SAPIENZA
UNIVERSITÀ DI ROMA

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF ORAL AND MAXILLOFACIAL SCIENCES

Director Prof. Ersilia Barbato

DOCTOR OF PHILOSOPHY IN INNOVATIVE TECHNOLOGIES IN THE DISEASES OF SKELETON, SKIN AND ORO-CRANIOFACIAL DISTRICT

-ODONTOSTOMATOLOGIC DISEASES-

XXX CYCLE

Director Prof. Antonella Polimeni

VAPORIZATION TECHNIQUE BY CO_2 LASER AS A TREATMENT OF THE TRUE ORAL LEUKOPLAKIA: CLINICAL STUDY

Tutor
Prof. Umberto Romeo
Supervisor
Dr. Gaspare Palaia

Candidate
Dr. Mohamed Mohsen
N. Matricola 1506557

ACADEMIC YEARS
2016 - 2017

“A writer is a person for whom writing is more difficult than it is for other people”

Thomas Mann

Acknowledgements

I would like to extend my gratitude to the many people who helped bring this research project to fruition.

First, I would like to thank Professor Umberto Romeo for giving me the opportunity to be a part of this PhD program. I am deeply grateful for his help, professionalism, valuable guidance and support throughout this research and through my entire program of study that I do not have enough words to express my deep and sincere appreciation.

Special appreciation goes to my supervisor, Doctor Gaspare Palaia, for his supervision and constant support. His invaluable help of constructive comments and suggestions throughout the experimental and thesis works has contributed to the success of this research. Not to forget to thank Doctor Amelia Bellisario; as her collaboration, support, and perpetual enthusiasm were of a great help for me.

Finally, I must express my profound gratitude to my parents, my wife, and my brother for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them, Thank you.

Abstract

Aim

To determine the sufficient safety margins during laser vaporization of oral leukoplakia as a trial to reduce the recurrence.

Introduction

Definitive treatment of oral leukoplakia is essential because of its recurrence and potentiality to the malignant transformation.

CO_2 laser vaporization is characterized by being with minimal damage to the adjacent tissues, limited scarring, little wound contraction, and low post-operative complications.

Materials and Methods

This study was conducted on 36 true leukoplakia lesions and diagnosed in 34 patients (20 Females and 14 Males). The range of the patients age was between 39 and 79 years.

The lesions were divided into three groups; Group A: 11 lesions in 11 patients, in which the laser vaporization was done for the entire lesion adding a maximum of 1 mm of safety margins; Group B: 9 lesions in 7 patients, in which the laser vaporization was done for the lesion adding at least 3 mm of safety margins; and finally the Control Group: consists of 16 lesions in 16 patients.

During six months after the laser vaporization, four follow-up visits were performed in order to evaluate the healing course and to evaluate the recurrence rate and its degree.

Results

Among all the completely healed lesions, 75% of which were in groups A and B while 25% were in the Control Group.

In this study, it was observed that some of the vaporized lesions which showed partial or complete recurrence after 6 months of follow-up, have shown the initial recurrence after 3 weeks of laser vaporization.

The best results were obtained in patients with no history of smoking habits as the complete healing was 87.5% (7 of 8 lesions) and the complete recurrence was 12.5% (1 of 8 lesions). However, in ex-smokers, the complete healing was 41.5% (5 of 12 lesions), the partial recurrence was 41.5% (5 of 12 lesions), and complete recurrence was 17% (2 of 12 lesions).

Discussion

The primary treatment of oral leukoplakia 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, after 6 months of follow-up, it was 45% in Group A and 33% in Group B.

Conclusion

The recommended optimal safety margins should be at least 3 mm in width; in addition, deep surgical margins may be related to the recurrence of oral leukoplakia.

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

Contents

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: _____

Date: _____

Chapter 1 : Introduction

1.1. DEFINITION OF ORAL LEUKOPLAKIA

The term leukoplakia was defined by the World Health Organization (WHO) as “white patch or plaque that cannot be characterized clinically or histologically as any other disease”. [1, 2]

WHO in collaboration with the center for oral cancer and pre-cancer in the United Kingdom, in May 2005, replaced the term “pre-cancerous lesions” with “Potentially Malignant Disorders” (PMD) which include Oral Leukoplakia (OL) among other diseases. [3]

In 2012, a new definition was proposed which seems more opportune as it includes the histological confirmation “A predominantly white lesion or plaque of questionable behaviour having excluded, clinically and histopathologically, any other definable white disease or disorder”. This one hasn’t been assessed yet by WHO, but it has good chances for acceptance.

In 2017, Villa [4] used the term leukoplakia for describing a white lesion that is pre-cancerous while recently WHO defined it as “a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer.”

It is one of several potentially malignant oral lesions, including erythroplakia and submucous fibrosis. As such, it is essential to be recognized because of its premalignant potentiality and managed accordingly and differently from other white lesions.

There is continuous confusion on the use of the term leukoplakia, especially on how to manage leukoplakia in a patient whose diagnosis shows ‘hyperkeratosis with no evidence of dysplasia’.

There is also no consensus on the guidelines for the management and treatment of dysplastic lesions, much less leukoplakias without dysplasia. [4]

1.2. DIFFERENTIAL DIAGNOSIS

Oral white lesions, including leukoplakia, are commonly encountered in daily practice by oral health care providers, especially oral and maxillofacial surgeons.

They are often investigated by biopsy examination to rule out the presence of dysplastic changes or cancer. [1]

Most white lesions are benign frictional keratosis or keratosis from inflammatory conditions, (e.g. Lichen Planus (LP)) and the diagnosis is usually evident from the histopathology.

Taking a careful history, clinical and histopathological examinations of all white lesions by an oral and maxillofacial pathologist is required to achieve the final diagnosis.

It is extremely important to accurately diagnose and differentiate between reactive or inflammatory keratotic conditions and True Leukoplakia (TL).

Because of its pre-cancerous nature, the management of TL should be performed through a close follow-up or complete removal. [4]

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: [4]

- 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).
- Damage of epithelial cells from direct and/or identifiable contact injury.

These changes can occur because of a genetic dyskeratotic disease (Cannon white sponge nevus, a very rare condition), immune-mediated disease (LP), bite, trauma, or oncogenic mutations (e.g. Leukoplakia with dysplasia). (Table 1)

Table 1 Classification of white lesions of the oral cavity

Developmental	Cannon White Sponge Nevus Hereditary Benign: intraepithelial dyskeratosis Other Congenital: genodermatoses (e.g. pachyonychia congenita)
Reactive or Frictional	Leukoedema Contact desquamation Frictional Keratosis: MMO, BARK Hairy Tongue Associated with tobacco use: nicotinic stomatitis, smokeless tobacco keratosis
Infectious	Candidiasis Hairy leukoplakia (associated with EBV)
Immune Mediated	LP Lichenoid lesions Benign migratory glossitis
Autoimmune	Lupus erythematosus Chronic graft vs. host disease
Metabolic	Uremic stomatitis Palifermin-associated hyperkeratosis
Malignant and OPMD	Keratosis of unknown significance (KUS) Dysplastic leukoplakia SCC Verrucous carcinoma

I. Developmental

These lesions are extremely uncommon and all have specific and distinctive histopathologic features.

- White Sponge Nevus:

Present as diffuse bilateral white plaques of the oral mucosa; could involve the buccal mucosa in particular, tongue; esophageal and genital mucosa but not the skin. (Fig. 1)

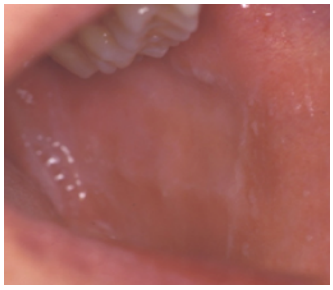

Figure 1 White sponge nevus

- Hereditary Benign Intraepithelial Dyskeratosis:

Present as bilateral thick white plaques of the oral mucosa and as gelatinous plaques of the conjunctiva without involvement of the skin.

- Pachyonychia Congenita:

Oral plaque, but always accompanied by the presence of thickened skin lesions. Congenital dyskeratosis causes leukoplakias and oral cancer at a young age. [4]

II. Reactive or frictional

- Leukoedema:

Occurs in up to 90% of the population and can occur after exposure to mildly irritating substances (e.g. mouthwash, toothpaste, or tobacco and marijuana smoke).

It is presented as delicate gray-white lacy lines on the buccal mucosa or ventral tongue that disappear with stretching of the mucosa and it shows histopathologically edema of epithelial cells.

These are rarely submitted for biopsy examination because they are readily recognized and no treatment is necessary except for stopping the habit.

- Morsicatio Mucosae Oris (MMO):

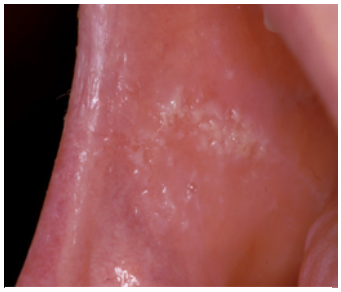

Figure 2 Morsicatio Mucosae Oris

Is usually self-induced and manifests as white plaques and papules with poorly demarcated 'fading' margins. (Fig. 2)

Affected sites are those that are easily traumatized by teeth, such as the lower lip mucosa, lateral or ventral surface of the tongue, and buccal mucosa.

Patients are usually unaware of this parafunctional habit, especially if the habit is nocturnal.

- Benign Alveolar Ridge Keratosis (BARK):

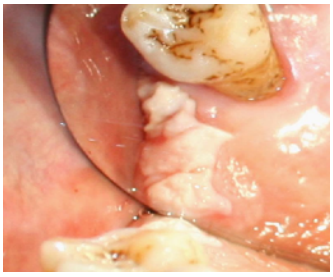

Figure 3 Benign Alveolar Ridge Keratosis

Is due to constant trauma to the edentulous alveolar ridge; it is commonly seen on the retromolar pad and underneath ill-fitting dentures.

This is a common traumatic frictional keratosis that constitutes approximately 75% of all biopsy results of white lesions and has distinct and readily recognized histopathological features. (Fig. 3)

The diagnosis on the pathology report should be 'benign frictional keratosis'. [4]

- Hairy Tongue:

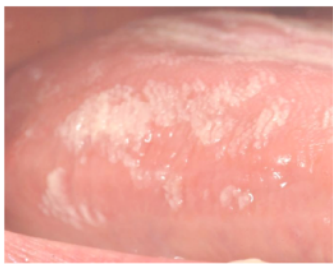

Figure 4 Hairy Tongue

It is a benign retention keratosis caused by decreased exfoliation of keratin and the development of elongated filiform papillae 'hairs'. (Fig. 4)

The dorsum of the tongue is presented as a white, coated, or a hairy appearance. It can become pigmented from intrinsic bacteria or food; hence, the 'black hairy tongue'.

Patients might also complain of sticky and mucinous saliva with an associated pasty, metallic taste and gagging (when the coating is localized in the posterior third of the tongue).

The most common cause of this condition is dehydration and hyposalivation. This is seen in patients who have had recent illness (often associated with antibiotic therapy), use of alcohol-containing rinses, or smoking.

Conditions that cause dry mouth, such as polypharmacy, chronic anxiety, radiotherapy, and Sjögren syndrome, may also cause the hairy tongue.

This condition is also seen in patients with poor diet for the consumption of mainly soft foods (common in hospitalized patients). [4]

III. Infectious

- Oral Candidiasis:

Is the most common opportunistic fungal infection. It is usually caused by Candida-albicans, a commensal present in 20 to 30% of patients.

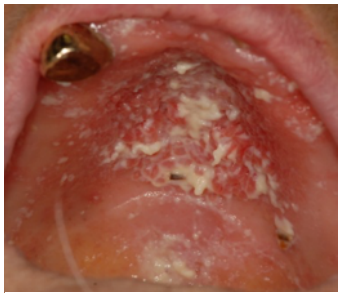

Figure 5 Acute pseudomembranous candidiasis

Oral lesions occur when the normal flora is altered (as in patients with hyposalivation, wear dentures, smokers or are on immunosuppressive agents).

Additional contributing factors include anaemia, endocrine dysfunction, immunosuppression (e.g. acquired immunodeficiency syndrome, human immunodeficiency virus (HIV)), prolonged antibiotic intake, diabetes mellitus, infancy, or advanced age.

Candidiasis also can develop overlying dysplastic lesions. Pseudomembranous candidiasis, the most common form, is characterized by thick white plaques and papules that can be rubbed off, which often leave a raw and bleeding surface. (Fig. 5)

Other forms include erythematous and hyperplastic candidiasis. The latter is a rare chronic variant that manifests as white plaques that cannot be rubbed off, mimicking leukoplakia.

Treatment consists of topical and systemic anti-fungal agents. The most commonly used topical medications include: nystatin suspension (100,000 U/mL) swished in the mouth 4 to 5 times a day, clotrimazole troches (10 mg) dissolved in the mouth 4 to 5 times a day for 7 to 10 days, or systemic therapy with fluconazole 100 to 200 mg/day for 7 days. (Fig. 6)

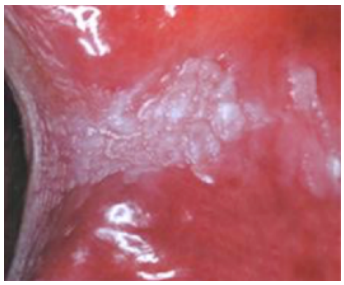

Figure 6 Chronic hyperplastic candidosis, nodular form

- Epstein-Barr virus:

Is mostly seen in immunocompromised patients, in particular those with HIV and a low CD4+ T-cell count and those after undergoing organ transplantation, although it can also be seen in healthy older individuals likely from immune senescence. In this condition, the term leukoplakia is not related to malignancy or to dysplastic changes.

IV. Immune-Mediated Keratotic Lesions [4]

- Oral Lichen Planus (OLP):

Is an immuno-mediated chronic condition present in 1 to 2% of the population, usually middle-aged women. 10 to 15% of patients with OLP have cutaneous lesions.

OLP can be idiopathic or may be associated to local or systemic conditions and in particular to medication intake, such as antihypertensive and hypoglycemic drugs.

Oral lesions are typically symmetric and bilateral and there is a controversy regarding the clinical types.

Although 6 distinct forms, reticular, atrophic, erosive, papular, plaque, and bullous, have been described, this is not universally accepted. (Fig. 7- 8)

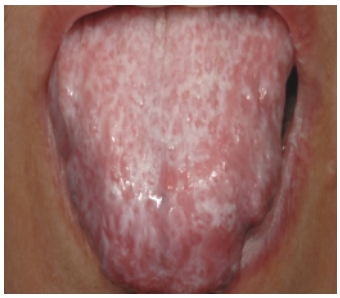

Figure 7 Oral lichen planus, Reticular form

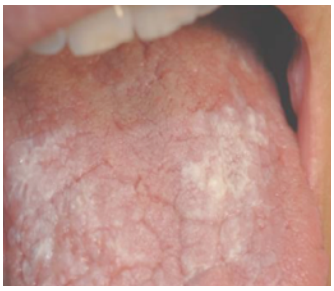

Figure 8 Oral lichen planus, Plaque form

The 3 most recognizable forms are reticular, erosive, and ulcerative. Bullous (rarely seen), atrophic, and erosive forms are likely better considered clinical entity because they present a spectrum of disease and the most common appearance is an erythematous or erosive lesion because of ruptured bullae or thin, atrophic red appearing mucosa.

The reticular form is characterized by classic white (keratotic) reticular lesions. The plaque type of LP may not be readily distinguishable from a leukoplakia. Lupus erythematous and chronic graft-versus-host disease are 2 conditions that can clinically resemble OLP.

Biopsy and histopathological examinations are usually indicated. Treatment includes topical corticosteroids (e.g. 0.05% fluocinonide or clobetasol gel) or 0.1% tacrolimus ointment, and for refractory cases, treatment may include systemic corticosteroids.

1.3. ORAL LEUKOPLAKIA AND LEVEL OF CERTAINTY

- Epidemiology

The estimated reported prevalence of OL, worldwide, is approximately 2%. However, when viewed in relation to an annual malignant transformation rate of 1%, this prevalence figure would result in development of oral cancer in 20 per 100,000 population per year.

Obviously, this cancer incidence figure, based on the malignant transformation of OL alone, is much too high. Probably, the prevalence of OL has to be set at a more realistic figure of less than 0.5%. There are some geographical differences with regard to the gender distribution.

Leukoplakia is six times more common among smokers than non-smokers. Alcohol is an independent risk factor, regardless of the beverage type or drinking pattern. There are conflicting results of studies related to the possible role of human papilloma virus infection. [5]

- Clinical Aspects

Leukoplakia may affect any site of the oral and oropharyngeal cavity. Clinically, leukoplakias are divided into homogenous and non-homogeneous lesions.

The homogeneous type is usually a thin, flat, and uniform white plaque with at least 1 area that is well demarcated with or without fissuring. (Fig. 9)

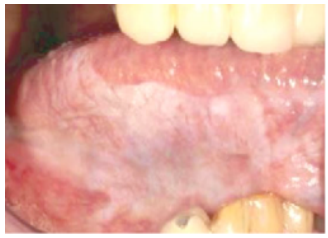

Figure 9 Homogeneous Leukoplakia

The non-homogeneous type has been defined as a mixed white and red lesion, that may either be irregularly flat (speckled) or nodular ‘erythroleukoplakia’. (Fig. 10)

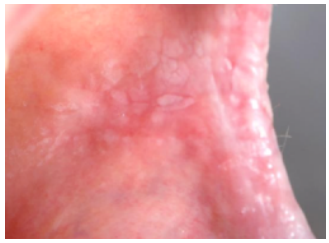

Figure 10 Non-homogeneous, nodular form

Erythroleukoplakia can be misdiagnosed as OLP because of its white and red components. However, other clinical signs can guide the

clinician to the correct diagnosis. Erythroleukoplakia does not possess the typical white reticular changes and is usually unilateral and associated with well-demarcated white plaques.

Verrucous leukoplakia is yet another type of non-homogeneous leukoplakia. Although verrucous leukoplakia usually has a uniform white appearance, its verrucous texture is the distinguishing feature from homogeneous (flat) leukoplakia. Verrucous leukoplakia is clinically indistinguishable from the clinical aspect of verrucous carcinoma.

Proliferative Verrucous Leukoplakia (PVL) is a sub-type of verrucous leukoplakia, characterized by multifocal presentation, resistance to treatment, and a high rate of malignant transformation. (Fig. 11)

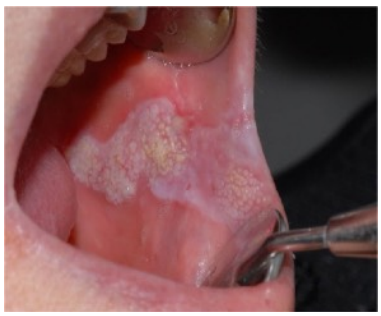

Figure 11 PVL

PVL seems more prevalent among elderly female. There may or may not be a history of tobacco use. [1]

Areas of firmness or induration should always be submitted for periodic biopsy examination.

PVL is usually multifocal or affects contiguous areas and is characterized by relentless progression and spread with the gingiva, being the most frequently affected site. [5, 6]

Most cases of PVL are non-homogeneous with a verrucous, nodular, or erythroleukoplakia-like appearance. Similar to erythroleukoplakia, the erythroleukoplakia form of PVL can be misdiagnosed as LP because it is multifocal and bilateral.

Multiple biopsy examinations show no evidence of cytologic dysplasia but often exhibit verrucous hyperplasia, hyperkeratosis, or parakeratosis with epithelial atrophy. There are many differences between the localized lesions of OL.

- Level of Certainty

Leukoplakia is mostly used as a clinical term. Provisional diagnosis is made when a predominantly white area at clinical examination cannot be clearly diagnosed as any other disease in the oral mucosa, its definition is usually modified after the histopathological evaluation.

“For example, a clinical impression of leukoplakia at biopsy examination might show candidiasis, bite keratosis, or LP; being aware of the diagnostic criteria of the other lesions can help in recognizing the type of the lesion”. [5] (Table 2)

Table 2 The most common white or predominantly white benign diseases of the oral mucosa and their main diagnostic criteria

Lesion	Main diagnostic criteria
Aspirin burn	History of local application of aspirin tablets
Candidiasis, pseudomembranous	Clinical aspect (pseudomembranes, often symmetrical pattern)
Frictional lesion	Presence of mechanical irritation (e.g. habit of vigorous tooth brushing)
Hairy leukoplakia	Clinical aspect (bilateral localization on the tongue); histopathology (EBV)
Leukoedema	Clinical aspect (symmetrical pattern)
Linea alba	Clinical aspect (location on the line of occlusion in the cheek mucosa)
Lupus erythematosus	History of skin lesions; clinical appearance (bilateral pattern); histopathology
Morsicatio (habitual chewing or biting of the cheek, tongue, lips)	History of habitual chewing or biting; clinical aspects
Papilloma and allied lesions	Clinical aspect; histopathology
Syphilis, secondary ‘mucous patches’	Clinical aspect; demonstration of T. pallidum; serology
Smoker’s palate (nicotinic stomatitis)	Clinical aspect; history of smoking
Snuff induced lesion	Clinical aspect; site where snuff is placed

The term leukoplakia can be used at different levels of certainty (C-factor). According to Van der Waal (2015), four steps of certainty may be useful in the diagnosis of OL: C1 and C2 which are clinical terms and C3 and C4 which are clinicopathological terms. [5] (Table 3)

The diagnosis of OL is thus made after the exclusion of other disorders and a biopsy is recommended when other disorders cannot be identified. The disorder is diagnosed as an OL with or without epithelial dysplasia.

It has been recommended to make a distinction between a provisional clinical diagnosis of OL and a definitive one.

Apparently, the recommendation to use a certainty factor has not been widely accepted in the recent literature, although it is a common practice to use such factor in cancer registries. [5]

Table 3 Certainty (C) factor for diagnosis of oral leukoplakia [5]

C_1	Evidence from a single visit, involving inspection and palpation, including a clinical picture of the lesion, ‘provisional clinical diagnosis’.
C_2	No evidence of resolution following elimination of etiological factors (eg. mechanical irritation) over 2- 4 weeks of follow-up or in the absence of any other etiological factors. ‘definitive clinical diagnosis’.
C3	As C2, but complemented by pretreatment incisional biopsy in which, histopathologically, no definable lesion is observed, ‘provisional histopathological diagnosis’.
C4	Evidence from histopathological examination of completely excised lesions, ‘definitive histopathological diagnosis’.

1.4. STAGING AND CLASSIFICATION OF ORAL LEUKOPLAKIA

For the purpose of a standardized reporting system of OL management, staging and classification is highly recommended.

The size, age, gender, causative factors, histopathology, and localization of OL should be taken in consideration at the time of diagnosis.

According to the treatment plan, if the management of OL will be performed only through clinical follow-up (observation), the size, colour, and/or texture of the individual lesion should be taken in consideration.

The clinical sub-types of OL may not be useful, because the clinical appearance of OL may vary and does not always allow a clear classifications.

Considering assessment after surgical intervention, biopsy reveals that the staging system is an important consideration; and it is mainly dependent on size and pathology.

The system takes into account the highest pathological score in case of multiple biopsies of single or multiple lesions of OL and the lowest size if there is any uncertainty in considering size category.

For classification and staging purposes it is recommended to record the size of a single OL, and to add together the sizes of multiple OL.

The oral subsite should be specified according to the International Classification of Diseases Application to Dentistry and Stomatology (ICD-DA) codes for the oral cavity

In this system, three size categories have been proposed, analogous to the TNM system of oral cancer:

I. L represents the size of a single or multiple OL as follows:

L1 < 2cm

L2 = 2-4cm

L3 > 4cm

Lx size not specified.

II. P represents the pathology of OL as follows:

P0 No epithelial dysplasia

P1 Mild or moderate dysplasia

P2 Severe dysplasia

Px Absence or presence of dysplasia not specified

III. Accordingly, four stages have been proposed in this system:

Stage I L1 P0

Stage II L2 P0

Stage III L3 P0 or L1 L2 P1

Stage IV L3 P1 or any L P2

1.5. PROGNOSIS OF ORAL LEUKOPLAKIA

Various oral mucosal lesions, have a potential for malignant transformation; the most common white lesions have the lowest risk of malignant transformation. Practitioners will see many oral white lesions, but a few carcinomas.

However, they must be able to recognize lesions at particular risk; several features help to assess the likelihood of malignant transformation.

The accuracy of such prediction is low, but the process of identifying the 'at risk' lesions is fundamental for diagnosis and treatment planning. Important factors are listed and information on each of these should be sought.

The best predictor of the potential for malignant transformation is the degree of dysplasia seen histologically. For this reason, few lesions will already be malignant, biopsy of white patches is mandatory.

The term dysplasia (literally, abnormal growth) is given to cytological abnormalities seen in both malignant and premalignant cells.

Premalignancy is distinguished from malignancy only by the invasiveness and release of metastases.

It must be emphasized that the implications of epithelial dysplasia are quite different from fibrous dysplasia, in which there is no risk of carcinomatous change.

Deep cell keratinization (dyskeratosis) refers to individual cells which start to keratinize before the surface is reached and show eosinophilic change deeply within the epithelium.

With loss of intercellular adherence, the cells become separated. A lymphoplasmacytic infiltrate of highly variable intensity is usually present in corium.

Dysplasia is usually graded as mild, moderate or severe as a guide for patient management. (Fig. 12- 17)

'Carcinoma in-situ' is a term sometimes used for the most severe dysplasia where the abnormalities extend throughout the thickness of the epithelium, a state sometimes graphically called 'top-to-bottom change'.

In such a lesion, all the cellular abnormalities characteristic of malignancy may be present, only invasion is absent.

The histological assessment of oral epithelial dysplasia is notoriously unreliable because it is subjective and changes are not reliably correlated with behaviour. [1]

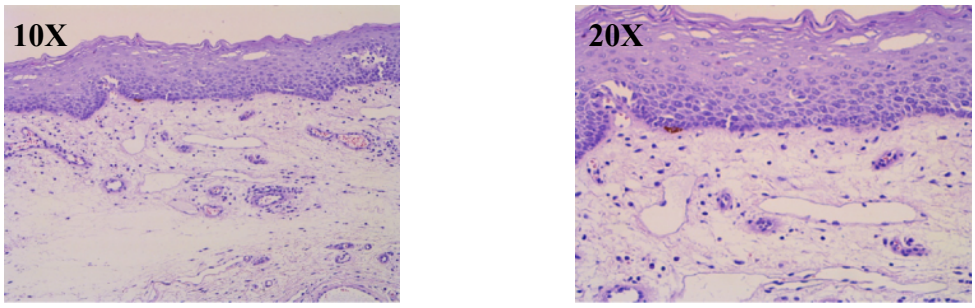

Figure 12- 13 Mild dysplasia (10X- 20X)

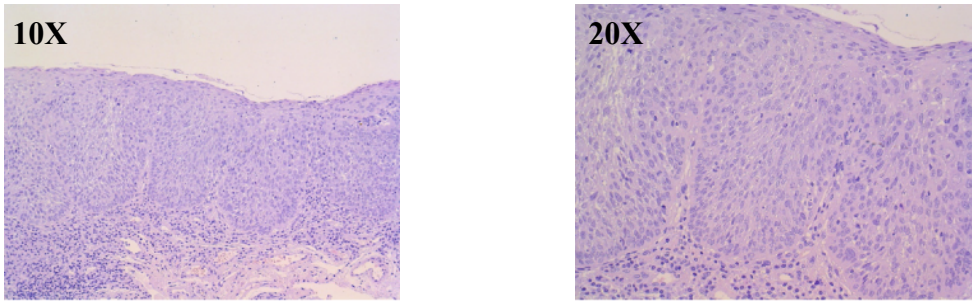

Figure 14- 15 Moderate dysplasia (10X- 20X)

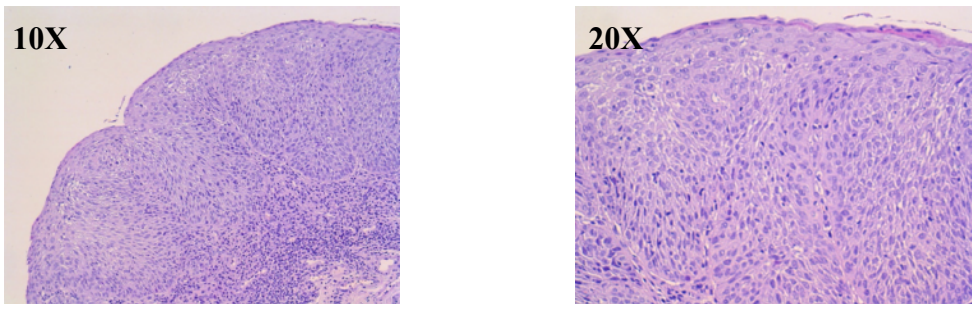

Figure 16- 17 Severe dysplasia (10X- 20X)

1.6. TREATMENT OF ORAL LEUKOPLAKIA

Clinicians treated OL with vitamin A, vitamin E and beta-carotene. However, the toxicity of vitamin A and the unsatisfactory response to vitamin E and beta-carotene caused them to discontinue to use such drugs. [9, 10]

Other treatment modalities for OL are scalpel excision or electrocautery and cryosurgery, for which there is a recurrence rate of approximately 33%. [9, 11] Studies on the clinical usefulness of laser surgery in treatment of OL have shown that the laser surgery prevents not only the recurrence and malignant transformation, but also post-operative dysfunction. [9, 12]

I. Non-surgical treatment

In order to carry out a treatment for OL, the degree of epithelial dysplasia should be assessed. However, OL presenting low to moderate malignant transformation risk may or may not be completely removed and the decision should consider other factors, such as location, size in the case of smokers, and smoking cessation. [13, 15, 16]

In the presence of moderate or severe epithelial dysplasia, surgical treatment is recommended. [15] Surgical treatment of OL may be performed either through conventional surgery [13, 14, 17], electrocauterization, laser vaporization, [12, 18] or cryosurgery. [19, 20] The recurrence rate of OL after surgical treatment has been reported to vary between 10 and 35%. [21, 22]

Non-surgical treatment may also be considered for the management of OL. This modality offers minimal adverse effects to patients, especially for patients with widespread OL that involves a large area of the oral mucosa or patients with medical problems and consequently high surgical risks. [19, 20, 23, 24]

Additionally, potential advantages of the non-surgical treatment of OL include an easy application that does not require treatment at a medical center and a relatively low cost. [25]

- Carotenoids

Beta-Carotene. The carotenoids are a group of extremely hydrophobic molecules with little or no solubility in water. [26] Beta-carotene is a carotenoid commonly found in dark green, orange or yellowish vegetables, such as spinach, carrots, sweet potato, mango, and oranges. [34]

Beta-carotene is a vitamin A precursor. The only known effect of excessive beta-carotene intake is a state in which the skin becomes strongly yellowish, the so-called ‘carotenodermy’, which disappears in a few weeks after the reduction of consumption. [26, 27, 28]

In other studies, the supplement diet based on beta-carotene caused headaches and muscle pain in some of the patients. Patients with OL can be treated with beta-carotene in oral doses of 90 mg/day, for three cycles of 3 months. The use of beta-carotene has been recommended in order to prevent OL and possibly oral cancer. [29, 40]

The potential benefits and protective effects against cancer are possibly related to its antioxidizing action. [30, 31] This function is accomplished through a ligation between beta-carotene and oxygen, which is an unstable reactive molecule; thus, diminishing the damaging effects of free radicals. [31, 32]

A diet supplemented with beta-carotene can prevent changes in the oral mucosa, especially in smoking patients, who present low serum levels of vitamin C and beta-carotene when compared to non-smokers.

It has also been shown that beta-carotene has a better therapeutic clinical response in the prevention of OL lesions in smoking patients than in the non-smoking ones. [33]

Lycopene. is a carotenoid without provitamin A action. This is a fat-soluble red pigment found in some fruits and vegetables; the greatest known source of licopene is tomatoes. [35] There is a positive relationship between lycopene consumption and a reduction in the risk of the development of degenerative diseases caused by free radicals, such as cancer and cardiovascular diseases. [36, 37]

The supplementation of lycopene (8 mg/day and 4 mg/day) in a three month time period reduces hyperkeratosis. [40] No systemic significant toxic effect of lycopene has been observed and there is no evidence of side effects from the treatment with lycopene. [36]

- Vitamins

L-Ascorbic Acid (L-AA) (Vitamin C): so-called vitamin C, is found in citrus fruits, such as kiwi, strawberries, papaya, and mango. [41] It is suggested that a daily intake of at least 140 mg/day is required for smokers because they usually present a reduction of the L-AA concentration in serum leukocytes. [42]

L-AA has antioxidizing properties and reacts with superoxide produced as a result of the cells normal metabolic processes. This inactivation of superoxide inhibits the formation of nitrosamines during protein digestion and helps avoid damage to DNA and cellular proteins. [43]

L-AA toxicity does not occur, since vitamin is water-soluble, and a decrease in absorption efficiency occurs when the consumption exceeds 180 mg/day. [44] There are no studies regarding the efficacy of the use of *L-AA* alone for OL treatment.

α-Tocoferol (AT) *(Vitamin E):* is the most common and most active form of vitamin E. Tocoferol is an effective antioxidant at high levels of oxygen, protecting cellular membranes from lipidic peroxidation. [45, 46, 47, 48, 49]

Retinoic Acid 13-cis-retinoic acid (13- cRA): the current definition of retinoid includes all the natural and synthetic compounds with an activity similar to that of Vitamin A.

Retinoic acid is obtained from carotene and animal products, such as meat, milk, and eggs, which are converted in the intestine respectively into retinal and retinol. [33, 48]

The use of systemic retinoids is not indicated in cases of: pregnancy or probability of pregnancy, non-compliance with the use of contraceptives, breastfeeding, and hypersensitivity to parabeno.

It is relatively contraindicated in cases of: leukopenia, hypothyroidism (patients using bexarotene), high levels of cholesterol and triglyceride, hepatic malfunction, and renal malfunction. [48, 50]

This treatment is not widely accepted due to its side effects: hypervitaminosis, toxicity, teratogenic effects, and alterations in various organic systems. [51]

13-CRA is the retinoid recommended for OL treatment. However, the high recurrence rate after short periods of discontinuance together with its side effects are limiting factors. [30, 51, 52, 53] OL recurred upon the discontinuance of medication; some patients ceased treatment due to its side-effects.

Fenretinide (4-HPR) or N-(4-hydroxyphenyl) Retinamide is a vitamin A analogue that was synthesized in the United States during the late 1960s.

This retinoid shows a preferential accumulation in breast instead of liver. [54] Systemic use of 4-HPR with 200 mg/day for 3 months demonstrated partial clinical resolution of OL.

- Bleomycin

Bleomycin is a cytotoxic antibiotic used for the treatment of squamous cell carcinoma (SCC) of the head and neck region, esophagus, and skin. [55]

After 12 to 15 applications, the white patch peeled off and the resultant raw surface was epithelialized over the following 14 days. Repeated biopsies showed a significant reduction of dysplasia and keratinisation. [33]

Topical bleomycin in treatment of OL was used in dosages of 0.5% /day for 12 to 15 days or 1% /day for 14 days.

- Photodynamic Therapy

Photodynamic therapy (PDT) is a non-invasive method for the treatment of premalignant lesions of head and neck cancers. [56, 57, 144]

The principle of PDT is a non-thermal photochemical reaction, which requires the simultaneous presence of photosensitizing drug (photosensitizer), oxygen, and visible light.

After a period of allowing the photosensitizer to be collect in the target tissue, the photosensitizer is activated by the exposure to low-power visible light of a drug-specific wavelength.

Mainly, the light source consists of portable diode laser and the light is transmitted via laser fibers to or into the tumor. Illumination of the tumor by light at the activating wavelength results in the destruction of cells by a non-free radical oxidative process.

These reactive oxygen species may damage crucial cell components, such as structural proteins, enzymes, DNA, and phospholipids. PDT is a cold photochemical reaction and the photosensitizing agents are of inherently low systemic toxicity.

PDT damage heals mainly by regeneration rather than scarring. Due to the organ preserving principle of PDT, important structures are maintained by good functional and cosmetic outcome. [57, 58] (Fig. 18-23)

Several photosensitizers have been developed during the past years, haematoporphyrin and haematoporphyrin derivatives were the first photosensitizer.

Four photosensitizers have been approved so far:

- Photofrin has been approved in many countries for the treatment of esophagus and lung cancers.
- 5-Aminolaevulinic acid (5-ALA) is also approved in several countries for the treatment of skin cancer.
- Verteporfin for the treatment of macular degeneration.
- Foscan is the only photosensitizer that has been approved for the treatment of advanced SCC of the head and neck in Europe in the year 2001. [59]

The 5-ALA is a naturally occurring compound in the haem biosynthetic pathway, which is metabolized to photosensitive product ‘protoporphyrin IX’.

The major advantage of 5-ALA when compared to synthetic photosensitisers is the rapid metabolism, which significantly reduce the period of cutaneous photosensitivity; mostly indicated in head and neck surgery.

The photosensitizer is administered systemically by intravenous injection. [58, 145] Only for very superficial skin lesions or premalignant lesions of the oral mucosa, the 5-ALA can be applied topically. For all other indications, an intravenous application is mandatory. [59]

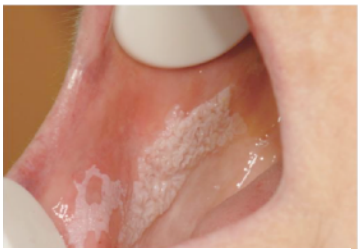

Figure 18 PVL, first session

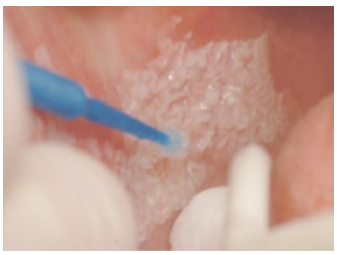

Figure 19 Application of 5-ALA

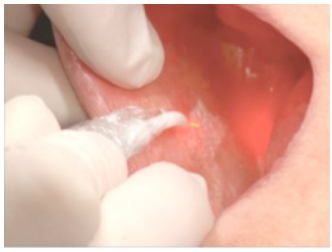

Figure 20 Laser application (635 nm)

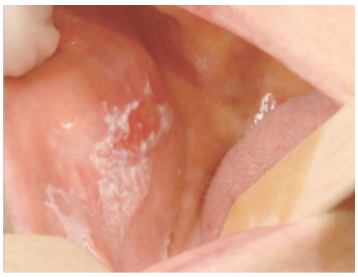

Figure 21 PDT, second session

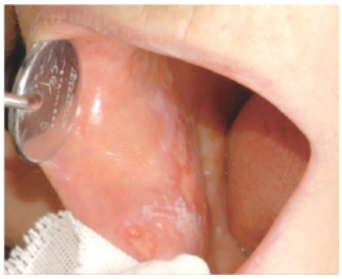

Figure 22 PDT, third session

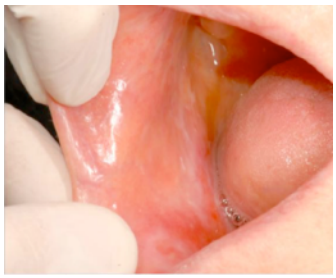

Figure 23 PDT, fourth session

II. Surgical Treatment

Although removal of a lesion still seems to be the predominant method of treatment by the majority of relevant health care professionals, no randomised controlled trials have been undertaken to test the hypothesis that excision either by scalpel or laser greatly influences the potential for later malignant transformation.

Surgical treatment have a beneficial effect. Theoretically, this effect may be reduced by the elimination of risk factors, such as tobacco usage and alcohol consumption. However, further studies are needed to prove such theory. [60]

The first aim in the management of OL is to avoid malignant transformation; and as a consequence, it might be appropriate to consider the outcomes of therapy of OL in terms of later expected Oral Squamous Cell Carcinoma (OSCC) incidences. [61]

Elimination or reduction of the size of the lesion (extent) cannot be considered an acceptable indicator of treatment efficacy as recurrence is

a common event (can be as high as 30% in the same or another oral mucosal site) and it cannot be excluded as the recurring lesions represent a high-risk group.

In addition, histopathological change (i.e. lessened or resolution of dysplastic features) cannot be considered a robust and useful outcome. [62] Although surgery is the first choice in the management of OL by most relevant specialists [63, 64], the hypothesis that removing potentially malignant oral lesions by different surgical techniques (scalpel, laser, and cryosurgery) can prevent the onset of oral cancer remains unproven.

Till now, there are a few reported randomized controlled trials evaluating surgical treatment of OL that underwent surgical (e.g. scalpel excision) or laser treatment.

Results are hardly comparable because of the differences in diagnostic and inclusion criteria, follow-up time intervals, patient characteristics, and surgical techniques employed.

The selection of patients may have been determined by the site, size, nature, or histopathology of the lesion as well as the medical history and desires of the patients.

There is no evidence that the incidence of oral carcinoma can be diminished by surgical removal of OL. This does not mean that surgical removal should be abandoned mainly for histologic diagnosis. [69]

In fact, an important issue in discussing the role of surgical removal in the management of OL is the potential relevance of excisional biopsy as a diagnostic tool. Even if excisional biopsy of OL is not effective as an intervention of primary prevention (i.e. to prevent malignant transformation), it may have a role as an intervention of secondary prevention. [65, 66, 67]

- Scalpel surgical excision

The surgical removal of OL may not reduce the risk of malignant transformation. [68, 69, 70]

Nevertheless, surgical excision does allow the opportunity for examination to ensure that all areas of dysplasia have been identified and excised.

- Cryotherapy

It is well received by patients due to a relative lack of discomfort; cryotherapy is the deliberate destruction of tissue by application of extreme cold, with minimal scarring and an absence of bleeding. [78]

Clinical advantages include the ease of application, preservation of inorganic structure of bone, and a very low incidence of infection. It can be repeated without permanent side effects. [79, 80]

Perhaps its greatest advantage is its usefulness in candidates for whom surgery is contraindicated due to either age or medical history.

Disadvantages of cryotherapy include an unpredictable degree of swelling and lack of precision with the depth and area of freezing. It is also highly dependent on the operator's skills and experience.

The value of histopathological examination of frozen sections to ensure adequate clearance of the disease at surgical margins as with OSCC may not be helpful as it will not detect the ploidy areas (if relevant) and surgeons may have to be more aggressive, than their usual practice, in the excision of the disease. [71, 72]

The basic technique of cryotherapy stresses rapid cooling, slow thawing, and repetition of the freezing process to maximize tissue destruction. [80] The two recognized methods are a closed system with the use of probes and nitrous oxide or an open system with the use of a liquid nitrogen spray or a cotton tip. [78, 80]

The nitrous oxide technique is useful for the treatment of various benign and malignant lesions of the oral cavity where the depth of necrosis is necessary.

Liquid nitrogen sprays and cotton swabs are more accessible to clinicians but are not suitable for use in the oral cavity. Their disadvantage is a lack of control over the temperature achieved within cells and the area of freezing, which makes them hazardous for the intraoral use.

During the freeze cycle as the temperature drops, it is believed that extracellular water undergoes crystallization. In addition, membrane lipids harden at low temperatures decreasing the cell resistance to shrinkage. As extracellular stores of water diminish, the electrolyte concentration increases.

In order to counteract this concentration gradient, intracellular water moves out of the cell and becomes involved in the crystallization process. Intracellular electrolytes reach toxic levels, which become lethal to the cell. Re-epithelialization occurs within 7- 12 days in the mouth and 10- 20 days on the skin. [81]

Cryotherapy is not considered to be the first line of treatment for OL, the reported recurrence rate varies from 20 to 71.4% and the malignant transformation varies from 7 to 25%; therefore, cryotherapy does not seem to be of a particular benefit. [12, 73]

There is a lack of widespread clinical availability because of the risk of post-operative scarring, tissue contraction, and importantly the resultant inability to observe signs of clinical recurrence. [73, 75, 76]

At the present time, no physical method of local excision of OL guarantees long-term resolution of relevant OL or the possibility of OSCC development. [77]

- Laser treatment

Laser beam differs from white light in its coherence. A high density of power is produced when the laser beam is focused by a lens because it has properties that neither diverge nor interfere. [84]

Several kinds of laser beams have been developed since the ruby laser was clinically used for skin lesions for the first time. The absorbed energy causes vaporization of intra and extracellular fluid with a minimal destruction of cell membrane and its surrounding tissue. [87, 88]

Laser surgery has a haemostatic effect, [82, 83] which makes it easier to maintain the operative field; it has the ability to remove lesions accurately. [84, 86]

The damage to the adjacent tissue is minimal, which reduces acute inflammatory reactions and post-operative pain. Also, wound healing after laser surgery is excellent because of the limited contraction. [83, 84, 85]

There have been reports over the past 30 years that among the different surgical techniques proposed for the treatment of OL, laser surgery has received the greatest attention.

Unfortunately, as with other surgical techniques, most studies have major methodological flaws and are very low in the hierarchy of evidence.

CO_2 and KTP lasers have been employed with various vaporization or excision techniques for the treatment of OL.

The main advantages of laser therapy are the potential haemostatic effects, limited tissue contraction, and post-therapy scarring; which may permit the treatment of lesions of large dimensions.

In addition, laser therapy may include reduced post-operative pain, swelling, and infection.

Wounds may take longer to re-epithelialize, small granulomas can complicate healing, and histopathological confirmation of the nature of the excised lesion will not be possible if ablation techniques have been employed. [89]

The excision technique can be performed by either the CO_2 laser or KTP laser in patients with a localized OL occurring in the field on the non-keratinized epithelium.

In the excision technique, both the CO_2 and KTP lasers were used in a non-contact application. As a role, the excised edges of the wound made by the excised technique shall be unsutured.

The vaporization technique can be performed using either CO_2 laser or Nd:YAG laser in patients who had comprehensive OL, or OL on the gingiva and hard palate. In this technique, CO_2 laser should be used in a non-contact mode using an average power output between 4 and 10 Watts, while Nd: YAG laser should be used in a contact mode using an average power output between 3 and 12 Watts. [18]

1.7. RECURRENCE OF ORAL LEUKOPLAKIA

It has been suggested that a recurrence of OL should be defined when a lesion subsequently occurs at the primary lesion site after it was confirmed that the condition had been reached with no evidence of a lesion for a definite period.

Actually, a thin white plaque can remain in the surrounding tissue of the primary lesion and subsequently increase its size and thickness.

Frame et al. [85] reported that the new epithelium that migrates from the periphery to cover the wound may originate from an area of potentially unstable mucosa; that may explain why new patches of OL develop adjacent to the margins of previously treated areas.

The recurrence rate differed significantly among the types of treatment procedures. [90] It was reported, from previous studies, that the recurrence after laser surgery was 7.7- 38.1%. [85, 86, 90, 91] Variation of recurrence is attributed to the difference in the variety, conditions of the laser beams, race, and the follow-up period.

Chiesa et al. [92] reported that the probability of developing recurrence and new occurrences was 23% within the first year of surgery and 40% after 3 years of follow-up.

On the basis of these findings, it is important to ascertain the border of the lesion. It is strongly recommended that vital tissue staining, such as iodine [94, 95] or toluidine blue staining immediately preoperatively, is necessary to reduce local recurrence and malignant transformation, because multi-centric or micro-invasive cancer may develop from OL. [18, 91]

It was also reported that all dysplastic and malignant oral mucosal lesions were detected as unstained by the iodine staining method [95] and that only 1.9% false negatives were defined by toluidine blue staining. [96]

It was found that the recurrence of OL is irrespective of complete excision by laser or conventional surgery.

This suggests that the origin of the cells in recurrence of OL as potentially premalignant lesions is located in adjacent epithelium of the OL visualized as normal oral mucosa before treatment. Despite complete removal or laser vaporization, the adjacent or peripheral epithelium may proliferate in the recurrence phenomenon and it is proposed that these epithelial tissues, that show clinically normal features, consist of highly active cells which are probably abundantly widespread in the basal cell layer.

It has been accepted that the ‘field of cancerization’ (FC) or ‘field change’ of oral mucosal cancer is very important in explaining the presence of dysplastic cells adjacent to SCC and recurrence following complete laser vaporization. [97]

It is suggested to include a wide FC for the lateral side of the tongue since it is characterized by a marked proportion of dysplastic changes and also a high recurrence rate.

Potentially, premalignant cells that are present adjacent to OL or oral carcinoma usually reveal normal clinical features under inspection, irrespective of pathologic and immunohistochemical abnormalities.

As asserted by the concept of FC or widespread carcinogenesis in oral mucosa, when a recurrence following laser surgery is visible, microscopic examinations in those lesions should permit a detailed diagnosis for the management of such patient and for long-term predictive assessments.

1.8. MALIGNANT TRANSFORMATION OF ORAL LEUKOPLAKIA

Oral cancer is the sixth most frequent leading cause of cancer death worldwide; as a result, the early detection of malignant events is of a high priority.

OSCC is the most frequent form for 90% of oral cancer, with 5-year survival rate of 50% despite various management in the past 3 decades. [96, 97]

It is widely accepted that development of OSCC in potentially malignant lesions evolve through a multi-step process followed by varying grades of epithelial dysplasia and invasive carcinoma.

Thus, biopsy is considered as the standard for detecting dysplasia and carcinoma in oral lesions, and histologic assessment of oral dysplasia is currently the gold standard for determining the risk of malignant transformation.

The grade of oral dysplasia significantly associated with malignant transformation remains a considerable source of debate. [100, 101] Therefore, further studies are necessary to improve the histological assessment of the dysplasia.

OL is the most common OPMD, with a higher tendency of malignant transformation increased with follow-up years. Unfortunately, the risk of OL malignant transformation is difficult to assess.

The role of tobacco and alcohol use as carcinogens related to OL malignant transformation remains controversial. Hence, assessment of these potential risk factors for oral cancer development in patients with OL is still needed.

For this reason Napier and Speight [99] recently reviewed the clinical risk factors (e.g. gender, age, lesion type, and location) for the

malignant transformation of OL, and the study was done on different populations.

The literature focusing on a longitudinal observational study of a comprehensive analysis of the clinicopathological factors predictive of outcome in patients with OL is not robust; whereas, a large cohort of patients are required to produce meaningful data supported by robust statistical analysis. [102, 103, 104]

There are six alterations and levels of OL:

I. Hyperkeratosis

II. Parakeratosis

III. Acanthosis which is increase of thickness of the spinous layer with handling the limit between the epithelium and lamina propria.

IV. Dysplasia

V. Carcinoma in-situ lesion with cancer characteristics, which does not exceed the basement membrane.

VI. Invasive carcinoma

The first three alterations are benign as in the majority of the cases of OL, only a small percentage of alterations are dangerous.

1.9. LASER TISSUE INTERACTIONS

In clinical dentistry, laser light is used to perform controlled and precise changes in the target tissue through the transfer of electromagnetic energy. [105]

Light energy interacts with a target medium (e.g. oral tissue) in one of four ways: [106] (Fig. 24)

I. Transmission

Laser beam enters the medium and emerges distally without interacting with the medium. The beam exits either unchanged or partially refracted.

II. Reflection

When either the density of the medium or angle of incidence is less than the refractive angle, total reflection of the beam will occur. The incident and emergence angles of the laser beam will be the same for true reflection or some scatter may occur if the medium interface is non-homogenous or rough.

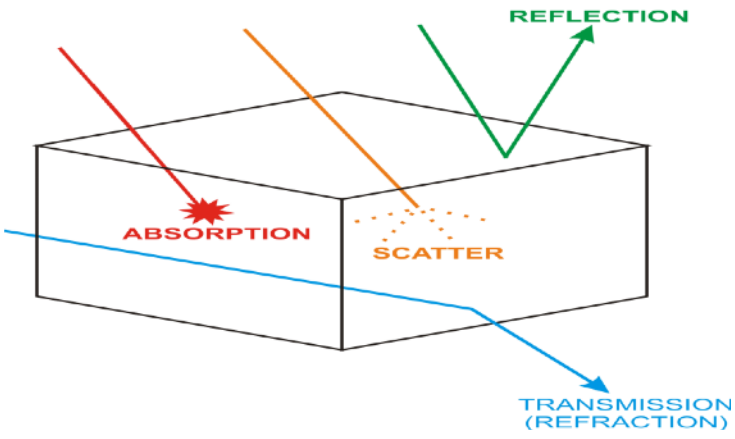

Figure 24 Possible laser light - tissue interactions

III. Scatter

There is an interaction between the laser beam and the medium. This interaction is not intensive enough to cause complete attenuation of the beam.

The result of light scattering is a decrease of laser energy with distance, together with a distortion in the beam (rays travel in an uncontrolled direction through the medium).

IV. Absorption

The incident energy of the laser beam is attenuated by the medium and converted into another form. With the use of dental diode lasers, the most common form of conversion of laser energy into heat or in case of very low energy values, biomodulation of receptor tissue sites seems to occur. [107, 108] Heat transfer mediated physical change in target tissue is termed ‘photothermolysis’.

In any desired laser-tissue interaction, the goal is to achieve the maximum absorption of laser light by the target tissue, as this will allow the maximum control of the resultant effects.

Absorption is determined by matching incident laser beam energy (wavelength) to the electron shell energy in the target atoms.

Absorption of laser energy in the target tissue leads to generation of heat. Rising heat levels leads to dissociation of covalent bonds (in tissue proteins), phase transfer from liquid to vapour (in intra and inter-cellular water), onto phase transfer to hydrocarbon gases and production of residual carbon. [109]

Secondary effects can occur because of heat generation (through conduction). When predicting the conversion of electromagnetic energy to heat effects in target tissue, unwanted change through conductive thermal spread must be taken into account and reduced to the lowest possible level.

The ability to control a progressively increasing heat loading of target tissue is termed ‘thermal relaxation’. [110]

Thermal relaxation rates are proportional to the area of tissue exposed and inversely proportional to the absorption coefficient of the tissue, assuming fixed values of thermal and light diffusivity for the tissue in question.

There are many different mechanisms by which laser light can interact with tissue; these have been categorized in a number of different ways.

For the purposes of these facts, the most common interaction mechanisms for therapeutic and surgical applications will be divided into five broad classes:

- Photochemical reaction

A molecule absorbs a photon of sufficient energy, the energy can be transferred to one of the molecule's electrons.

An electron with higher energy can more easily escape the nuclear forces, keeping it close to the nucleus and so excited molecules (which are molecules with an electron in a higher energy state) are more likely to undergo chemical reactions (exchange or share electrons) with other molecules.

In photodynamic therapy, for instance, a photosensitizing drug (a concoction of molecules when they absorb light, causing reactive oxygen species to form) is used to cause necrosis (cell death) and apoptosis (‘programmed’ cell death). Photodynamic therapy is increasingly widely used in oncology to destroy cancerous tumors.

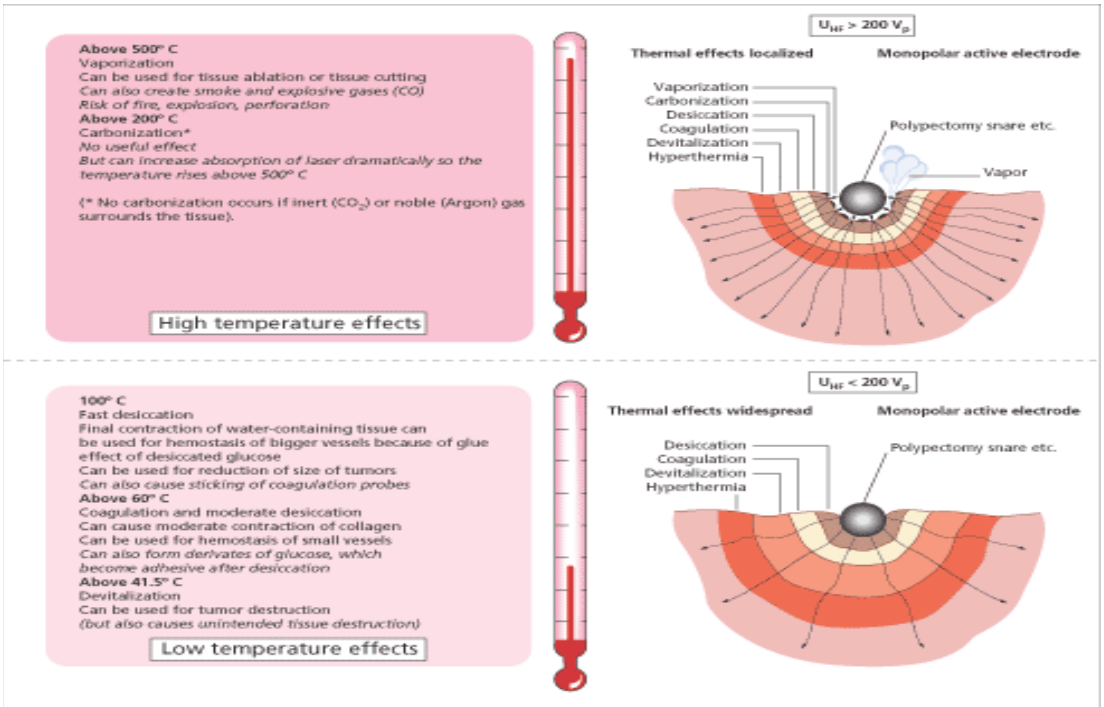

Figure 25 Laser tissue interaction

- In photothermal interaction

The energy of the photons absorbed by chromophores (a term used to refer to any light-absorbing molecules) is converted into heat energy via molecular vibrations and collisions, which can cause a range of thermal effects from tissue coagulation to vaporization.

A denaturation of proteins occurs for temperatures ranging from 42°C to 60°C; the water inside the tissues vaporizes at 100°C. Over 200°C, the carbonization with vaporization of the tissues takes place. It represents the last stage of the laser tissue interaction. (Fig. 25)

Applications include tissue cutting and welding in laser surgery and thermal effects which are explained as:

T = 40- 60°C: edema, functional imbalance of metabolism

(up to 50°C reversible)

T = 60- 100°C: denaturation of the proteins, coagulation, and contraction because of dehydration

T > 100°C : disruption, evaporation of water, and ablation

T > 150°C : vaporization, and ablation

T > 300°C : carbonisation

- In photoablation interaction

Ultraviolet (UV) photons are absorbed by electrons, raising them from a lower energy 'bonding' orbital to a higher energy 'non-bonding' orbital; thereby, causing virtually immediate dissociation of the molecules.

This naturally leads to a rapid expansion of the irradiated volume and ejection of the tissue from the surface.

This is used in eye (corneal) surgery, among other applications.

- In plasma-induced photo ablation

A free (sometimes called ‘lucky’) electron is accelerated by the intense electric field which is found in the vicinity of a tightly focused laser beam. When this very energetic electron collides with a molecule, it gives up some of its energy to the molecule. When sufficient energy is transferred to free a bound electron, a chain reaction of similar collisions is initiated, resulting in a plasma: a soup of ions and free electrons.

One application of this is in lens capsulotomy to treat secondary cataracts.

- The final set of related mechanisms

Grouped under the term ‘photo disruption’, are the mechanical effects that can accompany plasma generation, such as bubble formation, cavitation, jetting, and shockwaves. These can be used in lithotripsy (breaking up kidney or gall stones). [111]

1.10. TYPES OF LASER

Various laser devices are available in the market, all of them have particular features in relation to different parameters: the type of cut to be performed in the tissue, the lapse of time in which to operate, the depth of surgical wounds, and the absorption of laser wavelengths by the tissue. (Table 4)

Table 4 Common laser types used in dentistry

Laser type	Construction	Wavelength(s)	Delivery system(s)
KTP laser	Solid state	532 nm	Optical fiber
Argon laser	Gas laser	488, 515 nm	Optical fiber
Helium-neon laser	Gas laser	633 nm	Optical fiber
Diode laser	Semiconductor	635, 670, 810, 830, 980 nm	Optical fiber
Nd: YAG laser	Solid state	1064 nm	Optical fiber
Er: YAG laser	Solid state	2940 nm	Optical fiber, Waveguide, Articulated arm
CO_2 laser	Gas laser	9600, 10600 nm	Waveguide, Articulated arm

- Nd:YAG laser

(Neodymium: Yttrium Aluminum Garnet) is surely one of the most versatile devices for the wide range of emission frequencies it allows (corresponding to the values of 1064 nm and 1320 nm).

The light ray is mainly absorbed by melanin and haemoglobin. The using pattern can be pulsing or continuous with variable time.

This device is particularly indicated for the thermal destruction of vascularized or sessile lump tumors and in the surgery of vascular lesions.

The employment of this type of device is not suitable for lesions located in thermic sensible anatomical district (as periosteum) because of the heat release by deep (in depth).

- Er: YAG laser

(Erbium: Yttrium Aluminum Garnet) the emission frequencies are of 2940 nm. It is greatly absorbed by water and used in a pulsed pattern. The possibility to work using very short impulses (ranging between 50 and 100 microseconds) allows the mechanical removal of superficial vascularized or sessile lumps and the recovery of wide oral lesions in extremely temperature sensible zones as well.

The higher the water content of the tissue, the greater the laser removing effect. On the contrary, regarding the hemostasis, its effect is minimal, except when using longer impulses and high frequencies. The laser can be transmitted by different devices.

- KTP laser

(Potassium, Titanium, Phosphate) It is a solid-state laser that emits a green light with the wavelength of 532 nm and is used in oral surgery, in the dental bleaching, and the dentine hypersensitivity treatment. [112]

- He-Ne laser

(Helium- Neon) It is a gas laser whose active medium consists of a mixture of helium and neon inside a small-bore capillary tube. Its wavelength is of 633 nm and it emits in the infrared light.

- Argon laser

Its active medium is gas and it emits at 13 wavelengths through the visible ultraviolet and near-visible spectrum, including: 351.1 nm, 363.8 nm, 454.6 nm, 457.9 nm, 465.8 nm, 476.5 nm, 488.0 nm, 496.5 nm, 501.7 nm, 514.5 nm, 528.7 nm, and 1092.3 nm.

- CO_2 laser

(Carbon Dioxide) laser is considered the best laser from the surgical point of view. It is the most used laser because of its high absorption by water. Its emission frequency is 10600 nm and it is possible both to choose two use patterns, pulsing or continuous and to diminish the impulse range down to few nanoseconds. [119]

This type of laser is particularly indicated for surgical procedures (in those regions extremely difficult to reach), for a good superficial hemostasis and thermal therapy in some solid tumors. Since the thermal effect could be important, during the laser treatment, extreme caution is needed in those highly sensible zones to the temperature.

- Diode laser

The nucleus of a diode laser consists of a semi-conductive material (Indium Gallium Arsenic). [114]

The most common laser used in oral dental surgery, has a wavelength of 810 nm or 980 nm and shows a high affinity for the hemoglobin.

This type of laser is particularly devoted to the treatment of vascular lesions both by direct removal or by means of lesion clotting. Moreover, all the other surgical procedures, concerning both the major and minor oral surgeries, can be performed with diode laser. [120]

1.11. THE CO_2 LASER

The carbon dioxide laser (CO_2) was one of the first models of gas lasers to be invented by Kumar Patel of Bell Labs in 1964. This type of laser device emits in the infrared light and its principal wavelength is between 9.4 and 10.6 μm. (Fig. 26)

Figure 26 The CO_2 laser

The active medium is represented by a discharge tube gas-cooled air or with water in high power applications.

The gas is constituted by: carbon dioxide (CO_2), about 10- 20%, nitrogen (N_2) about 10- 20%, hydrogen (H_2), and/or xenon (Xe) 1- 2% and helium (He) (the remaining part of the gas mixture).

The necessary inversion of population is obtained by passing an electric discharge in the gaseous mixture, which causes the following chain of events: [113]

- The impacts of the electrons excite the vibrational modes of the nitrogen molecule. This molecule cannot rid the energy acquired by emitting a photon and its excited state is metastable and persists for a long time.

- The collisions between gas molecules transfer the energy from the excited molecules of nitrogen to those of CO_2, with the sufficient efficiency to produce the desired inversion of population.

- The excited CO_2 molecule comes back to the ground state emitting a photon and contributing to the establishment and the emission of the laser beam.

Medical and dental researchers soon began to study different types of lasers for extra and intraoral surgical procedures. Because of its affinity for water-based tissues, the CO_2 laser has become a favourite

instrument for oral surgeons for treatment of pathologic conditions of the oral mucosa. [115]

The CO_2 laser has been recommended to treat benign oral lesions, such as fibromas, papillomas, hemangiomas, gingival hyperplasia with different causes (idiopathic or due to side effects of medications), aphthous ulcers, mucosal frenula, or tongue ties (ankyloglossia), as well as premalignant lesions, such as OLs. [115, 116]

Some reports on the use of the CO_2 laser also support the possibility of treating malignant oral diseases in early stages (e.g. T1N0 carcinomas) with excisional biopsies. [117]

A study demonstrated dissemination of cancer cells into the blood circulation upon incisional biopsies with the scalpel, resulting in an increased risk of metastasis.

The CO_2 laser, with its sealing effect on vessels smaller than 500 µm in diameter, could be an advantage and therefore prevent occult micrometastasis. [118]

1.12. OBJECTIVES OF THE STUDY

Determining the anatomical distribution of the lesions to realize the sites attacked by OL.

Recognizing the effects of associated risk factors.

Dividing large lesions into multiple sections for laser vaporization to observe if it will reduce the post-operative symptoms and malfunctions.

Determining the sufficient safety margins during laser vaporization of OL as a trial to reduce the recurrence.

Trying to improve the management of OL which may prevent not only the recurrence rate and the malignant transformation, but also post-operative dysfunction.

Chapter 2 : Materials and Methods

Laser technology has made rapid progress over the past few decades. Because of its many advantages, it has been widely used in oral and maxillofacial surgeries.

Soft tissue laser is a state of the art tool that creates predictable esthetic results within a general dental practice. Ease of use and affordability makes it simple to incorporate in general practice.

All the patients in this study were treated by the same surgeon, they were informed about the advantages and disadvantages of laser surgery; also, side effects of laser surgery were explained for the patients and they signed an informed consent prior intervention.

Questions concerning carbonization, coagulation, cutting speed, pain, swelling, bleeding, need for drugs, functional reduction, and fibrin layer on wounds during treatment were discussed with every patient.

Full clinical examination, consisted of medical and dental history, were performed and the clinical treatment was documented by photos and questionnaires for patients and surgeons.

Elimination of associated risk factors was done for all the selected patients in the three groups.

An oral swab was done for all the lesions to exclude any fungal infection.

Patients were always photographed with the same equipment (Nikon D200, Nikon Corporation, Tokyo, Japan)

Local anesthesia was performed by 1.8 ml of Mepivacaine solution (Mepivacaina Pierrel, 30 mg/ml, injection solution 1.8 ml, Pierrel S.p.A., Milan, Italy).

No suturing was necessary after tissue laser vaporization. Even if laser wounds were left to secondary healing, the pain was found to be significantly lower after a laser vaporization compared to a scalpel resection with conventional wound care (e.g. dressings or suturing).

It is assumed that sealing the dissected nerve endings modifies the stimulation of pain on the wound surface.

Patients were informed to avoid hot, spicy, and sharp food; also, to eat cold, soft food, and to stop brushing the surgical area.

Conventional blade biopsy was performed on all the lesions and multiple biopsies were performed in large lesions more than 20 mm.

All biopsies samples were fixed in a 4% neutral-buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for conventional histopathological evaluation, conforming that all the TL lesions were without dysplasia. The collected analysis was evaluated and clinical diagnosis for every lesion was performed.

Selection of the patients was done according to the presence of TL without dysplasia which was confirmed by the conventional blade biopsy and histopatholgical evaluation.

This study was performed on 36 lesions, diagnosed in 34 patients (20 Females and 14 Males) (Fig. 28). The range of the patients age was between 39 and 79 years. The selected patients were divided into three different groups: Control Group, Group A and Group B.

The lesions were divided into the three groups as follows:

- Control Group (CG): 16 TL lesions in 16 patients, no treatment was prescribed; however, a periodic six months of follow-up was done.
- Group A (GA): 11 TL lesions in 11 patients, the laser vaporization was done for the entire lesion adding a maximum of 1 mm of safety margins.

- Group B (GB): 9 TL lesions in 7 patients, the laser vaporization was done for the lesion adding at least 3 mm of safety margins.

Large lesions, more than 20 mm, were divided into sections that were vaporized with a distance of at least 2 months.

All the lesions and extended borders were ascertained using a sterile dermographic pen and the safety margins were measured using a periodontal probe.

In Control Group, only a periodic six months of follow-up was done.

The laser machine employed was CO_2 Laser; model (SmartXide®, 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 then defocused and the wound surface was vaporized in scanning movements, vertical and horizontal, as a mean of haemostasis and for the elimination of any remnants of the lesion. [85]

Re-epithelialization was completed after 4- 6 weeks and the newly formed epithelium appeared healthy in most patients.

There was little post-operative scarring and tissues were soft on palpation. In some elderly patients, the newly formed epithelium appeared rather thin and atrophic.

The only prescribed medication, after vaporization, for the patients in groups A and B was a 0.2% chlorexidine spray called (Corsodyl, GlaxoSmithKline Consumer Healthcare S.p.A, Baranzate, Milan, Italy). (Fig. 27)

Figure 27 Corsodyl spray

The application of this drug was prescribed for three times a day for one week.

Four follow-up visits were performed according to a schedule: one week, three weeks, three months, and six months after the laser vaporization in order to evaluate the healing course, check the healing mode, and to evaluate the recurrence rate and its degree.

It was important to register the anatomical distribution of the lesions to realize that in our study the gingival mucosa has more potentiality to be attacked by OL. (Fig. 29)

Recurrence rate evaluation was divided into three categories: Complete healing, Partial recurrence (recurrence is more than half of the lesion), and Complete recurrence.

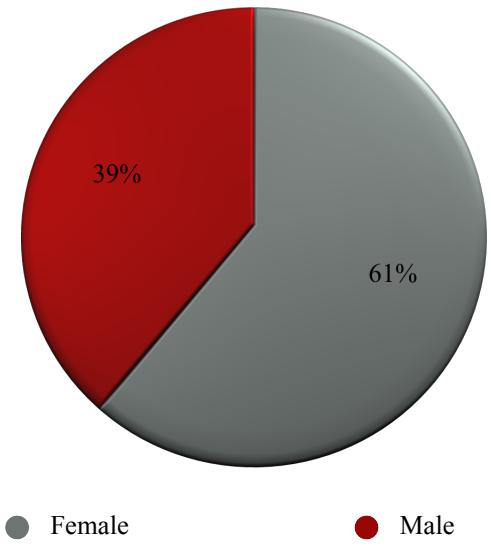

Figure 28 Distribution among the gender

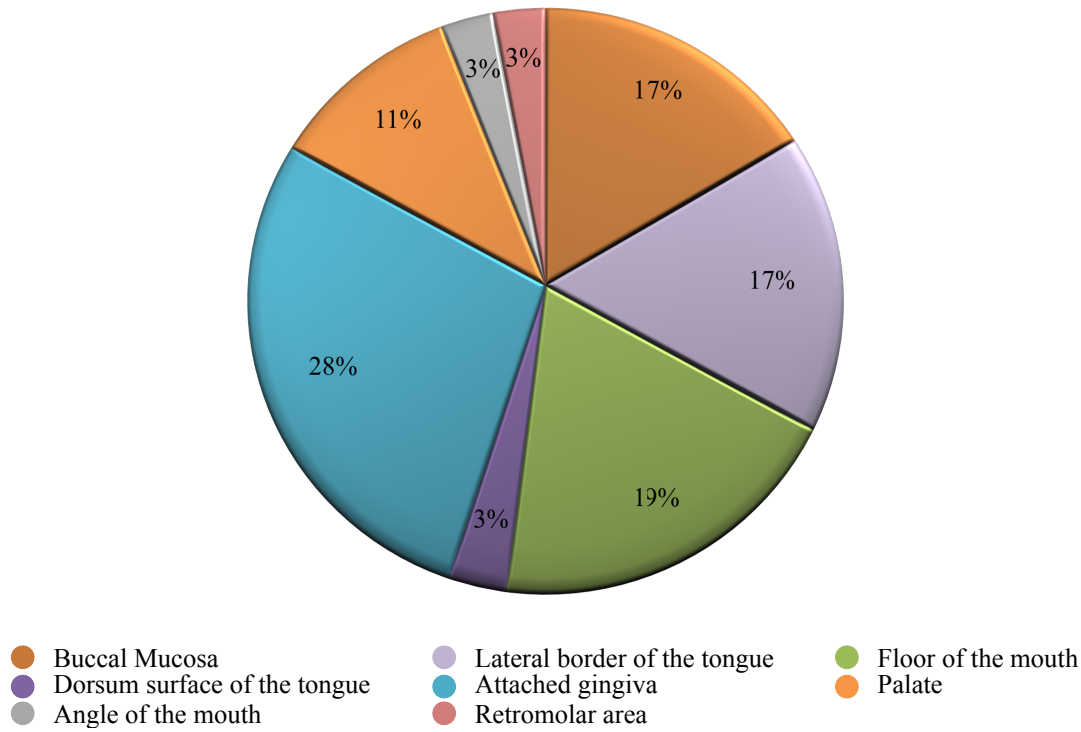

Figure 29 Anatomical distribution of the lesions in all the groups

Chapter 3 : Results

3.1. STATISTICAL RESULTS

The statistical analysis was done using variance ANOVA Software®.

This difference was statistically significant for the distribution of lesions in which complete healing occurred in the Control Group and the groups A and B with an F value of 0.016 and P value of 0.985. (Table 5)

	SS	df	MS	F	p
Between:	1.123	2	0.562	0.016	0.985
Within:	1,194.274	33	36.190		
Total:	1,195.397	35			

Table 5 Variance ANOVA analysis for distribution of lesions in which complete healing occurred between the Control Group and the groups A and B

This difference was statistically significant between the complete healing and the partial and complete recurrence occurred in groups A and B with an F value of 0.004 and P value of 0.952. (Table 6)

	SS	df	MS	F	p
Between:	0.111	1	0.111	0.004	0.952
Within:	538.177	18	29.899		

Table 6 Variance ANOVA analysis between the complete healing and the partial and complete recurrence occurred in groups A and B

This difference was statistically significant between completely healed lesions of previous smoking and non-smoking patients in groups A and B with an F value of 0.003 and P value of 0.997. (Table 7)

	SS	df	MS	F	p
Between:	0.478	2	0.239	0.003	0.997
Within:	2,626.314	33	79.585		
Total:	2,626.792	35			

Table 7 Variance ANOVA analysis for distribution of completely healed lesions based on the previous smoking habits in patients of groups A and B

3.2. RESULTS

No differences were clinically detected in the healing after CO_2 laser vaporization, the vaporized lesions fully healed without the formation of scar or dehiscence.

In general, good healing process and minimal post-operative pain or discomfort were shown by all the patients.

In addition, the group (CG) seemed to have more lesions than the other groups, while the group (GB) seemed to have less lesions than the other groups. (Table 8- 10)

It was observed that some of the vaporized lesions which showed partial or complete recurrence after 6 months of follow-up, have shown the initial recurrence after 3 weeks of laser vaporization.

The complete healing occurred in 16 lesions among all the groups of the study, with a percentage of 75% in both groups A and B and 25% in the Control Group. (Fig. 30)

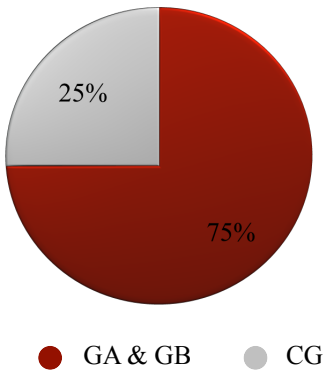

Figure 30 Complete healing in GA, GB, and CG

The results demonstrated that group (GB) achieved the greatest percentage of complete healing while group (GA) achieved the greatest percentage of complete recurrence. (Fig. 31- 32)

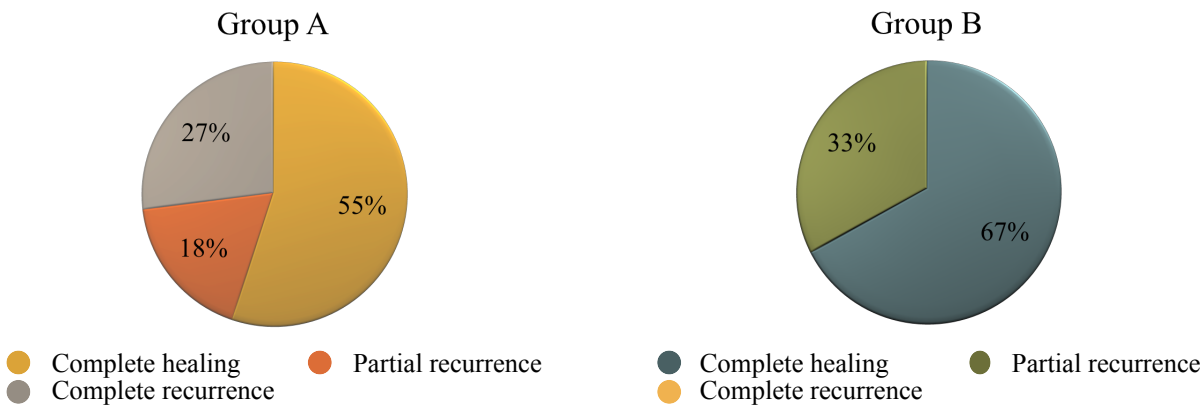

Figure 31- 32 Recurrence Rate Evaluation in GA and GB

Moreover, the evaluation of the recurrence rate after laser vaporization in groups A and B demonstrated that the complete healing occurred in 60% of the lesions, the partial recurrence occurred in 25% of the lesions, and the complete recurrence occurred in 15% of the lesions. (Fig. 33)

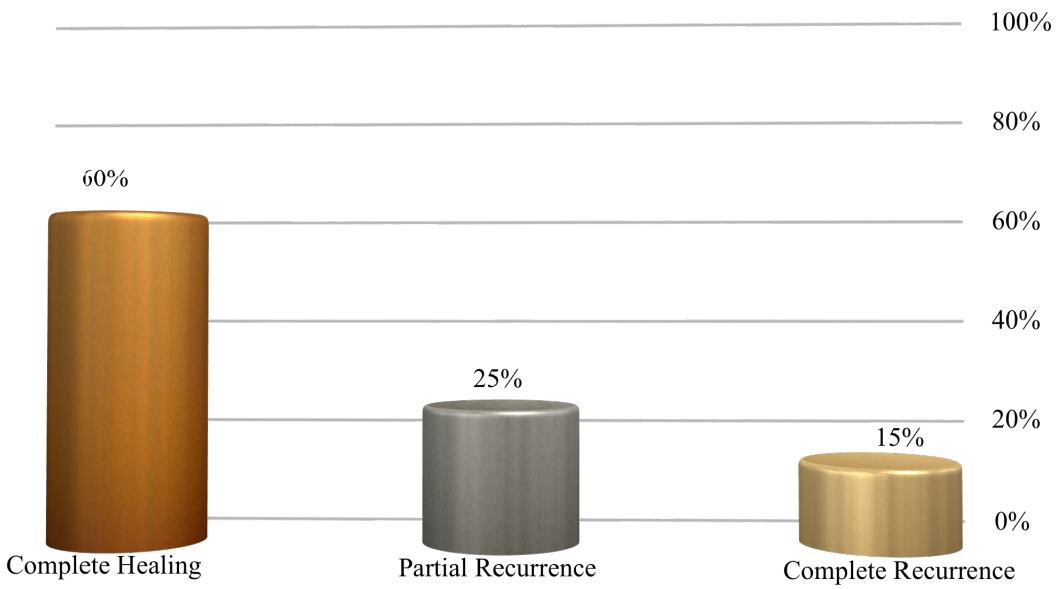

Figure 33 Recurrence rate evaluation in both groups A and B

The recurrence rate evaluation according to gender revealed that complete healing occurred in 75% of females and 37.5% of males, partial recurrence occurred in 17% of females and 37.5% of males, while the complete recurrence occurred in 8% of females and 25% of males. (Fig. 34)

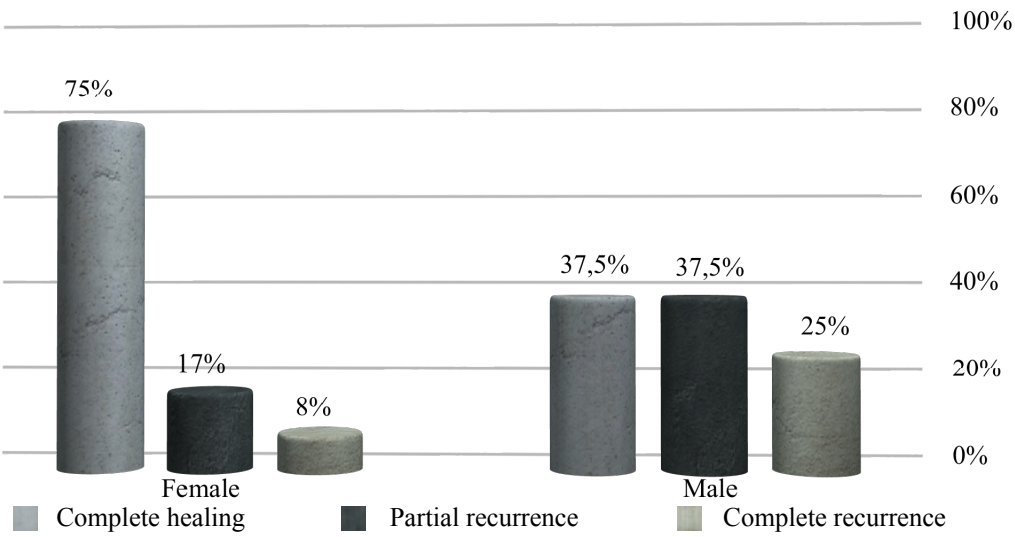

Figure 34 Recurrence Rate Evaluation According to the Gender

Furthermore, the smoking habits had a significant relation with the recurrence rate as it was found that the greatest percentage of patients who had the complete healing were the non-smoking patients. (Fig. 35)

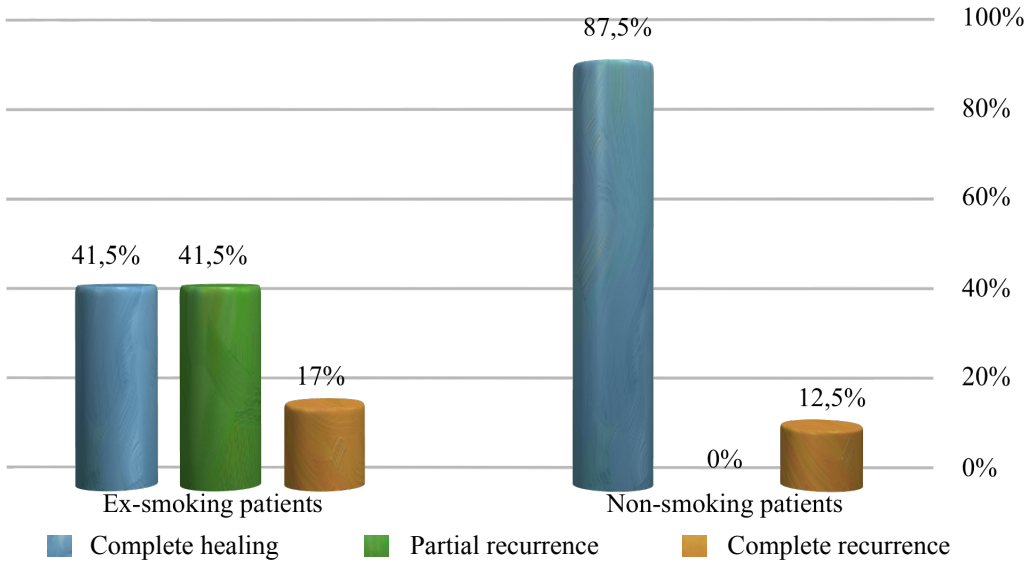

Figure 35 Recurrence rate evaluation according to smoking habits

Depending on the anatomical location of the lesion, the sites in which the complete healing was higher were the palate and floor of the mouth. (Fig. 36)

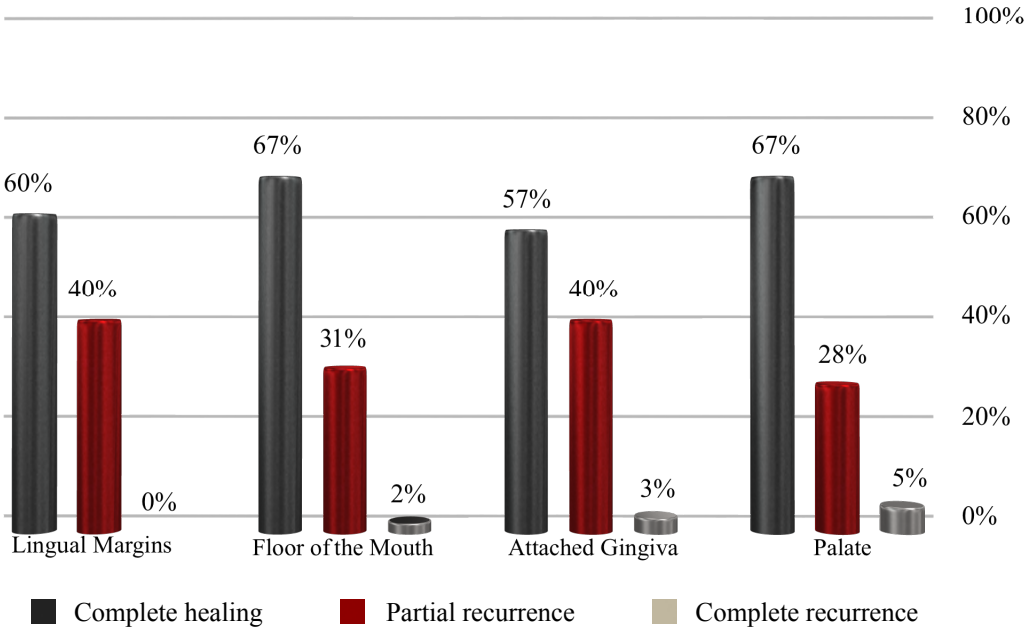

Figure 36 Recurrence rate evaluation according to anatomical site

Table 8 Control Group

Lesion No.	Gender	Anatomical site	Smoking habits	Follow-up	Results
1	F	Gingival mucosa zone 14- 15	Non-smoking	6 months	Complete healing
2	F	Floor of the mouth	Ex-smoker	6 months	No change
3	M	Angle of the mouth	Non-smoking	6 months	Partial healing
4	M	Buccal mucosa	Non-smoking	6 months	Partial healing
5	F	Floor of the mouth	Non-smoking	6 months	Partial healing
6	F	Gingival mucosa zone 23- 27	Ex-smoker	6 months	No change
7	F	Floor of the mouth	Non-smoking	6 months	Partial healing
8	F	Hard palate	Ex-smoker	6 months	Partial healing
9	M	Lateral border of the tongue	Non-smoking	6 months	No change
10	F	Buccal mucosa	Non-smoking	6 months	Partial healing
11	M	Retromolar pad	Ex-smoker	6 months	Complete healing
12	F	Floor of the mouth	Ex-smoker	6 months	Complete healing
13	M	Gingival mucosa	Ex-smoker	6 months	Partial healing
14	F	Buccal mucosa	Non-smoking	6 months	Complete healing
15	M	Buccal mucosa	Non-smoking	6 months	Partial healing
16	F	Buccal mucosa	Non smoking	6 months	Partial healing

Table 9 Group A

Lesion No.	Gender	Anatomical site	Smoking habits	Follow-up	Results
1	F	Gingival mucosa zone 27- 28	Non-smoking	6 months	Complete recurrence
2	M	Floor of the mouth	Ex-smoker	6 months	Partial recurrence
3	M	Hard palate	Ex-smoker	6 months	Complete healing
4	M	Lateral border of the tongue	Ex-smoker	6 months	Complete healing
5	F	Buccal mucosa	Ex-smoker	6 months	Complete healing
6	F	Gingival mucosa zone 44- 45	Non-smoking	6 months	Complete healing
7	M	Gingival mucosa zone 35	Ex-smoker	6 months	Complete recurrence
8	F	Floor of the mouth	Non-smoking	6 months	Complete healing
9	M	Hard palate	Ex-smoker	6 months	Complete recurrence
10	M	Gingival mucosa zone 37- 47	Ex-smoker	6 months	Partial recurrence
11	F	Hard palate	Non-smoking	6 months	Complete healing

Table 10 Group B

Lesion No.	Gender	Anatomical site	Smoking habits	Follow-up	Results
1	F	Lateral border of the tongue	Ex-smoker	6 months	Partial recurrence
2	F	Dorsal surface of the tongue	Ex-smoker	6 months	Partial recurrence
3	M	Floor of the mouth	Ex-smoker	6 months	Complete healing
4	M	Lateral border of the tongue	Ex-smoker	6 months	Partial recurrence
5	F	Lateral border of the tongue	Non-smoking	6 months	Complete healing
6	F	Gingival mucosa zone 13- 17	Non-smoking	6 months	Complete healing
7	F	Gingival mucosa zone 41- 44	Non-smoking	6 months	Complete healing
8	F	Gingival mucosa zone 44- 47	Non-smoking	6 months	Complete healing
9	F	Lateral border of the tongue	Ex-smoker	6 months	Complete healing

3.3. CLINICAL RESULTS

I. Control Group (CG): The figures below show two clinical cases included in this group. (Fig. 37- 40)

- First clinical case

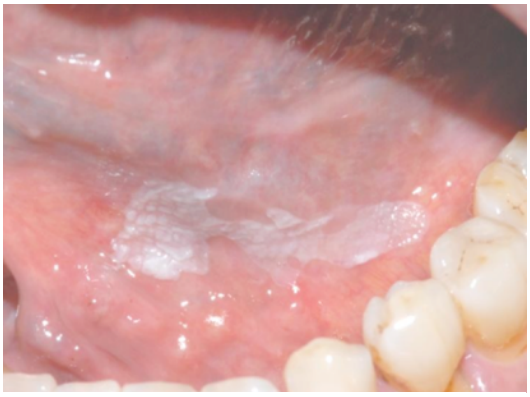

Figure 37 TL on the lateral surface of the tongue

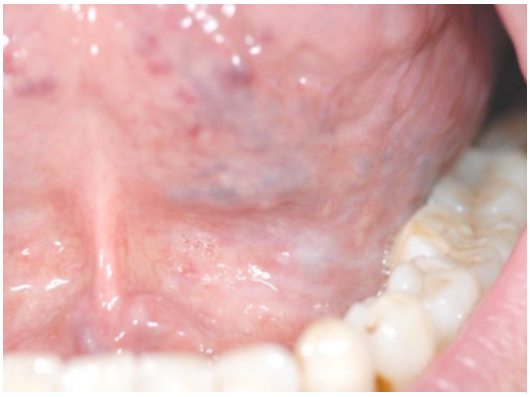

Figure 38 After 6 months of follow-up

- Second clinical case

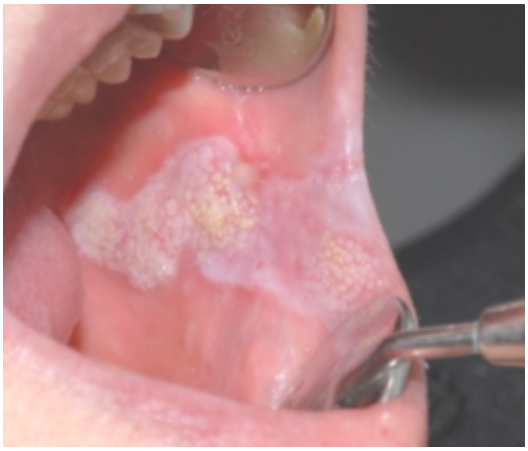

Figure 39 TL on the left cheek mucosa

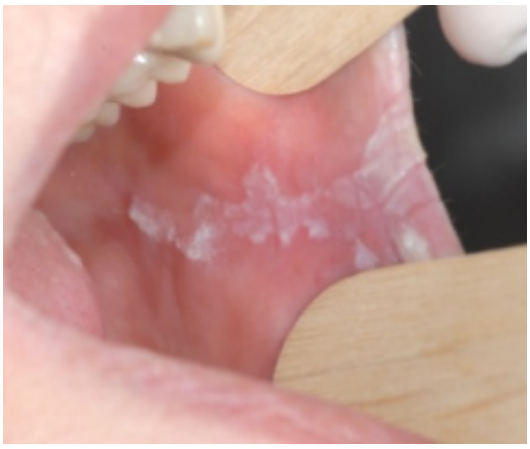

Figure 40 After 6 months of follow-up

II. Group A (GA): The figures below show a clinical case included in this group. (Fig. 41- 44)

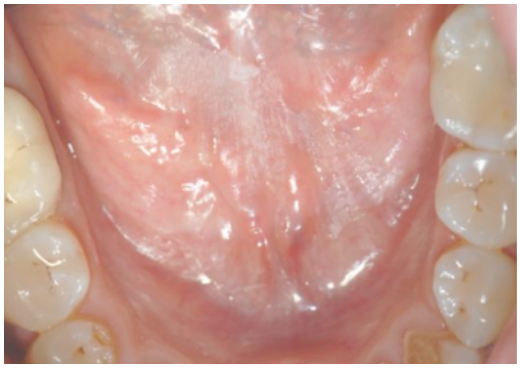

Figure 41 TL on the floor of the mouth

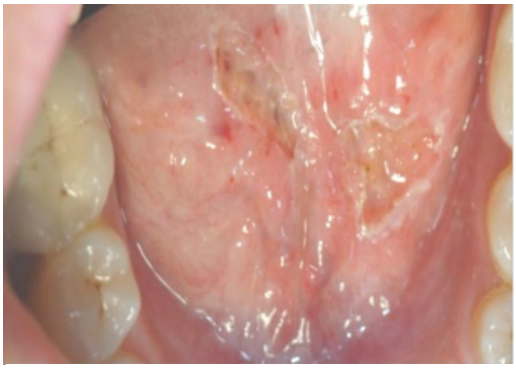

Figure 42 Post vaporization

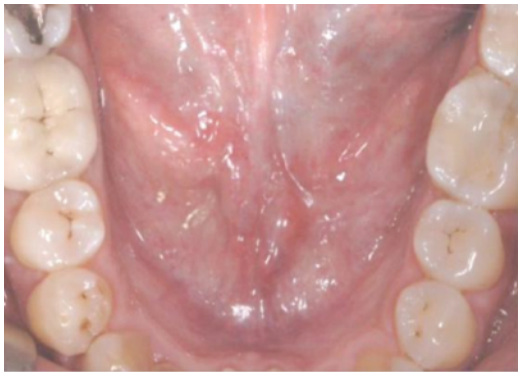

Figure 43 Follow-up after three months

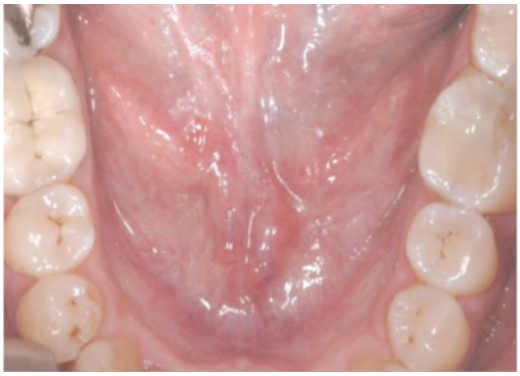

Figure 44 Follow-up after six months

III. Group B (GB): The figures below show a clinical case included in this group. (Fig. 45- 48)

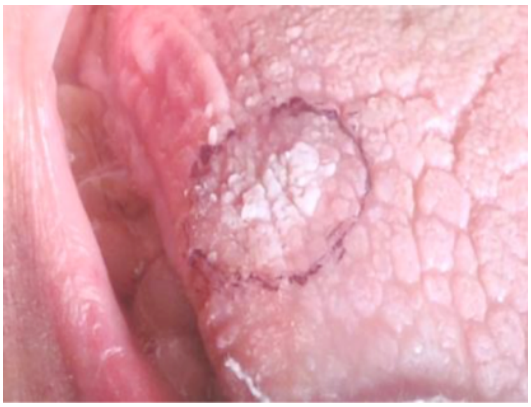

Figure 45 TL on the dorsum of the tongue

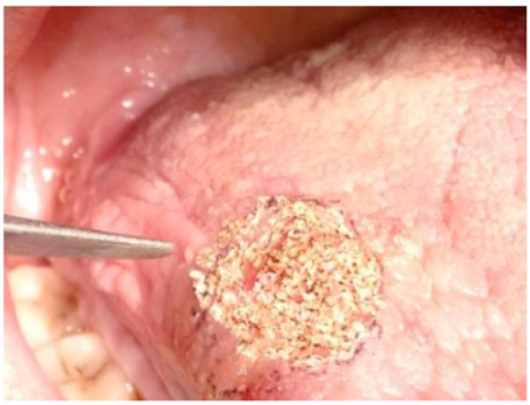

Figure 46 Post vaporization

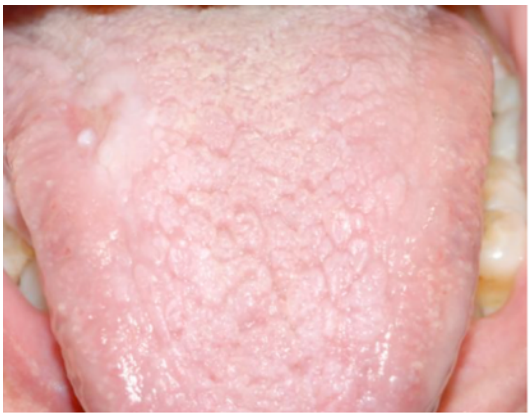

Figure 47 Follow-up after 3 weeks

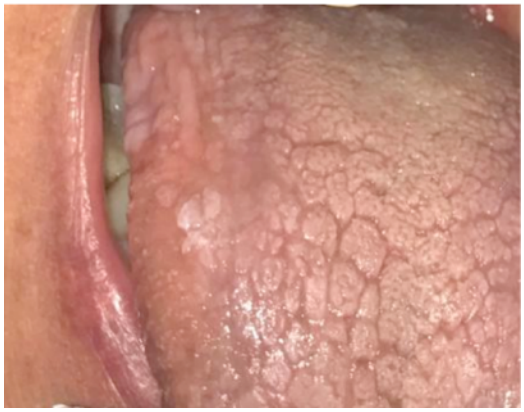

Figure 48 Follow-up after six months

Chapter 4 : Discussion

Leukoplakia monitoring showed that the lesion may regress unpredictably, remain stable, or may progress to carcinoma.

The primary treatment of OL focuses on the suppression of associated etiological factors (smoking, local irritating factors, etc.). [121]

In case of persistence of the hyperkeratosis, an oral biopsy is necessary. OL is potentially malignant lesion and some leukoplakias will unpredictably progress to carcinoma; therefore, OLs should be treated. [122, 123]

The prevalence of OL in the general population varies from <1% to >5%. [124] Moreover, the natural annual limit of OL malignant transformation rate is unlikely to exceed 1%. [125]

Non-homogeneous OL carries a higher risk of malignant transformation, when compared to the homogeneous variance. [127, 142]

Regarding non-surgical treatments (retinoid, bleomycin, local use of corticosteroid, etc.), the available evidence on medical and complementary interventions for treated OL is very limited. The relapses and adverse effects are common; [126] the recurrence of OL after surgical treatment has been reported in 10- 35% of cases. [128]

Further, Kuribayashi et al. [129] observed a long-term outcome (10 years) of non-surgical treatment of OLs. Their results indicated that on 237 observed lesions, 135 lesions (57%) remained unchanged, 30 (12.7%) lesions were characterized by a reduction in size, and 44 (18.6%) lesions had disappeared. While another 11 (4.6%) lesions developed OSCC. [129] 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. [129] Those results show the necessity to develop a more efficient prophylactic and acceptable permanent therapy for OL patients.

In an observational retrospective study published by Schepman et al., [130] they compared the incidence of SCCs in two groups of OL patients, one of which patients received some active treatments (medical and/or surgical) while the other, patients were kept under clinical follow-up. No significant difference was observed in the risk of malignant transformation between the two groups. These results perhaps suggested that the natural history of OLs might be independent of the treatment received and that there is a sub-group of lesions destined to undergo a malignant transformation regardless of the therapeutic strategy adopted. The recurrence rate varies for excision from 10 to 34% and for cryosurgery from 12 to 25%. These modalities cause scarring, contraction, and can mask early signs of recurrence. [131, 142]

Our study was conducted on 34 patients diagnosed with TL lesions. The diagnosis of TL may be provisional or definitive and associated with a variable degree of certainty factor.

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 has been 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.

Only TL lesions with no epithelial dysplasia were studied with a total of 36 lesions, 61% of which were female and 39% were male patients.

It was important to register the anatomical distribution of the lesions to realize that in our study the gingival mucosa has more potentiality to be attacked by OL, 28% of cases (10 lesions of 36); whereas in a study conducted by Brouns et al. [132] and in one conducted by Mogedas-Vegara et al. [133], the most common anatomical site was the lateral border of the tongue.

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 16 lesions among all the groups of the study, with a percentage of 75% in both groups A and B, in which laser vaporization was done, and 25% in the Control Group, after six months of follow-up.

The presence of statistically significant difference suggests that laser vaporization treatment of OL without epithelial dysplasia is preferable than the elimination of risk factors and follow-up for the lesion.

Lasers have been used for oral soft tissue dental procedures for more than 30 years and have been researched since the mid 1960s. [134] As compared to conventional surgery, they offer benefits, such as efficient and precise cutting with a calculated depth, good haemostasis (nearly or completely without bleeding), a shorter treatment time, bactericidal action, decontamination of the tissues, and no need for suturing. [135]

Laser vaporization is one of the treatment modalities available for the management of OL which guarantees a less complicated post-operative pain, edema, minor inflammatory complications, preservation

of the mobility and functionality of the treated tissue, and based on our results, it shows a lower recurrence rate. [62, 134]

CO_2 laser, wavelength of 10600 nm, is a superficial laser. It is absorbed by the epithelial cells with a superficial penetration of 0.1- 0.5 mm that can be used in superpulsed mode to achieve precise vaporization of the treated area. In addition, the high pulse rate allows an adequate thermal relaxation of the tissue, with a minimal damage to the adjacent healthy tissues. [146]

The advantage of the CO_2 laser, in comparison to the cold surgical blade, is the control of bleeding by thermal coagulation, which allows operators to observe the presence of eventual tissue abnormalities (white/ gray appearance involving connective tissues) that still remain after the removal of carbonized areas. In this case, the abnormalities can be easily localized and removed by vaporization. [62]

In our study, it was decided to use the CO_2 laser, the most used laser in the treatment of OL, for groups A and B.

The Nd:YAG laser is the second most used laser for vaporization of OL and is recommended especially for patients with high haemorrhagic risks, even though it has been reported that the post-operative pain levels are higher than those treated using CO_2 laser. [18]

Vivek et al. [9] reported a rate of recurrence of 10.71% for the Nd:YAG laser after three years of follow-up. While, White et al. [143] reported a recurrence rate of 27.2% for the Nd:YAG laser and 23.5% for the CO_2 laser.

The KTP laser penetrates deeper into the tissues and is frequently used to coagulate oral vascular malformations. According to Lim et al., the comparison between KTP laser and CO_2 laser in vaporization of OL showed a recurrence rate of 25% and 39.5% and a percentage of malignant transformation of 13.3% and 6.6%, respectively. [136]

In our study, the recurrence rate after 6 months for lesions subjected to vaporization using the CO_2 laser was 40% (25% partial recurrence, and 15% of the complete recurrence). During six months after the laser vaporization, four follow-up visits were performed in order to check the healing course and to evaluate the recurrence rate and its degree.

There has been a difficulty in comparing the percentages of the recurrence rate after vaporization, reported in the literature, due to the presence of many differences related to the diagnosis of TL, the criteria for inclusion of patients and lesions, and the aspects of the surgical operation phase. Another difficulty is that there are studies that report the recurrence rate considering the individual patient, while others consider the single lesion. This study reports the recurrence rate considering the single TL lesion.

Despite these difficulties, one of the first studies based on the use of CO_2 laser in the treatment of OL, in 1984 Frame et al., reported a recurrence rate of 13.6% after 10 months of laser treatment, where the laser was used for both vaporization and lesion excision. [137]

Chiesa et al. [92] evaluated the results after 3 years for 145 OL lesions treated with CO_2 laser, 140 excisions and 5 vaporizations. Evaluating the recurrence rate, the appearance of new lesions, and development of carcinoma was reported in 40% of the cases (58 lesions of 145).

A retrospective study, conducted by Gooris et al., [138] on the results of the vaporization of lip Leukoplakia by CO_2 laser for 27 patients, a complete re-epithelization was observed after 4 weeks of treatment without interfering with normal labial function. In the long-term follow-up, 4 recurrences (14.8%) were observed.

Van der Hem et al., [67] examined 200 patients with 282 OL lesions, subjected to CO_2 laser vaporization between 1976 and 2001. The follow-up lasted between 1 and 219 months; 251 lesions (89%) had no

recurrence, 29 (9.9%) had recurrence within 5 to 168 months, and 3 (1.1%) developed OSCC in the treated area, respectively 7, 17, and 19 months after vaporization.

Pedrosa et al. [139] conducted a study on patients with OL, treated with CO_2 laser vaporization between January 2006 and March 2013 with a lateral extension of 2 mm to the apparent clinical margins of the lesion reaching a depth of about 5 mm; while on the gingival and hard palate lesions, the surgical depth was about 2 mm and the recurrence rate was 40.7% (24 lesions of 59).

A recent article in 2015 reported a study by Mogedas-Vegara et al. [133] for 65 patients diagnosed with OL vaporized with CO_2 laser between January 2010 and April 2013, evidencing the recurrence in 22 of 65 patients (33.8%).

In our study, the results for groups A and B were compared for a total of 20 lesions. Group A with 11 TL lesions, the laser vaporization was done for the entire lesion adding a maximum of 1 mm of safety margins; while group B with 9 TL lesions, the laser vaporization was done for the lesion adding at least 3 mm of safety margins.

The selection of the 3 mm safety margins for group B was done based on a retrospective study conducted by Kuribayashi et al., [140] in which 53 TL lesions were present in 52 patients treated by surgical excision between 2004 and 2009. It was found that the cumulative recurrence-free rate in patients with resection margins >3 mm in width was significantly higher than that in patients with resection margins <3 mm; thus, advising that the optimal safety margins should be at least 3 mm in width and a deep surgical margin may relate to the recurrence of OL.

OL may be a complex lesion, which is a clinically and histologically visible area, derived from one or several clones of cells within an extended oral mucosal area comprising other clones of cells

characterized by clinically invisible changes involved in Field of Cancerization' (FC).

The new epithelium that migrates from the periphery to cover the wound might originate from potentially unstable mucosa 'Field of Cancerization'.

This explains the development of new patches of OL, adjacent to the margins of previously treated areas.

"The visible lesions may thereby be surrounded by genetically altered, cancer stigmatized epithelial cells unrevealed by routine clinical inspection and histological examination." [141]

Group A: after six months of follow-up, the recurrence rate evaluation was 55% for complete healing (6 of 11 lesions), 18% for partial recurrence (2 of 11 lesions), and 27% for complete recurrence (3 of 11 lesions).

While in Group B, the recurrence rate evaluation was 67% for complete healing (6 of 9 lesions), 33% for partial recurrence (3 of 9 lesions), with an absence of the complete recurrence.

These results highlight the best outcome achieved by adding 3 mm of safety margins beyond the clinical limit of the lesion since the complete healing at 6 months was higher for group B than group A; also, the variance ANOVA analysis highlighted the statistical significance of this difference.

The anatomical site in which the highest prevalence of recurrence was recorded is the gingival tissue; according to Kuribayashi et al., [140] the mean marginal thickness for gingival tissue is usually only 1.25 mm 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.

Regarding the smoking habits, all smoking patients were asked to stop the habit, at least two weeks before vaporization; thus, becoming ex-smokers.

Nevertheless, the best results obtained after 6 months of follow-up were confirmed by the statistical analysis for the lesions belonging to patients who had never smoked during their lifetime; with a complete healing percentage of 87.5% (7 of 8 lesions) and complete recurrence of 12.5% (1 of 8 lesions). However, in ex-smokers, the complete healing was 41.5% (5 of 12 lesions), partial recurrence 41.5% (5 of 12 lesions), and complete recurrence of 17% (2 of 12 lesions). Moreover, female patients showed better results than male patients where complete healing occurred in 75% of lesions, while it was 37.5% in male patients.

Chapter 5 : Conclusions

Before starting further treatment of TL, we should focus on the associated risk factors like the smoking habits, alcohol abuse, local irritating factors, and chronic infections.

It is preferred to treat TL without dysplasia not to leave it for 'wait and see'.

It is important to ascertain the borders of the lesions to detect if the peripheral recurrence occurred.

The beneficial effects of using CO_2 laser in the surgical procedures of soft tissues provided adequate and rapid surgical procedure, minimal damage to adjacent tissues, a hemostatic control, and a good visibility of the operating range.

1 mm safety margins is not sufficient enough, the recommended optimal safety margins should be at least 3 mm in width. Also, deep surgical margins may be related to the recurrence of oral TL.

Dividing large lesions, more than 20 mm, into multiple sections for laser vaporization could be a solution to reduce the post-operative symptoms and malfunctions.

The post-operative tissue contraction was significantly reduced as well as the elasticity in the region of laser vaporized tissue. Particularly, the mobility of the tongue has a major impact on speech production and swallowing which are two of the essential parameters of quality of life.

Six months of follow-up is not a sufficient period to evaluate the recurrence rate.

The lesion should be kept under close supervision with frequent follow-up and biopsies.

More cases treated with these considerations are needed for a better evaluation of the recurrence rate.

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

It should be clarified that the management of TL must be entrusted to professionals who know the clinical characteristics and the behaviour of this lesion in depth. In other words, the vaporization of TL by laser is a specialized therapeutic method that should be performed by experts who know how to discriminate whether to proceed with vaporization or to require a more invasive approach.

Finally, improving the management of TL will prevent not only recurrence and malignant transformation, but also post-operative dysfunction.

Chapter 6 : Bibliography

1. Cawson RA, Odell EW. Cawson's Essentials of Oral Pathology and Oral Medicine. Edinburgh; Churchill Livingstone/Elsevier, 2008.; 2008.

2. Pindborg J, Reichart P, Smith C et al. Histological Typing of Cancer and Precancer of the Oral Mucosa, In Collaboration with L.H.Sobin and Pathologists in 9 Countries. Springer Science & Business Media; 2012.

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(10):575-80.

4. Villa A, Woo SB. Leukoplakia-A Diagnostic and Management Algorithm. J Oral Maxillofac Surg. 2017;75(4):723-734.

5. Van der waal I. Oral leukoplakia, the ongoing discussion on definition and terminology. Med Oral Patol Oral Cir Bucal. 2015;20(6):e685-92.

6. Sudbø J, Reith A. The evolution of predictive oncology and molecular-based therapy for oral cancer prevention. Int J Cancer. 2005;115(3):339-45.

7. Bánóczy J, Gintner Z, Dombi C. Tobacco use and oral leukoplakia. J Dent Educ. 2001;65(4):322-7.

8. Bagan JV, Jiménez Y, Murillo J, et al. Epstein-Barr virus in oral proliferative verrucous leukoplakia and squamous cell carcinoma: A preliminary study. Med Oral Patol Oral Cir Bucal. 2008;13(2):E110-3.

9. Vivek V, Jayasree RS, Balan A, Sreelatha KT, Gupta AK. Three-year follow-up of oral leukoplakia after neodymium:yttrium aluminum garnet (Nd:YAG) laser surgery. Lasers Med Sci. 2008;23(4):375-9.

10. Epstein JB, Gorsky M. Topical application of vitamin A to oral leukoplakia: A clinical case series. Cancer. 1999;86(6):921-7.

11. Saito T, Sugiura C, Hirai A, et al. Development of squamous cell carcinoma from pre-existent oral leukoplakia: with respect to treatment modality. Int J Oral Maxillofac Surg. 2001;30(1):49-53.

12. Ishii J, Fujita K, Munemoto S, Komori T. Management of oral leukoplakia by laser surgery: relation between recurrence and malignant transformation and clinicopathological features. J Clin Laser Med Surg. 2004;22(1):27-33.

13. Van der waal I, Schepman KP, Van der meij EH, Smeele LE. Oral leukoplakia: a clinicopathological review. Oral Oncol. 1997;33(5): 291-301.

14. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin. 2002;52(4):195-215.

15. 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.

16. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol. 1978;46(4):518-39.

17. Lin HP, Chen HM, Cheng SJ, Yu CH, Chiang CP. Cryogun cryotherapy for oral leukoplakia. Head Neck. 2012;34(9):1306-11.

18. Ishii J, Fujita K, Komori T. Laser surgery as a treatment for oral leukoplakia. Oral Oncol. 2003;39(8):759-69.

19. Omaye ST, Krinsky NI, Kagan VE, Mayne ST, Liebler DC, Bidlack WR. beta-carotene: friend or foe?. Fundam Appl Toxicol. 1997;40(2):163-74.

20. Girod SC, Pfahl M. Retinoid actions and implications for prevention and therapy of oral cancer. Int J Oral Maxillofac Surg. 1996;25(1):69-73.

21. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79(3):321-9.

22. Vedtofte P, Holmstrup P, Hjørting-hansen E, Pindborg JJ. Surgical treatment of premalignant lesions of the oral mucosa. Int J Oral Maxillofac Surg. 1987;16(6):656-64.

23. Gorsky M, Epstein JB. The effect of retinoids on premalignant oral lesions: focus on topical therapy. Cancer. 2002;95(6):1258-64.

24. Wong F, Epstein J, Millner A. Treatment of oral leukoplakia with topical bleomycin. A pilot study. Cancer. 1989;64(2):361-5.

25. Epstein JB, Gorsky M, Wong FL, Millner A. Topical bleomycin for the treatment of dysplastic oral leukoplakia. Cancer. 1998;83(4): 629-34.

26. Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995;9(15):1551-8.

27. Krinsky NI. Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med. 1992;200(2):248-54.

28. Parker RS. Absorption, metabolism, and transport of carotenoids. FASEB J. 1996;10(5):542-51.

29. Sankaranarayanan R, Mathew B, Varghese C, et al. Chemoprevention of oral leukoplakia with vitamin A and beta carotene: an assessment. Oral Oncol. 1997;33(4):231-6.

30. Liede K, Hietanen J, Saxen L, et al. Long-term supplementation with alpha-tocopherol and beta-carotene and prevalence of oral mucosal lesions in smokers. Oral Dis. 1998;4(2):78-83.

31. Kaugars GE, Silverman S, Lovas JG, Thompson JS, Brandt RB, Singh VN. Use of antioxidant supplements in the treatment of human oral leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996;81(1):5-14.

32. Erhardt JG, Mack H, Sobeck U, Biesalski HK. beta-Carotene and alpha-tocopherol concentration and antioxidant status in buccal mucosal cells and plasma after oral supplementation. Br J Nutr. 2002;87(5):471-5.

33. Malaker K, Anderson BJ, Beecroft WA, Hodson DI. Management of oral mucosal dysplasia with beta-carotene retinoic acid: a pilot cross-over study. Cancer Detect Prev. 1991;15(5):335-40.

34. Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J. 1996;10(7):690-701.

35. Lycopene. Monograph. Altern Med Rev. 2003;8(3):336-42.

36. Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. J Am Coll Nutr. 2000;19(5):563-9.

37. Riccioni G, Mancini B, Di ilio E, Bucciarelli T, D'orazio N. Protective effect of lycopene in cardiovascular disease. Eur Rev Med Pharmacol Sci. 2008;12(3):183-90.

38. Boileau TW, Boileau AC, Erdman JW. Bioavailability of all-trans and cis-isomers of lycopene. Exp Biol Med (Maywood). 2002;227(10):914-9.

39. Michael mcclain R, Bausch J. Summary of safety studies conducted with synthetic lycopene. Regul Toxicol Pharmacol. 2003;37(2): 274-85.

40. Ribeiro AS, Salles PR, Da silva TA, Mesquita RA. A review of the nonsurgical treatment of oral leukoplakia. Int J Dent. 2010;2010:186018.

41. Naidu KA. Vitamin C in human health and disease is still a mystery? An overview. Nutr J. 2003;2:7.

42. Kallner AB, Hartmann D, Hornig DH. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. Am J Clin Nutr. 1981;34(7):1347-55.

43. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA. 1989;86(16):6377-81.

44. Levine M, Conry-cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci USA. 1996;93(8):3704-9.

45. Barth TJ, Zöller J, Kübler A, Born IA, Osswald H. Redifferentiation of oral dysplastic mucosa by the application of the antioxidants beta-carotene, alpha-tocopherol and vitamin C. Int J Vitam Nutr Res. 1997;67(5):368-76.

46. Ingold KU, Bowry VW, Stocker R, Walling C. Autoxidation of lipids and antioxidation by alpha-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: unrecognized consequences of lipid particle size as exemplified by

oxidation of human low density lipoprotein. Proc Natl Acad Sci USA. 1993;90(1):45-9.

47. Azzi A, Ricciarelli R, Zingg JM. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). FEBS Lett. 2002;519(1-3):8-10.

48. Horwitt MK. Vitamin E: a reexamination. Am J Clin Nutr. 1976;29(5):569-78.

49. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. J Physiol Biochem. 2001;57(2):43-56.

50. David M, Hodak E, Lowe NJ. Adverse effects of retinoids. Med Toxicol Adverse Drug Exp. 1988;3(4):273-88.

51. Olson JA. Carotenoids and human health. Arch Latinoam Nutr. 1999;49(3 Suppl 1):7S-11S.

52. Kaugars GE, Silverman S, Lovas JG, et al. A clinical trial of antioxidant supplements in the treatment of oral leukoplakia. Oral Surg Oral Med Oral Pathol. 1994;78(4):462-8.

53. Dimery IW, Hong WK, Lee JJ, et al. Phase I trial of alpha-tocopherol effects on 13-cis-retinoic acid toxicity. Ann Oncol. 1997;8(1):85-9.

54. Sporn MB, Newton DL. Chemoprevention of cancer with retinoids. Fed Proc. 1979;38(11):2528-34.

55. Bennett JM, Reich SD. Drugs five years later: Bleomycin.Ann Intern Med. 1979;90:945–948.

56. Sieron A, Namyslowski G, Misiolek M, Adamek M, Kawczyk-krupka A. Photodynamic therapy of premalignant lesions and local recurrence of laryngeal and hypopharyngeal cancers. Eur Arch Otorhinolaryngol. 2001;258(7):349-52.

57. A. C. K¨ubler. Photodynamic therapy. Medical Laser Application, vol. 20, no. 1, pp. 37–45, 2005.

58. Konopka K, Goslinski T. Photodynamic therapy in dentistry. J Dent Res. 2007;86(8):694-707.

59. Kelty CJ, Brown NJ, Reed MW, Ackroyd R. The use of 5-aminolaevulinic acid as a photosensitiser in photodynamic therapy and photodiagnosis. Photochem Photobiol Sci. 2002;1(3):158-68.

60. Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. J Oral Pathol Med. 2008;37(2):63-9.

61. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37(1):1-10.

62. Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med. 2008;37(3):127-33.

63. Marley JJ, Cowan CG, Lamey PJ, Linden GJ, Johnson NW, Warnakulasuriya KA. Management of potentially malignant oral mucosal lesions by consultant UK oral and maxillofacial surgeons. Br J Oral Maxillofac Surg. 1996;34(1):28-36.

64. Marley JJ, Linden GJ, Cowan CG, et al. A comparison of the management of potentially malignant oral mucosal lesions by oral medicine practitioners and oral & maxillofacial surgeons in the UK. J Oral Pathol Med. 1998;27(10):489-95.

65. Schwarz F, Maraki D, Yalcinkaya S, Bieling K, Böcking A, Becker J. Cytologic and DNA-cytometric follow-up of oral leukoplakia after CO2- and Er:YAG-laser assisted ablation: a pilot study. Lasers Surg Med. 2005;37(1):29-36.

66. Chandu A, Smith AC. The use of CO2 laser in the treatment of oral white patches: outcomes and factors affecting recurrence. Int J Oral Maxillofac Surg. 2005;34(4):396-400.

67. Van der hem PS, Nauta JM, Van der wal JE, Roodenburg JL. The results of CO2 laser surgery in patients with oral leukoplakia: a 25 year follow up. Oral Oncol. 2005;41(1):31-7.

68. Schepman KP, Van der meij EH, Smeele LE, Van der waal I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol. 1998;34(4):270-5.

69. Einhorn J, Wersall J. Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer. 1967;20(12):2189-93.

70. Vedtofte P, Holmstrup P, Hjørting-hansen E, Pindborg JJ. Surgical treatment of premalignant lesions of the oral mucosa. Int J Oral Maxillofac Surg. 1987;16(6):656-64.

71. Ribeiro NF, Godden DR, Wilson GE, Butterworth DM, Woodwards RT. Do frozen sections help achieve adequate surgical margins in the resection of oral carcinoma?. Int J Oral Maxillofac Surg. 2003;32(2):152-8.

72. Weijers M, Snow GB, Bezemer DP, Van dr wal JE, Van der waal I. The status of the deep surgical margins in tongue and floor of mouth squamous cell carcinoma and risk of local recurrence; an analysis of 68 patients. Int J Oral Maxillofac Surg. 2004;33(2): 146-9.

73. Sako K, Marchetta FC, Hayes RL. Cryotherapy of intraoral leukoplakia. Am J Surg. 1972;124(4):482-4.

74. Saito T, Sugiura C, Hirai A, et al. Development of squamous cell carcinoma from pre-existent oral leukoplakia: with respect to treatment modality. Int J Oral Maxillofac Surg. 2001;30(1):49-53.

75. Bekke JP, Baart JA. Six years' experience with cryosurgery in the oral cavity. Int J Oral Surg. 1979;8(4):251-70.

76. Gongloff RK, Gage AA. Cryosurgical treatment of oral lesions: report of cases. J Am Dent Assoc. 1983;106(1):47-51.

77. Farah CS, Savage NW. Cryotherapy for treatment of oral lesions. Aust Dent J. 2006;51(1):2-5.

78. Pogrel MA. The use of liquid nitrogen cryotherapy in the management of locally aggressive bone lesions. J Oral Maxillofac Surg. 1993;51(3):269-73.

79. Toida M, Ishimaru JI, Hobo N. A simple cryosurgical method for treatment of oral mucous cysts. Int J Oral Maxillofac Surg. 1993;22(6):353-5.

80. Salmassy DA, Pogrel MA. Liquid nitrogen cryosurgery and immediate bone grafting in the management of aggressive primary jaw lesions. J Oral Maxillofac Surg. 1995;53(7):784-90.

81. Leopard PJ, Poswillo DE. Practical cryosurgery for oral lesions. Br Dent J. 1974;136(5):185-96.

82. Ben-Bassat M, Kaplan I, Shindel Y, Edlan A. The CO_2 laser in surgery of the tongue. Br J Plast Surg. 1978;31(2):155–156. 19.

83. Strong MS, Vaughan CW, Healy GB, Shapshay SM, Jako GJ. Transoral management of localized carcinoma of the oral cavity using the CO2 laser. Laryngoscope. 1979;89(6 Pt 1):897-905.

84. Tuffin JR, Carruth JAS. The carbon dioxide surgical laser. Br Dent J. 1980;149(9):255-258.

85. Frame JW. Removal of oral soft tissue pathology with the CO2 laser. J Oral Maxillofac Surg. 1985;43(11):850-5.

86. Flynn MB, White M, Tabah RJ. Use of carbon dioxide laser for the treatment of premalignant lesions of the oral mucosa. J Surg Oncol. 1988;37(4):232-4.

87. Goldman L, Igelman JM, Richfield DF. Impact of the laser on nevi and melanomas. Arch Dermatol. 1964;90:71-5.

88. Rossmann JA, Brown RS, Hays GL, Lusk SS. Carbon dioxide laser surgical therapy for the management of oral leukoplakia: a case report. Tex Dent J. 1994;111(12):17-9, 21.

89. Gáspár L. The use of high-power lasers in oral surgery. J Clin Laser Med Surg. 1994;12(5):281-5.

90. Horch HH, Gerlach KL, Schaefer HE. CO2 laser surgery of oral premalignant lesions. Int J Oral Maxillofac Surg. 1986;15(1):19-24.

91. Pinheiro AL, Frame JW. Surgical management of premalignant lesions of the oral cavity with the CO2 laser. Braz Dent J. 1996;7(2):103-8.

92. Chiesa, F, Tradati, N, Sala, L, Cost, L, Podrecca, S, Boracchi, P et al. Follow-up of oral leukoplakia after carbon dioxide laser surgery. Arch Otolaryngol Head Neck Surg. 1990;116:177–180.

93. Chu FW, Silverman S, Dedo HH. CO2 laser treatment of oral leukoplakia. Laryngoscope. 1988;98(2):125-30.

94. Kurita, H, Kurashina, K, and Kotani, A. Lugol staining method for diagnosis of epithelial lesions of oral mucosa. J Jpn Soc Oral Tumor. 1995;7:319–325.

95. Nakanishi, Y, Ochiai, A, Shimoda, T et al. Epidermization in the esophageal mucosa (unusual epithelial changes clearly detected by lugol's staining) . Am J Surg Pathol. 1997;21:605–609.

96. Mashberg, A. Reevaluation of toluidine blue application as a diagnostic adjunct in the detection of asymptomatic oral squamous carcinoma (a continuing prospective study of oral cancer III). Cancer. 1980;46:758–763.

97. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4-5):309-16.

98. Ganly I, Patel S, Shah J. Early stage squamous cell cancer of the oral tongue--clinicopathologic features affecting outcome. Cancer. 2012;118(1):101-11.

99. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37(1):1-10.

100. Zini A, Czerninski R, Sgan-cohen HD. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. J Oral Pathol Med. 2010;39(4):299-305.

101. Arduino PG, Surace A, Carbone M, et al. Outcome of oral dysplasia: a retrospective hospital-based study of 207 patients with a long follow-up. J Oral Pathol Med. 2009;38(6):540-4.

102. Silverman S, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53(3):563-8.

103. Lind PO. Malignant transformation in oral leukoplakia. Scand J Dent Res. 1987;95(6):449-55.

104. Liu W, Shi L-J, Wu L, et al. Oral Cancer Development in Patients with Leukoplakia – Clinicopathological Factors Affecting Outcome. Gires O, ed. PLoS ONE. 2012;7(4):e34773.

105. Knappe V, Frank F, Rohde E. Principles of lasers and biophotonic effects. Photomed Laser Surg 2004;22:411-417.

106. RN KB. Lasers, The Perioperative Challenge. Mosby Incorporated; 1995.

107. Kujawa J, Zavodnik L, Zavodnik I, Buko V, Lapshyna A, Bryszewska M. Effect of low-intensity (3.75-25 J/cm2) near-infrared (810 nm) laser radiation on red blood cell ATPase activities and membrane structure. J Clin Laser Med Surg. 2004;22(2):111-7.

108. Kujawa J, Zavodnik L, Zavodnik I, Bryszewska M. Low-intensity near-infrared laser radiation-induced changes of acetylcholinesterase activity of human erythrocytes. J Clin Laser Med Surg. 2003;21(6):351-5.

109. Moshonov J, Stabholz A, Leopold Y, Rosenberg I, Stabholz A. [Lasers in dentistry. Part B--Interaction with biological tissues and the effect on the soft tissues of the oral cavity, the hard tissues of the tooth and the dental pulp]. Refuat Hapeh Vehashinayim (1993). 2001;18(3-4):21-8, 107-8.

110. Van gemert MJ, Lucassen GW, Welch AJ. Time constants in thermal laser medicine: II. Distributions of time constants and thermal relaxation of tissue. Phys Med Biol. 1996;41(8):1381-99.

111. Optics in Medicine, Introduction to Laser-Tissue Interactions. Ben Cox, October 2013.

112. Jones T, Fleming C, Llewelyn J. Management of vascular lesions of the mouth and lips using a potassium titanyl phosphate (KTP) laser: review of patient satisfaction. Br J Oral Maxillofac Surg. 2011;49(5):364-7.

113. Bornstein MM, Suter VG, Stauffer E, Buser D. [The CO2 laser in stomatology. Part 1]. Schweiz Monatsschr Zahnmed. 2003;113(5): 559-70.

114. Genovese WJ, Dos santos MT, Faloppa F, De souza merli LA. The use of surgical diode laser in oral hemangioma: a case report. Photomed Laser Surg. 2010;28(1):147-51.

115. Strauss RA. Lasers in oral and maxillofacial surgery. Dent Clin North Am. 2000;44(4):851-73.

116. Suter VG, Bornstein MM. Ankyloglossia: facts and myths in diagnosis and treatment. J Periodontol. 2009;80(8):1204-19.

117. Duncavage JA, Ossoff RH. Use of the CO2 laser for malignant disease of the oral cavity. Lasers Surg Med. 1986;6(5):442-4.

118. Kusukawa J, Suefuji Y, Ryu F, Noguchi R, Iwamoto O, Kameyama T. Dissemination of cancer cells into circulation occurs by incisional biopsy of oral squamous cell carcinoma. J Oral Pathol Med. 2000;29(7):303-7.

119. Van der hem PS, Egges M, Van der wal JE, Roodenburg JL. CO2 laser evaporation of oral lichen planus. Int J Oral Maxillofac Surg. 2008;37(7):630-3.

120. Goharkhay K, Moritz A, Wilder-smith P, et al. Effects on oral soft tissue produced by a diode laser in vitro. Lasers Surg Med. 1999;25(5):401-6.

121. Dosi T, Gupta D, Hazari A, Rajput R, Chauhan P, Rajapuri AS. 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.

122. Lodi G, Franchini R, Warnakulasuriya S, et al. Interventions for treating oral leukoplakia to prevent oral cancer. Cochrane Database Syst Rev. 2016;7:CD001829.

123. 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.

124. Reichart PA. Oral mucosal lesions in a representative cross-sectional study of aging Germans. Community Dent Oral Epidemiol. 2000;28(5):390-8.

125. Scheifele C, Reichart PA. Is there a natural limit of the transformation rate of oral leukoplakia?. Oral Oncol. 2003;39(5): 470-5.

126. Lodi G, Franchini R, Warnakulasuriya S, et al. Interventions for treating oral leukoplakia to prevent oral cancer. Cochrane Database Syst Rev. 2016;7:CD001829.

127. Warnakulasuriya S. Lack of molecular markers to predict malignant potential of oral precancer. J Pathol. 2000;190(4):407-9.

128. 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.

129. 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.

130. Schepman KP, Van der meij EH, Smeele LE, Van der waal I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol. 1998;34(4):270-5.

131. Axéll T. Occurrence of leukoplakia and some other oral white lesions among 20,333 adult Swedish people. Community Dent Oral Epidemiol. 1987;15(1):46-51.

132. Brouns ER, Baart JA, Karagozoglu KH, Aartman IH, Bloemena E, Van der waal I. Treatment results of CO2 laser vaporisation in a cohort of 35 patients with oral leukoplakia. Oral Dis. 2013;19(2): 212-6.

133. 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.

134. 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.

135. Abraham Reshma J, Arathy S Lankupalli. Laser Management Of Intraoral Soft Tissue Lesions A Review Of Literature. e-ISSN. 2014; Volume 13, Issue 1 Ver. X., PP 59-64.

136. Lim B, Smith A, Chandu A. Treatment of oral leukoplakia with carbon dioxide and potassium-titanyl-phosphate lasers: a comparison. J Oral Maxillofac Surg. 2010;68(3):597-601.

137. Frame JW, Gupta ARD, Dalton GA, Evans PHR. Use of the carbon dioxide laser in the Management of premalignant lesions of the oral Mucosa. *The Journal of Laryngology & Otology*. 1984;98(12): 1251-1260. doi:10.1017/S0022215100148364.

138. Gooris PJ, Roodenburg JL, Vermey A, Nauta JM. Carbon dioxide laser evaporation of leukoplakia of the lower lip: a retrospective evaluation. Oral Oncol. 1999;35(5):490-5.

139. Pedrosa A, Santos A, Ferreira M, Araújo C, Barbosa R, Medeiros L. 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.

140. Kuribayashi Y, Tsushima F, Sato M, Morita K, Omura K. 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.

141. Holmstrup P, Dabelsteen E. Oral leukoplakia-to treat or not to treat. Oral Dis. 2016;22(6):494-7.

142. Nammour S, Zeinoun T, Namour A, Vanheusden A, Vescovi P. Evaluation of Different Laser-Supported Surgical Protocols for the Treatment of Oral Leukoplakia: A Long-Term Follow-Up. Photomed Laser Surg. 2017;

143. White JM, Chaudhry SI, Kudler JJ, Sekandari N, Schoelch ML, Silverman S. Nd:YAG and CO2 laser therapy of oral mucosal lesions. J Clin Laser Med Surg. 1998;16(6):299-304.

144. Romeo U, Russo N, Palaia G, Tenore G, Del vecchio A. Oral proliferative verrucous leukoplakia treated with the photodynamic therapy: a case report. Ann Stomatol (Roma). 2014;5(2):77-80.

145. Del vecchio A, Palaia G, Tenore G, Russo N, Romeo U. The photodynamical treatment of oral verrucous proliferative leukoplakia. Case report at 18 months follow up. Ann Stomatol (Roma). 2013;4(Suppl 2):13-4.

146. 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. ScientificWorldJournal. 2014;2014:345685.

147. Van der waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45(4-5):317-23.

List of Abbreviations

OL	Oral Leukoplakia
TL	True Leukoplakia
WHO	World Health Organization
PMD	Potentially Malignant Disorders
OPMD	Oral Potentially Malignant Disorders
MMO	Morsicatio Mucosae Oris
BARK	Benign Alveolar Ridge Keratosis
LP	Lichen Planus
OLP	Oral Lichen Planus
HIV	Human Immunodeficiency Virus
KUS	Keratosis of Unknown Significance
SCC	Squamous Cell Carcinoma
OSCC	Oral Squamous Cell Carcinoma
EBV	Epstein-Barr Virus
PVL	Proliferative Verrucous Leukoplakia
ICD-DA	International Classification of Diseases Application to Dentistry and Stomatology
L-AA	L-Ascorbic Acid
13 - cRA	13-cis-Retinoic Acid
5-ALA	5-Aminolaevulinic Acid
AT	α-Tocoferol
FC	Field of Cancerization
CO_2	Carbon Dioxide Laser
Nd: YAG	Neodymium : Yttrium Aluminum Garnet
Er: YAG	Erbium: Yttrium Aluminum Garnet
KTP	Potassium-Titanium-Phosphate
GA	Group A
GB	Group B
CG	Control Group