ORIGINAL RESEARCH

Factors affecting Clinical Outcomes after Treatment of Oral Leukoplakia with CO$_2$ and Diode Laser

[1]Vivian Galletta, [2]Luciane Azevedo, [3]Giovanni Lodi, [4]Dante Migliari

ABSTRACT

Introduction: To analyze factors that may affect recurrence, development of new lesions, and malignant transformation in patients with oral leukoplakia (OL) following surgical treatment with lasers.

Materials and methods: A total of 40 patients were enrolled in this study, 17 females (mean age of 64.5 years; 33–88 years) and 23 males (mean age of 56.6 years; 28–84 years) with an overall mean age of 60.5 years. A total of 49 lesions were diagnosed and treated; 9 patients had more than one site affected. Mean time of follow-up was 22 months (6–71 months). Data were assessed by univariate Cox and multivariate Cox regression analyses.

Results: Recurrence (OL at the same site of the initial lesion) was observed in 11 patients (27.5%) while 4 patients (10%) developed new lesions, and 2 patients (5%) experienced malignant transformation. Only two clinical factors were statistically associated with the outcome for the development of new lesions: patients ≥ 60 years and female gender (p < 0.1). Neither of the outcomes of recurrences and malignant transformations was significantly correlated with any of the risk factors analyzed.

Conclusion: Surgical laser is not a deterrent for the outcomes evaluated; additionally, the design of this study did not allow us to determine whether the laser treatment had provided a great benefit by significantly reducing the rate of malignant transformation among the patients.

Clinical significance: It is highly important to inform patients with OL that their condition can be treated, when possible, by surgical laser, and that this treatment may be helpful in bringing down the odds of malignant transformation of their lesions. In addition, the patients should also be brought to the attention of the necessity of a continued clinical monitoring regardless of the outcome following a surgical intervention.

Keywords: Laser surgery, Malignization, Oral leukoplakia, Recurrence, Risk factors.

INTRODUCTION

The term "precancer" used to define oral lesions with predisposition to malignant transformation has been replaced by "potentially malignant disorder (PMD)," according to the workshop coordinated by the World Health Organization (WHO) held in 2005.[1] This means that not all oral lesions described under this term will develop into cancer and, additionally, that patients with PMD carry an increased risk of developing malignancies in clinically normal oral mucosa, not only at the lesion site.

Oral leukoplakia is the most common PMD in the clinical practice of oral medicine. Therefore, patients with OL should be monitored for early signs of malignancy with special attention given to lesions characterized in light of their location, nonhomogeneous surface, and degree of dysplasia. At the same time, clinicians must be aware that cancer may arise from "harmless" types of lesions, exhibiting mild or no dysplasia, localized at low-risk areas, or presenting a homogeneous surface.[1-4]

In considering whether or not to treat OL, it is important to bear in mind that there is no scientific evidence

[1,2]Clinic for Lasers in Dentistry (LELO), School of Dentistry University of São Paulo, São Paulo, Brazil

[3]Unit of Oral Medicine and Pathology, Department of Surgical Medicine and Dentistry, University of the Studies of Milan Milan, Italy

[4]Division of Clinic in Oral Medicine, Department of Stomatology School of Dentistry, University of São Paulo, São Paulo, Brazil

Corresponding Author: Dante Migliari, Av. Prof. Lineu Prestes 2227, Cidade Universitária, São Paulo, SP 05508900, Brazil Phone: +551130917883, e-mail: dantemigliari@gmail.com

that any type of treatment precludes the recurrences of OL or its malignant transformation.[5] However, except for widespread lesions, surgical excision has been advised since patients benefit from a detection of early cancer not detected by incisional biopsy[6] and, according to meta-analysis review, from a lower rate of malignant transformation as compared with nontreated cases.[7]

On a strictly surgical analysis, treatment with a high-power laser has advantages over conventional (scalpel) surgery in that it is quicker, results in less bleeding, and is prone to lower bacteremia. Moreover, when OL involves large areas of the mucosa, laser surgery is the first choice treatment, as it allows for healing by secondary intention.[8,9]

The purpose of this study was to assess the usefulness of treating OL lesions with CO_2 and diode lasers and to analyze factors that may affect recurrence, development of new lesions, and malignant transformation.

MATERIALS AND METHODS

Patient Selection

This study was conducted during the period of 2006 through 2013. The OL patients were selected according to the criteria established by the International Symposium of Uppsala.[10] The diagnosis of OL was based on the clinical characterization of white patches, which could not be defined as any other similar disease (e.g., white sponge nevus, lichen planus) followed by exclusion of any possible associated etiological factors, such as dental restoration materials, chronic trauma, and physical or chemical agents, except the use of tobacco or alcohol. Histopathological examination was made in all lesions following incisional biopsy. This procedure helped to exclude other similar diseases, such as plaque form of oral lichen planus and grade epithelial dysplasia, if present. This study was approved by the Committee on Ethics of the University of São Paulo; an informed consent was obtained from all participants.

Patients who had been previously treated for oral cancer were excluded from this study. The OL lesions on the lips were not considered for analysis since they may coexist with actinic cheilitis. Data regarding age, gender, and history of tobacco and alcohol consumption were registered.

Before the biopsy, patients were tested for the presence of superimposed candidosis by means of periodic acid–Schiff-stained smears. If positive for *Candida*, patients were treated for 2 weeks with topical antifungal agent (Nystatin oral suspension) and then retested. Areas selected for incisional biopsy were preferentially those with a red, nodular, or exophytic aspect (the nonhomogeneous type) or positively stained with toluidine blue.

Histopathological examination for degree of epithelial dysplasia was made according to the WHO criteria.[11]

Surgical treatment was performed using CO_2 laser (UM-L30, Union Medical Engineering Co, USA; continuous mode; 10,600 nm; 5–10 W) or diode laser (GaAlAs, ZAP softlase, ZAP Lasers Inc., USA; continuous mode; 808 nm; 2–3 W) under local anesthesia. The CO_2 laser was preferably used due to its properties of rapid incision and superficial thermal damage. Lesions localized at sites of difficult surgical access (e.g., the soft palate and lingual and palatal surfaces of the gingival mucosa) were removed with a diode laser, which provides precise control of the laser beam by use of optical fiber delivery in a contact mode. In general, lesions were initially outlined with a margin of 2 to 5 mm from their edge through vaporization, deepening to 3 mm in the connective tissue; the resection margin was lifted with forceps and excision was accomplished by undercutting at constant depth. Specimens excised through laser were submitted to histopathological analysis. Standard laser safety protocols were used in all cases.

Clinical Outcome

Patients were evaluated every 3 months, and only those with a minimum follow-up of 6 months had their data included for analysis. Three events were taken into consideration for the clinical outcome: Recurrence (reappearance of OL at the same site or adjacent to the initial lesion), development of a new lesion (recurrence of OL arising at a distant site from the initial lesion), and malignant transformation.

Statistical Analysis

Factors that may influence clinical outcomes were grouped to simplify statistical analysis. Age was split into ≥60 years and <60 years; lesion localization was grouped into high-risk sites (ventral and lateral tongue, floor of mouth, soft palate and palatoglossal arch) and low-risk sites (other sites of oral mucosa); lesion size was split into ≥2 and <2 cm; grade of dysplasia was grouped into high (intense dysplasia) and low (mild or moderate dysplasia) and no dysplasia; clinical aspect as homogeneous and nonhomogeneous; alcohol consumption was split into ≥200 and <200 gm/week; and tobacco consumption split into ≤20 and >20 cigarettes/day.

The Kaplan–Meier curve was used to assess cumulative free survival of clinical outcomes. The Kaplan–Meier curve followed by the log-rank test was used to evaluate factors that may have affected clinical outcomes. Risk (hazard ratio) of clinical outcomes for factors analyzed was assessed by univariate Cox regression analysis. Multivariate Cox regression analysis was performed to

verify any association between factors that presented $p < 0.1$ values on univariate analysis. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 40 patients were enrolled in this study, 17 females (mean age of 64.5 years; 33–88 years) and 23 males (mean age of 56.6 years; 28–84 years) with overall mean age of 60.5 years. A total of 49 lesions were diagnosed and treated; nine patients had more than one site affected. Mean time of follow-up was 22 months (6–71 months).

Risk factors were predominantly found in males: 7 (30.4%) were smokers and alcohol drinkers, 11 (47.8%) were smokers, 1 (4.3%) was only alcohol drinker, and 4 (21.7%) were neither smoker nor alcohol drinkers. Among the females, habit of smoking was found in six patients (35.3%), and there was no report of habitual consumption of alcohol (Table 1). Only two patients quit smoking during follow-up.

The majority of lesions had homogeneous surface (33/49; 67.3%) and measured <2 cm (28/49; 57.1%). The most affected sites were the lateral tongue (13/49; 26.5%), the buccal mucosa (10/49; 20.4%), and the floor of mouth (7/49; 14.3%). The clinical features and site of lesions are shown in Table 2.

Surgical removal by high-power lasers (CO_2 or diode) was the treatment of choice for most the cases (41 lesions). Laser vaporization was performed in eight lesions because of their proximity to salivary ducts (four lesions) or tooth surface (four lesions). During the postoperative evaluation, 27 patients complained of burning sensation or pain, which was controlled with nonsteroidal anti-inflammatory medication and analgesic. Symptomatic cases were predominantly associated with lesions located on the lateral tongue regardless of the size of the lesion. Healing with reepithelialization was complete within 4 to 5 weeks posttreatment.

Table 1: Age and risk habits (tobacco and alcohol consumption) according to gender of patients with OL

|	Gender	
	Males (n = 23)	*Females (n = 17)*
Age		
<60 years	15	6
≥60 years	8	11
Habits		
Only tobacco		
≤20 cig/day	8	6
>20 cig/day	3	0
Only alcohol*		
<200 gm/week	0	0
≥200 gm/week	1	0
Tobacco+alcohol	7	0
No habits	4	11

*Based on guidelines of alcohol safe consumption provided by Centers for Disease Control and Prevention, USA, 2005. (http://www.cdc.gov/alcohol), < 200 gm/week is equal to a can of beer, a glass of wine, or a shot of distilled spirits or liquor per day

Histopathological examination of the 49 biopsied lesions revealed 19 lesions with no dysplasia (38.8%) and 30 presenting dysplasia (61.2%), of which 14.3% (7/49) had mild dysplasia; 28.6% (14/49) had moderate dysplasia; while 18.3% (9/49) had severe dysplasia.

Recurrence (OL at the same site of the initial lesion) was observed in 11 patients (27.5%); four patients (10%) developed new lesions, and both events together (37.5%). No statistically significant association was found for these two clinical outcomes (recurrence or development of a new lesion) concerning the degree of epithelial dysplasia, location, size, or the clinical aspect of lesions ($p = 0.3$). Malignant transformation occurred in two patients (5%), though at different sites of initially treated lesions. The mean time for malignant transformation was 11.3 months, 6 to 19 months. In this particular event, one of the lesions, located on the palatoglossal arch, had a nonhomogeneous aspect, with intense dysplasia, and affected a 83-year-old male who was nonsmoker and nondrinker; while the

Table 2: Distribution of OL lesions according to clinical appearance and size

	Clinical appearance			Size (cm)			
Localization	*Homogeneous*	*Nonhomogeneous*	*Total*	*<2*	*2–4*	*>4*	*Total*
Lateral tongue	6	7	13	2	8	3	13
Buccal mucosa	9	1	10	8	2	0	10
Floor of mouth	5	2	7	4	3	0	7
Hard palate	3	1	4	4	0	0	4
Gingiva	1	1	2	1	0	1	2
Alveolar ridge	3	1	4	4	0	0	4
Soft palate	3	0	3	3	0	0	3
Palatoglossal arch	0	2	2	1	1	0	2
Dorsum tongue	1	1	2	1	1	0	2
Ventral tongue	0	1	1	0	1	0	1
Retromolar trigone	1	0	1	1	0	0	1
Subtotal	32	17	49	29	16	4	49
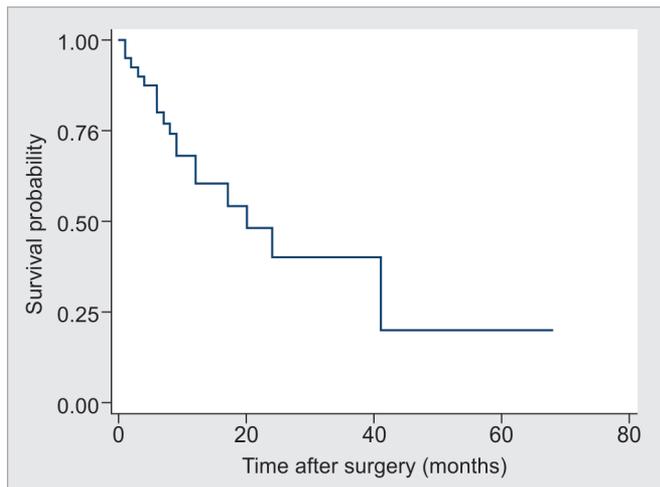
Graph 1: Kaplan–Meier survival curve for clinical outcomes of recurrence, development of new lesions, and malignant transformation
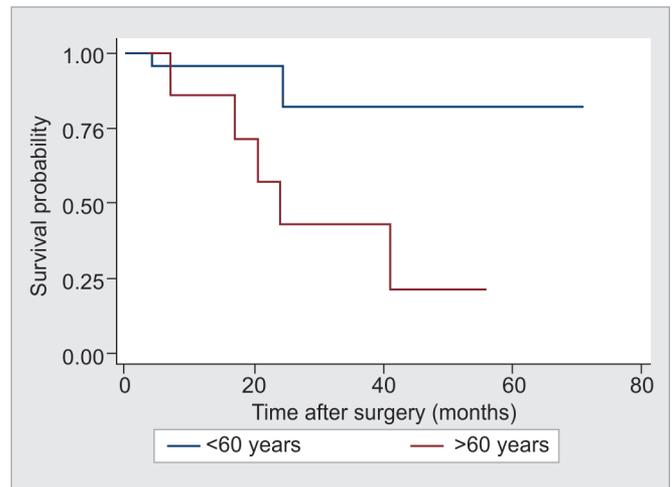
Graph 2: Kaplan–Meier curves for development of new lesions showing statistical association with age over 60 years (p = 0.024)

Table 3: Univariate Cox regression analysis of factors affecting recurrences and development of new lesions

Factors	Group	Hazard ratio	p-value	CI (95%)
Gender	Male		Reference	
	Female	4.22	0.059	0.94–18.85
Age	<60 years		Reference	
	≥60 years	5.32	0.037*	1.10–25.54
Lesion location	Low-risk sites		Reference	
	High-risk sites	0.64	0.459	0.20–2.04
Lesion size	<2 cm		Reference	
	≥2 cm	2.62	0.168	0.66–10.36
Clinical aspect	Homogeneous		Reference	
	Nonhomogeneous	0.40	0.244	0.09–1.83
Grade of dysplasia	Low		Reference	
	High	0.52	0.577	0.05–4.95
Alcohol consumption	<200 gm/week		Reference	
	≥200 gm/week	1.16	0.874	0.17–7.74
Tobacco consumption	No		Reference	
	Yes	0.38	0.167	0.09–1.49

*Statistically significant (p < 0.05)

other, located on the buccal mucosa, was dysplasia free, had a homogeneous aspect, and affected a 57-year-old female who was nonsmoker and nondrinker.

Overall, Kaplan–Meier survival curve showed the probability of patients being free of any clinical outcome (recurrence, new lesion, or malignant transformation) and was of approximately 50% after 20 months (Graph 1). Log-rank test showed significant association of patients aged ≥60 years (p = 0.024) with the clinical outcome of development of new lesions (Graph 2).

Univariate Cox regression analysis of factors influencing clinical outcomes showed that only patients ≥60 years had a higher risk of developing new lesions [hazard ratio = 5.32; confidence interval (CI) = 1.10–25.54; Table 3]. Multivariate Cox regression analysis of factors that presented p < 0.1 showed that female gender together with age ≥ 60 were factors associated with the risk for development of new lesions (Table 4). For the outcomes of recurrence or malignant transformation, the regression analysis did not show any statistically significant association between risk factors and these outcomes.

DISCUSSION

The OL is by far the most important lesion that affects the oral mucosa, since it harbors, indisputably, an

Table 4: Multivariate Cox regression analysis of factors (p<0.1) associated with recurrences and development of new lesions

Factor	Group	Hazard ratio	p-value	CI (95%)
Gender	Male		Reference	
	Female	3.10	0.138	0.69–13.90
Age	<60 years		Reference	
	≥60 years	4.14	0.101	0.75–22.64

innate potential for malignant transformation. Its most intriguing aspect, however, lies on the fact that there is no clear-cut evidence, either clinical or laboratorial, that can predict which OL lesion will transform into malignancy.[12-14]

The initial approach to OL should be mainly concerned with a proper diagnosis of this lesion. There have been many attempts to find a better definition of OL; however, all of them fell short, since this lesion does not show a proper histologic definition, and, clinically, it has been vaguely defined as the presence of a nonremovable white plaque.

Biopsy is a very important procedure to characterize OL, since it can both exclude lesions that have a specific histological feature (such as oral lichen planus) and provide (when present) an analysis of the degree of epithelial dysplasia. In addition, a clinician should exclude any etiological factor (especially physical or chemical trauma) that could be associated with the OL lesion. It has been mainly accepted that OL must be of idiopathic nature, but the presence of smoking and/or consumption of alcohol does not exclude the diagnosis of OL, according to all studies on OL classification.[5,10,13]

The diagnosis of OL in this study followed strictly the criteria proposed in the International Symposium of Uppsala,[10] which have been regarded as very instrumental for an accurate diagnosis and classification of OL.

A persistent and an unwelcome legacy after decades of studies on OL is the lack of adequate and reliable protocol for management of OL lesions. At present, there has been a growing consensus among authors that surgical excision is the most appropriate means for management of OL. In all studies, the authors clearly state that surgical intervention does not prevent malignant transformation of OL, but it can reduce significantly the odds ratio for a patient to develop cancer.[2,4,7,15,16]

In this respect, one specific study,[3] with a substantial number of cases (236 patients), did not find a statistically significant benefit for patients who underwent surgical excision (94 lesions), in terms of reducing the chance of malignant transformation (12%), as compared with those who did not undergo surgical treatment (175 lesions; malignant transformation in 4% of cases). One open criticism to the study is that the percentage of nonhomogeneous lesions in the surgically treated cases (49%) was substantially higher than that seen in those without surgical intervention (12%). This difference may account for the high rate of malignant transformation in patients undergoing surgical intervention.

Some other studies, however, also with a representative number of cases, such as Dost et al,[15] in which 368 patients were investigated, and van der Hem et al,[4] adding 200 OL patients treated with CO_2 laser, together with critical reviews,[7] firmly advocate treatment (when possible) for OL by surgical excision, which appears to decrease significantly (though not eliminating) the risk for OL patients to develop oral malignancy.

In this study, surgical excision was performed in all cases (40 patients) using a preferably CO_2 laser (diode laser was an option for lesions posing difficulty to surgical access). This surgical intervention was done not only for the proposition of this study but also, rather, it was an attempt to provide some degree of benefit to the patients by removing their lesions with minimal possible discomfort.

The main findings (or events) analyzed were recurrence (27.5%), development of new lesions (4%), and malignization (5%), all having occurred in a relatively short average follow-up time (22 months; 6–71 months). The percentage rates observed for recurrence and malignization were within the range reported in other studies.[2-4,8,17,18]

A consensus among authors has yet to be reached on which factor (or factors) would be associated with events, such as recurrence and malignant transformation after OL lesion being surgically removed by laser. One study pointed to the continuing alcohol intake (but not smoking) as the main factor for an increased risk for malignant transformation of OL[8]; other studies would relate the recurrence and malignant transformation rates to the homogeneous aspect of the OL lesions,[4,18] while others with the nonhomogeneous aspect,[3,17,19] and, still, others would find no correlation for malignant transformation with any factor at all.[2,9,15] One close agreement among authors is that the degree of dysplasia is of any relevance as a risk factor to be the cause of recurrence or malignant transformation in OL lesions ether surgically treated or not. In this study, there was no factor associated with recurrence and malignization, apart from the development of new lesions, this being the only outcome significantly affected by risk factors, such as age and gender (female gender and those aging over 60).

CONCLUSION

One criticism of this study is the relative short follow-up time and, arguably, the number of cases evaluated. Despite that, the data presented here have confirmed that either recurrence or malignant transformation of OL lesion cannot be avoided following a surgical intervention. The overall benefit to the patients is that whether the laser therapy could, in fact, provide a low rate of malignization could not be confidently estimated since a control group of OL patients without surgical treatment was not available. In this respect, a growing body of evidence points to surgical intervention, preferably by a laser,

as the best available treatment for OL by significantly reducing the odds of OL patient of developing squamous cell carcinoma. In addition to surgical treatment, patients should be instructed that a continued follow-up should be carried out since it stands to benefit greatly by detecting early signs of malignization.

ACKNOWLEDGMENT

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