Topical Tocopherol for treatment of reticular oral lichen planus: randomized, double-blind, crossover study

Received Date : 15-Apr-2016

Revised Date : 19-Jul-2016

Accepted Date : 27-Jul-2016

Article type : Original Manuscript

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Running title:

Tocopherol in oral lichen planus treatment

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Date of submission: 15 April 15, 2016

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ABSTRACT

Objective: This randomized, double-blind, placebo-controlled crossover study assessed the efficacy of topical tocopherol acetate compared with placebo in easing oral discomfort in patients with reticular oral lichen planus (ROLP).

Materials and Methods: 34 patients with clinically diagnosed and histologically confirmed ROLP were randomly assigned to two groups, which received first one of two treatments (treatment 1 or 2) for a month, then the other (treatment 2 or 1) for another month, with a two-week washout between them. One treatment contained tocopherol acetate, the other only liquid paraffin. The primary outcome was less discomfort, measured on a visual analog scale (VAS). Secondary outcomes were: length of striae measured and photographed at each follow-up; surface area of lesions; and a modified Thongprasom score.

Results: No statistically significant differences emerged between the two treatments (1 vs 2) in terms of VAS scores ($P>0.05$; 0.8624), or length of striae ($P=0.0883$). Significant differences were seen for surface area of lesions ($P<0.05$, $P=0.0045$), and modified Thongprasom scores ($P=0.0052$).

Conclusion: The two treatments differed only in terms of the surface area of the lesions and Thongprasom scores, not in VAS scores for discomfort or the length of patients' striae. Topical tocopherol proved effective in the treatment of ROLP.

Keywords: oral lichen planus, tocopherol, reticular oral lichen planus, ImageJ, Thongprasom scale

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disease affecting the stratified squamous epithelium (Eisen D et al.). It occurs quite frequently in the general population, with a prevalence of 1.27% and a male/female ratio of 2:3. It mainly involves adults from 30 to 60 years old (Lodi G et al.).

The etiology of OLP has yet to be fully clarified, but various hypotheses have been advanced, including the idea of an autoimmune condition: processing of the antigen by the dendritic cells (Langerhans cells) and its presentation to the T lymphocytes leads to activation of the CD4+ and CD8+ T cells and apoptosis of the basal cells. Some authors suggest a correlation with infectious factors such as HCV, HSV2, HIV, Candida, and Treponema pallidum, which prompt activation of the lymphocytes, which thus become cytotoxic for the keratinocytes of the basal layer. These infectious factors thus allow the disease to become manifest (Roopashree MR et al, Romero MA et al, Lodi G. et al.).

Psychosomatic and emotional factors also seem to play an important part in the onset of OLP. Its clinical manifestations often develop in subjects who have been under severe stress. This has been confirmed by epidemiological evidence of patients with OLP having high levels of anxiety and depression, and a greater vulnerability to psychological disorders (Soto Araya M et al.).

The lesions may appear clinically in a reticular pattern or form papules or plaques, or it may be described as atrophic and ulcerative, or bullous (erosive) (Lodi G et al.).

In 2003, van der Meij EH, van der Waal suggested a type of classification which reconsiders the classification WHO of 1978 (WHO 1978), on the basis of a scarce correspondence between the clinical suspicion of lichen and the histopathological observation of the biopsy (Van Der Meji & Van Der Waal, 2003).

The reticular form usually causes no symptoms (Neville BW et al), but sometimes it can cause severe discomfort to the patient, mainly when the tongue touches the lesions or the food bolus passes in the mouth. As stated by some authors (Yang H. et al.), the treatment of OLP is symptomatic, so the patients decided to participate to the study because they felt some kind of discomfort. Occasionally, however, patients may develop Candida superinfections, which cause a burning sensation in the oral mucosa, making anti-fungal therapy necessary in such cases. (Neville BW et al.).

On the other hand, erosive OLP gives rise to a distinct set of symptoms. In recent decades, various treatments have been attempted with a view to reducing the lesions and easing the associated pain, but no definitive treatment has been identified to date (Lodi G et al.), partly because of the refractory nature of this disease (Lozada-Nur F & Miranda C; Lodi G et al.; Eisen D et al.).

Topical corticosteroids have been amply used to treat vesicular-erosive diseases of the oral mucosa (including OLP) in an effort to reduce the related pain and inflammation (Neville BW et al.; Gonzalez-Moles MA & Scully C.). A certain utility has been demonstrated for some of these preparations, when applied directly to the lesions. They are beneficial in controlling the disease because of their anti-inflammatory effects and immunomodulation properties, making them capable of suppressing T cell function (Yoke PC et al.). Systemic therapy is necessary in some cases, and steroid dosage must be tailored to individual patients

depending on the nature of their lesions, the patients' weight, and their response to the treatment (Al-Hashimi I et al.).

Topical or systemic tacrolimus has proved effective, but this drug is potentially carcinogenic (Lozada-Nur FI & Sroussi HY).

For this type of disease, six-monthly follow-up is mandatory in order to monitor the patient's condition adequately (Epstein J.B. et al.)

An essential vitamin vital to human nutrition, tocopherol is a powerful liposoluble antioxidant contained in many plants. Vitamin E plays a fundamental part as an antioxidant factor in preventing oxidation of polyunsaturated fatty acids, a key event in the process of lipid peroxidation. (Kim SK et al., Heidari R et al, Niki E.) Since the onset of lipid peroxidation can give rise to profound changes in the cell membranes, vitamin E is acknowledged an important role in keeping these structures intact (Brigelius-Flohe R & Traber MG; Dutta A & Dutta SK). More detailed studies have demonstrated that tocopherol interacts directly with protein phosphatase 2A (PP-2A), which subsequently dephosphorylates various cell substrates, including some isoforms of protein-kinase C (PKC). Thanks to this mechanism, tocopherol can also behave as an antiproliferative agent against tumor cells. The existence of the universal mechanism of alpha-TPh cytoprotective action that does not depend on the nature of apoptogenes and realized on the general for the majority of them stage of the cells death induction is stated. (Petrova GV et al., Neuzil J et al.) Tocopherol has been used in the past for the prevention and treatment of oral mucositis in adults (Wadleigh RG et al.; Lopez I et al.) and children (El-Housseiny AA. et al.). In most studies, the role of vitamin E in maintaining and repairing the cell membranes has been well documented (Khurana H. et al.).

The present double-blind, randomized, placebo-controlled crossover study was devised to assess the efficacy of topical tocopherol acetate in improving the lesions and oral discomfort of patients suffering from reticular oral lichen planus (ROLP), comparing it with the results achievable by administering placebo. The term discomfort was used referring to "tactile discomfort", when the tongue impacted the lesion on the mucosa.

MATERIALS AND METHODS

The study involved patients with a clinical diagnosis of ROLP, histologically confirmed as established by some authors (Al-Hashimi I et al.), all routinely followed up at the Dental Clinic of Padua University Hospital (Ambulatorio di Medicina e Patologia Orale).
Patients were enrolled according to the following criteria:

Inclusion criteria

- Patients with lesions more than a year;
- patients who, after being informed in adequate detail, agreed to be examined and assessed at each follow-up;

- patients at least 18 years old and in full possession of their mental faculties;
- patients with ascertained oral lesions, but possibly also with cutaneous or genital lesions.
- Patients experiencing "tactile discomfort", when the tongue impacted the lesion on the mucosa or the food bolus touches the lesions

Exclusion criteria

- patients with a clinical or histological diagnosis of erosive, bullous, or plaque-forming oral lichen planus;
- patients under treatment with immunomodulatory drugs;
- patients who might have previously received conventional treatments (in the previous two weeks);
- patients refusing to give their informed consent to the trial;
- patients who might have previously taken antifungal drugs for the prevention or treatment of Candida superinfection (in the previous two weeks);
- Patients with concomitant oral pathologies (e.g. aphtosis).

Patients were considered independently from the localization of the lesions.
The detailed medical record was collected, particularly the eventual presence of other autoimmune and dermatological pathologies. Thus, it was also taken into account the fact to be a smoker or not.

Patients were divided into two groups (A or B), each of which was administered two different treatments sequentially (group A: tocopherol then placebo; group B: placebo then tocopherol). Patients were randomized to either group based on a list prepared with an appropriate software (SAS 9.2 for Windows, SAS Institute Inc., Cary, NC, USA). The treatment sequences for each patient were anonymized and sealed in envelopes marked with patient identification numbers, based on their order of enrolment in the study (patient 1, 2, 3, etc.) and the envelopes were only opened after a patient had been included in the study. The complete randomization list and the correspondence between the anonymous codes and the treatments was kept by a person uninvolved in the trial. Both groups were given their first treatment for one month, then waited a two-week interval (washout period) before starting the other. One treatment consisted of a gelly formulation of tocopherol acetate, the other (placebo) of a gelly formulation of Vaseline BFR070 FU (paraffinum liquidum) and its composition was identical to that of the other product except for the absence of tocopherol (Picture 1). The supplier identified the two products with two numerical codes (347/2 and 348/2) and the product corresponding to each code was not known to the experimenters or the patients. Group A received the treatment in the sequence 347/2 → 348/2, and group B vice versa.

Patients were instructed to apply the gel using the applicator given by the supplier in the entire inner part of the oral cavity, but more carefully in the sites indicated by us as most affected by the reticular lesions; this practice had to be made for three times per day, daily

until the next visit. Patients were also told neither to eat nor to drink in the thirty minutes following the application of the product.

The request for approval by the ethics committee was sent in August 2012, the final approval was obtained in December 2012 (Prot.n.2769P), the patient recruitment started in April 2013. For each patient, the study lasted a total of 10 weeks. Patients received no compensation for their participation in the study.

Assessment parameters

Patients were examined four times in all: at the baseline; after the first treatment (lasting four weeks); at the end of the washout period (after another two weeks); and at the end of the second treatment (lasting another four weeks). The primary outcome of interest was an improvement in patients' discomfort, measured using a visual analogue scale (on a range from 0 to 10) (Wu Y et al.). Any reported unpleasant sensation, pain, burning or itching was recorded, and contributed to defining the overall level of discomfort and the quality of disease control on the VAS (scores ranging from 0 to 10). A record was also kept of anything patients reported concerning their perception of flavours and any changes their condition had prompted in their diet and digestion. As secondary outcomes, we examined the trend of the reticular lesions. This was assessed by measuring the length and surface area of the lesions with a gauge, taking photographs at the time of each visit, and comparing them with the ImageJ software (Wayne Rasband, US National Institute of Health, Bethesda) This program standardizes images on the basis of a measure of known length, and has been used by several authors for the purpose digital image analysis). (Kerner S. et al, Lops D et al.). Eight standard photographs were taken: one frontal, one direct of each vestibular portion, two supported by the lateral mirrors of the attached gingiva and vestibular fornixes, another two under indirect view of the palate and floor of the mouth, and one of the tongue (Picture 2, 3, 4). Each photograph was also adjusted in relation to the measurements obtained clinically at each visit. In addition, a scale based on the clinical criteria developed by Thongprasom was adopted, generating a score where: 0= no lesions; 1 = white striae with no signs of erythema; 2 = white striae with areas of atrophy <1 cm^2; 3 = white striae with areas of atrophy >1 cm^2; 4 = white striae with areas of erosion <1 cm^2; 5 = white striae with areas of erosion >1 cm^2 (Thongprasom K et al.) This score was modified for the purposes of the present study, considering only grades 0 to 3, because any presence of erosive lesions was one of our exclusion criteria.

Statistical analyses

The numerosity of the sample was based on the expectation of a difference between the two treatments of one point on the VAS (primary variable) with a standard deviation of 2 (the standard deviation considered was for within-subject differences). The number of patients with ROLP needed for this percentage to be significant was estimated to be 34, with a power of 80% and an α of 5% bilaterally. The Shapiro-Wilk test was used to check the normality of the quantitative data.

The patients' baseline characteristics are presented by treatment sequence (1→2/2→1). Categorical variables were presented as counts and percentages in each sequence, and compared with the chi-square or Fisher's exact tests. For quantitative variables, median, minimum and maximum values were reported and analyzed with Wilcoxon's rank sum test because the distribution was not normal.

Results for the outcome variables are presented by treatment group, reporting the median, minimum and maximum values at the baseline and at the end of each treatment, and compared with a mixed-model analysis of covariance on the rank-transformed values, considering the baseline value as the covariate and the period and treatment as the factors. The level of significance was set at 5%. The analyses were performed with the SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) for Windows.

RESULTS

Of the 34 patients enrolled, 33 completed the study (one was uncooperative and failed to do it, so it was considered drop-out); of these patients, 22 were women, and 11 were men. In this sample, 32 patients were non-smokers and one female patient smoked. Four patients had a history of hepatitis (two were HBV-positive, and two HCV-positive). Seven patients had a positive clinical history of dermatological diseases such as seborrhoea, androgenetic hair loss, alopecia, contact dermatitis, eczema, and erythema. The localization of the lesions was posterior buccal mucosa, bilaterally; the back, the lateral margins and the belly of the tongue, the gums, the palate (Neville BW et al.) and the lips (Romero MA et al.). Three of our patients (9.09%) present also vulvo-vaginal lesions.

The results are summarized in Tables 1 and 2.

Baseline characteristics of the patients by sequence of randomization were presented at table 1.

Median (min-max) for VAS score was 1.85 (0.10-7.65) for all patients, 2.40 (0.10-7.65) for AB sequence and 1.73 (0.20-7.00) for BA sequence with p-value =0.6653.

Median (min-max) of Thongprasom score was 1(0-3) for all patients, 2(0-3) for AB sequence and 1(0-3) for BA sequence with p-value =0.1159;

Median (min-max) of lesion area (mm²) was 216.00 (0.00-1285.03) for all patients, 216.00 (0.00-989.60) for AB sequence and 283.80 (0.00-1285.03) for BA sequence with p-value = 0.4073.

Furthermore median (min-max) Lesion length (mm) was 35.0 (0.0-98.0) for all patients, 18.0 (0.0-75.0) for AB sequence and 49.5 (0.0-98.0) for BA sequence with p-value = 0.0110.

The analysis of covariance on outcomes for the treatment at baseline and follow-up for the treatment A and at baseline and follow-up for the treatment B is presented at table 2.

Median (min-max) of VAS score at baseline of treatment A was 1.70 (0.10-7.65) , at follow-up of treatment A was 1.75 (0.00-8.80) , while at baseline of treatment B was 2.45 (0.10-7.70) and at follow-up of treatment B was 2.28 (0.05-7.80) ,with p-value = 0.8624.

Median (min-max) of Thongprasom scale at baseline of treatment A was 2 (0-3), at follow-up of treatment A was 1 (0-3), while at baseline of treatment B was 1 (0-3) and at follow-up of treatment B was 1 (0-3), with p-value = 0.0052.

Median (min-max) of lesion area (mm^2) at baseline of treatment A was 265.7 (0-1208.9), at follow-up of treatment A was 122.7 (0-540.3), while at baseline of treatment B was 124.0 (0.0-1285.0) and at follow-up of treatment B was 231.8 (0.0-1497.6) with p-value = 0.0045.

Median (min-max) of lesion length (mm) at baseline of treatment A was 35 (0-141), at follow-up of treatment A was 29 (0-99), while at baseline of treatment B was36 (0-98) and at follow-up of treatment B was 40 (0-112) with p-value = 0.0883 .

None of the patients reported any side-effects of either of the two treatments. Seven patients reported finding it difficult to apply the products, and one of them judged them virtually impossible to apply.

DISCUSSION

We presented a study evaluating the efficacy of topical tocopherol acetate compared with placebo in easing oral discomfort in patients with reticular oral lichen planus (ROLP).
There are some studies in the literature (Gastaldi G et al., Petruzzi M et al., Petruzzi M. et al.) in which is described the use of tocopherol oil in OLP patients. Other studies state that any specific benefits of antioxidant micronutrients (retinol, alpha-tocopherol, zeaxanthin/lutein and cryptoxanthin, lycopene, alpha-carotene and beta-carotene) cannot be claimed for this inflammatory disorder (Nagao T. et al.).

The hypothesis was that this substance could improve disease control and reduce the related discomfort. The findings emerging from the study appeared to differ, depending on the variables taken into consideration.

As for the results of the present study, there was a reduction of one point on the VAS, but this difference did not appear to be statistically significant (P>0.05; 0.8624). It is important to bear in mind, however, that this is a somewhat subjective parameter, and the scores identified on the VAS by our patients were all very low. The VAS was used in the present study to quantify the patients' level of discomfort, where the term "discomfort" was used to mean the "tactile discomfort", when the tongue impacted the lesion on the mucosa, from mild tingling to real pain.

The variations in the length of the lesions measured directly, with the aid of a gauge, in the patients' oral cavity were not statistically significant (P = 0.0883). To document the dimensional changes in the lesions objectively at each follow-up visit, we obtained eight

digital photographs (Lozada-Nur F et Miranda C; Aguirre JM et al.; Radfar . et al.; van der Meij EHI & van der Waal I) and processed them with a software (ImageJ) already amply used in other medical fields (Alman AC et al., Grbatinić I & Milošević NT). Based on the lengths actually measured and adjusted to each different photograph, we calculated the surface area of the main lesions and compared the findings at the various follow-up visits. The analysis of these results revealed a statistically significant reduction in the dimensions of the lesions ($P = 0.0045$), and a greater reduction for tocopherol than for placebo. The drawback of this approach lies in that it gives areas based on two dimensions, not three; the advantage lies in that it considers a surface area rather than just a linear measurement (some lesions may be long and narrow, others broader and shorter), so it presumably affords a fairly good estimate of the boundaries of such areas.

The analysis of the modified Thongprasom scores (Salazar-Sànchez N et al.; Nolan A et al.), revealed a statistically significant difference ($P = 0.0052$) between the two treatments.
None of the patients reported any adverse effects during or after the administration of either of the treatments.

Judging from their VAS scores, 20 patients (60.60%) improved with the tocopherol treatment, while 18 (54.54%) improved with the placebo. When the length of the lesions was measured, 18 patients (54.54%) improved with the tocopherol treatment, and 18 (54.54%) with the placebo. The data obtained on the surface area of the lesions indicated that 26 patients (78.78%) benefited from the tocopherol preparation and 14 (42.42%) from the placebo. Finally, when we considered the modified Thongprasom scale, it emerged that 12 patients (36.36%) experienced an improvement with tocopherol and only 2 (6.06%) with the placebo.

These figures point to a clearly evident placebo effect (Salazar-Sànchez N et al.) starting with the first treatment. This result would confirm the psychosomatic and emotional aspects associated with OLP (Soto Araya M et al.). This may relate partly to the fact that patients were followed up with more frequent clinical examinations and took part in a strict protocol, possibly inducing them to report an improved disease control irrespective of the type of treatment administered.

Seven patients (21.21%) complained of having difficulty with the application of the two products. This finding points to the utility of adopting a two-point scale (Nolan A et al.) on which patients are asked to comment on the ease of application of a product being tested (1 = easy to apply; 0 = difficult to apply).

As for the post-treatment follow-up beyond the experimental phase, our patients were seen again every three or four months in the more severe cases, and every six months in the cases most responsive to the treatment (Yoke PC. et al.)

Two patients included in the study were HCV positive, whereas other two patients were HBV positive. HCV and HBV may create occurences similar to the lichen (Lodi et al. 2004, Romero et al. 2002). In our study the patients alrealdy had clinical and histological diagnosis of lichen, since they all had already been biopsied.

CONCLUSION

This study aimed to test the efficacy of vitamin E in the treatment of ROLP, with a view to exploiting its antioxidant effect in actively preventing the oxidation of polyunsaturated fatty acids and developing the process of lipid peroxidation.

Surprisingly, statistically significant differences ($P<0.05$) between the two treatments adopted in the two successive periods only emerged in terms of the surface area of the lesions and the score on the Thongprasom scale. There was no evidence of any statistically significant differences ($P>0.05$) when we compared the VAS scores for discomfort or the length of the lesions in the oral cavity.

Topical tocopherol thus proved effective in reducing the dimension of the lesions in the course of OLP, but it does not improve VAS score.

Figure 1: tocopherol or placebo applicator

Figure 2, 3, 4: clinical features of ROLP

REFERENCES

Aguirre JM, Bagàn JV, Dìaz de Rojas F, Jimenez Y, Martìnez-Conde R, Ponte A, Rodriguez C, *Efficacy of mometasone furoate microemulsion in the treatment of erosive-ulcerative oral lichen planus: pilot study,* J Oral Pathol Med, (2004), **33**:381-385.

Ahmed S, Asian Lichen Planus Study Group, Bee WH, Chaimusik M, Kim MJ, Machin D, Rajaseharan A, Seldrup J, Suresh S, Thongprasom K, Tin GB, Yoke PC, *A randomized controlled trial to compare steroid with cyclosporine for the topical treatment of oral lichen planus*, Oral Surg Oral Med Oral Pathol Oral Radiol Endod*,* (2006), **102**:47-55.

Al-Hashimi I, Axéll T, Brennan M, Bruce AJ, Carpenter W, Eisenberg E, Epstein JB, Holmstrup P, Jontell M, Lockhart PB, Lozada-Nur F, Migliorati CA, Nair R, Schifter M, Silverman B, Thongprasom K, Thornhill M, van der Waal I, Warnakulasuriya S, Wray D, *Oral lichen planus and oral lichenoid lesions: diagnostic and therapeutic considerations,* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2007), **103**, Suppl 25, e 1-12.

Allam AA, El-Housseiny AA, El-Masry AA, Saleh SM, *The effectiveness of vitamin E in the treatment of oral mucositis in children receiving chemotherapy*, J Clin Pediatr Dent (2007), **31**:167-170.

Alman AC, Calverley DC, Grunwald GK, Hokanson JE, Johnson LR, Lezotte DC, *Validation of a method for quantifying carotid artery calcification from panoramic radiographs,* Oral Surg Oral Med Oral Pathol Oral Radiol, (2013), **4**:518-524.

Anderson A, Cohen MH, Graham ML, Krasnow SH, Redman RS, Wadleigh RG, *Vitamin E in the treatment of chemotherapy-induced mucositis,* Am J Med, (1992), **92**:481-484.

Arduino PG, Bezzo C, Broccoletti R, Caiazzo G, Carbone M, Carrozzo M, Conrotto D, Gandolfo S, *Topical clobetasol in the treatment of atrophic-erosive oral lichen planus: a randomized controlled trial to compare two preparations with different concentrations.* J Oral Pathol Med, (2009), **38**:227-233.

Badminton J, Maguire J, Nolan A, Seymour RA, *The efficacy of topical hyaluronic acid in the management of oral lichen planus,* J Oral Pathol Med, (2009), **38**:229-303.

Bagan Sebastian J, Carrozzo M, Eisen D, Thongprasom K, *Oral lichen planus: clinical features and management,* Oral Dis, (2005), **6:338**-349.

Bardellini E, Flocchini P, Gastaldi G, *Topical alpha-tocopherol in the treatment of decubitus ulcers due to prosthetic trauma in patients with oral lichen planus,* Doctor Os, (2003), **14**(8): 883-886.

Brigelius-Flohe R, Traber MG, *Vitamin E: function and metabolism,* FASEB J, (1999), **13**: 1145-1155.

Camacho-Alonso F, Lòpez-Jornet P, Salazar-Sànchez N, Sànchez-Siles M, *Efficacy of topical aloe vera in patients with oral lichen planus: a randomized double-blind study,* J Oral Pathol Med, (2010), **39**:735-740.

Carriero C, Petruzzi M, De Benedittis M, Carriero C, Giardina C, Parisi G, Serpico R, *Oro-vaginal-vulvar lichen planus: report of two new cases*, Maturitas. (2005), **14;50**(2):140-50.

Carrozzo M, Fuerness S, Lodi G, Thongprasom K, *Interventions for treating oral lichen planus: a systematic review*, Brit J Dermatol, (2012), **166**: 938-947.

Carrozzo M, Griffiths M, Lodi G, Scully C, Sugerman P.B, Thongprasom K, *Current controversies in oral lichen planus; report of an international consensus meeting. Part 2. Clinical management and malignant transformation,* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2005), **100**:164-178.

Dreyfus F, Goudou C, Hazebroucq G, Lopez I, Ribrag V, Sauvage C, *Treatment of mucositis with vitamin E during administration of neutropenic antineoplastic agents.* J Ann Med Interne, (1994), **145**:405-408.

Dutta A, Dutta SK, *Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review,* J Am Coll Nutr, (2003), **22**: 258-268.

Epstein JB, Gorsky M, Wan LS, Zhang L, *Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management*, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2003), **96**: 32–37.

Esguep A, Rojas Alcayaga G, Soto Araya M, *Association between psychological disorders and the presence of oral lichen planus, burning mouth syndrome and recurrent aphthous stomatitis*, Med Oral, (2004), **9**:1-7.

George J, Gondhalekar RV, Roopashree MR, Shashikanth MC, Shukla A, Thippeswamy SH, *Pathogenesis of oral lichen planus – a review,* J Oral Pathol and Med, (2010), **39**: 729-734.

Gonzalez-Moles MA, Scully C, *Vesciculoerosive oral mucosal disease management with topical corticosteroids: fundamental principles and specific agents available,* J Dent Res, (2005), **84**:294-301.

Grbatinic I, Milosevic NT, *Incipient UV-induced structural changes in neutrophil granulocytes: morphometric and texture analysis of two-dimensional digital images,* Microsc Microanal, (2016), **24**:1-7.

Heidari R, Esmailie N, Azarpira N, Najibi A, Niknahad H, *Effect of Thiol-reducing Agents and Antioxidants on Sulfasalazine-induced Hepatic Injury in Normotermic Recirculating Isolated Perfused Rat Liver,* Toxicol Res. (2016), **32**(2):133-40

Kerner S, Etienne D, Malet J, Mora F, Monnet-Corti V, Bouchard P, *Root coverage assessment: validity and reproducibility of an image analysis system,* J Clin Periodontol. (2007), **34**(11):969-76

Khurana H, Kumar A, Pandey RK, Saksena AK, *An evaluation of vitamin E and pycnogenol in children suffering from oral mucositis during cancer chemotherapy*, Oral Diseases, (2013), **19**: 456-464.

Kim SK, Im GJ, An YS, Lee SH, Jung HH, Park SY, *The effects of the antioxidant α-tocopherol succinate on cisplatin-induced ototoxicity in HEI-OC1 auditory cells,* Int J Pediatr Otorhinolaryngol. (2016), **86**:9-14

Lin M, Wu Y, Xiong C, Zeng H, Zhou G, Zhou H, *A randomized double-blind, positive-control trial of topical thalidomide in erosive lichen planus*, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2010), **110**:188-195.

Lodi G, Giuliani M, Majorana A, Sardella A, Bez C, Demarosi F, Carrassi A. *Lichen planus and hepatitis C virus: a multicentre study of patients with oral lesions and a sistematic review*, British Journal of Dermatology, (2004), **151**: 1172–1181.

Lops D, Bressan E, Parpaiola A, Sbricoli L, Cecchinato D, Romeo E, *Soft tissues stability of cad-cam and stock abutments in anterior regions: 2-year prospective multicentric cohort study*, Clin Oral Implants Res (2015), **26**(12):1436-42

Lozada-Nur F, Miranda C, *Oral lichen planus: topical and systemic therapy*, Semin Cutan Med Surg, (1997), **16**:295-300.

Lozada-Nur FI, Sroussi HY, *Tacrolimus powder in Orabase 0.1% for the treatment of oral lichen planus and oral lichenoid lesions, An open clinical trial*, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2006), **102**:744-9.

Luangjarmekorn L, Sererat T, Taweesap W, Thongprasom K, *Relative efficacy of fluocinolone acetonide compared with triamcinolone acetonide in treatment of oral lichen planus*, J Oral Patho Med, (1992), **21**: 456-458.

Nagao T, Warnakulasuriya S, Ikeda N, Fukano H, Yamamoto S, Yano M, Miyazaki H, Ito Y, *Serum antioxidant micronutrient levels in oral lichen planus*, J Oral Pathol Med. (2001), **30**(5):264-7.

Neuzil J, Weber T, Schröder A, Lu M, Ostermann G, Gellert N, Mayne GC, Olejnicka B, Nègre-Salvayre A, Stícha M, Coffey RJ, Weber C, *Induction of cancer cell apoptosis by alpha-tocopheryl succinate: molecular pathways and structural requirements,* FASEB J. (2001), **15**(2):403-15

Neville BW, Allen CM, Damm DD, Chi AC *Oral and maxillofacial pathology (Fourth edition.).* (2016). Chapter 16, pages 729-733 St. Louis, MO: Elsevier..

Niki E, *Evidence for beneficial effects of vitamin E*, Korean J Intern Med, (2015), **30**(5):571-9.

Petrova GV, Delemenchuk NV, Donchenko GV, *[Effects of alpha-tocopherol and its anologues on rat thymocytes programmed death induced by protein kinase inhibitors]*. Ukr Biokhim Zh (1999), (2012), **84**(6):86-95

Petruzzi M, De Benedittis M, Pastore L, Pannone G, Grassi FR, Serpico R, *Isolated lichen planus of the lip,* Int J Immunopathol Pharmacol. (2007), **20**(3):631-5.

Radfar L, Suresh L, Wild RC, *A comparative treatment study of topical tacrolimus and clobetasol in oral lichen planus*, Oral Surg Oral Med Oral Pathol Oral Radiol Endod, (2008), **105**:187-193.

Romero MA, Seoane J, Varela-Centelles P, Diz-Dios P, Otero XL, *Clinical and pathological characteristics of lichen planus in hepatitis C-positive and –negative patients*, Clin Otolaryngol Allied Sci, (2002), **27**(1):22-6.

van der Meij EH, van der Waal I, *Lack of clinical pathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications,* J Oral Pathol Med, (2003), **32**:507-12.

van der Meij EHI, van der Waal I, *Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently-available diagnostic criteria and suggestions for modifications*, J Oral Pathol Med, (2003), **32**(9):507-512.

Yang H, Wu Y, Ma H, Jiang L, Zeng X, Dan H, Zhou Y, Chen Q, *Possible alternative therapies for oral lichen planus cases refractory to steroid therapies,* Oral Surg Oral Med Oral Pathol Oral Radiol. (2016), **121**(5):496-509.

Statistical Analysis

Shapiro-Wilk test was used to check the normality of the quantitative data.

The patients' characteristics at baseline are presented by sequence of treatment (AB/BA). Categorical variables were presented as count and percentage in each sequence and compared with chi-square or Fisher's exact test, for quantitative variables median, minimum and maximum were reported and analyzed with Wilcoxon rank sum test since the distribution was not normal.

Results for the outcome variables were presented by treatment group reporting the median, minimum and maximum at baseline and end-of-treatment and compared with a mixed model analysis of covariance on the values rank-transformed and considering the baseline value as covariate and the factors period and treatment.

The level of significance was set at the 5% value. The analyses were performed with SAS 9.2 (SAS Institute Inc., Cary, NC, USA) for Windows.

Table 1 – Baseline characteristics of the patients by sequence of randomization.

	All patients (n=33)	Sequence		p-value
		AB (n=17)	BA (n=16)	
Gender F n (%)	22 (66.7)	11 (64.7)	11 (68.8)	0.8055
Smoke n (%)	1 (3.0)	0 (0.0)	1 (6.3)	0.4848
Diseases				
Hepatitis n (%)	4 (12.1)	2 (11.8)	2 (12.5)	1.0000
Stress n (%)	8 (24.2)	6 (35.3)	2 (12.5)	0.2245
Autoimmune n (%)	5 (15.2)	3 (17.7)	2 (12.5)	1.0000
Dermatologic n (%)	7 (21.2)	5 (29.4)	2 (12.5)	0.3983
Thongprasom scale Median (min-max)	1 (0-3)	2 (0-3)	1 (0-3)	0.1159
VAS Median (min-max)	1.85 (0.10-7.65)	2.40 (0.10-7.65)	1.73 (0.20-7.00)	0.6653
Lesion area (mm²) Median (min-max)	216.00 (0.00-1285.03)	216.00 (0.00-989.60)	283.80 (0.00-1285.03)	0.4073
Lesion length (mm) Median (min-max)	35.0 (0.0-98.0)	18.0 (0.0-75.0)	49.5 (0.0-98.0)	0.0110

Table 2 – Analysis of covariance on outcomes.

	Treatment				p-value
	A		B		
	Baseline	Follow-up	Baseline	Follow-up	
Thongprasom scale Median (min-max)	2 (0-3)	1 (0-3)	1 (0-3)	1 (0-3)	0.0052
VAS Median (min-max)	1.70 (0.10-7.65)	1.75 (0.00-8.80)	2.45 (0.10-7.70)	2.28 (0.05-7.80)	0.8624
Lesion area (mm²) Median (min-max)	265.7 (0-1208.9)	122.7 (0-540.3)	124.0 (0.0-1285.0)	231.8 (0.0-1497.6)	0.0045
Lesion length (mm) Median (min-max)	35 (0-141)	29 (0-99)	36 (0-98)	40 (0-112)	0.0883