

Brush Biopsy Sample Quality: Preliminary Investigation of a Metal Brush Prototype

Pelin Güneri¹, Ceyda Gürhan², Umut Aykutlu³, Ali Veral⁴, Baris Oğuz Gürses⁵

¹Department of Oral and Maxillofacial Radiology, School of Dentistry, Ege University, Izmir, Turkey.  0000-0001-9423-9191

²Department of Oral and Maxillofacial Radiology, School of Dentistry, Ege University, Izmir, Turkey.  0000-0002-4101-4965

³Department of Pathology, Faculty of Medicine, Ege University, Izmir, Turkey.  0000-0002-9373-0912

⁴Department of Pathology, Faculty of Medicine, Ege University, Izmir, Turkey.  0000-0003-0343-9978

⁵Department of Mechanical Engineering, Faculty of Engineering, Ege University, Izmir, Turkey.  0000-0002-2755-3452

Author to whom correspondence should be addressed: Ceyda Gürhan, Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Ege University, Izmir, Turkey. Phone: +90 232 3112700. E-mail: cydgrhn@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 17 February 2019 / Accepted: 01 April 2019 / Published: 03 April 2019

Abstract

Objective: To qualitatively investigate whether a prototype brush composed of metal bristles collects oral epithelial cells effectively for cytological evaluation of oral mucosal lesions. **Material and Methods:** Twenty patients with suspicious oral mucosal lesions were enrolled. Patients were asked to gargle with saline and to deposit the oral rinse into specimen cup. Then, oral mucosal cell samples were collected using a metal oral brush, via sweeping motion. Punch biopsy was performed for histological examination. All samples were evaluated with liquid based cytology (LBC) according to the cellularity, the depth of the epithelial layer, cellular integrity by an oral pathologist. **Results:** Oral rinse provided samples with 100% cellular integrity and cellularity, mostly from the intermediary layers. With metal brush, both inadequate cellularity and cellular integrity was observed in 25% of the cases. Cellular integrity was adequate in 65%, cellularity was adequate in 45% of the lesions. Samples were dominantly from the intermediary layers, but in one case, metal brush collected cells from the parabasal layer. **Conclusion:** The narrow spiral pitch and width of metal bristles may have resisted to release the cellular samples collected. With adjustment of the spiral pitch and diameter of metal brush bristles, its' efficacy could be enhanced.

Keywords: Mouth Neoplasms; Cytodiagnosis; Early Diagnosis; Cytological Techniques.

Introduction

Oral Potentially Malignant Disorders (OPMD) are morphologically altered oral mucosal tissue with higher risk of progression to cancer and usually revealed during clinical examination [1-3]. In these lesions, varying degrees of cellular atypia and tissue dysmaturation restricted to the epithelium may be observed and are termed as oral epithelial dysplasia [4,5].

The major histological criteria for diagnosis of an epithelial dysplasia are abnormal patterns of keratinization [6], hyperplastic basal cells, enlarged and hyperchromatic nuclei, and drop-shaped rete ridges [7], which may be present at the same site before the malignant changes develop [3,8]. Even though these alterations may be observed within the whole thickness of epithelium, they do not destroy the basement membrane to invade the underlying stromal tissues [6].

Depending upon the extent of cytologically immature cells, OPMDs are usually grouped as low-grade dysplasia (combining mild and moderate dysplasia) or high-grade dysplasia (combining severe dysplasia and carcinoma in situ) [6]. Although controversial [3], the greatest likelihood for malignant transformation usually depends on the severity of dysplastic changes of the tissue [2,9,10]. OPMD might turn into carcinoma in a percentage varying between 5-18% [11,12], but the presence of moderate or severe dysplasia has been accepted to have the highest risk for malignant transformation [13,14], ranging from 11-36% with a mean time of 33.6 months [13].

The presence of symptoms that are not strongly indicative of carcinoma might result in diagnostic delay and poor prognosis [2,15]. Even the expert oral medicine specialists report difficulties in assessment of the risk of malignant transformation of OPMDs [16] and non-invasive diagnostic adjuncts including vital staining, optical imaging and brush biopsy cytology have been proposed in order to improve early detection of OPMDs with risk of malignant progression in oral mucosa [3,17-19]. Unfortunately, their efficacy is yet to replace the histological examination, which is still accepted as the gold standard in oral mucosal lesion diagnosis [3,18-20].

Cyodiagnosis is a minimally invasive technique whereby individual cells are gathered from their tissue of origin and transferred to a cytology slide for microscopic examination [19,21,22]. However, lack of a complete sampling containing cells from all layers of the stratified epithelium is a major drawback of the technique, especially in highly keratinized mucosal lesions [3,21,23], and in order to overcome this problem, newer collection devices or “cytobrushes” in addition to conventional plastic cytobrush have been developed [24,25].

The physical features of the brushes are different from each other: the Orcellex® Brush (Rovers Medical Devices B.V., Oss, the Netherlands) has a specially designed head that is composed of five segments of high-density fibres and clinically, it is used similar to conventional cytobrush [19]. Oral CDx® Brush Biopsy (CDX Laboratories Inc., Suffern, NY, USA) is used for oral mucosal cell sampling with specialized designed rigid hairs of biopsy brush which enables sampling of cells from deeper epithelial layers and aid to decrease the false negative and inadequate results [17,26]. Unfortunately, the clinical efficacy of these equipment is still debatable [24] due to their low specificity [22,26], low availability and high cost [26].

Utilization of dermatological curette as a biopsy sampling device from oral mucosa has presented favorable results due to its' ability to collect cellular samples from deeper epithelial layers and to provide micro-biopsies with the remnants of tissue particles within the samples [1,27,28]. By means of a dermatological curette to scrape the oral epithelium, more cellular specimen was delivered for cytological evaluation and also, fragments of tissue were supplied for histological examination, when needed [27].

In line with this approach, the aim of the present study was to qualitatively investigate whether a brush totally composed of metal bristles collects oral epithelial cells effectively for cytological evaluation of oral mucosal lesions.

Material and Methods

Sample

Twenty patients who were referred to Ege University School of Dentistry, Department of Oral and Maxillofacial Radiology for diagnosis and treatment of their oral mucosal lesions were enrolled. After the patients provided their informed consents, they were examined clinically and radiologically at the outpatient clinic. Thorough extra- and intra-oral examinations were performed by a specialist with expertise on oral mucosal lesions under standard conditions, using incandescent light and routine dental examination instruments.

Data Collection

Saline oral rinse was used as a control prior to oral brush biopsy procedure. All patients were requested to refrain eating or drinking at least 1 hour before collecting buccal cells. As done previously [8], the patients were asked to massage the lesion with their tongue for 30 seconds, and to take 10 milliliters of phosphate buffered saline solution with pH 7.2 into their mouths, to swish and gargle for 45 seconds rigorously and then deposit the oral rinse into 50 milliliters specimen cup which was filled with fixative solution for liquid based cytology (LBC).

In order to reach the deeper layers of the epithelium and to collect cells without cellular damage, a novel oral brush containing 25 spiral metal bristles which were made of biocompatible 304V half round, smooth wires with 0.091 mm thickness and 0.2 mm width (Fort Wayne Metals, Indiana, USA) on its head was developed by a team of dentists and engineers, and was used to collect the oral mucosal cell samples. After observing pinpoint bleeding which is the indicator of reaching the basal layer, the brush was immersed in the fixative solution for LBC and was agitated for 10 seconds.

For each oral mucosal lesion, considering the ulcerated, white, red or mixed colored, and verrucous areas, the site that requires biopsy was determined. The brush was placed on the lesion and cells were collected with a sweeping motion until pinpoint bleeding. Afterwards, punch biopsy was performed under local anesthesia using standard procedures and equipment (5 mm punch, Kai Europe GmbH, Solingen, Germany) by a specialized oral surgeon simultaneously and exactly from

the same area where the brush was applied. The biopsy sample with a diameter of 5 mm was immersed in 10% formalin glass tube supplied for transportation of the material to the pathology laboratory.

A single ThinPrep slide from each vial was prepared using the ThinPrep 5000 processor (Hologic Inc., Marlborough, MA, USA) according to the manufacturer's instructions. Briefly, the slide preparation process involves collection of the cytology material on a membrane using a gentle vacuum, trapping cells on the filter and transfer filtered cells to the glass slide. After these procedures, slides were stained with Papanicolaou in Leica XL autostainer (Leica Biosystems Nussloch GmbH, Nussloch, Germany). Oral biopsy specimens were fixed in formalin, embedded in paraffin, and processed for routine hematoxylin and eosin evaluation using standard techniques.

All samples were examined by a pathologist informed about clinical diagnosis, but blind to the histopathological results; using Leica BME (Leica Microsystems Inc., Buffalo Grove, IL, USA) and Olympus BX51(Olympus Corp., Tokyo, Japan) microscopes in 4X,10X, 20X and 40X magnifications.

The characteristics of the cytological samples were analyzed according to the following criteria, modified from the literature [9]: 1) Cellularity (defined as the number and the quality of the structure of collected cells, and was assessed by analyzing the number, distribution and the homogeneity of the cells): a) inadequate; b) barely adequate; c) adequate; 2) The depth of the epithelial layer: a) inadequate; b) superficial; c) intermediary; d) parabasal/basal; and 3) Cellular integrity: a) inadequate; b) barely adequate; c) adequate.

The samples were considered "inadequate" when poor cellularity, poor fixation (air dried), and/or thick or obscured spread were observed. Cellularity was evaluated by measuring the average cell count within ten discontinuous fields across the middle diameter of each preparation. An average of at least seven cells/ field was required in order to consider the specimen "adequate" [7].

Cellularity and cellular integrity were quantified by scoring: If the sample was acellular, it was scored as 0, the inadequate sample received score 1, barely adequate ones received score 2, and adequate sample received score 3. The depth of the sampled epithelial layer was also scored: samples with inadequate cells scored as 0, cells of superficial layers scored as 1, intermediary layers scored 2, and parabasal/basal layers scored 3.

Ethical Aspects

The study design has been approved by the Ethical Committee of Ege University (Protocol No. #17-7.2/4).

Results

Of 20 patients, 7 were diagnosed as squamous hyperkeratosis, 4 as squamous cell carcinoma, 2 as oral lichen planus, 2 as verrucous carcinoma, 1 as granulation tissue, 1 as pemphigus, 1 as inflammatory inflammation, 1 as ulcerous inflammation, and 1 as healthy squamous epithelial tissue with non-specific findings.

All tested cellular parameters were scored lower with metal brush when compared with those of the oral rinse (Table 1). With oral rinse, cell samples were collected from intermediary layers of oral mucosa in 65% of the cases, whereas 35% of the samples were from superficial layers. Cellular integrity and cellularity were complete for all cases (100% for both parameters).

Table 1. The scores of the tested cellular parameters for the cell samples obtained with metal brush and oral rinse, and the final diagnoses of the lesions determined after histological evaluation.

Patient	Brush (N = 119)			Oral Rinse (N = 153)			Histological Diagnosis
	Cellular Integrity	Cellularity	Depth of Epithelial Layer	Cellular Integrity	Cellularity	Depth of Epithelial Layer	
# 1	2	2	1	3	3	2	Squamous Hyperplasia
# 2	3	3	1	3	3	2	Squamous Hyperplasia
# 3	2	2	2	3	3	2	Squamous Cell Carcinoma
# 4	3	1	0	3	3	2	Squamous Cell Carcinoma
# 5	3	2	1	3	3	2	Lichen Planus
# 6	2	2	3	3	3	2	Ulcerous Inflammation
# 7	3	3	2	3	3	1	Squamous Hyperplasia
# 8	3	2	1	3	3	2	Squamous Hyperplasia
# 9	3	3	2	3	3	2	Squamous Hyperplasia
# 10	3	3	2	3	3	2	Squamous Epithelium with non-Specific Findings
# 11	3	3	2	3	3	1	Granulation Tissue
# 12	1	1	0	3	3	1	Squamous Hyperplasia + Hyperkeratosis
# 13	3	3	2	3	3	2	Pemphigus Vulgaris
# 14	3	2	1	3	3	1	Verrucous Carcinoma
# 15	1	1	0	3	3	1	Chronic Inflammatory Infiltration
# 16	1	1	0	3	3	1	Squamous Cell Carcinoma
# 17	1	1	0	3	3	1	Thick Hyperkeratosis
# 18	3	3	2	3	3	2	Lichen Planus
# 19	3	3	2	3	3	2	Squamous Cell Carcinoma
# 20	3	3	2	3	3	2	Verrucous Carcinoma
Total	49	44	26	60	60	33	

In one case (5%), metal brush collected cells from the parabasal layer of the epithelium, and it was the deepest layer among all samples obtained with brush and oral rinse. However, with metal brush, the samples were inadequate in 5 cases (25%), and inadequate cellular integrity was observed in those cases as well. Of the cases, 45% were from intermediary layers whereas 25% were from superficial layers of the epithelium. Cellular integrity was inadequate in 20%, barely adequate in 15%, and adequate in 65% samples. Likewise, cellularity was inadequate in 25%, barely adequate in 30%, and adequate in 45% of the cases.

Discussion

The physical features of the brushes which are developed in order to overcome drawback of the cytodagnosis are different from each other: the Orcellex® Brush has a specially designed head that is composed of five segments of high-density fibres and clinically, it is used similar to conventional cytobrush [1]. Oral CDx® is used for oral mucosal cell sampling with specialized

designed rigid hairs of biopsy brush which enables sampling of cells from deeper epithelial layers and aid to decrease the false negative and inadequate results [10,11]. Unfortunately, the clinical efficacy of this equipment is still debatable [6] due to their low specificity [3,11], low availability and high cost [11].

Utilization of dermatological curette as a biopsy sampling device from oral mucosa has presented favorable results due to its' ability to collect cellular samples from deeper epithelial layers and to provide micro-biopsies with the remnants of tissue particles within the samples [12-14]. By means of a dermatological curette to scrape the oral epithelium, more cellular specimen was delivered for cytological evaluation and also, fragments of tissue were supplied for histological examination, when needed [13].

Today, use of minimally invasive brush biopsies as an adjunct for diagnosis of oral mucosal lesions with risk of malignancy has regained interest because of technical improvements in cytological analyses and development of adjuvant diagnostic tools and liquid-based cell preparation techniques. Conventional cytology presents with drawbacks such as poor air-drying artifact, entrapment of cells in blood clot, and crushing of cells [2,6]. Spreading out in a thin layer eliminates a great part of the inflammatory cells, necrosis, and red blood cells, thus avoiding the majority of superimposition artifacts found in conventional cytology [9,15,16].

The advantages of LBC include rapid and better fixation, even distribution of cells over a smaller slide area, decreased obscuring background elements such as blood, inflammation, and mucus [15-17], the reduction of unsatisfactory samples [13,16] and the possibility to perform DNA analysis and molecular biology testing with sampled cells [13,16]. These eventually lead to improved quality and speed of interpretation [9,15], and diagnostic improvement by 9.61% [15].

On the other hand, the higher cost and the need to train professionals in the new technique are main drawbacks of LBC [9,16]. It's also reported that by targeted isolation of cells, LBC causes destruction of collections of squamous epithelial cells; thus, impairs the examination of epithelial layers [2,18]. Considering that clinically high-risk lesions are referred for prompt histological examination via scalpel biopsy [13,19], any adjunct diagnostic test is expected to be effective in the rest of oral mucosal lesions appearing clinically suspicious.

Some reports stated the value of brush cytology [1,13,20-22] and immediate scalpel biopsies, which were obtained after brush biopsies from oral lesions revealed 96.3% sensitivity and 100% specificity of brush cytology for dysplasia or carcinoma [2]. Another study with Orcellex® brush revealed 60% sensitivity, 99% specificity, 67% positive predictive value and 99% negative predictive value of brush cytology for OSCC [1]. On the contrary, high false positive and false negative results of brush biopsy ranging between 30-84% for OSCC [23] and 63% for dysplastic lesions [13] also have been reported. Similarly, the range of sensitivity of Oral CDx® varied between 71.4 to 100% [23], specificity between 25 to 100% [19,23], positive predictive value between 33 to 86% [23].

However, it has been stressed that the exact value of the method still needs further investigation since the lesions with negative brush biopsy results do not usually receive scalpel

biopsy for histological examination [13,22,24]. It's established that brush biopsies can only assess a small region of the oral mucosa [18], require time to assess the diagnosis due to preparation process [25], and are not reliable for evaluating OPMDs with thick keratin layers [2,7,19]. Also, in low risk lesions, reduced accuracy of OralCDx® cytologic test and increased rate of false-positive findings have been reported [3]. Because of the presence of necrosis and/or infection, transepithelial access may be unmanageable in OSCC [19] and accompanying inflammation may cause misdiagnosis of oral lesions [19,23].

As stated above, the place of cytology/cytological instruments and tests in oral mucosal lesion diagnosis has been investigated in the literature, by assessment of the sensitivity, specificity, positive and negative predictive values and accuracy of the diagnostic system. In our paper, instead of the efficacy of the oral brush biopsy method, the ability of the sampling instruments was investigated. Our results revealed that the cellularity and cellular integrity of samples obtained with oral rinse were better than the specimen of the brush. Considering the non-invasive nature of the cell collecting technique with rinsing, and the high number of exfoliated cells from the whole oral mucosa, these findings were not unexpected. In the literature, the non-rigid structure of the conventional cytobrush has been considered as the potential reason of inadequate material and false negative results [25]. The lack of cells from basal and parabasal layers might be the result of minimally invasive properties of the collecting device, indicating the need for a more invasive brush to obtain samples including the deeper cells [7].

The ability to obtain trans-epithelial samples of basal, para-basal and superficial cell layers which is vital for cytological examination of lesions with thickened keratin layers has been reported as an advantage of Orcellex® Brush [1]. Similarly, special design of the rigid bristles of CDx® Brush has been attributed as the reason of collection of cell samples from deep epithelial layers [10]. Stiffness of the bristles has been required to collect the cells of all diagnostically relevant cell layers [18]. Similarly, others authors utilized metal dermatological curette instead of a brush and reported that not only the cells, but also small fragments of tissue were sampled with microcurette [13,14]. In the present study, metal bristles of the brush managed to collect cells from parabasal layer in one case, revealing its potential to reach to the deeper layers of the epithelium. This was an encouraging finding since receiving samples from basal and parabasal layers are vital for accurate diagnosis of dysplastic changes [5].

Cellular integrity was adequate in 65% samples, and cellularity was adequate in 45% of the cases. Even though the pinpoint bleeding was observed in all cases during brush application, it was unusual to notice low rate of cellularity and cellular integrity. The authors suggest that the spiral metal bristles of the brush collect the cells from mostly the intermediary layers, but the narrow spiral pitch and width of metal bristles have resisted to release the cellular samples collected. Thus, lower rate of cellularity and cellular integrity were observed. It is suggested that the highly fibrotic nature of oral epithelium prevents exfoliation of the dysplastic cells to the surface [2], and also, only 20% of cells collected with different devices can be transferred to a glass slide [26]. However, in a

recent report that used conventional cytology for oral mucosal lesion evaluation, inadequate samples have been reported as 22.7% [5]. On the contrary, due to the nature of oral epithelium, oral rinse managed to collect more flaked off epithelial cells, which held their original shape and were not disrupted by external forces, i.e. the application pressure during brush biopsy. However, the number of the cells with diagnostic value of the flaked off cells within oral rinse would be very limited, considering the large number of epithelial cells shed into the saliva [27].

The limitations of the present study are the small sample size and the lack of utilization of standard cytobrush for comparison. Both these factors are planned to be eliminated in ongoing subsequent trials. The metal brush used in this investigation seems to hold promise after adjusting the spiral pitch and diameter to provide better release of the collected cells, but its value needs confirmation in further studies with larger group of patients.

Considering that oral dysplasia is an ominous process starting from the basal layer and extending to the top, involving all layers, [28] minimally invasive brush biopsy which can be used as a chairside adjunct to use for mass screenings, to evaluate clinically suspicious oral lesions or to examine oral epithelium after oral oncological treatment may be beneficial for the practitioners [28]. Using a less invasive method than surgery may encourage the oral health care providers to attempt to evaluate oral lesions, which may appear suspicious, prior to referral for surgical biopsy [28]. A device that collects cells from both the deeper and superficial layers has been advocated for this purpose [1,28].

Conclusion

The narrow spiral pitch and width of metal bristles may have resisted to release the cellular samples collected. With adjustment of the spiral pitch and diameter of metal brush bristles, its efficacy could be enhanced.

Financial Support: None.

Conflict of Interest: The authors declare no conflicts of interest.

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