World Cancer Research Journal
WCRJ 2023; 10: e2712
DOI: 10.32113/wcrj_202312_2712

ASSOCIATION BETWEEN *C. ALBICANS* AND LEUKOPLAKIA AND ITS TREATMENT WITH PHOTODYNAMIC THERAPY: A REVIEW OF THE LITERATURE AND A CASE REPORT

C. CASU[1,2], M. PINNA[1], G. DENOTTI[1], M.S. MURGIA[1], G. ORRÙ[1,3]

[1]Department of Surgical Sciences, Oral Biotechnology Laboratory, Cagliari State University, Cagliari, Italy
[2]International PhD in Innovation Sciences and Technologies, Cagliari State University, Cagliari, Italy
[3]National Research Council of Italy CNR – ISPA Sassari, Sassari, Italy

Corresponding Author
Cinzia Casu, MD; e-mail: ginzia.85@hotmail.it;
Germano Orrù, MD; e-mail: orru@unica.it

ABSTRACT – ***Objective:*** This paper deals with oral leukoplakia, a potential oral malignant disorder that often increases in malignancy due to an associated infection supported by the fungus Candida albicans. The work is aimed at describing this dual condition through a literature review and an unusual clinical case treated with Photodynamic Therapy..

Materials and Methods: We used PubMed as a research engine in order to detect the most recent papers (2014-2023) written in English. Our main goal was to obtain more information about oral leukoplakia, its colonization by C. albicans and its rate of malignant transformation. We also searched the database in order to evaluate the efficacy of Photodynamic therapy against Candida infections.

Case Presentation: The case presentation refers to a 37-year-old man with a diagnosis of tongue leukoplakia with a co-infection of *C. albicans*, treated with Photodynamic Therapy instead of conventional antifungal drugs.

Results: The literature review was based on a total of 17939 articles, which were reduced to only 25 after setting the inclusion and exclusion criteria in several steps. Oral leukoplakia is an idiopathic condition that can be considered a precancerous lesion; its co-infection with *C. albicans* increases the chances of its malignant transformation. Photodynamic therapy is a new approach in terms of non-conventional therapies, and there is growing evidence that it can be used in the treatment of oral diseases, too.

Conclusions: We eradicated the presence of C. albicans strains on our patient's leukoplakia by using a mixture of photo-activated curcumin and H_2O_2, decreasing the chances of malignant transformation of our patient's lesion, who is still undergoing a six-month control protocol.

KEYWORDS: *Candida albicans*, Leukoplakia, Photodynamic therapy, Photosensitizers, Oral Candidiasis.

ABBREVIATIONS: CHC = Chronic Hyperplastic Candidiasis; CFU = Colony Forming Unit; MT = Malignant Transformation; OC = Oral Candidiasis; OL = Oral Leukoplakia; OPMDs = Oral Potential Malignant Disorders; OSCC = Oral Squamous Cell Carcinoma; PDT = Photodynamic Therapy; ROS = Reactive Species of Oxygen.

INTRODUCTION

Oral Leukoplakia (OL) is demonstrated to be a potentially malignant disorder[1-9], with a rate of transformation of about 9.5%[10]. Leukoplakia, literally meaning "white patch", presents as a white lesion, not scrapable or due to anything else. WHO states as the definition of leukoplakia a "white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk for cancer" [1-9]. So, it can be easily said that leukoplakia is idiopathic[3] and it is a diagnosis of exclusion[1,3,5,10,11]. Oral Candidiasis (OC) is a disease due to fungal infection, in particular Candida albicans, which is the most common fungus we can find in the oral microbiota. It can be responsible for many diseases, from the thrush of the newborn child to severe infections that can lead to malignant transformation[1,7,12-16], such as Chronic Hyperplastic Candidiasis (CHC), which is reported in the literature with a rate of transformation of about 12% [10,14]. Sometimes it could happen that C. albicans could colonize OL, increasing its rate of malignant transformation [10,12]. It is clear that it is mandatory to reduce or neutralize the fungal load in order to prevent the cancerization of the lesion itself. Different approaches can be used in the management of OL, the traditional ones – from a pharmacological approach up to surgery – or non-invasive ones such as LASERs and Photodynamic Therapy (PDT)[11,17,18]. Photodynamic Therapy consists of the activation of a photosensitizer with a light at a specific wavelength, which can damage only the rotten cells thanks to the production of ROS [11-18]. For the first time in dermatology, in the past two decades, dentistry has entered as an innovative and non-invasive therapy used most of all in the cure of malignant lesions, with a rate of success undeniable. Its field of application is nowadays even against bacterial, viral, and fungal infections. The purpose of our paper is, hence, to report a case of an Oral Potential Malignant Lesion (OPML) colonized by *C. albicans* treated with PDT and to assess its effectiveness, as sustained by the literature.

MATERIALS AND METHODS

We performed a short review of the literature using PubMed as a research engine. The keywords we used to perform our research were the following: "Leukoplakia" "Oral candidiasis" and "Oral candidiasis and leukoplakia". We set some inclusion and exclusion criteria (Figure 1) in order to fulfill our review. The inclusion criteria we set were the following: we included only articles written from 2014 until 2023,

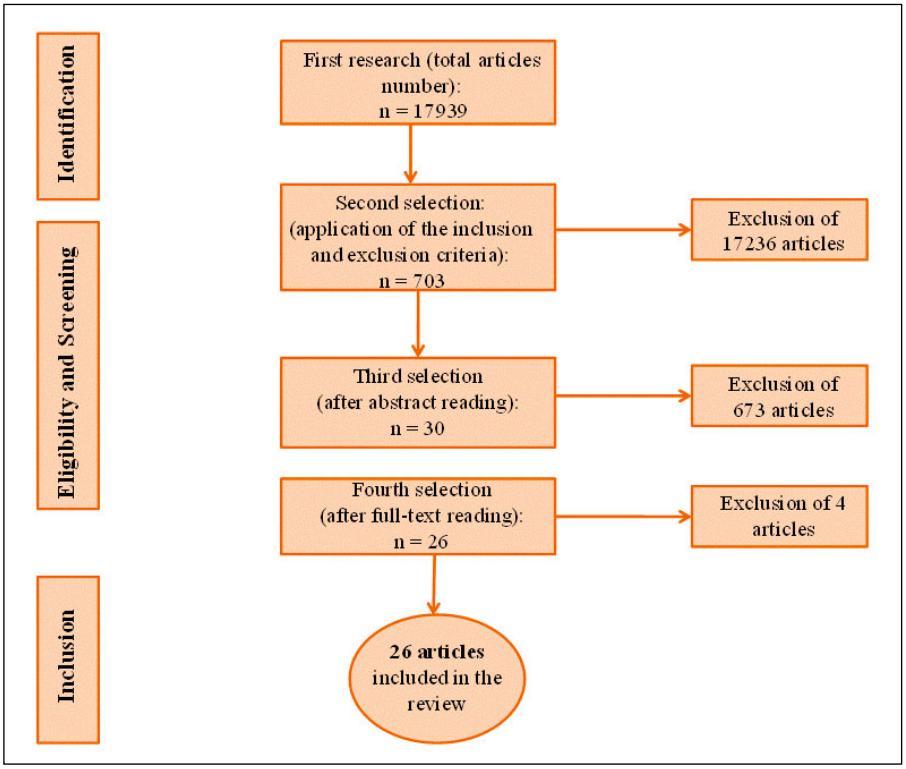

Figure 1. Flow chart illustrating the choice of the articles.

in English, and with full text. We considered case reports, clinical trials, meta-analyses, reviews, and systematic reviews of the literature. We excluded from our research every kind of article that presented patients with candidiasis in other districts of the human body except the oral cavity, with comorbidities; even associations between candidiasis or leukoplakia and other microorganisms such as viruses, bacteria, or immune disorders were not taken into consideration. No distinction in age, sex, or ethnicity was considered.

CASE PRESENTATION

We present a case of a 37-year-old man who came to our attention with a suspected leukoplakia on the right side of the tongue of about 2 x 3 cm in dimension (Figure 2). After signing the informed consent, the patient has been submitted to an incisional biopsy on the ventral right side of the tongue. The histological report confirms the suspected leucoplakia and describes the lesion as a flap of squamous epithelium with hyper-parakeratotic aspects, free from dysplasia but rich in microbial and fungal flora, with the indication for the clinician to evaluate the load with a cultural investigation. Hence, according to the patient, we decided to perform a brush on the remaining lesion in order to detect what kind of microorganism could have colonized it. The brush revealed a high presence of C. albicans tires. Due to what is reported in the literature about the association between Oral Candidiasis (OC) and Leukoplakia (OL) and its opportunity to become a potential malignant lesion, we decided to try to eradicate the microorganism using Photodynamic Therapy (PDT). The protocol used was the following: we used a mixture of curcumin and H_2O_2 at 3% as a photosensitizer; we applied it to the entire surface of the lesion (Figure 3), and then we activated it with a blue diode light at a wavelength of 460 nm and 7 watts of power (FlashMax P7, CMS Dental, Copenhagen, Denmark) for about 5 minutes, handling the light about one centimeter far from the lesion (Figure 4). After that, the photosensitizer was washed away with a huge quantity of water. We immediately perform a brush (scaped for 30 seconds on the tongue mucosa) in order to evaluate *C. albicans* colonies. We repeated another brush five minutes later in order to compare the two analyses and assess their effectiveness over time. We sent the samples to the Molecular Biology Laboratory (University of Cagliari, Cagliari, Italy), for a cultural microbiological analysis, where both of the two brushes were used to inoculate two different Petri dishes with Sabouraud agar that were successively put in incubation for 48 hours at 37°C. After 48 hours of incubation, both samples revealed no presence of a fungal colonies load after the treatment, assessing the effectiveness of PDT with curcumin and hydrogen peroxide (Figure 5). We communicated the results to our patient, explaining to him that we drastically reduced the possibility of malignant transformation of his lesion, but we decided, in accordance with the patient, to keep monitoring him with six-month controls in order to immediately recognize a possible change in the sense of malignant transformation in his lesion.

Figure 2. Diagnosed Leukoplakia of the ventral right side of the tongue of our patient.

Figure 3. Application of the photosensitizer on the lesion: a mixture of curcumin and H_2O_2 at 3% was used. H_2O_2 was sperimentally added to curcumin in order to boost up the efficacy of curcumin and the production of ROS.

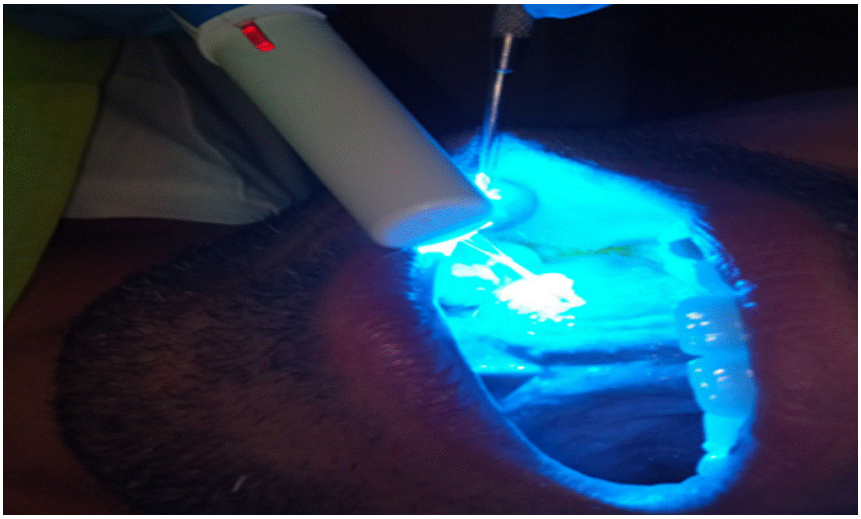

Figure 4. Activation of the photosensitizer with a blue diode laser (wavelength of 460 nm; power 7 W). The protocol used was one activation of about 5 minutes, handling the light beam one centimeter far from the lesion.

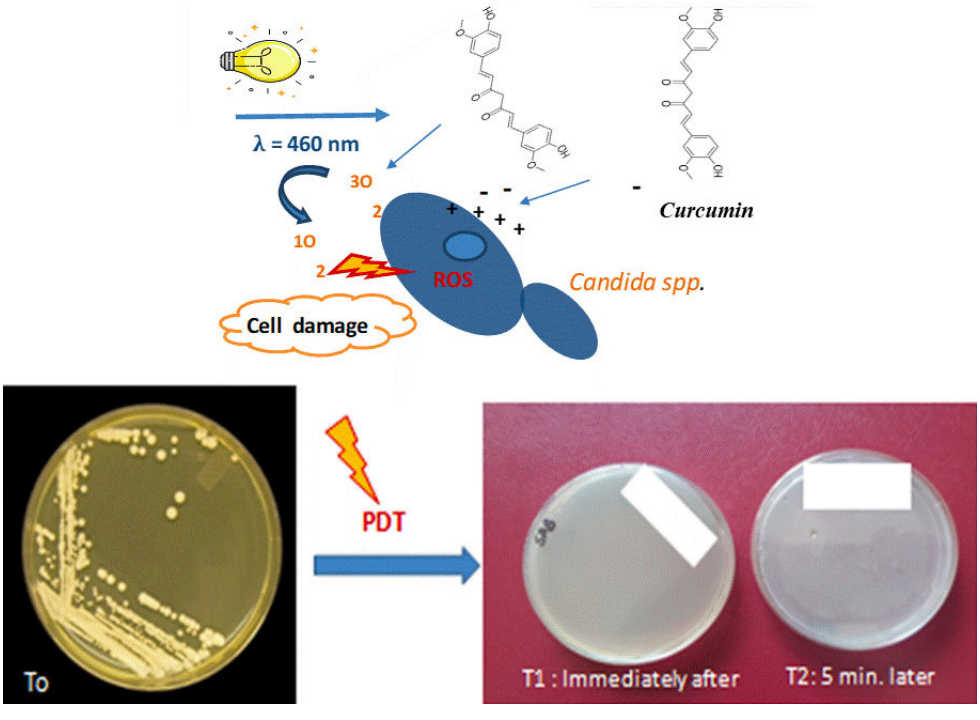

Figure 5. Schematic representation of antifungal PDT linked with a laboratory result PDT anti-Candida effect. In this case, the cultural results on samples recruited at time 0 (before PDT), at time 1, and at time 2 (after PDT) showed how the fungal load was totally reduced after the treatment.

RESULTS

The flow chart in Figure 1 illustrates schematically the procedure for choosing the articles for our review. We now report the first results for every keyword we put on the research engine once we set the inclusion and exclusion criteria: "Leukoplakia" brought up 7594 results; "oral candidiasis" brought up 9663 results; "oral candidiasis and leukoplakia" brought up 682 results, for a total of 17939 articles. After setting the inclusion and exclusion criteria, we obtained the following results: 257 articles for "Leukoplakia", 420 articles for "Oral candidiasis" and 26 articles for "Oral candidiasis and Leukoplakia," for a total of 703 articles. After reading the abstract, we considered 16 articles for the "Leukoplakia" keyword, 12 articles for "Oral candidiasis" and only 2 for "Oral candidiasis and leukoplakia". The final choice of the articles used for this paper was then obtained by reading the full text of the chosen articles, which brought up a total of 25 articles for our purpose.

DISCUSSION

We decided to organize our paper focusing on the three main topics of our case report, i.e., Photodynamic Therapy (PDT), the role of *Candida albicans* in the oral microbiota, the description of Oral Leukoplakia and the presumed connection between these two latter topics. We analyzed them with a short review of the literature in order to present our conclusions with strong evidence.

Focus on topic (1): Photodynamic Therapy

Photodynamic Therapy (PDT) can be considered a new approach in the treatment of oral diseases. Its use is mainly in dermatology, where precancerous lesions and cancer skin are treated. Even in the gynecology and urology branches, PDT has taken place [11,17], and from a decade on, its use is developing in dentistry, too. The existence of chemical substances that can be activated by a specific light in order to produce ROS (*reactive oxygen species*) in an aerobic environment is known from the past century, when in 1904 Professor Hermann von Tappeiner, head of the Pharmacological Institute of the Ludwig-Maximilians University in Munich, for the first time talked of a "photodynamic action" in order to describe the interaction between a chemical substance and a light in the presence of oxygen[17,18]. Since then, more studies have developed, and the photodynamic action first described became what we now know as PDT. Moreover, more molecules have been found to be well activated by specific lights.

PDT consists of three main agents: a photosensitizer, a specific light, and the presence of oxygen.[11,17,18]. Photosensitizer is a molecule derived chiefly from natural substances, which is divided into groups by their chemical structure and origin; some examples include porphyrin, methylene blue, toluidine blue, curcumin, and 5-ALA (5-aminolevulenic acid)[11]. Every type of photosensitizer is activated by a specific light at its specific wavelength. The most used lights are diode, argon, and Nd/YAG lasers[11,17], because of the monochromatic nature of the light, which gives a more stable beam (11). Diode LASERs for PDT work on a range of 600–850 nm; Argon LASERs between 448–515 nm[11,17]. 600–800 nm is considered the "therapeutic window"[11] in which PDT gives its best results. In this field of short wavelengths, tissue penetration is optimal in terms of depth penetration and efficacy of ROS, reaching the epithelial damaged cells in a safer way and carrying lesser adverse effects[11]. In a way that is still not very well known, the molecule can bind selectively only to damaged cells, and its activation can induce the apoptosis of that targeted cell thanks to the production of ROS in the presence of oxygen[11,17,18]. Figure 5 shows how PDT works. 5-ALA (5- Aminolevulenic acid) is the most commonly used photosensitizer in the treatment of OPMDs[11,18]. In the treatment of OL, its use represents the 4th non-surgical approach in its management, after chemotherapy, surgery, and CO_2 laser ablation [11]. It is the most studied photosensitizer for this kind of lesion, and its effectiveness is established even in the treatment of recurrences[18]. 5-ALA is a second-generation photosensitizer and a precursor of porphyrin (11,17). It carries a minimally invasive nature, low adverse effects, and tested efficacy on OPMDs and cancer[11,17,18]; it is also water-soluble, which is optimal considering the presence of saliva in the oral mouth, and it can even be administered intravenously.

Not only 5-ALA is used in the treatment of OL. Prazmo et al[17] in their review investigated the effectiveness of methylene blue as a photosensitizer, in particular against the most common dental and oral pathologies. They obtained good results in periodontal disease and in the disinfection of endodontic

canals. Moreover, they assessed fungicidal efficacy against C. albicans, which is the most common fungus causing opportunistic infections in the oral cavity[13-15,19-23]. In order to minimize the toxicity of the photosensitizer, molecules that derive from natural products are used in PDT. Nowadays, there is a huge interest in literature towards curcumin and its beneficial properties. Curcumin is a yellow derivative plant that belongs to the ginger family; in particular, it is taken from Curcuma longa (turmeric)[23-26]. It is well established that it is a powerful antioxidant and anti-inflammatory agent, with antiangiogenetic, antiseptic, analgesic, and even anticarcinogenic action[23-26]. It is well assessed for its action against viruses, bacteria, and fungi, too [23,25]. Its oral administration in OPMDs such as OL gave optimal both clinical and histopathological responses when compared to chemoprevention [26]. It is able to prevent DNA damage and increase antioxidant and Vitamin C levels in OL [25]. The real problem with curcumin is its poor solubility in water [24], which represents a big issue for its topical use in the oral cavity. This problem can be overcome using nanocurcumin, which is soluble in water and shares the same characteristics as curcumin. Anwar et al[24] in their study on mice infected with C. albicans, reported approximately the same therapeutical effects of nano-curcumin against oral candidiasis in terms of remission of the disease and duration of the treatment compared with nystatin, which represents the gold standard therapy used for the cure of oral candidiasis. Moreover, they report that curcumin can be used in cases of recurrences and against drug-resistant species[23-26]. It is stated in the literature that using PDT instead of the conventional pharmacological approach in the treatment of OPMDs and OL can reduce the treatment session[18]. Moreover, it represents a non-pharmacological therapy useful in cases of refractory OC or MDR species, which is an extremely important issue of our time.

Focus on topic (2): Candida spp.

Specifically talking about Candida spp., Oral Candidiasis (OC) is one of the most common oral infections, which can be found at every age in the worldwide population. It is principally due to C. albicans[13-15,19-23], which is the most common fungus being part of the oral microbiota. It is stated in fact that up to 30–50% of healthy people—some authors reporting up to 80% (14) - carry this microorganism as a harmless inhabitant of the oral environment[15,20,23], meaning that hosting C. albicans in our own mouth does not signify being affected by OC. C. albicans is, in fact, an opportunistic pathogen whose disease onset depends on several factors, but principally on an imbalance between the host immune system and the virulence factors of the fungus[2,14-16,19-23]. Poor oral hygiene, smoking, reduced salivary flow, broad-spectrum antibiotic therapies, nutritional deficiencies (such as iron, zinc, vitamin A, B6, B12, and folic acid), and immune disorders such as diabetes or endocrinopathies can lead to the onset of OC[13-15,19-21,23]. Immunodepression plays a fundamental role in the assessment of Candida's spectrum of illnesses; in HIV-positive patients, OC can be the first sign of the development of AIDS. Patients diagnosed with tumors, due both to the type of cancer and the adverse side effects of chemotherapy or radiotherapy, can develop OC, too[13-15,19-23]. Dental prosthetics or orthodontic patients can develop OC in the absence of accurate oral hygiene practices[15]. Even if it is estimated that about 95% of OC has to be attributed to C. albicans [14,21], it is important to remember that other species of Candida are found in the oral microbiota, and that can establish the same disorders as C. albicans does. Nowadays, molecular analysis of the oral microbiota reports that C. glabrata, C. kruseii, C. tropicalis, and C. dubliniensis can be very common findings in adults[2,13,15,22-23]. The real issue with these other species is that more of C. albicans are able to induce drug resistance to the common antifungal therapies, such as nystatin or azoles[14,21,23]. From here, it is necessary to find other therapeutic tools to annihilate the fungal load in the event of the onset of the disease. Clinically, OC is divided into primary and secondary manifestations and Candida-associated lesions [14,15,20,22]. Table 1 schematizes the subdivisions and their main characteristics.

We already said that Candida spp. are normally found in healthy people, and it is important to note that carrying these fungi cannot signify having a disease. We already explained that the onset of the various diseases attributable to Candida is due to an imbalance between the host immune system and the virulence factors expressed by the fungus. Virulence factors are not expressed when they are harmless parts of the oral microbiota; they are considered the key to its pathological transformation. C. albicans lives in the oral environment as a yeast, and its transformation to hyphae status is believed to be the key to pathological transformation[14,15,20]. Hyphae, being filamentous, facilitate the colonization of the epithelial cells. Colonization is the first step towards pathological transformation together with the expression of its virulence factors, and it is mediated by cell wall receptors, which are able to express several adhesive proteins[14,15,20] such as ALS-3 (Agglutinin-line Sequence) and Hwp1[14,15].

Table 1. Primary and secondary manifestation and Candida-associated lesions and their main features.

Timing and Symptoms	Clinics	Definition	Appearance	Affected	Age sites	Signs
	Pseudomembranous	most common type, it presents as a white plaque on the oral mucosa	white plaque easily detached by gentle scraping, signifying the presence of the hyphae of the fungus (transition to virulence). Only the upper layer of the epithelium is interested	tongue, buccal mucosa, palate, it can be localized or generalized	newborns, aged adults, immunocompromised patients	bad taste, dysgeusia, sometimes can be asymptomatic
First manifestation	erythematous/ atrophic	adverse effect of systemic therapy: broad-spectrum antibiotics, corticosteroids, immunosuppressive drugs	red lucent/atrophic areas caused by depopulation of the tongue	dorsum of the tongue (most of all)	generally adults	burning, dysgeusia
	pseudomembranous	same as the first manifestation but with recurrence				
	erythematous/ atrophic	also known as denture candidiasis: typical of patients with full upper prosthetic appliance	red atrophic area underneath the prosthetic appliance. Lesion is in most cases limited to where the prosthetic lies	most of all hard palate	aged population	burning, swelling, impossibility to wear the appliance, sometimes asymptomatic
Second manifestation	hyperplastic	also known as candida leukoplakia	white hard plaque that cannot be removed by scraping of unknown cause. It carries an increased risk of MT	lateral sides of the tongue, buccal mucosa (especially where commisure forms)	adults, men smokers have increased risk; increased risk in non-smoker women (idiopathic)	asymptomatic
Candida - associated lesions	angular cheilitis	Candida infection of the commissures of the lips	erythematous areas, Candida can be found or not, sometimes co-infection with S. aureus	commissures of the lips	newborn, elder people (loss of vertical height), immucompromised patients, non-collaborative patients	asymptomatic
	median rhomboid glossitis	white lesion on the back side of the tongue	white rhomboidal area due to anatomical predisposition and Candida infection which presents a mold correspondence on palate	back dorsum of the tongue, palate	adults	asymptomatic, bad taste
	chronic mucocutaneous candidiasis	several systemic disorders caused by Candida infection which the organism is not able to defeat	candidemia and involvement of different organs	various systems	elder adults	various (depending on the affected site)

After colonization and adhesion occur, the growth of the fungus and the construction of a biofilm[14,15] protect it from immune system responses and make it spread through epithelial cells. The damage to the cells is attributable to the expression of hyphae-specific toxin, first of all candidalysin[14,15], which creates pores on the surface of the targeted cell. The creation of a biofilm is important not only because C. albicans is protected from the host immune system[14], but even because it facilitates the co-existence of other pathological bacteria. There is a strong association in the literature between the interaction of Candida spp. with anaerobic bacteria, in particular P. gingivalis and F. nucleatum, which are two of the imputative bacteria responsible for periodontitis and whose CFU levels are higher even in OPMDs and OSCC[2].

The very intent of this paper is to note the existing connection between *C. albicans* and OL. After the short consideration we had before in order to frame the protagonists of our work, our focus is now on OL and the interaction with C. albicans in that entity called Chronic Hyperplastic Candidiasis.

Focus on topic (3): Oral Leukoplakia and Candida spp. co-infection: Chronic Hyperplastic Candidiasis

In their last consensus on oral potential malignant disorders published in 2020, Warnakulasuriya et al[1] define OL as a predominantly white plaque of questionable risk, having excluded other known diseases or disorders that carry no increased risk for cancer[1-9]. OL has a worldwide estimated prevalence ranging from 1 to 4%[3-5,7,8]. The biggest concern of OL is its rate of MT, estimated between 0.13 and 34%[11], lower for some authors (around 2-3.5%)[7,9], higher for others (13%)[4] but generally accepted around 9.5%[10], percentage that rises if risk factors are present. Even if, from the definition given, it is clear it can be considered an idiopathic lesion (i), a strong association with tobacco is found. Smoke of cigarettes represents one of the most common risk factors for MT of OPMDs as a rule, and even if there is still no certain evidence, their association is strongly detectable. This could be the reason why in the Asian population the incidence of OL is higher than in others, attesting to around 7.7% [3], because of cultural habits of smoking and chewing tobacco. Other known and associated risk factors are alcohol consumption, male gender, and elder age[1-4,7,8]. Conversely, when the lesion is considered idiopathic and not associable with the over-mentioned risk factors, women and non-smokers have a major possibility of developing a more aggressive lesion[1,4,7,12]. The most affected sites of the oral cavity are the lateral sides of the tongue, the buccal mucosa, and the floor of the mouth[1,3,7,8,12,13,15]. Its presentation can vary and it can be homogeneous, everywhere white-colored and with well-defined borders, or non-homogeneous, where borders are vanished and co-presence of white lesion and red areas can be found, giving rise to an erythron leukoplakia[1-4,7,8,12]. A grade of dysplasia can be found when a biopsy of the lesion is performed. Histopathological analysis is the only method to asset an OL, once excluded all other oral pathologies which can be put on a differential diagnosis – remember that OL is a clinical diagnosis of exclusion[1,3,5,11]. OL with dysplasia, which can be mild, moderate, or severe, is another risk factor that increases the possibility of MT[1-3,8-10,12,13]. OL with a severe or high grade of dysplasia shares some histological features with OSCC, such as altered telomerase activity, positivity to p53, an increase in CD8+ cells, and a general increase in proinflammatory cytokines[1,3,7,9,10,16], so it is easy to understand how important it is to maintain this lesion under surveillance.

Summarizing:

- Tobacco habits and alcohol consumption
- Male gender
- Elder age (>60)
- Site of the lesion: tongue and floor of mouth
- Not-defined borders
- Presence of ulcerations or red areas in the lesion

Dysplasia

In this context, idiopathic lesions are more common in older females and non-smokers and have the highest rate of MT. The dimension of the lesion and its persistence in the oral cavity are two other important factors, adding to the over-cited ones. The rate of MT in OL increases when a co-infection with *C. albicans* is found [10,14], adding 12% of potentiality to the initial one of the OL[10]. Co-infection perpetrated by C. albicans gives rise to an entity called Chronic Hyperplastic Candidiasis (CHC). This illness represents the rarest form of infection of C. albicans[12,16] and is already known as “candida leukoplakia”[10,12,15,16].

About this latter classification, there is no complete acceptance in literature. Warnakulasuriya et al[1] in their Consensus assert that candida leukoplakia and CHC share some aspects but are not the same entity; the same is reported by van der Waal et al[6]. In our opinion, this definition of conflict has no concern because the main intent should be to assume an increased possibility of MT if a co-infection with the fungus is established. Strengthening this fact, Shah et al[16] report that about 7–50% of OL are associated with C. albicans. The sites that are more interested in developing CHC are the same as those in OL: first, the lateral sides of the tongue, followed by the buccal mucosa and floor of the mouth, even if, as with OL, every part of the oral cavity can be affected. CHC develops primarily in young or middle-aged adult men [16], and a co-infection with a *Candida spp*. is found. When OL presents a moderate to severe grade of dysplasia and is accompanied by a fungal infection, the rate of MT rises up to 28% [1]. Moreover, Shah et al[16] report that up to 15% of CHC carries a grade of dysplasia, highlighting the importance of detecting this microorganism on the lesion. *C. albicans* contributes to induced MT by producing carcinogenic substances such as N-nitrosobenzylmethylamine, acethaldehyde, and several other proteinases that are able to degrade the basal membrane of the cell, promote deep infiltration in the epithelial cells, and cause histological changes that bring about aberrant features typical of cancer[2,10,16]. Hence, it is understandable how important it is to defeat the fungal load in order to lower the chances of MT. OL, in fact, has an unpredictable behavior: not all lesions presenting dysplasia progress to cancer, but, at the same time, not carrying features of dysplasia does not represent a minor risk of developing a tumor nearby the lesion or somewhere else in the oral cavity [4,6].

Treatment of OL is based primarily on attesting to the real nature of the lesion. Being OL, a diagnosis of exclusion requires a period of 4–8 weeks where every other possible factor generating a white lesion, such as dental appliances that can cause frictional keratosic irritation, is removed[6,11]; if the lesion presents red areas or ulceration or is symptomatic, an immediate biopsy is mandatory[6,12]. Pharmacological treatment foresees the use of topical bleomicin, Vitamin A and systemic rhetinoids[3,18,26]; surgical excision is the gold standard of therapy when the dimension and the anatomical site where the lesion is found allow[11,18]. Surgery can be performed traditionally, or it could be cryosurgery or LASER ablation[3]. However, it does not lower the risk of recurrence or the risk of developing OSCC [7,26]; from these circumstances, the need to find new non-surgical approaches to cure OL, such as PDT, is undeniable. As already discussed in the first paragraph, PDT against OPMDs and OL gives brilliant results in terms of efficacy and can reduce treatment sessions[18]. PDT with 5-ALA as a photosensitizer is the best acknowledged approach against OL[11,18]. Side adverse effects and traumas to noble structures that could be nearby the lesion are minimally noticed with PDT as opposed to surgical treatment. Pain, swelling, hyperhaemia, and bleeding are little attested[11]. Treatment of OC is, instead, the primary topic and requests three classes of antifungal drugs: polyenes, such as nystatin and amphoterycin B, azoles (miconazole, clotrimazole, and fluconazole) and echinocandins (only in systemic and resistant cases)[13-15,19,24]. Table 2 summarizes their main characteristics. The main issue, as already said before, is that more and more *Candida spp*. are developing resistance to these traditional antifungal drugs[14,21,23,24]. From here, the importance of taking into consideration new non-pharmacological approaches, such as, again, PDT. We already discussed in paragraph one its effectiveness in treating oral diseases, and there is more and more evidence that it can be safely used not only against tumor cells but even against bacteria, viruses, and fungi. Noteworthy and extremely recent is the use of curcumin as a photosensitizer[23], taking advantage of its natural properties as an anti-inflammatory, antioxidant, anti-angiogenetic, anti-septic, anticarcinogenic, and analgesic agent[23-26]. Cessation of risk factors such as smoking and alcohol consumption is the best way to reduce the MT rate of an OL[3,7,16], adding to the annihilation of the fungal load. In the scientific literature, no case is currently reported of eradication of fungal load within lingual leukoplakia, diagnosed with histological examination on biopsy through PDT. Chronic hyperplastic candidiasis is a rare type of candidiasis that causes the formation of a non-removable white plaque, mono or bilateral, much more frequent in the buccal mucosa than in other locations. Our case could be associated with a picture of somewhat atypical chronic hyperplastic candidiasis, although we cannot establish whether it was the fungal infection that caused the onset of the plaque or whether the fungal infection occurred after the formation of leukoplakia. Very few recent works report the use of PDT through 5-ALA as a photosensitizer in cases of chronic hyperplastic candidiasis, alone 27 or in association with topical anti-fungals[27,28]. In one case, the combined use of PDT and laser excision is reported on an 85-year-old patient refractory to pharmacology [29]. The use of a curcumin-based photosensitizer has not been documented in the *in vivo* treatment of this form of candidiasis, although it has proven to be effective on MDR *Candida spp.*[23]. In no work has the fungal load within leukoplastic lesions been evaluated before and after photodynamic therapy. Even in works on the use of PDT in chronic hyperplastic candidiasis, the healing observed is essentially clinical. Evaluation through intraoral brush sampling and cooperation with a Molecular Biology Department could be protocols to be repeated in the case of leukoplakia and more generally in potentially malignant disorders. The association between Candida and Leukoplakia, in fact, highlights

Table 2. Main characteristics of the 3 class of drugs most commonly used in the treatment of OC.

Class of drug	Main characteristics	Clinical notes
Poliens *(Nistatyn* *Amphotericin B)*	Fungicidal action Bind to ergosterol of Candida's cell wall, causing loss of K +	Broad spectrum of action Clinical efficacy against biofilm Nephrotoxicity! To be not used in non compensated diabetes patients
Azoles Imidazoles *(Myconazol,* *Cotrimazol)* Triazoles *(Fluconazol)*	Fungistatic action Inhibit enzymes which bring to the production of ergosterol of the wall cell, causing augmented permeability to the membrane cell High efficacy Low cost No great interaction with other drugs (topic use) Fluconazol is mainly used in severe systemic mycoses	Less side adverse effect Limited absorption when topically used Synergic effect with anticoagulant drugs
Echinocandines	Fungicidal action against *Candida spp.*; fungistatic action against other species; no action against fungi with no cell wall Inhibit enzymes which bring to the production of ergosterol of the wall cell	Mainly used for relapses, systemic involvement and resistant species

a greater predisposition of the patient to cancer. The control of the fungal infection within the leucoplakias and the management with PDT could be part of a prevention protocol that drastically reduces the percentage of cancer risk. Against oral infections the PDT could be considered an integrative approach in association with the conventional therapy. This work highlights a new clinical approach that could reduce fungi infection symptoms, especially when this method is combined with conventional therapy. Indeed, a vision inside integrative medicine is now widely considered in different areas of infective and degenerative diseases[30-41].

CONCLUSIONS

The protocol we decided to use on our patient gave the best result. As already mentioned, we obtained the total elimination of the fungal load from his OL, incredibly lowering the chances of MT. Our patient is, of course, under periodic surveillance, with visits every six months. He was warned, according to literature, that if he notes variations on the surface of the tongue, he has to come immediately in order to assess every possible mutation of the condition. Even if a clear protocol in the treatment of CHC and in the management of OL is not yet assessed in the literature, this short review we performed strengthened our non-pharmacological therapeutic approach. The use of PDT in dentistry, and in particular in OL, is nowadays well documented, even if there is a growing need to find scientific evidence of its efficacy with other photosensitizers, too. We specifically decided to use a mixture of curcumin because of its magnificent therapeutic properties against tumor cells, well supported by the literature. The boost of H_2O_2 we empirically added to the formula was made in order to maximize the production of ROS. Our results, although preliminary, confirmed the efficacy of our protocol. There is still, anyway, the need for more studies in order to scientifically establish new knowledge and a rigid protocol.

Ethical approval:

This study was approved by the Ethic Committee of Cagliari University Hospital, (approval number: NP/2023/2551 del 31/05/2023, Comitato etico indipendente AOU Cagliari). In any case, the Helsinki guidelines were observed.

Informed consent:
The patient was informed about the nature of this research and signed the informed consent.

Availability of data and materials:
The datasets generated during the current study are available from the corresponding author on reasonable request to orru@unica.it

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

Funding:
This study received no external funding.

Author Contributions:
MP participated in the conception and design of the study, in bibliographic research and in the selection of the articles, in the acquisition and analysis of data and in the drafting and revision of the manuscripts and of the images. CC participated in bibliographic research and in reviewing of the manuscript. CC and GO participated in the photographic collection of the presented case. GD and MSM participated in critical revision. CC and GO participated in the correction of the paper and in the final approval of the manuscript. All authors have read and accepted the published version of the manuscript.

ORCID ID:
Cinzia Casu (https://orcid.org/0000-0002-7962-2712); Martina Salvatorina Murgia (https://orcid.org/0000-0002-0504-098X); Gloria Denotti (https://orcid.org/0000-0001-6946-9876); Germano Orrù (https://orcid.org/0000-0003-1032-443).

REFERENCES

1. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MÁ, Kerr AR, Lodi G, Mello FW, Monteiro L, Ogden GR, Sloan P, Johnson NW. Oral potentially malignant
2. disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. Oral Diseases 2021; 27: 1862-1880.
3. Pietrobon G, Tagliabue M, Stringa LM, De Berardinis R, Chu F, Zocchi J, Carlotto E,
4. Chiocca S, Ansarin M. Leukoplakia in the Oral Cavity and Oral Microbiota: A Comprehensive
5. Review. Cancers 2021; 13: 4439.
6. Kumari P, Debta P, Dixit A. Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. Front. Pharmacol 2022; 13: 825266.
7. Saldivia-Siracusa C, González-Arriagada WA. Difficulties in the Prognostic Study of
8. Oral Leukoplakia: Standardization Proposal of Follow-Up Parameters. Front Oral Health 2021; 2: 614045.
9. Woo SB. Oral Epithelial Dysplasia and Premalignancy. Head Neck Pathol 2021; 13: 423-439.
10. van der Waal I. Oral leukoplakia, the ongoing discussion on definition and terminology. Med Oral Patol Oral Cir Bucal 2015; 20: e685-92.
11. van der Waal I. Oral potentially malignant disorders: Is malignant trans¬formation predictable and preventable? Med Oral Patol Oral Cir Bucal 2014; 19: e386-90.
12. Masthan K, Babu NA, Sankari SL, Priyadharsini C. Leukoplakia: A short review on malignant potential. J Pharm Bioall Sci 2015; 7: S165-6.
13. Grigolato R, Bizzoca ME, Calabrese L, Leuci S, Mignogna MD, Lo Muzio L, Leukoplakia and Immunology: New Chemoprevention Landscapes?. Int J Mol Sci 2020; 21: 6874.
14. Lorenzo-Pouso AI, Pérez-Jardón A, Caponio VCA, Spirito F, Chamorro-Petronacci CM, Álvarez-Calderón-Iglesias Ó, Gándara-Vila P, Lo Muzio L, Pérez-Sayáns M. Oral Chronic Hyperplastic Candidiasis and Its Potential Risk of Malignant Transformation: A Systematic Review and Prevalence Meta-Analysis. J Fungi 2022; 8: 1093.
15. Qianming C, Hongxia D, Fan T, Jiongke W, Xiaoying L, Junxin C, Hang Z, Xin Z. Photodynamic therapy guidelines for the management of oral leucoplakia. Int J Oral Sci 2019; 11: 14.
16. Moon-Jong K, Hong-Seop K. Changes in the lesion surface suggesting transformation of oral potentially malignant disorders to malignancy – a report of eight cases. BMC Oral Health 2023; 23: 268.
17. Hellestein JW, Marek CL. Candidiasis: Red and White Manifestations in the Oral Cavity. Head Neck Pathol 2019; 13: 25-32.
18. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral Candidiasis: A Disease of Opportunity. J Fungi 2020; 6: 15.
19. Talapko J, Juzbaši´c M, Matijevi T, Pustijanac E, Beki´c, S, Kotris I, Škrlec I. Candida albicans—The Virulence Factors and Clinical Manifestations of Infection. J Fungi 2021; 7: 79.
20. Shah N, Ray JG, Kundu S, Sardana D. Surgical management of chronic hyperplastic candidiasis refractory to systemic antifungal treatment. J Lab Physicians 2017; 9: 136-139.
21. Prażmo EJ, Kwaśny M, Łapiński M, Mielczarek A. Photodynamic Therapy as a Promising Method Used in the Treatment of Oral Diseases. Adv Clin Exp Med 2016; 25: 799-807.

22. Choudhary R, Reddy SS, Nagi R, Nagaraju R, Kunjumon SP, Sen R. The effect of photodynamic therapy for oral-premalignant lesions: A system¬atic review. J Clin Exp Dent 2022; 14: e285-92.
23. Ferreira GL, Pérez AL, Rocha ÍM, Pinheiro MA, de Castro RD, Carlo HL, Lima Ede O, Castellano LR. Does Scientific Evidence for the Use of Natural Products in the Treatment of Oral Candidiasis Exist? A Systematic Review. Evid Based Complement Alternat Med 2015; 2015: 147804.
24. Singh A, Verma R, Murari A, Agrawal A. Oral candidiasis: An overview. J Oral Maxillofac Pathol 2014; 18: 81-85.
25. Quindós G, Gil-Alonso S, Marcos-Arias C, Sevillano E, Mateo E, Jaure¬gizar N, Eraso E. Therapeutic tools for oral candidiasis: Current and new antifungal drugs. Med Oral Patol Oral Cir Bucal 2019; 24: e172- 180.
26. Baumgardner DJ. Oral fungal microbiota: to thrush and beyond. J Patient Cent Res Rev 2019; 6: 252-261.
27. Casu C, Orrù G, Scano A. Curcumin/H2O2 photodynamically activated: an antimicrobial time-response assessment against an MDR strain of Candida albicans. Eur Rev Med Pharmacol Sci 2022; 26: 8841-8851.
28. Anwar SK, Elmonaem SNA, Moussa E, Aboulela AG, Essawy MM. Curcumin nanoparticles: the topical antimycotic suspension treating oral candidiasis. Odontology 2023; 111: 350-359.
29. Salehi B, Lopez-Jornet P, Pons-Fuster López E, Calina D, Sharifi-Rad M, Ramírez-Alarcón K, Forman K, Fernández M, Martorell M, Setzer WN, Martins N, Rodrigues CF, Sharifi-Rad J. Plant-Derived Bioactives in Oral Mucosal Lesions: A Key Emphasis to Curcumin, Lycopene, Chamomile, Aloe vera, Green Tea and Coffee Properties. Biomolecules 2019; 9: 106.
30. Ahmad T, Khan I, Rizvi MM, Saalim M, Manzoor N, Sultana A. An overview of effect of lycopene and curcumin in oral leukoplakia and oral submucous fibrosis. Natl J Maxillofac Surg 2021; 12: 316-23.
31. Li B, Fang X, Hu X, Hua H, Wei P. Successful treatment of chronic hyperplastic candidiasis with 5-aminolevulinic acid photodynamic therapy: A case report. Photodiagnosis Photodyn Ther 2022; 37: 102633.
32. Al Ghamdi AS, Qamar Z, AlSheikh R, Al Hinai MTA, Abdul NS, Aljoghaiman EA, Ali S. Clinical efficacy of 5-aminolevulinic acid-mediated photodynamic therapy versus topical antifungal agent and surgical excision for the treatment of hyperplastic candidiasis. Photodiagnosis Photodyn Ther 2023; 41: 103258.
33. Zhang W, Wu S, Wang X, Wei P, Yan Z. Combination treatment with photodynamic therapy and laser therapy in chronic hyperplastic candidiasis: A case report. Photodiagnosis Photodyn Ther 2022; 38: 102819.
34. Berretta M, Dal Lago L, Tinazzi M, Ronchi A, La Rocca G, Montella L, Di Francia R, Facchini BA, Bignucolo A, Montopoli M. Evaluation of Concomitant Use of Anticancer Drugs and Herbal Products: From Interactions to Synergic Activity. Cancers (Basel) 2022; 14: 5203.
35. Berretta M, Morra A, Taibi R, Monari F, Maurea N, Ippolito M, Tirelli U, Fiorica F, Montella L, Facchini G, Quagliariello V, Montopoli M. Improved Survival and Quality of Life Through an Integrative, Multidisciplinary Oncological Approach: Pathophysiological Analysis of Four Clinical Cancer Cases and Review of the Literature. Front Pharmacol 2022; 13: 867907.
36. Berretta M, Rinaldi L, Taibi R, Tralongo P, Fulvi A, Montesarchio V, Madeddu G, Magistri P, Bimonte S, Trovò M, Gnagnarella P, Cuomo A, Cascella M, Lleshi A, Nasti G, Facchini S, Fiorica F, Di Francia R, Nunnari G, Pellicanò GF, Guglielmino A, Danova M, Rossetti S, Amore A, Crispo A, Facchini G. Physician Attitudes and Perceptions of Complementary and Alternative Medicine (CAM): A Multicentre Italian Study. Front Oncol 2020; 10: 594.
37. Berretta M, Bignucolo A, Di Francia R, Comello F, Facchini G, Ceccarelli M, Iaffaioli RV, Quagliariello V, Maurea N. Resveratrol in Cancer Patients: From Bench to Bedside. Int J Mol Sci 2020; 21: 2945.
38. Berretta M, Della Pepa C, Tralongo P, Fulvi A, Martellotta F, Lleshi A, Nasti G, Fisichella R, Romano C, De Divitiis C, Taibi R, Fiorica F, Di Francia R, Di Mari A, Del Pup L, Crispo A, De Paoli P, Santorelli A, Quagliariello V, Iaffaioli RV, Tirelli U, Facchini G. Use of Complementary and Alternative Medicine (CAM) in cancer patients: An Italian multicenter survey. Oncotarget 2017; 8: 24401-24414.
39. Berretta M, Quagliariello V, Bignucolo A, Facchini S, Maurea N, Di Francia R, Fiorica F, Sharifi S, Bressan S, Richter SN, Camozzi V, Rinaldi L, Scaroni C, Montopoli M. The Multiple Effects of Vitamin D against Chronic Diseases: From Reduction of Lipid Peroxidation to Updated Evidence from Clinical Studies. Antioxidants (Basel) 2022; 11: 1090.
40. Bozali K, Guler EM, Celikten M. Investigation of the effect of curcumin on cytotoxicity, genotoxicity, and apoptosis on breast cancer cells. World Cancer Res J 2022; 9: e2149.
41. Bozorgi M, Karami A, Khazaei F, Khazaei M. In vitro survey on the synergistic effect of Cichorium intybus L. and doxorubicin on apoptotic induction in myeloid (KG-1) and lymphoid (NALM-6) cell lines. World Cancer Res J 2022; 9: e2157.