

# Clinical and Histopathological Evaluation of Photodynamic Therapy Associated with The Low-Level Laser Therapy on Patients with Actinic Cheilitis - A Six-Month Follow-Up Trial

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• **Conflicts of interest:** none declared.

## ABSTRACT

**Objective:** the aim of the present study was to evaluate the clinical and histopathological efficacy of photodynamic therapy (PDT) associated with the low-level laser therapy (LLLT) in the treatment of patients with Actinic Cheilitis (AC) after a six-month follow-up. **Material and Methods:** patients with active AC undergone through two sessions of PDT, associated with the low-level laser therapy using the Methyl 5-aminolevulinate as a photosensitizer drug. After a six-month follow-up, a new incisional biopsy was performed in order to evaluate the response to treatment. **Results:** excellent efficacy rates were observed in the improvement of symptoms in this study, however, histopathological analysis was not so promising and the improvement of dysplasia degree could only be seen in 20% of the sample. **Conclusion:** according to the results of the present study, we concluded that the photodynamic therapy associated with low-level laser therapy is an option to be considered when treating Actinic Cheilitis. However, patients should be clinically followed-up in a severing agenda and eventually, histopathologically.

**Keywords:** Actinic cheilitis; Photochemotherapy; Low-level light therapy; Biopsy.

## Introduction

Actinic cheilitis (AC) consists of a potentially malignant disorder often shown on the vermilion of lower lip, first described in 1923, due to excessive and chronic exposure to ultraviolet radiation from sunlight, especially Ultraviolet B rays.<sup>1</sup>

AC commonly occurs on white-skinned male individuals, over 45 years-old, mainly in those who work in open-air activities, such as sailors and farm workers.<sup>2,3</sup> Initial alteration often consists in a vermilion atrophy, marked by an even surface, with white plaques; its progression results in the occurrence of squamous surfaces on the vermilion. AC can appear as located or diffuse lesions. These lesions - usually asymptomatic - are white, red, or white with reddish areas.<sup>2,3</sup>

With the evolution of the disease, chronic ulcerations occur in one or more sites and may last months, deeply suggesting a progression to squamous cell carcinoma (SCC). Previous studies suggest that 95% of vermilion squamous cell carcinoma evolve from an actinic cheilitis.<sup>3</sup>

At the histopathological exam, it can be observed the atrophy of the stratified squamous epithelium, with a remarkable production of keratin. Subjacent connective tissue shows degeneration of collagen fibers named as solar elastosis. Multiple grades of epithelial dysplasia can appear and the chances to evolve into a SCC are directly proportional to its severity.<sup>4</sup>

In cases presenting absence of malignant transformation at sight, a chirurgical procedure by vermilionectomy might take

place, consisting in a total or partial vermilion excision. Other options of treatment include CO<sub>2</sub> or erbium laser ablation: YAG, electrosurgery, topical 5-fluorouracil and topical trichloroacetic and photodynamic therapy (PDT).<sup>5</sup>

Photodynamic therapy has been often applied for multiple and diffuse lesions treatments, mainly in actinic cheilitis treatment. It consists in the interaction of three agents: light, the photosensitizer and oxygen. Cellular death is induced by the formation of reactive oxygen species (EROs), formed through the photoactivation of the photosensitizer. Your main limitation is the warranty of the presence of light, oxygen and photosensitizer in sufficient amounts on the tissue targeted by the photodynamic effect.<sup>6,7,8</sup>

Methyl 5-aminolevulinate is a compound commonly applied at PDT, being the engine of the Protoporphyrin IX - which is a photosensibilizer. When choosing the source of light for PDT, it must be considered the light penetration rate in the tissue, but also it must adapt to excite the photosensibilizer.<sup>6,7,8</sup>

Regarding the PpIX, the 630 nm red-light spectrum presents deeper penetration in tissues and absorption by the Protoporphyrin IX, thus producing a higher quantity of PpIX in the targeted tissues, which justifies the link with the low-level laser in this treatment. When the light interacts with the photosensibilizer, it ignites a series of chemical reactions.<sup>6,7,8</sup>

The main reaction happens through the transference of the photosensibilizer molecular energy to the molecular oxygen

in the tissue, leading to the formation of ROS in the tissue. The most important is the singlet oxygen, capable of oxidizing cells, leading to necrosis or apoptosis.<sup>6,7</sup>

The molecular oxygen concentration inside the cells are a key activity for PDT. This concentration decreases during the treatment, because the oxygen molecules are consumed on photochemical reactions, in addition to the own photosensibilizer molecule. The consumption rate of these substances by the tissues can be monitored through the blue-light fluorescence diagnostic. Therefore, PDT is considered a minimum invasive treatment for Actinic Cheilitis.<sup>7</sup>

Thus, the goal of the present study was to evaluate the clinical and histopathological efficacy of PDT, associated with the low-level laser therapy (LLLT) on the treatment of patients with AC after a six-month follow-up.

### Material and Methods

This research was an experimental and prospective study, based on the analysis of the medical records of patients with actinic cheilitis, from 2016 to 2020, which had been treated with PDT, associated with the low-level laser therapy in the Stomatology clinic of the Dentistry school of State University of Rio de Janeiro.

#### Ethical Considerations

This study was submitted and approved by the Research Ethics Committee of the Hospital Universitário Pedro Ernesto, Rio de Janeiro State University, under protocol No. 3.177.086 (CAAE: 91956616.3.0000.5259).

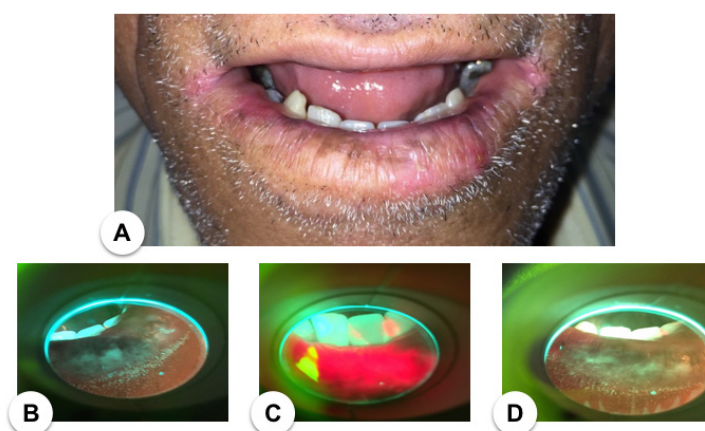
#### Study Design and Protocol

Patients with the clinical hypothesis of AC were submitted to an incisional biopsy and the specimen was driven to the oral pathology lab located in the Dental School of the State University of Rio de Janeiro for histopathological exam. After diagnosis, those who showed AC with severe, moderate or mild degree of dysplasia met the eligibility criteria of this study - a total of twenty patients - and were submitted to the PDT associated with the low-level laser therapy.

At the first session, patients were informed regarding the lesion nature and PDT. They signed an Informed Consent Form and answered to a standard medical record which contained the following items: 1) Identification; 2) Phototype (regarding Fitzpatrick classification); 3) The use of lip balm; 4) Solar exposition during work, sub-classified on: Always; Often; Rarely; None; 5) Habits (tobacco or alcohol consumption).

Also at the first PDT session, a standardized photography of the lesioned area was taken and the blue light photodiagnosis - which is a non-invasive optical diagnosis method, capable of identifying real-time differences between a healthy tissue and the lesioned one - was performed. When applying the

medication, detrits were removed from the lip using gauze compresses soaked in cold saline solution and then, a 1.0mm layer of the photosensibilizer cream MAL (PDT-PHARMA, Brazil) was spreaded in all semi-mucosa of the lower lip. Then the surface was occluded as the drug was covered by a thin layer of film paper, laminated paper and finally a bandage with gauze. Patient kept waiting for three hours. After this period, the bandage was removed and a new blue light photodiagnosis was performed in order to check the formation of protoporphyrin IX in the region. Finished these steps, PDT treatment with LLLT carried out with a wavelength between 630-640 nm. The light intensity was manually chosen on the device - LINCE (MMOptics LTDA) - at 125 mW / cm<sup>2</sup>, radiated over eighteen minutes. Figure 1 shows the blue light photodiagnosis sequence.



**Figure 1.** Blue light photodiagnosis sequence (A) Clinical findings of Actinic Cheilitis at the first PDT (photodynamic therapy) session. (B) Blue light photodiagnosis before applying the photosensibilizer. (C) Blue light photodiagnosis after the 3-hour period 5-Aminolevulinic acid methyl ester hydrochloride use. (D) Blue light photodiagnosis right after photodynamic therapy (PDT).

Pain data were collected with the aid of a visual analog scale from zero (no pain) to ten (unbearable pain), during the irradiation every two minutes. A final blue light photodiagnosis was performed to check the consumption of PpIX in the tissue, along with a standardized photograph. At the end of the session, the patient was instructed regarding the care and received a table containing a pain scale from zero to ten - which should be completed during the next seven days - and also if there was the need for the use of oral analgesics such as dipyrone for pain.

The second session was scheduled after seven days, following the same steps as described for the first one.

One month after the second session, the patients returned for a clinical follow-up. After six-month follow-up, a new incisional biopsy at the same area as the previous one was performed for histopathological analysis in order to confirm healing, persistence or worsening of the initial lesion.

### Data Analysis

From the data collected in the research, the following variables were analyzed: Sex; Age; Phototype; Use of lip balm; Initial diagnosis; Average pain score; Presence of painful symptoms after the procedure; Clinical appearance six months after PDT and the final diagnosis, which were organized in the form of a table at the Microsoft's Excel program.

### Results

Our sample first included 20 patients initially who undergone PDT in which: one patient left after the first session; seven did not return after the six-month period to be submitted for the final biopsy and two other patients are still on follow-up, resulting on a final sample of ten patients (n = 10). Male patients prevailed (70%), with an average age of 57.6 years. The most prevalent phototype was type I (70%), followed by type II (20%) and only one case (10%) presented with type III. It was observed that half of the patients did not use lip balm prior to diagnosis. Table 1 summarizes the epidemiological profile of the patients.

**Table 1.** Epidemiological profile of the patients included in the sample.

Patient	Age	Sex	Phototype	Lip Balm use	Habits/Addictions	Solar Exposure
1	43	Female	I	Yes	None	Rarely
2	53	Male	III	No	None	Often
3	62	Male	I	Yes	None	Rarely
4	67	Male	I	No	Alcoholism	Often
5	66	Male	II	Yes	None	Often
6	52	Female	I	Yes	None	Rarely
7	62	Male	II	No	Alcoholism	Always
8	58	Male	I	No	None	Often
9	58	Male	I	Yes	None	Often
10	55	Female	I	No	None	Rarely

All irradiated patients obtained their data collected through a visual pain scale during therapy, resulting in an average score of 4.5 - considering values from both sessions - and higher levels of pain were reported at the first five minutes of the therapy and then progressively decreased until the eighteenth minute. In addition, 60% (n = 6) felt pain after the procedure, with a mean score of 7.1 and oral analgesic administration (one 500mg Dipyron Sodium tablet, every 6 hours) was necessary for symptom relieving.

The response to PDT was evaluated, first in relation to clinical symptoms, which can be classified as pain, dryness or asymptomatic. For the 10 patients evaluated, 50% showed improvement of clinical symptoms (Figure 2) and 30% remained asymptomatic, totalizing 80% of patients with asymptomatic results after 6 months. Besides this, in two cases (20%), the persistence and/or worsening of symptoms



**Figure 2.** Initial and Final clinical aspect of Actinic Cheilitis. A, C ,E - Before photodynamic therapy (PDT) and B, D ,F - After photodynamic therapy (PDT)

such as pain and dryness of the lip vermilion were reported. Table 2 summarizes the symptoms reported by patients before and after treatment.

Despite the effectiveness of PDT in relieving symptoms, some difference of pigmentation can appear in the treated region and were still observed after the final period of treatment: in 40% of the cases it was possible to observe the presence of hypochromic scars; 20% showed hypertrophic scars at the treated site and 10% showed an atrophic scar. Superficial mucosal ulceration could be seen in 10% of patients and in only 20% some level of residual lesion was visible.

In addition to the clinical evaluation, a histopathological exam was performed. After a six-month follow-up, 30% of patients showed a reduction in the degree of dysplasia - 2 patients progressed from mild dysplasia to their absence

after PDT; 60% of the patients did not change the level of dysplasia - remaining with mild dysplasia; Only 10% showed exacerbation in levels of dysplasia, evolving from moderate into intense. Table 3 shows the levels of dysplasia before and after PDT.

**Table 2.** Presented symptoms before and after the photodynamic therapy (PDT) associated with the low-level laser therapy. The results show the improvement regarding the symptoms in 50% of patients.

Patient	Before PDT	After PDT
1	Dryness	Assymptomatic
2	Assymptomatic	Assymptomatic
3	Pain; Dryness	Assymptomatic
4	Dryness	Assymptomatic
5	Assymptomatic	Assymptomatic
6	Dryness	Assymptomatic
7	Pain	Pain
8	Pain	Pain
9	Dryness	Assymptomatic
10	Assymptomatic	Assymptomatic

Patients in which the degree of dysplasia had not been altered or who had achieved the remission of dysplasia, kept on follow-up with periodic tests of Toluidine Blue every six months; patients who had severe dysplasia at the end of the treatment were referred for vermilionectomy surgery and are on clinical follow-up.

**Table 3.** Degrees of dysplasia before and after the PDT associated with LLLT (low-level laser therapy).

Patient	Before PDT	After PDT
1	Mild	Mild
2	Mild	Mild
3	Moderate	Mild
4	Mild	Absence
5	Mild	Mild
6	Mild	Mild
7	Moderate	Severe
8	Severe	Severe
9	Mild	Absence
10	Mild	Mild

## Discussion

Actinic cheilitis is a potentially cancerous disorder that commonly affects white-skinned men in their fifth decade of life. Furthermore, the lower lip is the most affected region, precisely due to its greater exposure to UV sun rays.<sup>1,2</sup> In our study, most patients were male; white-skinned and all the

AC lesions were located at the lower lip. Early diagnosis and effective treatment of actinic cheilitis is essential to prevent its malignant progression to the squamous cell carcinoma.

Photodynamic therapy is a non-invasive therapeutic modality which has the ability of treating large and injured areas at a single time and has good cosmetic results. It is often considered in the treatment of actinic keratosis - as a conservative option - and its use has increased on the treatment of Bowen's disease and basal cell carcinoma.<sup>8,9,10,11</sup>

The early cases report of researches using PDT in the treatment of actinic cheilitis showed optimistic results. However, these studies only evaluated the clinical perspective of therapy, without further in-depth follow-up.<sup>8,9,10,11</sup> The present study analyzes the use of PDT on the treatment of actinic cheilitis from a clinical and histopathological point of view, with a six-month follow-up.

Immediate pain during the procedure is an expected adverse effect of PDT. A prospective study that treated 141 lesions in 108 patients has associated phototypes I and II as those of most risk for high levels of pain, since body-skins with a higher concentration of melanin have less light penetration resulting in less singlet oxygen formation and less tissue damage.<sup>12</sup> The study carried out by *Chaves et al* used local anesthesia with 2% lidocaine prior to PDT, in order to minimize pain during the procedure which culminated in an average score of 3.94 in the first session and 4.0 in the second.<sup>13</sup> In our research, we did not perform local anesthesia before irradiation which may have contributed to a higher score of pain during therapy, in addition to the fact that we obtained the majority of patients with phototype I and II - related to higher levels of pain. Despite the presence of pain after PDT was reported as one of the most common adverse effects, our study obtained a higher score than other studies,<sup>11,13</sup> with an average rate of 7.1.

*Chaves et al*<sup>13</sup> treated 16 patients with AC and observed the complete absence of clinical signs in 62.5% of their sample. Another study by *Berking et al*<sup>14</sup> treated 15 patients, using the same photosensitizing agent used in our study, detecting a total absence of signs in 47% and a partial absence in another 47%. The present study did not reveal complete remission of the clinical signs of AC - a fact that perhaps can be explained by the number of our sample were lower than those reported by others. Although other studies have also reported improvement in symptoms,<sup>7,14</sup> our study obtained an excellent efficacy regarding the improvement of the symptoms - specially the painful sensation and dryness - reported by the patients, since persistence or exacerbating was only observed in 20% of cases.

Despite the evident clinical improvement using the PDT, other studies that also evaluated their patients histopathologically, reported the persistence in the degrees of dysplasia. *Berking et al* obtained histological cure in only 38%

of their sample.<sup>14</sup> *Sotirou et al* reported a favorable histological response in 73% of patients, however in their study, PDT was combined with the use of 5% imiquimod (3 days a week for 4 weeks), which may have contributed to this result.<sup>6</sup> In contrast to these two studies, *Chaves et al* reported that none of their patients were cured after histopathological analysis.<sup>13</sup> In our study, 20% were successful - showing absence of dysplasia after the therapy.

Studies using PDT in the treatment of actinic keratosis used the Methyl 5-aminolevulinic acid as a photosensitizer drug, demonstrating positive clinical and histopathological responses.<sup>15,16,17</sup> According to the obtained results, it is evident that PDT does not have the same histopathological response for actinic cheilitis as it does for actinic keratosis, due to the fact that the lips are a very moist anatomical region and that the patient is hardly able to remain immobile, which can impair the retention of the medication in the desired site.

The standard treatment for AC includes invasive options such as vermilionectomy - indicated for diffuse cases of AC and with severe dysplasia - and others less-conservative forms for those presenting mild to moderate dysplasia, such as the CO<sub>2</sub> laser ablation.<sup>18-20</sup> It is observed that higher cure rates are obtained with these therapeutic modalities, despite their adverse effects.<sup>21</sup> Although 20% of the patients in this study were referred for vermilionectomy, PDT showed excellent clinical improvement in most presented symptoms. However, the histopathological analysis showed the need of improving the protocol of photodynamic

therapy associated with low-level laser therapy in the treatment of actinic cheilitis. This search for an efficient PDT protocol for AC can bring a non-invasive treatment and can benefit several patients with this type of lesion.

## Conclusion

According to the results of the present study, it can be concluded that the photodynamic therapy associated, with the low-level laser therapy seems to be an option to be considered when treating Actinic Cheilitis. However, patients should be clinically followed-up in a severing agenda and eventually, histopathologically.

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