Clinical Trial

Clin Ter 2020; 171 (3):e209-215. doi: 10.7417/CT.2020.2215

CO_2 laser ablation of oral leukoplakia: with or without extension of margins?

U. Romeo, M. Mohsen, G. Palaia, A. Bellisario, A. Del Vecchio, G. Tenore

Department of Oral Sciences and Maxillofacial Surgery, Sapienza University of Rome, Italy

Abstract

The purpose is to determine the sufficient extension of margins during laser ablation of oral leukoplakia and observe its short-term recurrence rate.

Materials and Methods. The study was designed as a randomized controlled clinical trial was conducted on 33 oral leukoplakia lesions diagnosed in 30 patients (16 Females and 14 Males) with an age range between 39 and 79 years. The lesions were divided into three groups; Group A: 11 lesions in 11 patients, in which the laser ablation was done for the entire lesion without extension of margins; Group B: 11 lesions in 8 patients, in which the laser ablation was done for the lesion adding at least 3mm extension of margins; and finally the Group Control: consists of 11 untreated lesions in 11 patients, in which only "wait and see" approach was done.

Results. Complete healing of 13 lesions occurred in both groups A and B. Complete regression of 3 lesions occurred in Group Control. After 6 months of follow-up, 6 of 9 lesions in both groups A and B that showed the recurrence, had shown an initial recurrence after 3 weeks of the laser ablation. Patients with no history of smoking habits showed complete healing of 87.5%, while in ex-smokers complete healing was 42.8%. The statistical analysis was performed, and the averages of all groups are significantly different ($p < 0.00001$).

Discussion. The primary treatment focuses on the elimination of associated risk factors (smoking, alcohol, and local irritating factors). In the literature, the recurrence rate varies between 13.6% and 40.7%, while in our study, it was 45.5% in Group A and 36.4% in Group B.

Conclusion. The recommended extension of margins should be at least 3mm in width. Further research can be performed to evaluate the immediate re-ablation of the lesions which showed an initial recurrence after 3 weeks of laser ablation. ***Clin Ter 2020; 171 (3):e209-215. doi: 10.7417/CT.2020.2215***

Key words: *Oral leukoplakia, CO_2 laser, Oral Potentially Malignant Disorders, Laser ablation*

Introduction

The term Oral Leukoplakia (OL) was defined by the World Health Organization (WHO) as "white patch or plaque that cannot be characterized clinically or histologically as any other disease". This clinical term can be used when other white oral lesions have been excluded through clinical differential diagnosis and histological examination. (1, 2) Recently, the working group on potentially malignant disorders, coordinated by the WHO Collaborating Centre for Oral Cancer and Precancer, presented new definition of leukoplakia "The term leukoplakia should be used to recognize white plaques of questionable risk having excluded other known diseases or disorders that carry no increased risk for cancer". This is a novel relevant element that emphasizes the real risk of malignant change during follow-up of certain alterations identified at the first assessment as ''leukoplakia'' in some areas of the oral tissue. (3) Its appearance in oral cavity usually takes place after the age of 30 years, resulting in a peak of incidence above the age of 50. (4, 5)

The elimination of suspected etiological factors should be accomplished. In case of persistent lesions (no signs of regression within 2 weeks), an identification by histopathological examination should be performed to have a definitive clinical diagnosis of OL.

The prevalence of OL in the population is 1-5%, vary among the oral anatomical sites and demographic groups. (6) The OL annual malignant transformation rate is 1%, however many authors suggest that this percentage would be higher following the proper diagnosis of OL. In a study by Woo et al, (7) 57.1% of cases of True Leukoplakia (TL) were diagnosed as "Keratosis of Unknown Significance" (KUS) when all frictional keratosis were excluded.

OL is one of the Oral Potentially Malignant Disorders (OPMDs), thus its diagnosis is essential because of the premalignant potential and its need to be managed differently from other white lesions (8).

The management of OL includes various treatment modalities with a low success rate in a long follow-up such as: surgical treatment, medical therapy, and nonsurgical treatments such as vitamin A, retinoid, beta carotene or

Correspondence: Mohamed Mohsen, Department of Oral Sciences and Maxillofacial Surgery, Sapienza University of Rome
Address: Via Caserta 6, 00161, Rome, Italy. Tel.: +393478048688. Email: mohamed.mohsen@uniroma1.it

ISSN 1972-6007

carotenoids, non-steroidal anti-inflammatory drugs, herbal extracts, bleomycin, and Bowman–Birk inhibitor. (9)

The conventional types of surgical treatments are done by scalpel excision, electrosurgery, and cryosurgery. The recurrence rate varies from 10% to 34% for scalpel excision and from 12% to 25% for cryosurgery. These modalities cause tissue scarring and contraction, which can mask early signs of recurrence. (10)

The use of laser for surgical treatment of OL is reported. Different laser wavelengths are proposed: CO_2, Nd:YAG, Er:YAG, and diode laser. The CO_2 laser in comparison with the other laser wavelengths can superficially remove the soft tissues by ablation with minimal thermal damage to adjacent tissue and good haemorrhage control (11, 12), which results in a minimal tendency to scar and little postoperative pain and edema. (13, 14)

The surgical procedures and their success rates are different and multiple. However, a gold standard surgical protocol for the treatment of OL by laser beam is still needed to improve the success rate. (13) It was preferred to use the expression "laser ablation" to indicate the complete removal of the lesion by laser.

The aim of this study is to determine the adequate Extension of Margins (EM) during CO_2 laser ablation of OL and observe its short-term recurrence rate.

Materials and Methods

The study was designed as a randomized controlled clinical trial (CE Prot. n.729). Patients were recruited in the Department of Oral Sciences and Maxillofacial Surgery, Sapienza University of Rome. All the interventions were performed by the same oral surgeon and conducted according and passing the online examination for the Certificate in Essential Good Clinical Practice (GCP) (Certificate number: EGCP19/3015) and following the guidelines of the World Medical Association Declaration of Helsinki (15). All participants underwent the same procedures without any deviation from protocol during the entire trial and were informed about the methods of treatment (with or without EM) and the randomization procedure and gave written informed consent.

Full clinical examination consisted of medical and dental history were performed. Patients were photographed with the same equipment (Nikon D200, Nikon Corporation, Tokyo, Japan). Elimination of associated risk factors was done for all the patients. An oral swab was done for all the lesions to exclude any fungal infection, moreover conventional blade biopsy was performed on all the lesions. Multiple biopsies were performed for large lesions more than 20mm. All surgical specimens were preserved in neutral-buffered formalin solution (10%) and sent for histopathological evaluation to confirm the diagnosis of TL by a pathologist. The histopathological evaluation confirmed that all the lesions were "hyperkeratosis with no evidence of dysplasia".

One month after the histological diagnosis, patients were treated following the protocol for the complete ablation with the CO_2 laser. Local anesthesia was performed by 1.8 ml of Mepivacaine solution with adrenaline (Mepivacaina Pierrel, 30 mg/ml, injection solution 1.8 ml, Pierrel S.p.A., Milan, Italy) and was applied around the OL lesion.

The inclusion criteria for the lesions to be enrolled in the study were: the presence of white lesion in the oral cavity for two weeks after the elimination of associated risk factors, oral swab to exclude any fungal infection, conventional blade biopsy to confirm histopathologically that the lesion is "hyperkeratosis with no evidence of dysplasia" to confirm the clinical diagnosis of "Keratosis of Unknown Significance" (KUS)

The exclusion criteria: hyperkeratosis with evidence of dysplasia and any other white lesions.

Patient's enrollment was performed following the CONSORT (Consolidated Standards of Reporting Trials) criteria (Table 1); 43 OL lesions in 17 males and 19 females. Ten lesions were excluded (not matching the inclusion criteria (n = 5), declined to participate (n = 4), or for other reasons (n = 1)).

This study was performed on 33 lesions, in 30 patients. The patients age range was between 39 and 79 years old, of which 14 were males (47%) and 16 females (53%). Patients were randomly distributed into three groups according to a computer-generated series. The web Research Randomizer® free resource for researcher was used for randomization:

- Group A (GA): 11 OL lesions in 11 patients. The laser ablation was done for the entire lesion without EM.
- Group B (GB): 11 OL lesions in 8 patients. The laser ablation was done for the lesion adding at least 3mm of EM.
- Group Control (GC): 11 OL lesions in 11 patients. No treatment was prescribed. Only a periodic six months follow-up "wait and see" approach was done.

Large lesions (more than 20mm) were divided into sections that were ablated with a distance of at least 2 months (compartmentalization of the lesion). All the lesions and extended borders were ascertained using a sterile dermographic pen. The EM was measured using a periodontal probe (Sklar's®, Goldman-Fox Probe, West Chester, PA, USA)

The laser device employed was CO_2 Laser (SMART US-20D®, DEKA, Florence, Italy, 10600 nm) using a power of 4.5 Watts on PW, 80 Hz, fluence of 44.78 J/cm2, and a spot diameter of 400 μm.

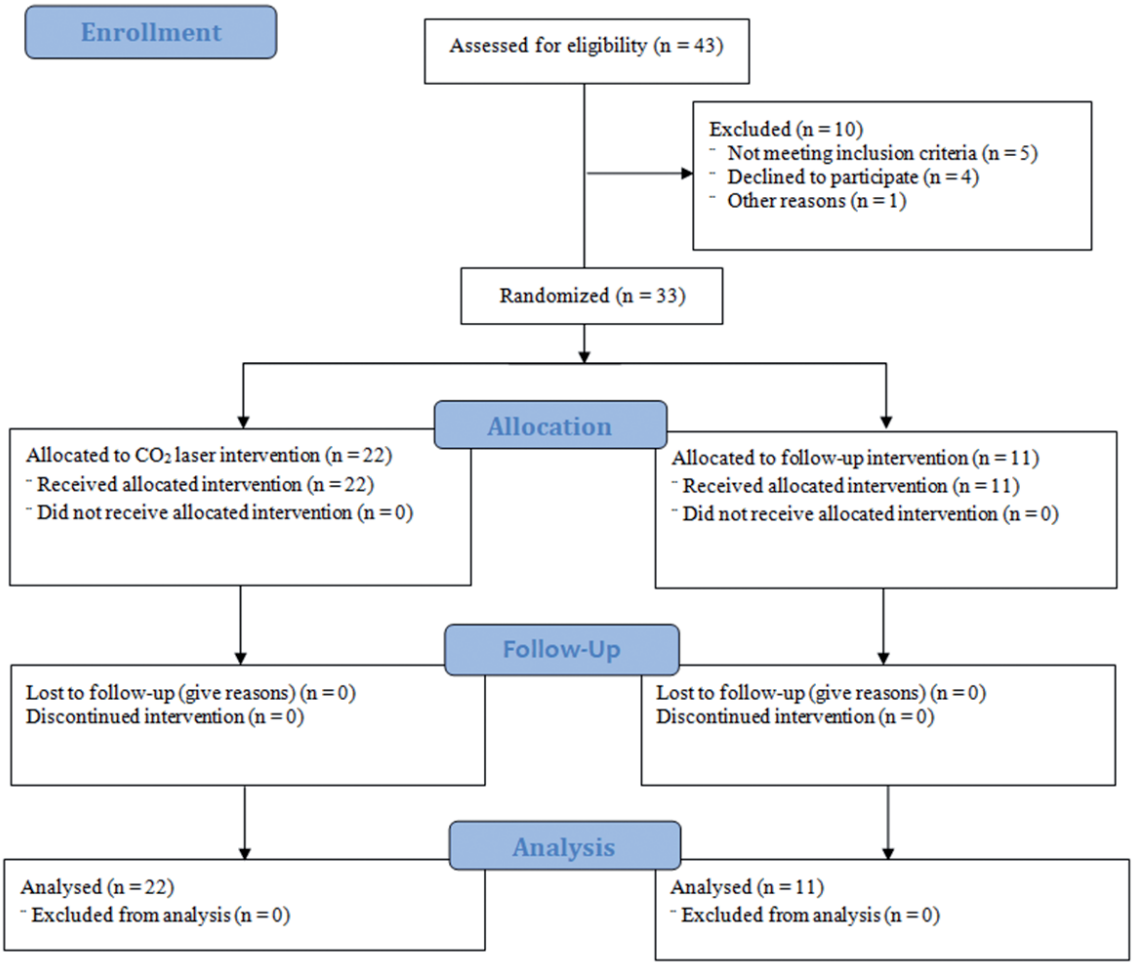
The laser was used in defocused mode (1-2 mm distance to the mucosa). The wound surface was ablated in scanning movements, vertical and horizontal, to remove at least 3mm in depth except in gingival lesions the maximum depth was 1mm for the elimination of any remnants of the lesion.

All laser ablated wounds were left to open granulation and secondary epithelialization. The prescribed medication for the patients in GA and GB was 0.2% Chlorhexidine spray (Corsodyl®, GlaxoSmithKline Consumer Healthcare S.p.A, Baranzate, Milan, Italy) prescribed for three times a day for one week.

Four follow-up visits were performed according to this schedule: one week, 3 weeks, 3 months, and 6 months.

Recurrence rate evaluation in GA and GB was divided into two categories: complete healing and recurrence, while in GC was divided into two categories: complete regression and no change. The collected data of the study was analysed and the statistical analysis was performed (Student-Newman–Keuls multiple comparison).

Table 1. CONSORT (Consolidated Standards of Reporting Trials) of the study



Results

The one-way analysis of variance statistical analysis coupled to the Student-Newman–Keuls multiple comparison between the success rate (Complete healing and complete regression) post-hoc test showed that the averages of all groups are significantly different ($p < 0.00001$; F: 3.292). Thus, the averages of success rates in all the groups are significantly different (Table 2).

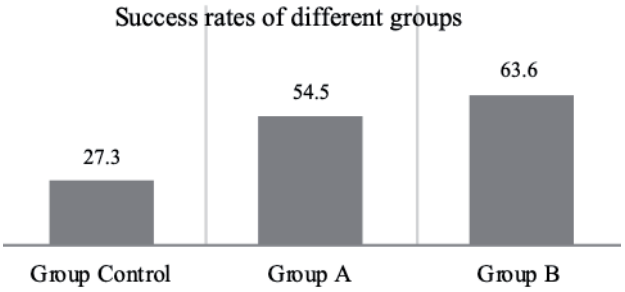
The results of different groups (n=33) pointed out a percentage of regression of 27.3% for the GC (only follow-up), percentage of healing of 54.5% for the GA (ablation without EM) and 63.6% for the GB (ablation with EM of 3mm) (Table 3).

No differences were clinically detected in the healing process. In general, good healing process and minimal post-operative pain or discomfort were shown by all the patients.

Table 2. The one-way analysis of variance statistical analysis coupled to the Student-Newman–Keuls multiple comparison.

Groups	**N**		**Subset for alpha = 0.05**	
		1	2	3
Group Control	11	27.27 %		
Group A	11		54.54 %	
Group B	11			63.63 %
Sig.		1.000	1.000	1.000

Table 3. The different rates of healing are shown for each group



After 6 months of follow-up, 6 of 9 lesions in both GA and GB that showed the recurrence, had shown an initial recurrence after 3 weeks of the laser ablation (Fig. 1).

The complete healing occurred in 13 lesions (59%) among the GA and GB. While the complete regression was observed in 3 lesions (27.3%) in the GC.

In GA, the complete healing occurred in 6 lesions (54.5%) and the recurrence occurred in 5 lesions (45.5%) (Fig. 2). While in GB, the complete healing occurred in 7 lesions (63.6%) (Fig. 3) and the recurrence occurred in 4 lesions (36.4%).

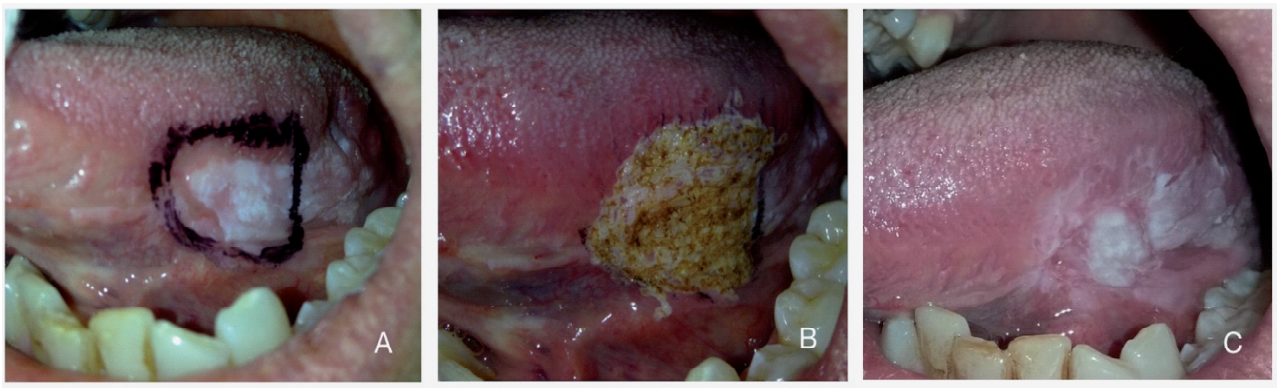


Fig. 1. (A). Oral leukoplakia lesion on the tongue; (B) Immediately after CO_2 laser ablation with EM; (C) Follow-up after 3 weeks shows initial recurrence.

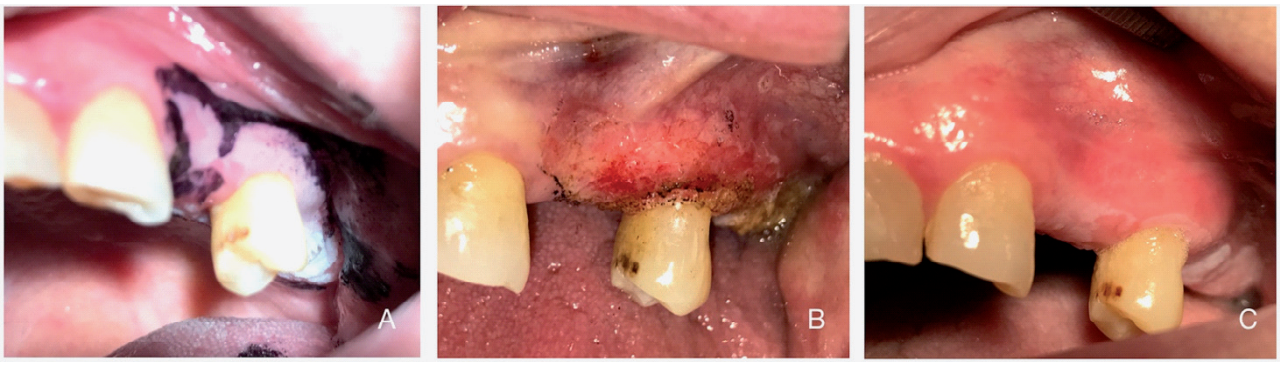


Fig. 2. (A) Oral leukoplakia on the gingiva; (B) Immediately after CO_2 laser ablation without EM; (C) Follow up after 6 months shows the recurrence.

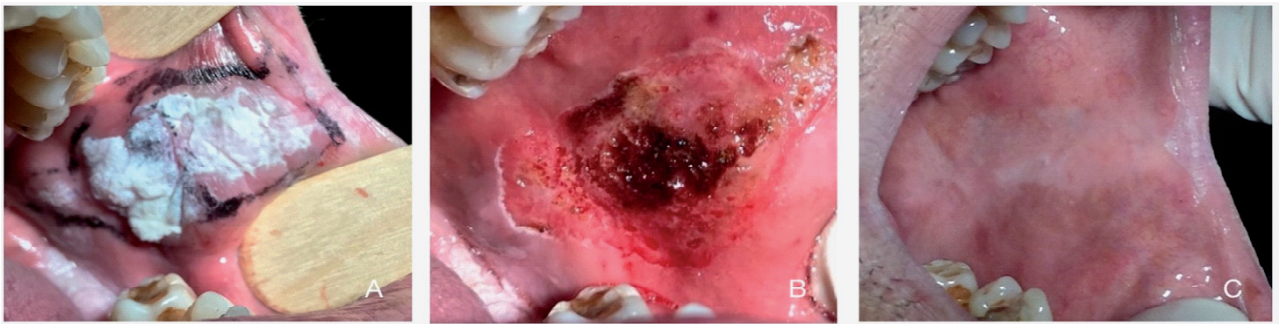


Fig. 3. (A) Oral leukoplakia lesion on the buccal mucosa; (B) Immediately after CO_2 laser ablation with EM; (C) Follow-up after 6 months shows complete healing.

Table 4. An overview of the study.

	Group Control	Group A	Group B
Number of lesions	11	11	11
Gender			
Male	4 (36.4 %)	6 (54.5 %)	4 (50 %)
Female	7 (63.6 %)	5 (45.5 %)	4 (50 %)
Smoking habits			
Non smoking	8 (72.7 %)	4 (36.4 %)	4 (36.4 %)
Ex-smoker	3 (27.3 %	7 (63.6 %)	7 (63.6 %)
Anatomical Site			
Tongue	1 (9.1 %)	1 (9.1 %)	5 (45.4 %)
Palate		3 (27.3 %)	
Buccal mucosa	4 (36.3 %)	1 (9.1 %)	2 (18.2 %)
Attached gingiva	3 (27.3 %)	4 (36.3 %)	3 (27.3 %)
Floor of the mouth	3 (27.3 %)	2 (18.2 %)	1 (9.1 %)
Laser technique	No laser	Ablation without EM	Ablation with EM
Healing / Regression Rate	3 (27.3 %) Complete regression 8 (72.7 %) No change	6 (54.5 %) Complete healing 5 (45.5 %) Recurrence	7 (63.6 %) Complete healing 4 (36.4 %) Recurrence
Follow-up	6 months	6 months	6 months

The complete healing was observed in 75% of females and 44% of males. Furthermore, the smoking habits had a significant relation with the complete healing as it was found that the percentage of complete healing in ex-smoking patients was 42.8% (6 of 14 lesions).

The highest percentage of complete healing according to the anatomical site was observed in lesions of the palate and floor of the mouth (Table 4). During the follow-up period, none of the patients showed an oncological oral transformation, and no reported complications were observed.

Discussion

OL monitoring showed that the lesion may regress unpredictably, remain stable, or may progress to oral squamous cell carcinoma (16). The primary treatment of OL focuses on the suppression of associated etiological factors (smoking, local irritating factors, etc.) (17).

The main advantages of laser therapy are the potential haemostatic effects especially for patients with contraindications to the traditional surgery, the potential for limited tissue contraction and post-therapy scarring. Which may permit the treatment of lesions with large dimensions.

It is extremely important to accurately diagnose and differentiate between reactive or inflammatory keratotic conditions and OL. Because of its pre-cancerous nature, the management of OL should be performed through a close follow-up or complete removal. Not all white keratotic lesions on the oral mucosa are OL, as noted in the WHO definition. The oral mucosa becomes white for the following reasons: Excess production of keratin as a response to injury (e.g. friction or biting), excess production of keratin intrinsically from benign keratotic diseases (e.g. genodermatoses) or dysplasia, thickening of the epithelium (acanthosis), and/or damage of epithelial cells from direct and/or identifiable contact injury (6, 18).

In case of persistence of the hyperkeratosis, an oral biopsy is necessary. OL is potentially malignant lesion, therefore, OL should be treated (9, 19).

Non-homogeneous OL carries a higher risk of malignant transformation, when compared to the homogeneous variance. (20) Regarding non-surgical treatments (retinoid, bleomycin, local use of corticosteroid, etc.), the available evidence on medical and complementary interventions for treating OL is very limited. The relapses and adverse effects are common; the recurrence of OL after surgical treatment has been reported between 13.6 and 40.7% (21).

Further, Kuribayashi et al. (22) showed a long-term outcome (10 years) of non-surgical treatment of OL. Their results indicated that on 237 observed lesions, 135 lesions (57%) were remained unchanged, 30 (12.7%) lesions were characterized by a reduction in size, and 44 (18.6%) lesions were disappeared. While another 11 (4.6%) lesions developed oral squamous cell carcinoma. In their conclusion, the authors indicated that the development of appropriate treatments for OL is required, which will enable successful differentiation between surgical and observational treatment plans. Those results show the necessity to develop a more efficient prophylactic and acceptable permanent therapy for OL.

According to Van der Waal (18), four steps of certainty C factor (C1, C2, C3, C4) may be useful in the diagnosis of OL including C1 (provisional clinical diagnosis), C2 (definitive clinical diagnosis), C3 (definitive clinicopathological diagnosis in case of incisional biopsy) and C4 (definitive clinicopathological diagnosis in case of excisional biopsy or surgical excision after an incisional biopsy). So that the diagnosis of OL may be provisional or definitive and associated with a variable degree of certainty factor.

Our study was conducted on 33 lesions diagnosed with OL. A certainty factor of C3 was assigned to all the lesions, based on the evidence obtained by non-regression of the pathological framework after the elimination of the associated risk factors for a period of two weeks.

In addition to incisional biopsy for small lesions and multiple incisional biopsies for large lesions, a histopathological report confirmed that no other clearly defined lesions were revealed. Although histopathological diagnosis was obtained through an incisional biopsy, there are studies in the literature that show the possible differences between the results of an incisional biopsy and those related to an excisional biopsy causing, in some cases, the failure to diagnose dysplastic or neoplastic lesions.

The anatomical distributions of the lesions in this study showed a predominant appearance on the gingival mucosa 30.3% (10 of 33 lesions). On the other hand, in a study conducted by Brouns et al. (23) and in another conducted by Mogedas-Vegara et al. (24), the most common anatomical site was the lateral border of the tongue.

Laser ablation is one of the treatment modalities available for the management of OL which guarantees a less complicated postoperative pain, edema, minor inflammatory complications, preservation of the mobility and functionality of the treated tissue (25, 26, 27, 28), and based on our results, it shows a lower recurrence rate.

In our study, there were some limitations that should be considered. Including OL lesions with the same site and size for each group was one of the major limitations of the study. Another limit may be related to the presence of some normal variables such as gender and age, that might increase the difficulty to standardize the results for the population treated in normal life. In fact, the major limitation of any randomized clinical trials is their restriction to interventions that are supposed to have a positive effect. In addition, clinical trials can not usually provide complete solid answers to the questions asked by practitioners and deciders. (29)

Although there is still no unanimity about the most suitable treatment for OL, the collected data from our study demonstrated that complete healing occurred in 13 lesions (59%) among both GA, GB and complete regression of 3 lesions (27.3%) in the GC after six months of follow-up.

Pedrosa et al. (30) conducted a study on patients with OL, treated with CO_2 laser with a lateral extension of 2mm to the apparent clinical margins of the lesion reaching a depth of about 5mm; while on the gingival and hard palate lesions, the surgical depth was about 2mm and the recurrence rate was 40.7% (24 of 59 lesions).

The selection of the 3mm of EM for GB was done based on a retrospective study conducted by Kuribayashi et al. (31), in which 53 lesions of OL were present in 52 patients treated by surgical excision. It was found that the free recurrence rate in patients with resection margins >3mm in width was significantly higher than that in patients with resection margins <3mm; thus, advising that the optimal safety margins should be at least 3mm in width and a deep surgical margin may relate to the recurrence of OL.

OL may be a complex lesion. It is derived from one or several clones of cells within an extended oral mucosal area comprising other clones of cells characterized by clinically invisible changes “Field of Cancerization”. The new epithelium of potentially unstable mucosa that migrates from the periphery to cover the wound might be the cause of the recurrence. This may explain the development of new patches of OL, adjacent to the margins of previously treated areas. (32)

The results of this study highlight a better outcome achieved by adding 3mm of EM beyond the clinical limit of the lesion since the complete healing at 6 months was higher for GB than GA.

The anatomical site in which the highest prevalence of OL in our study was the attached gingiva; the mean marginal thickness for gingival tissue is usually only 1.25mm. So that it is too thin to resect with sufficient safety margins. As a result, the lesions on the gingival mucosa were of a significant risk factor for recurrence.

Conclusion

It is preferred to treat OL not to leave it for “wait and see” or “watch-and-see” approaches. It is important to ascertain the borders of the lesions to detect if the peripheral recurrence occurred.

The recommended EM should be at least 3mm in width. Also, deep surgical margins may be related to the recurrence of OL. Dividing large lesions, more than 20mm, into multiple sections for laser ablation could be a solution to reduce the post-operative symptoms and malfunctions.

Further research can be performed to evaluate the immediate re-ablation of the lesions which showed an initial recurrence after 3 weeks of ablation.

Conflict of interest

There is no conflict of interest in this study and it did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Staines K, Rogers H. Oral leukoplakia and proliferative verrucous leukoplakia: a review for dental practitioners. Br Dent J. 2017; 223(9):655-661.
2. Arduino PG, Bagan J, El-Naggar AK, Carrozzo M. Urban legends series: oral leukoplakia. Oral Dis. 2013 Oct; 19(7):642-59
3. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007; 36:575–580
4. Reichart PA. Oral mucosal lesions in a representative cross-sectional study of aging Germans. Community Dent Oral Epidemiol 2000; 28:390–398
5. Axell T. Occurrence of leukoplakia and some other oral white lesions among 20,333 adult Swedish people. Community Dent Oral Epidemiol 1987; 15:46–51
6. Nammour S, Zeinoun T, Namour A, et al. Evaluation of Different Laser-Supported Surgical Protocols for the Treatment of Oral Leukoplakia: A Long-Term Follow-Up. Photomed Laser Surg. 2017
7. Woo SB, Grammer RL, Lerman MA. Keratosis of unknown significance and leukoplakia: a preliminary study. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014; 118(6):713-24
8. Villa A, Woo SB. Leukoplakia-A Diagnostic and Management Algorithm. J Oral Maxillofac Surg. 2017; 75(4):723-734
9. Lodi G, Franchini R, Warnakulasuriya S, Varoni EM, et al. Interventions for treating oral leukoplakia to prevent oral can-

cer. Cochrane Database of Systematic Reviews 2016, Issue 7. Art. No.: CD001829. DOI: 10.1002/14651858.CD001829.pub4.
10. Brennan M, Migliorati CA, Lockhart PB, et al. Management of oral epithelial dysplasia: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103 Suppl:S19:1–12
11. Palaia G, Del Vecchio A, Impellizzeri A, et al. Histological Ex Vivo Evaluation of Peri-Incisional Thermal Effect Created by a New-Generation CO2 Superpulsed Laser, The Scientific World Journal, vol. 2014; Article ID 345685, 6 pages, 2014. https://doi.org/10.1155/2014/345685
12. Romeo U, Russo C, Palaia G, et al. Biopsy of Different Oral Soft Tissues Lesions by KTP and Diode Laser: Histological Evaluation, The Scientific World Journal, 2014; Article ID 761704, 6 pages, 2014
13. Ishii J, Fujiita K, Komori T. Laser surgery as a treatment for oral leukoplakia. Oral Oncol 2003; 39:759–769
14. Romeo U, Palaia G, Del vecchio A, et al. Effects of KTP laser on oral soft tissues. An in vitro study. Lasers Med Sci. 2010; 25(4):539-43
15. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191–2194. doi:10.1001/jama.2013.281053
16. Villa A, Sonis S. Oral leukoplakia remains a challenging condition. Oral Dis. 2018;24:179–183. https://doi.org/10.1111/odi.12781
17. Dosi T, Gupta D, Hazari A, et al. Assessment of micronuclei frequency in individuals with a habit of tobacco by means of exfoliated oral buccal cells. J Int Soc Prev Community Dent. 2016; 6(Suppl 2):S143-7
18. Van der waal I. Oral leukoplakia, the ongoing discussion on definition and terminology. Med Oral Patol Oral Cir Bucal. 2015; 20(6):e685-92
19. Gopinath D, Thannikunnath BV, Neermunda SF. Prevalence of Carcinomatous Foci in Oral Leukoplakia: A Clinicopathologic Study of 546 Indian Samples. J Clin Diagn Res. 2016;10(8):ZC78-83
20. Warnakulasuriya S. Lack of molecular markers to predict malignant potential of oral precancer. J Pathol. 2000; 190(4):407-9
21. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003; 14(1):47-62
22. Kuribayashi Y, Tsushima F, Morita K, et al. Long-term outcome of non-surgical treatment in patients with oral leukoplakia. Oral Oncol. 2015; 51(11):1020-5
23. Brouns ER, Baart JA, Karagozoglu KH, et al. Treatment results of CO2 laser vaporisation in a cohort of 35 patients with oral leukoplakia. Oral Dis. 2013;19(2):212-6
24. Mogedas-vegara A, Hueto-madrid JA, Chimenos-küstner E, Bescós-atín C. The treatment of oral leukoplakia with the CO2 laser: A retrospective study of 65 patients. J Craniomaxillofac Surg.2015; 43(5):677-81
25. Romeo U, Libotte F, Palaia G, et al. Oral soft tissue wound healing after laser surgery with or without a pool of amino acids and sodium hyaluronate: a randomized clinical study. Photomed Laser Surg. 2014; 32(1):10-6
26. Jerjes W, Upile T, Hamdoon Z, et al. CO2 laser of oral dysplasia: clinicopathological features of recurrence and malignant transformation. Lasers Med Sci. 2012; 27(1):169-79
27. Warnakulasuriya S, Reibel J, Bouquot J, et al. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med. 2008; 37(3):127-33
28. Staffoli S, Romeo U, Amorim RNS, et al. The effects of low level laser irradiation on proliferation of human dental pulp: a narrative review. Clin Ter. 2017;168(5):e320-e326.
29. Collet JP. [Limitations of clinical trials]. Rev Prat. 2000; 50(8):833-7
30. Pedrosa A, Santos A, Ferreira M, et al. Is carbon dioxide laser vaporization a valuable tool in the management of oral leukoplakia? A survey at an oncology hospital. Lasers Med Sci. 2015; 30(5):1629-30
31. Kuribayashi Y, Tsushima F, Sato M,et al. Recurrence patterns of oral leukoplakia after curative surgical resection: important factors that predict the risk of recurrence and malignancy. J Oral Pathol Med. 2012; 41(9):682-8
32. Holmstrup P, Dabelsteen E. Oral leukoplakia-to treat or not to treat. Oral Dis. 2016; 22(6):494-7