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Evaluation of the Effect of Low-Level Laser Therapy Versus Photodynamic Therapy on the Level of Serum IL-17 and Salivary IL-4 in Patients Suffering from Erosive/Atrophic Oral Lichen Planus: A Case Control Study

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Abstract

Background: One of the most common treatment modalities of oral lichen planus (OLP) is topical corticosteroids (TCS). However, it has several side effects which are harmful to the patients. One such promising treatment modality is low level laser therapy (LLLT) as well as photodynamic therapy (PDT) which are considered safe and reliable in reducing the painful symptoms of the disease with minimal side effects. The inflammatory mediators; cytokines as (IL- 4 & IL- 17) were found to play a crucial role in the pathogenesis of atrophic/erosive OLP.

Objective: The aim of this study is to investigate and compare the effect of PDT, LLLT & TCS by measuring the pain scale VAS, IL- 4 in saliva and IL- 17 in serum to detect the response of OLP patients to these different modalities.

Methods: The study was conducted on 30 patients divided into 3 groups. Each group received either of 3 treatments; PDT, LLLT or TCS, then VAS, salivary IL-4 and serum IL-17 were measured by ELISA at base line, immediately after the first session, at 2 and 4 months from base line to detect the efficiency of each type of treatment.

Results: The use of PDT and LLLT were effective and superior to TCS in treatment of OLP in adult patients. Cytokines were reduced in the 3 groups and VAS was markedly reduced by LLLT. No correlation was found between any of the used parameters, this shows that each one of the parameters was efficient on its own in monitoring the effectiveness of the three treatment modalities used.

Conclusions: the use of LLLT and PDT was effective in treatment of OLP in adult patients.

Keywords: Oral Lichen Planus; Photodynamic Therapy; Low-Level Laser Therapy, IL-4, IL-17, VAS.

1. Introduction

Lichen planus is known as a chronic common inflammatory muco-cutaneous disorder affecting middle-aged adults. Compared to cutaneous lesions, oral lichen planus (OLP) was more common and likely to be more treatment-resistant. OLP was categorized as erosive, reticular, plaque-like, atrophic, or bullous type [1] based on its clinical presentation. Patients with erosive/atrophic types of OLP experienced severe discomfort and required medical attention, whereas those with reticular lesions lacked symptoms and did not require therapy [2, 3]. The characteristic features of erosive/atrophic OLP are diffuse erythematous patches surrounded by fine white lines known as Wickham striae, which are associated with pain and burning sensation. Furthermore, certain lesions have the potential to turn malignant [4].

The gold standard effective treatment for OLP is corticosteroids, which may be applied locally, systemically, or in combination. OLP necessitated ongoing care and patient monitoring [5]. However, long-term corticosteroid therapy for chronic OLP resulted in numerous undesirable local and systemic complications, including oral candidiasis, hyperglycemia, hypertension, mucosal atrophy, and adrenal insufficiency [6]. It has been thought that other therapeutic methods like PDT and LLLT could overcome the side effects of corticosteroids. These two therapies have recently been suggested as OLP management approaches [7].

A photosensitizer, a light source, and oxygen are the three requirements for photodynamic therapy (PDT). The exposure of the photosensitizer to light at a certain wavelength in the presence of oxygen initiates a photochemical reaction. It acts as a cytotoxic agent on the stained tissues, selectively destroying cells by the oxidative process with the production of free radicals. Tissues that are not stained are unaffected by this photochemical process [8]. The photosensitizer not only induces apoptosis in mitochondria but also necrosis in lysosomes and cell membranes, causing cytotoxic effects on sub-cellular organelles and molecules such as mitochondria, lysosomes, cell membranes, and cell nuclei [9]. MB- PDT was reported to be effective in the management of signs and symptoms of OLP with limited side effects [10]. MB is an orally and topically administered heterocyclic aromatic chemical material with relatively minimal tissue toxicity. It is

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employed as a photosensitizer in PDT, as demonstrated in our study [11], because of its significant absorption at wavelengths longer than 620 nm, when light penetration into tissues is the highest. However, the biostimulatory and antiablation properties of laser are the basis of its application principle. In laser therapy, electromagnetic fields are intensified and stimulated by an external energy source, such as light. This produces a coherent, well-collimated, monochromatic laser beam that can promote tissue regeneration and healing without causing systemic disturbances or undesirable effects on healthy tissues [12]. Lasers operating at low levels can have primary or secondary physiological effects on many tissues. Vasodilation, improved blood flow, lymph drainage, cellular metabolism, and activation of neutrophils and fibroblasts are among the primary effects. The secondary effects include prostaglandin aggregation such as PG E2, immunoglobulins and lymphokines, as well as β-endorphins and encephalins in the tissues, resulting in reduction of inflammation, immune response and pain respectively [12].

The involvement of Th1 and Th2 CD4+ helper T cell subsets in the immunopathogenesis of OLP was widely acknowledged [12]. Additionally, it has been found that Th-helper 17 (Th17) contributes in the aetiology of several autoimmune and inflammatory illnesses, and Th17 cells' signature cytokine is interleukin-17 (IL-17) [13]. Due to its major role in both innate and adaptive immunity, IL-17 was also implicated in the etiopathogenesis of OLP [14, 15]. Th17 cells were later discovered to be prevalent in OLP lesions, particularly in erosive/atrophic types. Salivary IL-4 levels were observed to be higher in the erythematous/ulcerative group compared to the reticular group in a study by Liu et al. (2011), who also suggested that this biomarker might be helpful for tracking the severity of OLP [16].

2. The aim of our study was to compare the effects of PDT and LLLT with TCS application, before and after treatment of erosive/atrophic OLP, using three different parameters; VAS, salivary IL-4 and serum IL-17 in patients, and to detect efficiency and long- term effect of each method.

1. Methods

1.1. Study Population:

Thirty patients were recruited in this study (21 females, 9 males) with an age range from 35-55 years old and an average of 5-10 years duration of OLP. They were selected from the outpatient clinic of dermatology of the National Research Centre (NRC) or referred from Dermatology Hospital (El Hod El Marsoud).

3. The MREC of the Oral and Dental Research Institute National Research Centre approved this study (Clearance No. 15-023 / Date: 26 February 2015). The procedures of the current study followed the Declaration of Helsinki.

1.2. Inclusion Criteria:

Patients free from any systemic diseases with no history of taking systemic corticosteroids for the last 6 months were included in the study. Patients clinically and histologically diagnosed with symptomatic erosive/atrophic OLP by a punch biopsy to confirm diagnosis based on the modified definition of the World Health Organization (WHO) criteria [17]. Medical data of the patients were collected according to the detailed questionnaire of the Modified Cornell Medical Index [18].

1.3. Exclusion Criteria:

Patients with indefinite diagnosis such as lichenoid reactions, those suffering from any disease with similar features to OLP as graft versus host disease, lupus erythematosus, any dysplastic features [19], cutaneous lichen planus, pregnant, lactating females and smokers or patients who had received corticosteroids for the last 6 months were excluded from the study.

1.4. Treatment protocol:

Before starting treatment, proper scaling and oral hygiene instructions were given to all patients to minimize plaque accumulation.

The patients were randomly divided into three groups using a randomization software where blocked randomization was used to ascertain equal distribution of patients into each group.

Group I: Photodynamic Therapy Group (PDT);

10 patients were subjected to PDT where laser safety glasses were used for safety and protection of all personnel as well as the patients. 5% MB muco-adhesive oral gel was applied to the oral lesions using cotton swabs and left for 15 minutes after proper isolation of the oral cavity. Diode laser (red light) with a wave length of 650 ±10 nm, where the device power was 150 mW and the output power was 100 mW was applied to the oral lesions in a continuous non-contact mode for 2 minutes [20]. Laser irradiation was performed once every third day, 2 times per week for 4 weeks with a maximum of 10 sessions [21], where energy applied was 12 joules. The diameter of the working probe was 7 mm and that of the laser beam was 5mm. The lesions were irradiated using this laser device, 3mm of the peri-lesional tissues were exposed to laser in an overlapping manner so that energy will be evenly distributed all over the oral lesions. Laser device was manufactured and calibrated using power meter at the National Institute of Laser Enhanced Sciences (NILES), Cairo University with the serial number (S.N.15012).

Group II: Low Level Laser Therapy Group (LLLT);

10 patients were subjected to LLLT where laser safety glasses were used for safety and protection of all personnel as well as the patients. The same diode laser (red light) with a wave length of 650 ±10 nm and the device output power was 100 mW was used as for the PDT group exactly with the same criteria but without the use of MB photosensitizer.

Group III: Topical Corticosteroid Group (TCS) (control group);

10 patients were treated with topical corticosteroids (0.1% triamcinolone acetonide orabase, (Kenacort-A Orabase Pomad, DEVA HOLDING A.Ș, Istanbul, Turkey). The medication was applied 4 times per day for 4 weeks, food and fluid intake were restricted for one hour after application. If treatment was to be extended for longer

durations, miconazole antifungal oral gel was recommended four times a day for one week to prevent superimposed fungal infections [22].

A follow-up was performed for patients in the three groups after cessation of treatment.

Post-treatment instructions

The patients were instructed to take a cold diet and avoid salty, spicy and hot food following each laser session. Topical anesthetic, oracure oral gel was recommended post-operatively and the gel was applied twice a day for one week.

1.5. Assessment methods:

1.5.1. Clinical evaluation (Primary outcome):

VAS was used for measuring pain as it was used to measure the severity of symptoms of the lesions. Pain was recorded using the VAS which is a ten-centimeter horizontal line starting from zero to ten, where zero= no pain, and 10=extremely painful [23]. The VAS was recorded at baseline, immediately, 2 months and 4 months from baseline. Patients were asked to record the pain scale at each visit, pre and post laser sessions. Complete resolution of the symptoms (no symptoms) was defined as the absence of any discomfort, corresponding to a zero VAS score.

1.5.2. Laboratory analysis (Secondary outcome)

By analysis of salivary IL-4 and serum IL-17. Human interleukin -4 (IL-4) in saliva and human interleukin -17 (IL-17) in serum were analyzed using the human interleukin- 4/17 ELISA kits (KORIAN BIOTECH CO., LTD, Bioassay Technology Laboratory, Shanghai, China) for accurate quantitative detection of Human Interleukin -4 in saliva & Human Interleukin -17 in serum.

1.5.3. Blood sample collection:

Four blood samples were withdrawn from the patients. The first was withdrawn from all patients one week before starting treatment at the day they presented at the clinic (base line). A second blood sample was taken immediately after the first session (PDT, LLLT & TCS), and a third blood sample was taken two months from base line then a fourth one was withdrawn at four months from the base line. Centrifugation of all blood samples was performed at 400 g for 10 min at 4°C after 30 minutes from their collection to allow the samples to clot. Serum was withdrawn from all samples then stored at -80°C for laboratory procedures to be done.

1.5.4. Salivary sample collection:

Four saliva samples were collected from patients. The first was collected from all patients one week before starting treatment at the day they presented at the clinic (base line). A second saliva sample was collected immediately after the first session (PDT, LLLT & TCS), and a third saliva sample was collected two months from base line then a fourth one was collected at four months from the base line. Centrifugation was done for 20 minutes at 3500 xg, then aspiration of the supernatants was performed and kept at -80 °C until analysis of the samples. Collection of the whole unstimulated saliva (WUS) was performed between 8:00 and 10:00 a.m., using standard techniques described by Navazesh (1993) [24]. In brief, patients were instructed to stop eating and drinking for one hour before sample collection. Patients were asked to swallow first, tilt their head forward and then rest for 5 minutes and to minimize orofacial movements. Saliva was collected by suction method; saliva was continuously aspirated from the floor of the mouth into a sterile test tube by an aspirator for centrifugation.

1.6. Statistical analysis:

Statistical analysis was performed by Statistical Package for Social Science (SPSS version 20), while (Microsoft excel 2010) software was used for data handling and graphical presentation. The statistical results were presented in tables and charts describing and comparing the changes occurring in both IL-4, IL-17 & VAS for the different treatment modalities in different time intervals. Quantitative data were presented as mean and standard deviation (SD). Results were tested for normality, then one way ANOVA (Analysis of variance) followed by post-hoc Tukey were used for comparing more than two different groups of parametric data. Pearson's correlation coefficient was used to measure the correlation between VAS, IL-4 & IL-17. Significance level was considered at P- value less than or equal $\leq$ 0.05 and was considered statistically significant.

2. Results

The study included 30 patients suffering from erosive/atrophic OLP with an average duration 5-10 years of illness. Demographic characteristics of patients are described in Table 1.

Table 1: Descriptive statistics for the demographic data

Age (years)	Mean ± SD	45.9 ± 6.8
	Range	35 – 55
Gender	Female	21 (70%)
	Male	9 (30%)

Baseline measurements were taken before treatment for all patients, then immediately after application of the therapeutic modalities, then 2 months and 4 months from baseline. Measurements of IL-4 in saliva, IL-17 in serum and VAS were documented and statistically analysed and presented in Tables 2, 3, 4.

Our results showed a significant decrease in the level of salivary IL- 4 through whole time intervals of the study by using three treatments (p-value<0.001), there was significant difference in each column. However, by comparing the three treatments we couldn't detect any statistically significant differences between them at different time points except at 4 months after treatment (p-value<0.011).

We noticed that the lowest level of IL-4 was due to PDT treatment followed by LLLT and finally by TCS even after 4 month which enforces its long- term effect.

By comparing the three treatments using IL-17 statistically significant differences were detected in each treatment alone through the whole study as well. However, IL-7

failed to detect any differences between the three groups at different time points.

By comparing the three treatments using VAS, statistically significant differences were detected in each group alone (p- value <0.001). There was statistically significant difference between the three groups after 4 months of treatment (p-value<0.001). There was significant increase in VAS in TCS treated group as well and there was initial decrease immediately after treatment followed up by gradual increase in pain score.

Levels of IL-4, IL-17 & VAS were revealed in different groups at different time points respectively in Tables 5, 6, 7.

Table 2: ANOVA and post-hoc repeated ANOVA test for comparison of salivary IL-4 expression between the three study groups (Inter- group comparison)

		PDT	LLLT	TCS	ANOVA	
					p value	sig.
IL-4 at BL (pg/ml)		5363 ± 2469.49	4963.6 ± 1824.77	5178.3 ± 2900	0.935	NS
IL-4 Immediately after BL		3748 ± 2225.74	4156.3 ± 1778.31	3913.1 ± 2696.42	0.921	NS
IL-4 2 months from BL		2564 ± 1605.36	3848.5 ± 1739.04	4120.8 ± 2489.65	0.192	NS
IL-4 4 months from BL		1829 ± 1281.37	3415 ± 1589.2	4726.5 ± 2743.89	0.01	S
Repeated measure ANOVA	**p-value**	<0.001	<0.001	<0.001		
	significance	S	S	S		

Note: BL; Baseline, NS; Non- Significant, S; Significant, P-value <0.001 is significant.

Table 3: ANOVA and post-hoc repeated ANOVA test for comparison of serum IL-17 expression between the three study groups (Inter- group comparison)

		PDT	LLLT	TCS	ANOVA	
					p value	sig.
IL17 at BL (pg/ml)		534.2 ± 247.42	624.3 ± 232.92	579.9 ± 202.82	0.682	NS
IL17 Immediately		424.9 ± 206.27	498.9 ± 212.33	507.5 ± 196.29	0.618	NS
IL17 2 months from BL		332.3 ± 179.31	402.3 ± 191.91	441 ± 186.22	0.427	NS
IL17 4 months from BL		285.3 ± 143.86	328.9 ± 149.77	400.4 ± 163.43	0.252	NS
Repeated measure ANOVA	p value	<0.001	<0.001	<0.001		
	sig.	S	S	S		

Note: BL: Baseline, NS: Non- Significant, S: Significant, P-value <0.001 is significant.

Table 4: ANOVA and post-hoc repeated ANOVA test for comparison of VAS between the three study groups (Inter-group comparison) (scale 0-10).

		PDT	LLLT	TCS	ANOVA	
					p value	sig.
VAS at BL		7.5 ± 1.58	8.2 ± 1.32	7.6 ± 1.26	0.488	NS
VAS Immediately after BL		6.6 ± 1.71	7 ± 1.33	6 ± 1.05	0.288	NS
VAS 2 months from BL		5.8 ± 1.62	6.1 ± 1.2	7.2 ± 1.03	0.057	NS
VAS 4 months from BL		5 ± 1.7	4.1 ± 1.1	8.1 ± 0.88	<0.001*	S
Repeated measure ANOVA	p value	<0.001	<0.001	<0.001		
	sig.	S	S	S		

Note: BL: Baseline, NS: Non- Significant, S, sig: Significant, P-value <0.001 is significant.

Table 5: Post hoc test repeated ANOVA for comparison of salivary IL-4 level in different time intervals and different treatment modalities and their P-values (Intra-group comparison)

Post hoc test for Repeated measure ANOVA	**PDT**		**LLLT**		**TCS**	
	p value	**sig.**	**p value**	**sig.**	**p value**	**sig.**
IL 4: BL vs Immediate	<0.001	S	0.002	S	<0.001	S
IL 4: BL vs 2 months	<0.001	S	0.011	S	0.001	S
IL 4: BL vs 4 months	<0.001	S	0.001	S	0.011	S
IL 4: Immediate vs 2 months	0.002	S	0.395	NS	1.000	NS
IL 4: Immediate vs 4 months	0.002	S	0.018	S	0.001	S
IL 4: 2 months vs 4 months	0.006	S	0.924	NS	0.007	S

Note: NS: Non- Significant, S: Significant, P-value <0.001 is significant, BL: Baseline.

Table 6: Post hoc test repeated ANOVA for comparison of serum IL-17 level in different time intervals and different treatment modalities and their P-values (Intra-group comparison)

Post hoc test for Repeated measure ANOVA	PDT		LLLT		TCS	
	p value	sig.	p value	sig.	p value	sig.
IL17: BL vs Immediate	0.002	S	0.003	S	0.002	S
IL17: BL vs 2 months	0.001	S	<0.001	S	<0.001	S
IL17: BL vs 4 months	<0.001	S	<0.001	S	<0.001	S
IL17: Immediate vs 2 months	0.005	S	0.010	S	0.001	S
IL17: Immediate vs 4 months	0.001	S	0.001	S	0.001	S
IL17: 2 months vs 4 months	0.042	S	0.003	S	0.010	S

Note: NS: Non- Significant, S: Significant, P-value <0.001 is significant, BL: Baseline.

Table 7: Post hoc test repeated ANOVA for comparison of VAS in different time intervals and different treatment modalities and their P-values (Intra-group comparison)

Post hoc test for Repeated measure ANOVA	PDT		LLLT		TCS	
	p value	sig.	p value	sig.	p value	sig.
VAS: BL vs Immediate	0.004	S	<0.001	S	<0.001	S
VAS: BL vs 2 months	<0.001	S	<0.001	S	1.000	NS
VAS: BL vs 4 months	<0.001	S	<0.001	S	0.574	NS
VAS: Immediate vs 2 months	0.001	S	<0.001	S	<0.001	S
VAS: Immediate vs 4 months	<0.001	S	<0.001	S	<0.001	S
VAS: 2 months vs 4 months	0.001	S	<0.001	S	0.023	S

Note: BL: Baseline, NS: Non- Significant, S: Significant, P-value <0.001 is significant.

There was statistical significance within each group at different time points using the 3 parameters except in LLLT group, IL-4 failed to detect significant decrease between immediate vs 2 months & 2 months vs 4 months, and in TCS treated group also IL-4 couldn't detect significant decrease between immediate vs 2 months and VAS also couldn't detect significant decrease between base line vs 2 months and base line vs 4 months.

We detected the final rate of improvement after 4 months in every treatment by detection of rate of improvement % of all measured diagnostic markers (IL-17, IL-4 and VAS) comparing between different treatments by different markers. Figure 1.

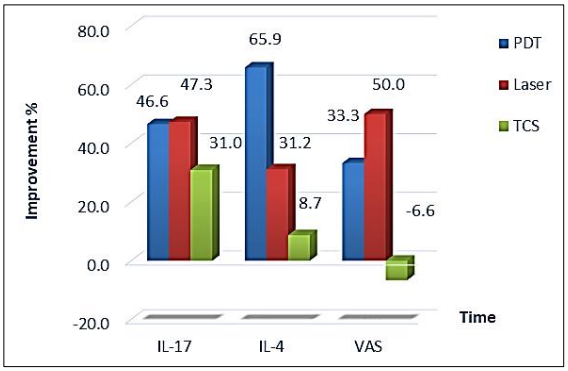

Figure 1: Represents the final improvement % of all measures (IL-17, IL-4 and VAS) by the 3 different treatment modalities by the end of the study (4 months).

Regarding improvement rate of PDT, IL-4 showed the best improvement compared to the other two measures (65.9% by IL-4 versus 46.6%, 33.3% by IL-17 and VAS respectively). IL-4 showed that PDT has the highest effect compared to the other two treatments (65% versus 31.2%, 8.7%). Concerning LLLT treatment, VAS showed the best improvement compared to the other two measures (50% by VAS versus 31.2%, 47.3% by IL-4 and IL-17 respectively). VAS showed that LLLT has the highest effect compared to the other two treatments (50% versus 33.3%, -6.6%). Concerning TCS, IL-17 showed the best improvement compared to the other two measures. (31% in IL-17 versus 8.7%, -6.6% in salivary IL-4 and VAS respectively).

The following charts show the changes of all measures as a % of improvement due to each treatment over time which will be illustrated in figures 2, 3 and 4.

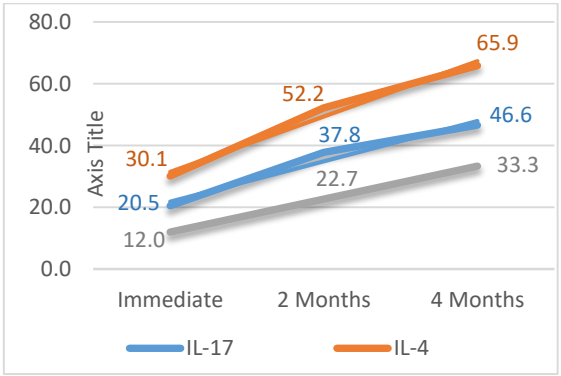

Figure 2: Chart showing improvement % by PDT over study durations (sessions).

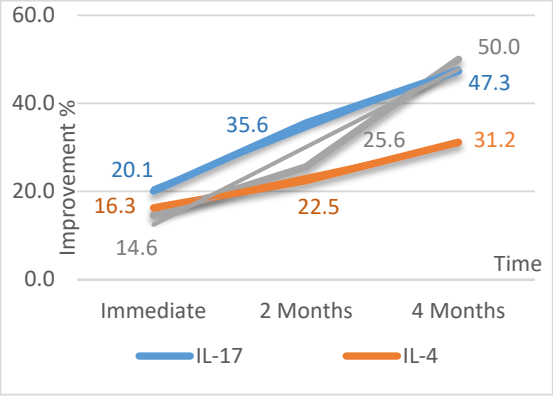

Figure 3: Chart showing improvement % by LLLT over study durations (sessions).

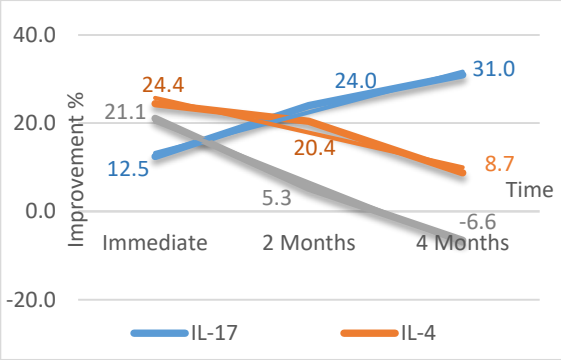

Figure 4: Chart showing improvement % by TCS over study durations (sessions).

In PDT treatment (figure 2), the efficiency of PDT on the measured markers was IL-4 > Il-17 > VAS i.e.: there is maximum increase in the rate of improvement in IL-4, thus recommending for extension of the duration of PDT treatment more than 4 months.

In LLLT treatment (figure 3), the efficiency of LLLT on the measured markers was VAS > Il-17 > IL-4 i.e.: there is max. increase in the rate of improvement in VAS, thus recommending for extension of the duration of LLLT treatment more than 4 months. Especially the VAS (pain scale) reflects patient comfort which has a priority apart from other measures (IL-4 & IL-17). In TCS treatment (figure 4), the efficiency of TCS on the measured markers was IL-17 > IL-4> VAS i.e. it has weak rates down to negative improvement. This in turn proved that TCS treatment is not reliable due to human error as it is applied by patients. The improvement was gradual from 12.5% immediately to reach 31% after 4 months. Decrease of the initial improvement from 24.4% & 21.1 in IL-4 and VAS respectively to reach 8.7% in IL-4 and ended up by negative deterioration -6.6 in VAS after 4 months.

3. Discussion

Oral Lichen Planus (OLP) is a chronic mucocutaneous inflammatory immune-related illness that is mediated by T-helper cells (Th1) [25]. Typically, the tongue and buccal mucosa are the most affected oral mucosal tissues [26-28].

All OLP lesions are asymptomatic except for erosive/atrophic lesions; our primary goal in this study, and ulcerative forms which have symptoms ranging from mild burning sensation to severe pain. The most common treatment modality of OLP is corticosteroids. Numerous research attempts took place to discover substitute therapies, such as PDT and LLLT, in order to avoid their adverse effects.

Our study included 21 females and 9 males as many researches pointed out that gender predilection was typically shifted 2:1 in favor of females over males [29] and OLP affects the Egyptian population at a ratio of 2.2:1, as proven by Mostafa et al., [30]. The average age of the participants was 45.9±6.8 years, which corresponds to the most prevalent age range for adults with symptomatic OLP. This is because the condition primarily affects adults in their fourth decade of life, according to research conducted by Pavlic and Aleksic in 2014 [31].

A total of 30 patients participated, where ten patients received PDT in group I, ten patients received LLLT in group II and ten patients received topical corticosteroids (TCS) in group III (control group) in this study.

Up to our knowledge no previous studies were conducted to detect the effect of the three different treatment modalities (LLLT, PDT & TCS) on the salivary level of IL-4 as an inflammatory marker in OLP patients, however, many studies have found that OLP affects the local site and influences cytokine secretion more than serum [32]. Malekzadeh et al., concluded that the salivary levels of IL-4 in OLP were increased compared to control group in their study [33], also the results of the study done by Liu et al., showed significant increase in the levels of IL-4 in saliva in ulcerative OLP compared to the reticular ones [34] and in 2014, Liu et al., found out that the levels of IL-4 in saliva were higher than their serum partners [35]. In this study, the level of IL-4 was estimated in saliva as it reflects the local vasculature, serum components derived from oral lesions, and serum and blood derivatives from oral lesions. That's why we investigated the level of IL-4 in saliva in our study as it is local; present at the site of the lesion, offers non-invasive procedure and ease of access.

Results in this study showed a significant decrease in the level of salivary IL-4 in all groups, however, it was markedly reduced in PDT group by the end of the study duration in comparison to LLLT group, while in TCS group it was reduced immediately then it was elevated by the end of the study. IL-4 was reduced along the course of the study which clarified the strong effect of PDT on this inflammatory mediator. Significant difference was detected between PDT vs LLLT and PDT vs TCS groups while the difference between LLLT and TCS was non-

significant. The improvement % revealed by IL-4 was highest in PDT (65%) compared to LLLT & TCS groups (31% & 8.7%), respectively.

Although it was accepted that OLP is a localized disease, an increasing number of studies indicated that many significant changes in the peripheral blood were implicated in the pathogenesis of OLP. Several investigations focused on the alteration of T-lymphocytes subsets in the peripheral blood of OLP patients [36]. A systemic review article in 2022 by Husein-ElAhmed et al., concluded that the level of IL-17 in serum was higher in OLP patients in comparison to controls [37]. In this study IL-17 was measured in serum as it has a great impact in the pathogenesis of the disease by enhancing T cell-mediated reactions stimulating the release of chemokines and other cytokines [38]. In 2017, Gueiros et al., conducted a study to find out that IL17A G197A was associated with a higher susceptibility of developing OLP and there was a considerable increase in IL17A in patients' serum. The results reinforced its role in disease pathogenesis and the role of Th17 immune response in inflammatory diseases which suggested that IL-17 may become a therapeutic target in further studies [39].

The level of IL-17 in serum is significantly decreased in the 3 groups during the whole duration of the study. However, improvement % revealed by IL-17 was highest in laser (47.3%), followed by PDT with nearly the same level of improvement (46.6%) and finally TCS (31%). This was in agreement with Mirza et al., who studied the effect of laser and found that laser action was mainly biostimualtion and antiablation. It enhances tissue healing and regeneration as it works locally, with no systemic side effects thus minimizing the undesirable effects on healthy tissues [40]. This was also in accordance with Cosgarea et al., (2020) who conducted a study on the effect of PDT on OLP where there was a decrease of peripheral Th-17 (IL-17a+) cells after PDT treatment. There was also clinical amelioration of OLP including decrease in burning sensation, improvement of lesion size and quality of life of the patient and quantitative reduction of oral bacteria [41].

Although the level of IL-17 in serum was reduced throughout the course of our study after applying three different types of treatment, no correlation was found between VAS and IL-17 levels [42].

Finally, regarding VAS (pain scores), it was significantly reduced in all groups with improvement % in PDT & LLLT groups, 33.3% & 50% respectively. However, in the TCS group, there was an immediate drop in the pain scores followed by its increase by the end of the study. Findings in this study of LLLT effect were matching the results of Dillenburg et.al, in which laser phototherapy was more effective than topical clobetasol for treating OLP lesions and preventing their exacerbation [43]. In 2018, Ferri et al., conducted a study on the effect of LLLT on OLP lesions using diode laser, 660 nm wavelength of 100 mW power, twice weekly for 4 weeks compared to standard corticosteroids and it was still debatable if photobiomodulation was more effective when compared to corticosteroids [44]. In 2011, a study carried out by Jajram et al., demonstrated that LLLT was as effective as topical corticosteroid therapy regarding pain score VAS [45], where in 2018, Jajram et al., carried out another study to compare the effect of PDT, LLLT and topical steroids in OLP patients and found out that LLLT was as effective as corticosteroids in decreasing pain levels and sign scores as well and that PDT failed to show any significant effect on treating the signs of OLP [46]. These results were in contrast to our findings regarding the VAS scores in PDT group, as there was significant decrease in pain scale about 33.3% which was maintained throughout the course of the study. These findings were in accordance with Mostafa et al., and Saleh et al., where there was a marked decrease in VAS scale and complete relief of pain in the PDT group compared to control (topical steroid) group [21, 47].

Our findings were against Mirza et al., who carried out a similar study comparing the effect of toluidine blue (TB)-PDT and LLLT to topical corticosteroids, where they found out that the highest reduction in pain scores was in TCS group compared to PDT and LLLT groups which was against our results where LLLT and PDT were superior to TCS that increased by the end of the study [40]. Also, Jajram et al., compared the effect of PDT using TB as a photosensitizer versus topical corticosteroids and the results were against our study as well, where topical steroids showed better results than TB-PDT [46]. This conflict in results could be due to the use of TB as a photosensitizer and the wavelength of the laser device used (630 nm) in both studies. However, in our study we used methylene blue (MB) mucoadhesive oral gel as a photosensitizer which allowed longer and better adherence to the oral mucosal tissues and the wavelength of the laser device used was 650 nm. The longer the photosensitizer stays on the lesions, the better the efficacy of PDT. Wavelength is the most important factor in all types of phototherapies, therefore the most appropriate wave length should be selected to obtain the best results. As regard topical steroid used in these two studies, it was dexamethasone mouth wash (0.5 mg in 5 ml), while in our study it was 0.1% triamcinolone acetonide orabase oral pomad allowing for better adherence to oral mucosa for longer durations and therefore sustained pharmacological effects. These findings were confirmed by Muhaxheri et al., in 2017 using TB-PDT and GaAlAr laser of 685 nm wavelength, they revealed that PDT with toluidine blue was not efficient in the treatment of refractory OLP, therefore it was not suggested to use TB mediated PDT in patients with OLP [48].

A recent study by He et al. in 2020 reported that PDT is as effective as topical steroids in the management of OLP,

as for the study by Lavaee & Shadmanpour, they concluded that PDT was statistically more effective in decreasing all scores except for VAS which was the score of our concern [49, 50]. This is in accordance with Bakhtiari et al., who compared the effects of PDT with topical steroid application and revealed that no significant difference existed between the two treatment modalities [51].

4.Conclusions

We concluded that the use of LLLT and PDT was effective in treatment of OLP in adult patients. Regarding VAS, LLLT was superior to PDT and TCS. Considering level of IL-17 in serum, LLLT & PDT yielded similar results however, PDT was more effective regarding IL-4 level in saliva. TCS showed effective results only in the early phase of treatment. The assessment of inflammatory mediators (IL-4 &IL-17) is more reliable than VAS, as it is a subjective measure giving a high amount of heterogenicity in the outcomes of this study, that's why we used two types of inflammatory mediators; one in serum (systemic) and one in saliva (local) together with the VAS seeking for reliable results. MB-PDT and LLLT were considered better treatment modalities for OLP in comparison with the TCS as they showed reduction in the levels of inflammatory cytokines, more remarkable pain reduction as a patient reported outcome and lesion regression. Besides, the immediate relief of pain after application of MB-PDT and LLLT and the needless use of anesthesia were very effective points in patients' psychological responses and expectations. PDT has a selective effect on superficial tissues without harming normal tissues, which makes it a safe treatment for patients with or without systemic diseases. PDT & LLLT do not interact with any drugs therefore they can be used with TCS for better and quicker results. However, the bad taste of MB dye, muscle tiredness due to prolonged mouth opening and the chair-time consumption are the disadvantages of MB-PDT as they were temporary manifestations. Like any other study, this study had certain limitations, as lesion size was not evaluated, in addition to the number of patients included in each group should be increased.

6.Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

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