIL	4 (25.0%)	**12 (75.0%)**	0	0
HSIL	0	2 (50.0%)	0	2 (50.0%)
OSCC	0	0	0	24 100%

NILM – negative for intraepithelial lesion or malignancy, LSIL - low-grade squamous intraepithelial lesion, HSIL - high-grade squamous intraepithelial lesion, OSCC - oral squamous cell carcinoma, SIN 0 - the absence of dysplasia, SIN I - mild epithelial dysplasia, SIN II – moderate epithelial dysplasia, SIN III- severe epithelial dysplasia/carcinoma in situ.

Sensitivity, as a measure of true positive (in this case diagnosis of the malignant lesion), was 92.85% for the cytological diagnosis. Specificity, as a measure of true negative results (in this case the diagnosis of the benign lesion), was 100% (Table 3). The evaluation of transepithelial cytology as a potential screening test was further measured as the positive predictive value (PPV) which was 100%. The negative predictive value (NPV) of transepithelial cytology was 93.54%

Table 3. Benign/malignant cytological and histopathological correlation

		Cytological diagnosis	
		Malignant	Benign
Hp dg	Malignant	**26** (True positive)	**0** (False positive)
	Benign	**2** (False negative)	**29** (True negative)

Hp dg = Histopathological diagnosis; Benign = NILM, LSIL, SIN 0, SIN I and SIN II, Malignant = HSIL, SIN III and OSCC

Discussion

The five-year OSCC survival rate reaches up to 82% when it is early detected[17]. In developing countries, such as Serbia, low socioeconomic status, lack of regular check-ups, as well as high-risk factors prevalence (alcohol consumption and cigarette smoking) may be the reasons for late diagnosis and consequently low survival rates[18]. Resource-challenging areas require a standardized, simple, inexpensive and reliable method for the early detection of malignant lesions. According to previously mentioned, we compared the oral brush biopsy to 'gold standard', i.e. surgical biopsy accompanied with histopathology.

Our study showed a good agreement (kappa= 0.791) between the cytological and histopathological results. Additionally, when comparing only benign and malignant diagnoses, our results showed high specificity and sensitivity. Even though there are many studies correlating oral cytology and histopathology, their methodology is heterogeneous, and it is hard to make a complete comparison. Some of them, unlike our study, used exfoliative cytology, where the sampling tools included a wooden stick, a tongue blade or a wooden end of cotton swab[19, 20] as a low-cost method suitable for low socioeconomic countries. Mentioned exfoliative cytology showed low sensitivity[21], due to inadequate sampling procedures[22]. An improvement of sampling procedures has been introduced using brush biopsy. Sampling is performed by specially designed 'Cytobrush', which obtains complete transepithelial samples from all layers of the oral epithelium[23]. However, modifications of brush biopsy have been also detected throughout the literature: a collection of cells were performed using a baby toothbrush[19], a cytobrush[24], as in our study, or by specially designed and expensive cell collectors[25]. Differences of preservation methods – from the air drying of smear to liquid-based biopsy[24-26], as well as for staining protocols were also observed[26-28]. Finally, in some studies, the interpretation of smears has been performed by pathologists[24], as in our study, or by software and reviewed by a pathologist[25]. Additionally, the majority of the studies compare results exclusively for leukoplakia/erythroplakia, and most frequently, biopsies were performed only for the cases with atypical cytology. In our study, the decision for the biopsy was made upon a clinical oral examination (COE), which means it's based on examiners' experience, since brush cytology has not been a standard diagnostic method for oral mucosal lesions. Cytological smears were collected immediately before the biopsy. By simultaneous sampling, discordance in cytological and histopathological results due to time delay, which has been reported in some studies was avoided[29]. A similar level of agreement using kappa statistic (kappa = 0.66) was shown in the study by Seijas – Naya *et al.*[25]. The mentioned study compared

histopathological with software-assisted cytology results, which is supposed to improve cytology. However, they compared these two diagnostic procedures only for the clinical diagnosis of leukoplakia. Some authors consider that sufficient cellularity, necessary for reliable cytological diagnosis cannot be achieved at high keratinized lesions, such as leukoplakia[10, 23, 29-31] and that may contribute to lower efficacy of cytological results in such studies. A high percentage of our samples (96.61%) showed desired sufficient cellularity. Such a level of successful sampling has been shown in the limited number of studies[27]. A high level of samples with satisfactory cellularity in our study may be due to two reasons: sampling was performed from both low and high keratinized lesions and cytological smears were fixed immediately after sampling which avoid air-drying artefacts. The sensitivity and specificity of oral brush cytology in previously published studies range between 70-100% and 90-100% respectively[23, 30, 32-34]. These high sensitivity and specificity results are similar to our results, albeit their methodology is heterogeneous, and some of them even performed more expensive liquid-based cytology.

In everyday clinical practice, the decision about the need for a biopsy is only up to examiners' experience and this may be the crucial point when early detection of malignant transformation may be neglected. Although the tissue biopsy remains the main method for the diagnosis, it may be described as expensive, invasive, and unfortunately unreliable for large, multifocal lesions[35, 36]. Considering these findings, there is a need for an adjuvant method for the detection of malignant transformation of OPMDs.

The brush biopsy stained by H&E may represent a desirable adjuvant diagnostic procedure. It seems to be quite a fast, simple and reliable method which accompanied with a clinical oral examination could improve early detection of the suspicious lesion. This methodology requires standardization before its clinical use. Additionally, our experience and results of previously published studies showed a deficiency of pathologists who are specialized in the oral cytology analysis[23, 25]. Lack of standardization of this methodology may be the reason for it.

Conclusions

Regarding our and other mentioned studies, it is clear that there is a need for an additional reliable method that can be beneficial in early diagnosis in developing countries where advanced cytological methods such as liquid-based cytology, modern laboratory tests and expensive optical devices are not affordable. This study shows that brush biopsy should be routinely performed as it can provide fast, cost-effective and efficient cytological results which are in good agreement with histopathological results.

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References

1. De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, Pierannunzio D, et al. Cancer survival in Europe 1999-2007 by country and age: results of EUROCARE--5-a population-based study. Lancet Oncol, 2014;15:23-34.
2. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma--an update. CA Cancer J Clin, 2015;65:401-421.
3. Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. Periodontol 2000, 2011;57:19-37.
4. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol, 2018;125:612-627.
5. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol, 2018;125:582-590.
6. Awadallah M, Idle M, Patel K, Kademani D. Management update of potentially premalignant oral epithelial lesions. Oral Surg Oral Med Oral Pathol Oral Radiol,Oral surgery, 2018;125:628-636.
7. Stell PM, Wood GD, Scott MH. Early oral cancer: treatment by biopsy excision. Br J Oral Surg, 1982;20:234-348.
8. Kowalski LP, Carvalho AL. Influence of time delay and clinical upstaging in the prognosis of head and neck cancer. Oral Oncol, 2001;37:94-98.
9. McCullough MJ, Prasad G, Farah CS. Oral mucosal malignancy and potentially malignant lesions: an update on the epidemiology, risk factors, diagnosis and management. Aus Dent J, 2010;55:61-65.
10. Yang EC, Tan MT, Schwarz RA, Richards-Kortum RR, Gillenwater AM, Vigneswaran N. Noninvasive diagnostic adjuncts for the evaluation of potentially premalignant oral epithelial lesions: current limitations and future directions. Oral Surg Oral Med Oral Pathol Oral Radiol, 2018;125:670-681.
11. Afrogheh A, Wright CA, Sellars SL, Wetter J, Pelser A, Schubert PT, et al. An evaluation of the Shandon Papspin liquid-based oral test using a novel cytologic scoring system. Oral Surg Oral Med Oral Pathol Oral Radiol, 2012;113:799-807.
12. Ramadas KLE, Thomas G, Mathew B, Balan A, Thara S, Sankaranarayanan R. A digital manual for the early diagnosis of oral neoplasia 2019 [cited 2019 20th October 2021]. Available from: https://screening.iarc.fr/atlasoral.php.
13. Patton LL, Epstein JB, Kerr AR. Adjunctive techniques for oral cancer examination and lesion diagnosis: a systematic review of the literature. J Am Dent Assoc, 2008;139:896-905.

14. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda SystemTerminology for Reporting Results of Cervical Cytology. JAMA. 2002;287:2114-2119.
15. Thompson LDR. World Health Organization Classification of Tumours: Pathology and Genetics of Head and Neck Tumours. Ear Nose Throat J, 2006;85:74.
16. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics, 1977;33:159-174.
17. Gómez I, Seoane J, Varela-Centelles P, Diz P, Takkouche B. Is diagnostic delay related to advanced-stage oral cancer? A meta-analysis. Eur J Oral Sci, 2009;117:541-546.
18. Stepovic MM, Stajic D, Sekulic M, Rajkovic Z, Djonovic N. Analysis of Mortality from Carcinomas Primary Localized in Region of Lip, Oral Cavity and Pharynx in Central Serbia, 1999-2015. Iran J Public Health, 2020;49:274-282.
19. Gupta S, Shah JS, Parikh S, Limbdiwala P, Goel S. Clinical correlative study on early detection of oral cancer and precancerous lesions by modified oral brush biopsy and cytology followed by histopathology. J Cancer Res Ther, 2014;10:232-238.
20. 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;24:52-56.
21. Diniz-Freitas M, García-García A, Crespo-Abelleira A, Martins-Carneiro JL, Gándara-Rey JM. Applications of exfoliative cytology in the diagnosis of oral cancer. Med Oral, 2004;9:355-361.
22. Sugerman PB, Savage NW. Exfoliative cytology in clinical oral pathology. Aust Dent J, 1996;41:71-74.
23. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. J Am Dent Assoc, 1999;130:1445-1457.
24. Fontes KB, Cunha KS, Rodrigues FR, Silva LE, Dias EP. Concordance between cytopathology and incisional biopsy in the diagnosis of oral squamous cell carcinoma. Braz Oral Res, 2013;27:122-127.
25. Seijas-Naya F, García-Carnicero T, Gándara-Vila P, Couso-Folgueiras E, Pérez-Sayáns M, Gándara-Vila R, et al. Applications of OralCDx® methodology in the diagnosis of oral leukoplakia. Med Oral Patol Oral Cir Bucal, 2012;17:e5-e9.
26. Remmerbach TW, Pomjanski N, Bauer U, Neumann H. Liquid-based versus conventional cytology of oral brush biopsies: a split-sample pilot study. Clin Oral Investig, 2017;21:2493-2498.
27. Jajodia E, Raphael V, Shunyu NB, Ralte S, Pala S, Jitani AK. Brush Cytology and AgNOR in the Diagnosis of Oral Squamous Cell Carcinoma. Acta Cytol, 2017;61:62-70.
28. Alsarraf A, Kujan O, Farah CS. Liquid-based oral brush cytology in the diagnosis of oral leukoplakia using a modified Bethesda Cytology system. J Oral Pathol Med, 2018;47:887-894.
29. Scheifele C, Schmidt-Westhausen AM, Dietrich T, Reichart PA. The sensitivity and specificity of the OralCDx technique: evaluation of 103 cases. Oral Oncol, 2004;40:824-828.
30. Mehrotra R, Singh MK, Pandya S, Singh M. The use of an oral brush biopsy without computer-assisted analysis in the evaluation of oral lesions: a study of 94 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2008;106:246-253.
31. Trullenque-Eriksson A, Muñoz-Corcuera M, Campo-Trapero J, Cano-Sánchez J, Bascones-Martínez A. Analysis of new diagnostic methods in suspicious lesions of the oral mucosa. Med Oral Patol Oral Cir Bucal, 2009;14:E210-216.
32. Poate TW, Buchanan JA, Hodgson TA, Speight PM, Barrett AW, Moles DR, et al. An audit of the efficacy of the oral brush biopsy technique in a specialist Oral Medicine unit. Oral Oncol, 2004;40:829-834.
33. Svirsky JA, Burns JC, Carpenter WM, Cohen DM, Bhattacharyya I, Fantasia JE, et al. Comparison of computer-assisted brush biopsy results with follow up scalpel biopsy and histology. Gen Dent, 2002;50:500-503.
34. Epstein JB, Güneri P, Boyacioglu H, Abt E. The limitations of the clinical oral examination in detecting dysplastic oral lesions and oral squamous cell carcinoma. J Am Dent Assoc, 2012;143:1332-1342.
35. Thomson P. Oral Carcinogenesis. Oral Precancer – Diagnosis and Management of Potentially Malignant Disorders. Chichester: Wiley-Blackwell; 2012:31-47.
36. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol, 2009;45:317-323.

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