Viability of Whole Tissue Microbiopsy (WTM) for the Study and Management of Oral Leukoplakia

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Abstract: *Introduction*: Leukoplakia is the most frequent potentially malignant disorder. Management and diagnosis requires clinical and histopathogical monitorization. Conventional biopsy generates patient morbidity and is considered a complex procedure for general dentists, which can delay initial diagnosis. To solve these problems, we have proposed a novel procedure denominated Whole Tissue Microbiopsy (WTM). The aim of this study is to evaluate the samples obtained with the WTM procedure and to test their viability; to check if they are applicable in all anatomic locations and compare the results with those obtained with conventional biopsy.

Methods: We studied 41 clinically compatible lesions with oral leukoplakia. A tissue sample was taken using the WTM technique, after which, a conventional biopsy was performed on the same location. Both samples were studied and compared in terms of viability and concordance.

Results: 100% of the samples obtained using the WTM procedure were viable. 95% of the samples were useful to detect dysplasia, and in 85% of cases the basal membrane was retained. Coincidence with conventional biopsy as to detect cancer-dysplasia was 78% and showed a 53.8% sensitivity regarding the detection of dysplasia-Cancer.

Discussion and Conclusion: The samples obtained by the WTM are viable for study. Conservation of all epithelial layers in the sample and the basement membrane in particular is not influenced by the anatomical area or by the clinical appearance of the lesion. The results that did not coincide with the conventional biopsy were due to the difference in size and not the quality of it.

Keywords: Whole Tissue Microbiopsy, leukoplakia, oral biopsy, microbiopsy, potentially malignant disorder.

INTRODUCTION

Leukoplakia is considered the most frequent potentially malignant disorder (OPMD). The annual malignant transformation is estimated between 1-3% [2] and the recurrence of the lesion in the same location or elsewhere is 30% [1]. For the diagnosis of this type of lesion, several clinical criteria have to been defined and histopathological analysis should be carried out to confirm this lesion or any other entities; and to determine the existence of any malignant or premalignant findings (epithelial dysplasia).

Several methods have been proposed to manage leukoplakia lesions, and although the most accepted treatment is the surgical removal of the lesion, this does not guarantee the development of a malignant lesion in the area [4]. Thus, several authors stress on the importance of monitoring these lesions [1] forcing the clinicians to control these lesions with histopathology by performing a surgical biopsy. Biopsy is considered the Gold Standard procedure for this type of lesions, and is also used to determine the malignancy and the prognosis [5,6]. The ideal biopsy technique should be a representative sample, as this will allow the Pathologist to interpret this sample and reduce the operative and postoperative discomfort [7]. The incisional or excisional biopsy is not problem-free and is a technique that requires certain surgical skills [8]. This makes it complicated for regular general dental practitioners, who have to refer the patient for the biopsy procedure [9.12]. On the other hand, it is an invasive technique associated with a healing time morbidity and presumes a certain risk for patients with systemic diseases. If we take this in mind and considering that patients may have multiple lesions and should have periodical biopsies to control these lesions [13], it may be difficult in many circumstances to perform this intervention [14].

If general dental practitioners and the patients would not delay the decision to perform the biopsy, the diagnosis would be accelerated and would allow to treat these disorders at early stages [15]. Thus, it would be advisable to find a technique that would reduce

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patient morbidity and at the same time allow to process a representative sample of the lesion.

Several non-invasive procedures have been proposed to manage leukoplakia lesions: from staining these lesions [16] to light examination [17] or cytology techniques with cellular or genetic analysis [19], but none of these procedures have been able to match, in terms of sensitivity, specificity or precision, the use of the traditional biopsy technique [20].

Navone *et al*. (2008) [21], proposed the microbiopsy procedure, consisting in obtaining small size samples with a dermatological curette. These samples were then inserted in cellular binding agent (similar to the liquid base cytology technique), after which they were fixed in formalin and histopathologically studied. This procedure obtained promising results in terms of dysplasia-cancer detection sensibility but had drawbacks such as a complex fixing procedure and the need of specific transport materials.

The whole tissue microbiopsy technique (WTM) proposed in this sutdy, consists in obtaining samples with a dermatological curette. These samples must be intentionally as integral as possible and of sufficient extension and thickness to be able to directly fix the sample in formalin and to perform the histopathological analysis. The aim should be to remove as little as possible to reduce both intraoperative and postoperative patient morbidity. Samples will be fixed and treated conventionally, without the use of specific materials for transportation and preservation, which would make its implantation easier for general dental practitioners.

The aim of the study is to evaluate samples obtained with the WTM technique, to examine feasibility, and application to other anatomical locations and to compare with the results obtained with the conventional biopsy.

MATERIAL AND METHODS

Forty one (41) lesions clinically compatible with oral leukoplakia lesions were studied in thirty nine (39) patients. All patients signed the applicable consent form and were included in a oral leukoplakia management protocol.

Before performing the biopsies, patients were urged to cease toxic habits such as smoking and alcohol intake, were treated to resolve any traumatic factors, such as refining and polishing prosthesis or/and teeth, and were prescribed anti-fungal topical application for a month.

All the samples were obtained by the same operator, both conventional biopsies and WTM microbiopsies. Under local anesthesia, a dermatological curette (Disposable dermal curette 3mm. Miltex® Gmbh Langes Gewand. Rietheim-Weilheim. Germany) was used to perform the WTM procedure. The most suspicious area of the lesion was inspected and curetted until there was bleeding to make sure the connective tissue was reached (Figure **1**). Measured were taken to ensure an integral sample of the lesion was obtained, not only a superficial scraping. This sample was inserted in a fixative 7.7pH 10% formalin. Once the bleeding had stopped, the conventional biopsy was performed including the area that had been chosen for the WTM procedure (Figure **2**). If the area is extensive, an incisional biopsy will be

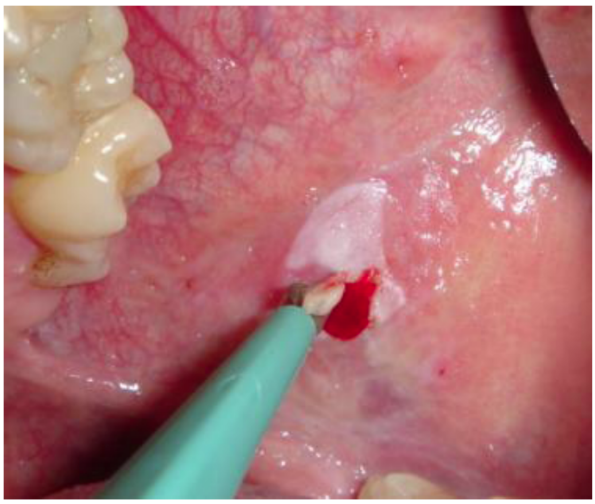

Figure 1: Whole Tissue Microbiopsy performed with curette.

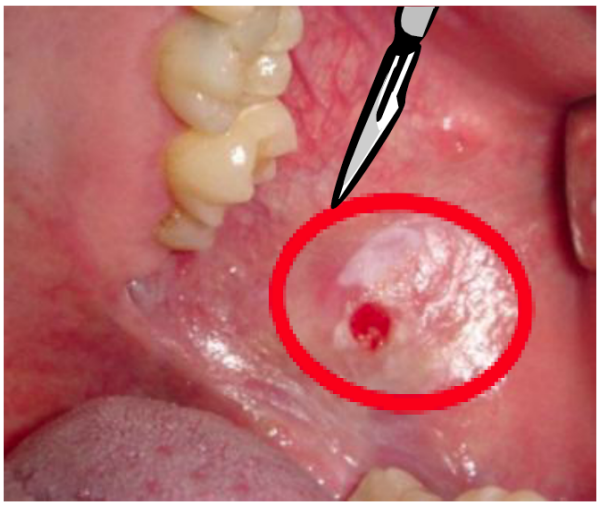

Figure 2: Conventional biopsy performed with scalpel.

preformed; other times an excisional biopsy will be preferred. The sample will also be inserted in a different vial with a fixative 7.7pH 10% formalin. Both vials will be labeled and sent for histopathology analysis by the same pathologist.

Both samples will be procesed and treated as conventional biopsies for their microscopic examination under hematoxylin-eosin staining. No further processing was perfomed (Figures **3** and **4**).

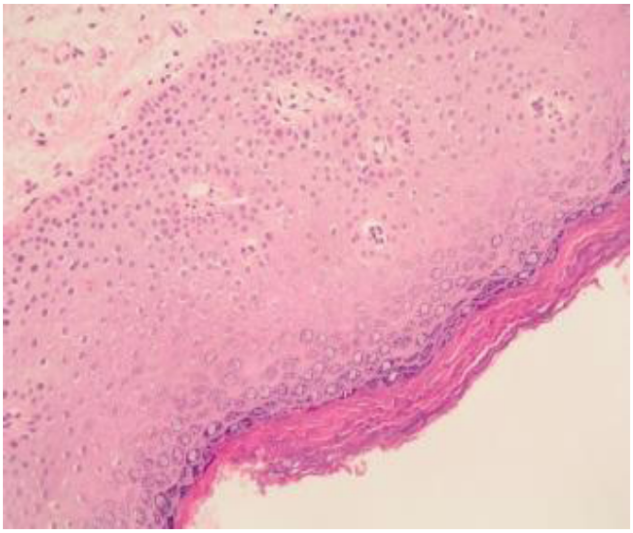

Figure 3: Histological section obtained with the whole tissue microbiopsy (H-E X 200).

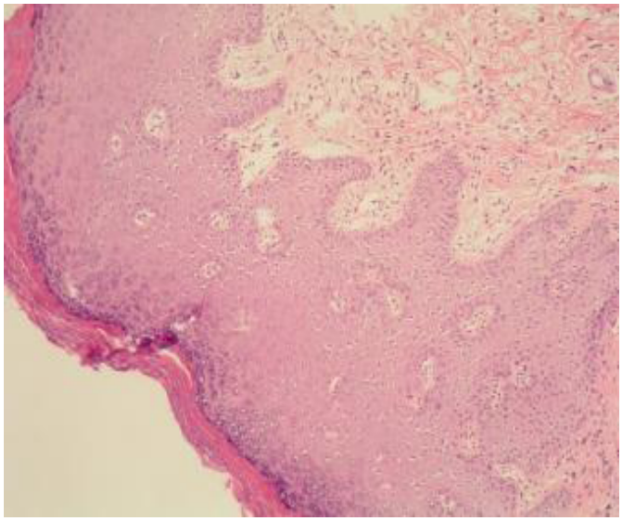

Figure 4: Histological section obtained with conventional biopsy (H-E X 100).

The following details were assessed in each studied sample:

- Sample viability: the amount of tissue presented on the plate should be acceptable for study, and the sample should include cells other than stratum corneum.
- Possibility of the sample to detect and determine dysplasia: the sample should preserve architecture and enough cells to determine if there is dysplasia and if so, what amount of dysplasia is present.
- Basal membrane preservation.
- Recognizable cell layers: how many epithelial layers are recognizable.
- Diagnostic match: conventional biopsy sample should coincide with the WTM sample.

WHO criteria (2005) were used to determine the dregree of epitelial displasia and/or the presence of cancer [3].

The histology diagnosis of the WTM samples was compared to the conventional biopsy samples using the Cohen Kappa test (K).

The reference value of the worst obtained result of each technique was used to obtain the sensibility and negative predictive value (NPV).

Chi-squared test with Yates' correction was used to analyse whether the location or the clinical type of lesion may influence the basal membrane conservation or the epithelial layers.

RESULTS

41 clinically compatible oral leukoplakia lesions from 39 patients were analysed. 21 patients were female and 18 were men. 34 lesions corresponded to a homogenous white patch (83%); 5 lesions were non-homogenous white patches (12,2%); and 1 was a verrucous lesion (4,8%). Regarding the location of the lesions; 13 were located ventro-laterally on the tongue (31,7%), 4 were on the dorsum of the tongue (9,7%), 1 was on the floor the mouth (2,4%), 11 were in keratinized gingiva (26,8%), and 12 were located in the buccal mucosa (29,3%). None of samples were considered as not viable for study, thus, all of the samples, were processed and presented for their microscopical examination.

WTM preserved the basal membrane in 35 samples (85,36%) and 39 samples (91,12%) were viable to analyze the presence of dysplasia. The two samples that were not viable for examination included only the

Table 1: Presence of Basement Membrane regarding biopsy location.

Basement membrane	Floor of the mouth (n=1)	Buccal mucosa (n=12)	Tongue dorsum (n=4)	Keratinised gingiva (n=11)	Ventro-lateral aspect of the tongue (n=13)	Total (n=41)
Si	1(100%)	10(83,3%)	3(75%)	10(90%)	11(91,8%)	35(85,4%)
No	0(0%)	2(16,7%)	1(25%)	1(10%)	2(18,2%)	6(14,6%)
						p=0.841

Table 2: Presence of Basement Membrane regarding clinical aspect of the lesion.

Basement membrane	Homogeneous White Patch (n=34)	Non Homogeneous White Patch (n=5)	Verrucous lesion (n=2)	Total (n=41)
Si	29(85,3%)	4(80%)	2(100%)	35(85,4%)
No	0(0%)	1(20%)	1(25%)	6(14,6%)
				p=1

most superficial layers of the epithelium, the granular and keratinized layers. In 33 samples (80.48%) all layers of the epithelium were found, in an orderly fashion and without suffering alterations or disorders in their architecture or orientation.

Tables **1** and **2** present the percentage of samples obtained with the WTM technique that retained the basement membrane relative to the anatomical location (ventro-laterally on the tongue, dorsum of the tongue, floor the mouth, keratinized gingiva and buccal mucosa) and clinical appearance.

The ratio of samples that preserved all layers of the epithelium respect to the anatomical location and clinical appearance can be seen in Tables **3** and **4**.

All conventional biopsy samples were viable, retained the basement membrane and preserved all layers of the epithelium, which could be used for comparison with the WTM technique.

Thirteen lesions (31.7%) had a positive diagnosis (11 dysplasias, 1 Carcinoma in situ and 1 Oral Carcinoma Squamous Cell (OSCC)) and 28 lesions (68.3%) were free of malignant-premalignant changes.

Table 3: Relationship between the samples that preserved all of the epithelial layers regarding location of the lesion.

Preservation of all of the epithelial layers	Floor of the mouth (n=1)	Buccal mucosa (n=12)	Keratinised gingiva (n=11)	Ventro-lateral aspect of the tongue (n=13)	Lingual Dorsum (n=4)	Total (n=41)
Yes	1(100%)	9(75%)	9(81,8%)	11(84,6%)	3(75%)	33(80,5%)
No	0(0%)	3(25%)	2(18,2%)	2(15,4%)	1(25%)	8(19,5%)
						p=0.957

Table 4: Relationship between the samples that preserved all of the epithelial layers regarding the clincial aspect of the lesion.

Preservation of all of the epithelial layers	Homogeneous White Patch (n=34)	Non Homogeneous White Patch (n=5)	Verrucous lesion (n=2)	Total (n=41)
Yes	27(65,8%)	4(80%)	2(100%)	33(80,5%)
No	7(34,6%)	1(20%)	0(0%)	8(19,5%)
				p=1

Table 5: Presence of dysplasia and carcinoma in the samples.

	WTM	Conventional biopsy	Definitive diagnosis[1]
(Dysplasia or in situ Ca + SCC)	7(6+1)	10(10+0)	13(12+1)
Negative	34	31	28
False negative[2]	6	3	—
Total.	41	41	41

[1]Worst diagnosis obtained with microbiopsy or conventional biopsy.
[2]Regarding the most serious diagnosis.

The presence of dysplasia or carcinoma in the samples compared to the final diagnosis can be observed in Table **5**. We consider the final diagnosis as the most serious or severe finding both with WTM and CB.

The CB samples showed a more severe diagnosis compared to the WTM technique in 9 (21.95%) samples. The WTM samples displayed a worse diagnosis in 3 (7.31%) samples. This assumes a diagnostic underestimation of 21,95% for the WTM technique and a 7,31% underestimation for the CB.

Regarding the sensitivity, the whole tissue microbiopsy had a sensitivity regarding the diagnosis of dysplasia and/or carcinoma of 0.538 (CI95%=0.251;0.807) and the sensitivity for the conventional biopsy was 0.796 (CI95%=0.461;0.949)

The NPV obtained regarding detection of dysplasias and/or carcinoma by the whole tissue microbiopsy was 0.896 (CI95%=0.8026;0.989) and in the case of conventional biopsy, the NPV was 0.945 (CI95%=0875;1000).

The last three results are summarized in Table **6**.

Table 6: Values obtained by both biopsy techniques.

	WTM	Conventional Biopsy
Underdiagnosis	21,95%	7,31%
Sensitivity	53,8%	79,6%
Negative predictive value NVP	89,6%	94,5%

Of the false negative cases obtained with the WTM technique with respect to the detection of dysplasia and/or carcinoma (6 false negatives cases), five of them had retained the basal membrane and in 4 samples, all layers of the epithelium were retained.

The presence or absence of basal membrane, or the conservation of all layers of epithelium, does not affect the ratio of false negatives of the whole tissue microbiopsy (*p=1 and p=0.578*).

As for the detection of epithelial dysplasia, the results of 32 of the 41 samples (78%) were in agreement, obtaining a matching Kappa (κ) coefficient for dysplasia detection of 0.34 (CI 95%= 0.96-0.03) which is a low degree of agreement on the Landis and Koch scale.

DISCUSSION

The biggest advantage of the WTM technique is that it allows to obtain small viable sample sizes. This allows the performance of biopsies in a simple and convenient way for the patient, but also allows us to study only a small amount of the lesion. We have to keep in mind that this same problem is present when incisional biopsies are performed regarding excisional biopsies [22, 23]. The fact that the only OSCC lesion found in the total samples was obtained with the WTM technique stresses the importance of chosing the location of the sample. In comparison studies between incisional and excisional biopsies, a 56% concordance level has been found with a diagnositic underestimation of 29,5% regarding the incisional biospsy [24]. This result is slightly worse than the results obtained in this study when comparing WTM vs conventional biopsy (78% concordance and 21,95% diagnostic underestimation). Some authors have considered the incisional biopsy as insufficient, although in reality the majority of specialists accept it [25]. In our study, the conventional biopsy presented a diagnostic underestimation of 7,31% and a 76,9% sensitivity. No distinction was made regarding the type of conventional biopsy (incisional or excisional) because we intended to compare de WTM technique with the indicated test for each lesion. The comparison of the type of biopsy was left for future studies.

As it is well known, an extensive lesion may have several zones with different diagnosis: OSCC in one area, dysplasia in another and hyperkeratosis in another [13]. To solve this concordance problem between the incisional biopsy and excisional biopsies, and thus minimize underdiagnosis, the performance of several incisional biopsies [26] is proposed. Thereby, the correlation between both types of biopsies when several samples are performed increases to 71% and decreases the diagnostic underestimation to 11.9% [24].

The WTM allows the clinician to take multiple biopsies from the same lesion, allowing the study of areas which are heterogeneous. This surely would be an improvement in the sensitivity and concordance of the WTM with respect to BC.

As for sample viability, which was the main objective of this study, the WTM technique has shown a 100% viability. 80.48% of the samples preserved all of the epithelial layers, preserving the basement membrane in 85% of the cases. In addition, there are no significant differences regarding the area where we want to take the biopsy, or the clinical aspect of the area, and the preservation of the basement membrane or epithelial layers, which is in accordance with the study by Navone *et al*. [21]. This enables it as a technique capable to obtain viable samples regardless of the location or the clinical aspect. The WTM samples present a viability equal to those obtained with conventional biopsies, and better than others obtained with non-invasive techniques such as cytology or oralCDX test [27,28].

A priori The WTM should not present the complications that conventional biopsy may have such as bleeding, infection or dehiscence [29]. Due to its non-aggressive nature, another advantage may be stress reduction and low rejection of both the patient and the general dentist [30]. Other advantages may be: no requirement of complex or expensive materials, specific or spacious storage, which would facilitate the availability in all general dental offices. Furthermore the laboratory to which the sample is sent does not need to perform different processing or analysis techniques compared to conventional biopsy. The WTM, also has inconvenients. First and foremost, due to the sample size, we do not study a large sample of the lesion, so if we take just one sample, we will leave much of the lesion unexamined [31]. This increases the risk of underdiagnosis or false negatives. To reduce this false negatives, several samples can be taken from the lesion, during the same intervention or in follow-up consultations thanks to the low morbidity and ease of the procedure.

Another point to consider is the operator sensitivity. This study was performed by a Specialist in Oral Medicine, so we do not know whether the results will be the same when the operator is a general dentist, although a study by Pentenero *et al*. [30] suggested that the results obtained with the scraping microbiopsy technique (similar to WTM) is not influenced by operator experience.

CONCLUSIONS

The samples obtained with the WTM technique are viable for study. Preservation of the epithelial layers and in particular of the basement membrane, is not influenced by the anatomical area or by the clinical appearance of the lesion.

Although the whole tissue microbiopsy is not a technique that is designed to replace conventional biopsy for the diagnosis of lesions, it could be considered for the control and monitoring of diagnosed lesions. Further studies with a larger sample size to assess both the operator's influence and the level of acceptance by general dentists and patients should be performed. In the same way, further studies should be able to determine the influence of obtaining several samples of the same lesion and its influence in terms of sensitivity and concordance regarding excisional biopsy.

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