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Topical photodynamic therapy is very effective for oral verrucous hyperplasia and oral erythroleukoplakia

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BACKGROUND: Oral verrucous hyperplasia (OVH) and oral erythroleukoplakia (OEL) are two oral precancerous lesions with relatively high malignant transformation potential. One of the best cancer prevention strategies is to use a conservative and effective treatment modality to eliminate oral precancers to stop their further malignant transformation. Our previous studies have shown that the topical 5-aminolevulinic acid-mediated photodynamic therapy (topical ALA-PDT) using the 635-nm light-emitting diode (LED) light is very effective for OVH and OEL lesions.

METHODS: Because the laser machine is a more-popular light source than the LED device in PDT clinics, in this study 40 OVH and 40 OEL lesions were treated once a week with the same PDT protocol but using the 635-nm laser light to evaluate whether this laser light-mediated topical ALA-PDT was also effective for OVH and OEL lesions.

RESULTS: We found that all the 40 OVH lesions exhibited complete response (CR) after an average of 3.6 PDT treatments. Of the 40 OEL lesions, 38 showed CR after an average of 3.4 PDT treatments and two showed partial response (PR). Better PDT outcomes were significantly associated with OVH and OEL lesions with the smaller size, pink to red color, epithelial dysplasia, or thinner surface keratin layer.

CONCLUSION: This study indicates that the laser light-mediated topical ALA-PDT is also very effective for OVH and OEL lesions. Therefore, we suggest that topical ALA-PDT using either the LED or laser light may serve as the first-line treatment of choice for OVH and OEL lesions.

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Introduction

Oral leukoplakia (OL), oral erythroleukoplakia (OEL), and oral verrucous hyperplasia (OVH) are three common oral precancerous lesions. OEL has a higher malignant transformation rate than OL (1). Histologically, approximately 90% of OL lesions show hyperkeratosis and/or epithelial hyperplasia, whereas nearly all OEL lesions reveal some degree of epithelial dysplasia, carcinoma *in situ* or superficially invasive carcinoma (1). In addition, OEL lesions have higher mitotic and apoptotic indices than homogeneous and nodular OL lesions (2). Immunohistochemical study also demonstrated a higher Ki67 or p53 expression in OEL than in homogeneous and nodular OL lesions (2). In Taiwan, both areca quid chewing and cigarette smoking are involved in multistate progression of oral premalignancies including OL and OEL lesions (3, 4). Cohort study found that the average dwelling times are 24 years for OL and 7 years for OEL. Furthermore, the risks of developing oral cancer after 20 years of follow-up are 42.2% for OL and 95.0% for OEL (4). These findings suggest that OEL lesions have higher malignant transformation potential than OL lesions. A retrospective clinical study showed a malignant transformation rate of 3.1% and a mean malignant transformation duration of 54.6 months for 324 OVH lesions arising from Taiwanese patients (5). Our recent study also demonstrated a 5-year malignant transformation rate of 3% for 30 plaque-typed and of 17% for 30 mass-typed OVH lesions (6). The high malignant transformation rates of OEL and OVH lesions highlight the importance of early detection and treatment of these two types of oral precancerous lesions.

Traditional treatment for oral precancers is total surgical excision that always leads to scar formation for a large precancerous lesion (7). Photodynamic therapy (PDT) is another effective treatment option for human precancerous lesions because it can be used repeatedly without cumulative side effects and results in little or no scar formation (8). 5-Aminolevulinic acid (ALA) is the biological precursor of the photosensitizer, protoporphyrin IX (PpIX), in the heme biosynthesis pathway. ALA is superior to other photosensitizers because it can be rapidly cleared from the tissues and the body within 48 h and patients after ALA-mediated PDT (ALA-PDT) treatment have no problem of prolonged skin photosensitivity (9, 10). During ALA-PDT treatment, the generated reactive oxygen species (ROS) can kill tumor cells directly or damage the tumor-associated vasculature, leading to thrombus formation and subsequent tumor infarction. Moreover, PDT can also activate an immune response against tumor cells (8).

Our previous studies have shown that the topical ALA-PDT using the 635-nm light-emitting diode (LED) light is very effective for treatment of OVH and OEL lesions (11–16). Because the laser machine is a more-popular light source than the LED device in PDT clinics, in this study we tried to evaluate whether the same PDT protocol but using the 635-nm laser light was also effective for treatment of OEL and OVH lesions. If this is true, then we will propose that topical ALA-PDT using either the LED or laser light may serve as the first-line treatment of choice for OEL and OVH lesions.

Materials and methods

Patients and oral precancerous lesions

Forty patients (39 men and one woman, aged 42–74 years, mean age 50 ± 6 years) with OVH and 40 patients (38 men and two women, aged 34–89 years, mean age 58 ± 12 years) with OEL were recruited from the Department of Oral and Maxillofacial Surgery, National Taiwan University Hospital (NTUH) from August 2006 to February 2010. The 40 OEL lesions included 26 OEL lesions which have been published before (16). At the patient's first visit, biopsy specimens were taken from the characteristic part of OEL and OVH lesions. Clinical and histological criteria for diagnosis of OVH and OEL including the histological criteria for diagnosis of mild, moderate, and severe dysplasia as well as carcinoma *in situ* have been described previously (12–16).

Clinicopathological data of 40 OVH and 40 OEL patients are shown in Table 1. The buccal mucosa was the most common site for OVH (25 cases, 63%) and OEL lesions (36 cases, 90%). Mild to severe dysplasia or carcinoma *in situ* was found in 15 (38%) OVH and 40 (100%) OEL lesions. The mean greatest diameter was 1.8 (range, 0.6–4.8) cm for OVH and 1.7 (range, 0.8–4.1) cm for OEL lesions. The surface keratin thickness of each oral precancerous lesion was measured from hematoxylin and eosin-stained tissue section by a built-in microscopic meter and expressed as a mean of five measurements from five randomly-selected areas.

Table 1 Clinicopathological data of 40 patients with oral verrucous hyperplasia (OVH) and 40 patients with oral erythroleukoplakia (OEL) lesions

	OVH (n = 40)	*OEL (n = 40)*	*Total (n = 80)*
Age			
30–39	0	2	2
40–49	22	10	32
50–59	16	12	28
60–69	1	11	12
70–79	1	3	4
80–89	0	2	2
Gender			
Men	39	38	77
Women	1	2	3
Location			
Buccal mucosa	25	36	61
Tongue	5	1	6
Labial mucosa	4	1	5
Alveolar mucosa	0	1	1
Palate	5	1	6
Floor of mouth	1	0	1
Pathological diagnosis			
Mild dysplasia	0	12	12
Moderate dysplasia	0	18	18
Severe dysplasia	0	7	7
Carcinoma *in situ*	0	3	3
OVH	25	0	25
OVH with mild dysplasia	9	0	9
OVH with moderate dysplasia	4	0	4
OVH with severe dysplasia	2	0	2

The mean surface keratin thickness was 46 (20–150) μm for OVH and 27 (10–50) μm for OEL lesions. In this study, informed consent was obtained from each patient before biopsy procedure and before PDT. This study was reviewed and approved by the Human Investigation Review Committee at the NTUH.

ALA preparation

The formulation of 20% ALA has been described previously (11, 12). This 20% ALA was packed into a needleless 1-ml syringe and used within 3 h after the preparation.

Fluorescence spectroscopy

ALA-induced PpIX fluorescence spectroscopy was used to monitor the conversion of ALA into PpIX in the lesional epithelial cells at the patient's second visit as described previously (12–16). In this study, 40 OVH or OEL patients participated in the kinetics study. We found that the PpIX reached its maximum level in oral lesional epithelial cells approximately 1.5–2 h after local ALA application. Therefore, the subsequent light treatments were set at 1.5–2 h after topical application of ALA onto all OVH and OEL lesions.

PDT

Topical ALA-PDT was performed once a week for all 40 OVH and 40 OEL lesions starting from the patient's third appointment. Each OVH or OEL lesion was irradiated with a 635-nm laser light generated by the Arts-Laser (Arts International Biotechnology Inc.,

Taipei, Taiwan) which is a power-adjustable (from 0 to 1200 mW) diode laser. The light treatment was given 1.5–2 h after topical application of 20% ALA onto oral precancerous lesions for a total of 1000 s (fluence rate, 100 mW/cm^2; light exposure dose, 100 J/cm^2). The 1000-s treatment period was divided into five 3-min and one 100-s irradiations separated by five 3-min rests as described previously (12–16). In 62 patients (32 OVH and 30 OEL patients) with severe throbbing pain or marked burning sensation during PDT, laser light treatments were carried out under local anesthesia using 2% lidocaine. In 64 patients (33 OVH and 31 OEL patients) with moderate to severe post-PDT pain, analgesics (acetaminophen, 500 mg/tablet, one tablet three or four times a day) were prescribed to the patients after PDT. Clinical photographs were taken at each patient's visit to evaluate the clinical outcome of PDT.

Clinical evaluation

Lesion response was characterized as follows: complete response (CR), lack of detectable lesion confirmed by clinical evaluation; partial response (PR), reduction of lesion by at least 20% in diameter; and no response (NR), reduction of lesion by less than 20% in diameter. All lesion responses were evaluated at the completion of eight treatments of ALA-PDT. If the lesion showed CR after less than eight treatments of PDT, the PDT was ended and the patient was arranged for a follow-up schedule once a month in the first post-PDT year and once two months thereafter. If the lesion showed PR or NR even after eight treatments of PDT, the patients might choose a plan of total excision of the residual lesion, a follow-up schedule once a month, or a continuous topical ALA-PDT using the laser light once a week.

Statistical analysis

The difference in efficacy of PDT treatment between two different groups was assessed for statistical significance by chi-square test (two rows by two or three columns). The difference in the mean treatment number of topical ALA-PDT to achieve a CR or in the mean surface keratin thickness of oral lesions between any two different groups was assessed for statistical significance by Student's *t*-test or one way analysis of variance, where appropriate. Univariate and multivariate linear or logistic regression analyses were performed to assess which clinicopathological parameter was the most important or independent factor that could influence the PDT treatment outcome. A P value of less than 0.05 is considered statistically significant.

Results

Oral verrucous hyperplasia

All the 40 OVH lesions showed CR (Fig. 1) after an average of 3.6 (range, 1–6) treatments of ALA-PDT. Correlation between the mean treatment number of topical ALA-PDT to achieve a CR and the clinicopathological parameters of 40 laser light-treated OVH lesions or those of 36 LED light-treated OVH lesions published previously is shown in Table 2 (15). We found that the mean treatment number of PDT to achieve a CR for OVH lesions with the clinical appearance of a mass (2.8 ± 1.1), with the greatest diameter < 1.5 cm (2.2 ± 0.7), with the pink color (3.0 ± 1.2), with epithelial dysplasia (2.5 ± 1.1), or with the surface keratin layer ≦ 40 μm (3.0 ± 1.2) was significantly fewer than for OVH lesions with the clinical appearance of a plaque or a combination type (4.4 ± 0.8, $P = 0.000$), with the greatest diameter ≧ 1.5 cm (4.3 ± 0.8, $P = 0.000$), with the white color (4.4 ± 0.8, $P = 0.000$), without epithelial dysplasia (4.2 ± 0.8, $P = 0.000$), or with the surface keratin layer > 40 μm (4.4 ± 0.8, $P = 0.000$), respectively (Table 2). Univariate logistic regression analyses discovered that the clinical appearance, size, color, epithelial dysplasia, and surface keratin thickness of the lesion were all significant factors influencing the PDT treatment number (Table 3). However, multivariate logistic regression analyses showed that only the size of OVH lesions was the independent factor influencing the PDT treatment number ($P = 0.006$, Table 3). No recurrence of the 40 OVH lesions was found after a follow-up period of 8–37 (mean, 20) months. In addition, although the mean treatment number of PDT to achieve a CR for the 40 laser light-treated OVH lesions (3.6 ± 1.2) was lower than that for the 36 LED light-treated OVH lesions (3.8 ± 1.5), no significant difference was found ($P = 0.521$, Student's *t*-test) (15).

Histologic examination demonstrated that the mean surface keratin thickness of OVH lesions was significantly thinner in lesions with pink color (30 ± 8 μm) or with epithelial dysplasia (28 ± 8 μm) than in lesions with white color (68 ± 30 μm, $P = 0.000$) or without epithelial dysplasia (57 ± 30 μm, $P = 0.000$), respectively.

Oral erythroleukoplakia

The 40 OEL lesions treated with topical ALA-PDT by the laser light once a week showed CR in 38 and PR in 2. The former 38 CR OEL lesions required an average of 3.4 (range, 2–6) treatments of ALA-PDT to achieve CR of the lesions (Fig. 2). Correlation between the mean treatment number of topical ALA-PDT to achieve a CR and the clinicopathological parameters of 38 laser light-treated OEL lesions or those of 17 LED light-treated OEL lesions published previously is shown in Table 4 (16). We found that the mean treatment number of PDT to achieve a CR for OEL lesions with the greatest diameter < 1.5 cm (2.7 ± 1.0) or with the surface keratin layer ≦ 30 μm (2.7 ± 0.9) was significantly fewer than for OEL lesions with the greatest diameter ≧ 1.5 cm (4.4 ± 1.3, $P = 0.000$) or with the surface keratin layer > 30 μm (4.5 ± 1.2, $P = 0.000$), respectively (Table 4). After a follow-up period of 6–30 (mean, 18) months, eight (21%) of the 38 CR OEL lesions recurred. These eight OEL lesions recurred 6–14 (mean, 9) months after the last PDT treatment. The eight OEL recurrent lesions were treated by the same PDT protocol as before and showed complete regression after 1–3 (mean, 2) treatments. Although the mean treatment

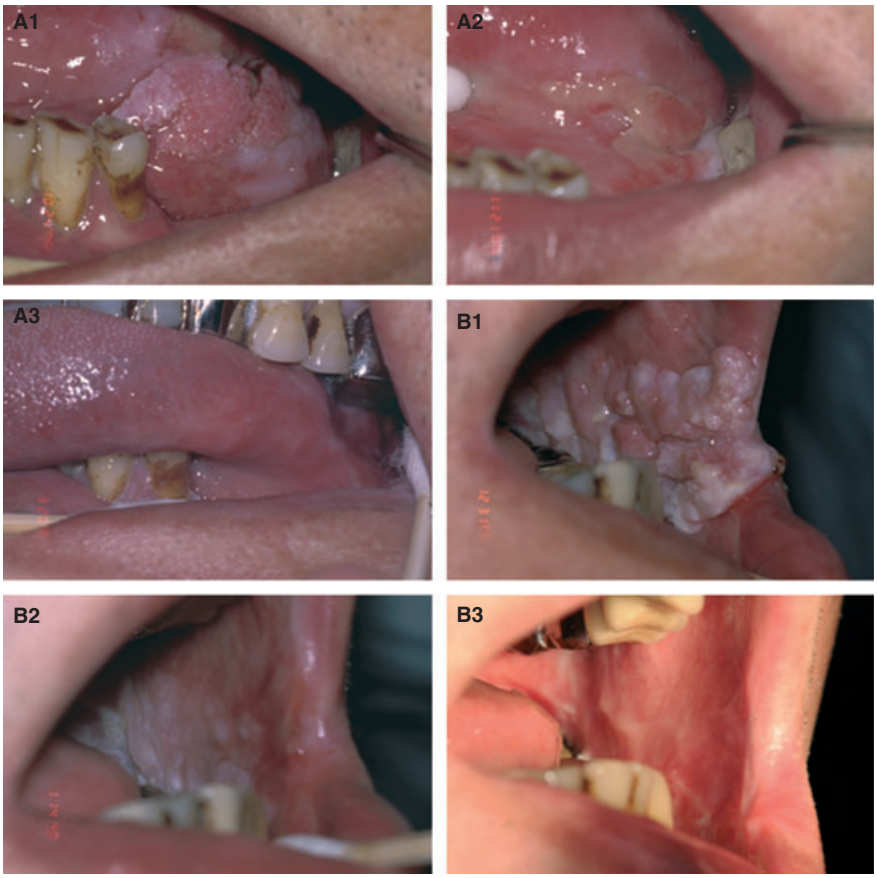

Figure 1 Clinical photographs of patients with oral verrucous hyperplasia (OVH) before and after the laser light-mediated topical ALA-PDT treatment. A: An OVH lesion on the left posterior lateral border of the tongue before biopsy (A1), after four treatments of PDT showing partial response (PR) (A2), and after six treatments of PDT showing complete response (CR) (A3). B: An OVH lesion on the left buccal mucosa before biopsy (B1), after four treatments of PDT showing PR (B2), and after six treatments of PDT showing CR (B3).

Table 2 Correlation between the mean treatment number of topical ALA-PDT to achieve a complete response and the clinicopathological parameters of 40 laser light-treated oral verrucous hyperplasia (OVH) lesions or those of 36 LED light-treated OVH lesions published previously (15)

	Mean treatment number ± SD			
	Laser light group (n = 40)	*P-value*[a]	*LED light group (n = 36)*	*P-value*[a]
Age		0.179		0.101
< 50 years	3.4 ± 1.3 (*n* = 22)		4.3 ± 1.3 (*n* = 16)	
≧ 50 years	3.9 ± 1.1 (*n* = 18)		3.5 ± 1.5 (*n* = 20)	
Location		0.454		0.446
Buccal mucosa	3.7 ± 1.2 (*n* = 25)		3.7 ± 1.5 (*n* = 24)	
Other oral mucosal sites	3.4 ± 1.4 (*n* = 15)		4.1 ± 1.4 (*n* = 12)	
Clinical appearance		0.000		0.000
Mass type	2.8 ± 1.1 (*n* = 20)		2.9 ± 1.3 (*n* = 17)	
Plaque or combination type	4.4 ± 0.8 (*n* = 20)		4.7 ± 1.1 (*n* = 19)	
Size (greatest diameter)		0.000		0.011
< 1.5 cm	2.2 ± 0.7 (*n* = 13)		3.0 ± 1.3 (*n* = 12)	
≧ 1.5 cm	4.3 ± 0.8 (*n* = 27)		4.3 ± 1.4 (*n* = 24)	
Color		0.000		0.000
Pink	3.0 ± 1.2 (*n* = 23)		3.2 ± 1.4 (*n* = 22)	
White	4.4 ± 0.8 (*n* = 17)		4.8 ± 1.1 (*n* = 14)	
Epithelial dysplasia[b]		0.000		0.043
With	2.5 ± 1.1 (*n* = 15)		3.1 ± 1.5 (*n* = 9)	
Without	4.2 ± 0.8 (*n* = 25)		4.2 ± 1.3 (*n* = 26)	
Surface keratin thickness[b]		0.000		0.003
≦ 40 μm	3.0 ± 1.2 (*n* = 24)		3.4 ± 1.4 (*n* = 23)	
> 40 μm	4.4 ± 0.8 (*n* = 16)		4.8 ± 0.7 (*n* = 12)	

[a]Student's *t*-test was used to compare the mean treatment numbers between any two groups.
[b]For the LED light group, biopsy was not done in one patient, because the lesion was too small.

Table 3 Univariate and multivariate analyses between PDT treatment number and clinicopathological parameters of 40 oral verrucous hyperplasia lesions by logistic regression and stepwise logistic regression, respectively

Factor	*Hazard ratio (95% CI)*	*P value*
Univariate		
Clinical appearance (Mass type vs. plaque or combination type)	0.037 (0.006, 0.219)	< 0.001
Size (greatest diameter) (< 15 mm vs. ≧ 15 mm)	0.019 (0.002, 0.181)	0.001
Color (Pink vs. white)	0.071 (0.013, 0.392)	0.002
Epithelial dysplasia (With vs. without)	0.063 (0.013, 0.310)	0.001
Surface keratin thickness (≦ 40 μm vs. > 40 μm)	0.086 (0.016, 0.468)	0.005
Multivariate (stepwise)		
Size (< 15 mm vs. ≧ 15 mm)	0.035 (0.003, 0.377)	0.006
Epithelial dysplasia (With vs. without)	0.149 (0.021, 1.041)	0.055

number of PDT to achieve a CR for the 38 laser light-treated OEL lesions (3.4 ± 1.4) was lower than that for the 17 LED light-treated OEL lesions (3.7 ± 1.4), no significant difference was found ($P = 0.466$, Student's t-test).

Discussion

This study showed that the laser light-mediated topical ALA-PDT is also very effective for OVH and OEL lesions. We suggest that the successful clinical outcomes for OVH and OEL lesions treated by the laser light-mediated topical ALA-PDT may be due to the ALA preparation, the topical ALA-PDT protocol used, and the characteristic morphological, histologic and biological features of the OVH and OEL lesions themselves. In brief, our 20% ALA preparation was a gel form, which was adhesive to the oral mucosa, was partially resistant to the dilution of the saliva, and in turn helped the absorption of ALA from the mucosal surface. Moreover, this study used a fractionated protocol to deliver light treatment, the lesional epithelial cells might regenerate new PpIX and obtain new oxygen during multiple 3-min resting periods, finally resulting in a more successful clinical outcome for our OVH and OEL lesions (12–16).

The verrucous appearance of the OVH lesion provided a large area for good retention and absorption of

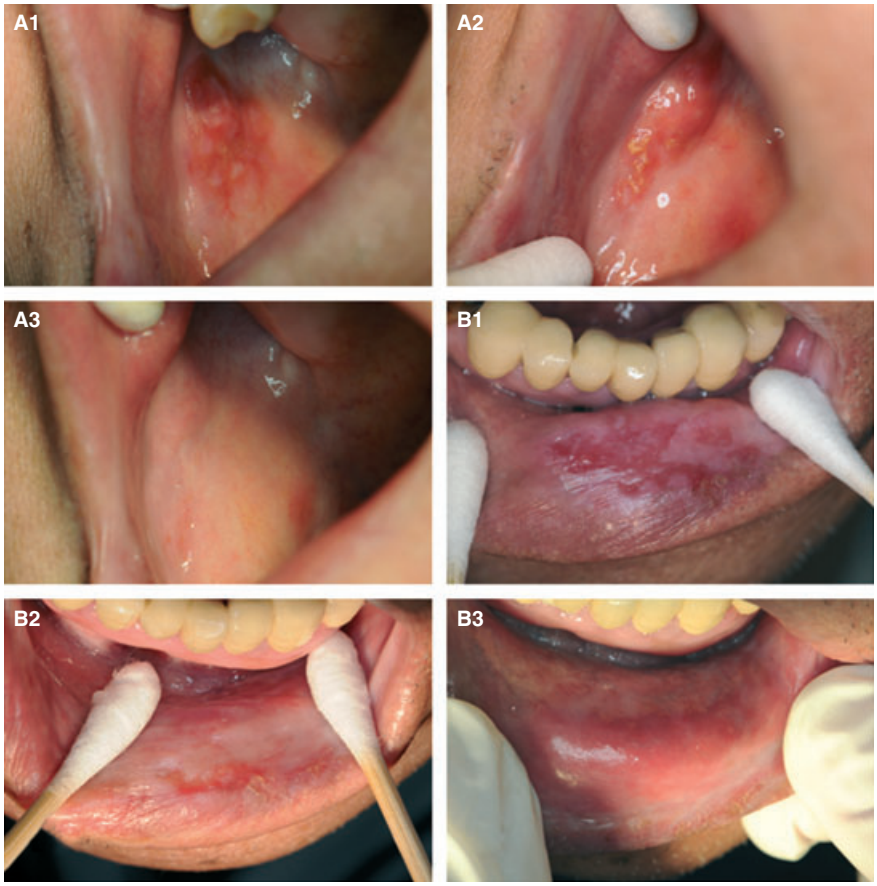

Figure 2 Clinical photographs of patients with oral erythroleukoplakia (OEL) before and after the laser light-mediated topical ALA-PDT treatment. A: An OEL lesion on the right posterior buccal mucosa before biopsy (A1), after one treatment of PDT showing partial response (PR) (A2), and after four treatments of PDT showing complete response (CR) (A3). B: An OEL lesion on the left lower labial mucosa before biopsy (B1), after two treatments of PDT showing PR (B2), and after three treatments of PDT showing CR (B3).

Table 4 Correlation between the mean treatment number of topical ALA-PDT to achieve a complete response and the clinicopathological parameters of 38 laser light-treated oral erythroleukoplakia (OEL) lesions or those of 17 LED light-treated OEL lesions published previously (16)

	Mean treatment number ± SD			
	Laser light group (n = 38)	*P-value*[a]	*LED light group (n = 17)*	*P-value*[a]
Age		0.227		1.000
< 50 years	3.8 ± 1.4 (*n* = 12)		3.7 ± 1.4 (*n* = 7)	
≧ 50 years	3.2 ± 1.4 (*n* = 26)		3.7 ± 1.5 (*n* = 10)	
Location		0.895		0.460
Buccal mucosa	3.4 ± 1.4 (*n* = 34)		3.8 ± 1.4 (*n* = 15)	
Other oral mucosal sites	3.5 ± 1.7 (*n* = 4)		3.0 ± 1.4 (*n* = 2)	
Size (greatest diameter)		0.000		0.023
< 1.5 cm	2.7 ± 1.0 (*n* = 22)		3.0 ± 0.9 (*n* = 9)	
≧ 1.5 cm	4.4 ± 1.3 (*n* = 16)		4.5 ± 1.5 (*n* = 8)	
Pathological diagnosis		0.750		0.070
Mild dysplasia	3.3 ± 1.5 (*n* = 12)		2.8 ± 1.0 (*n* = 4)	
Moderate dysplasia	3.6 ± 1.3 (*n* = 17)		4.4 ± 1.3 (*n* = 9)	
Severe dysplasia and carcinoma in situ	3.2 ± 1.5 (*n* = 9)		3.0 ± 1.2 (*n* = 4)	
Surface keratin thickness		0.000		0.006
≦ 30 μm	2.7 ± 0.9 (*n* = 23)		2.9 ± 0.9 (*n* = 9)	
> 30 μm	4.5 ± 1.2 (*n* = 15)		4.6 ± 1.3 (*n* = 8)	

[a]The mean treatment numbers were compared between any two groups by Student's *t*-test or one way analysis of variance (ANOVA), where appropriate.

ALA on the surface. In general, OVH and OEL lesions with smaller size, pink to red color, epithelial dysplasia, and thinner surface keratin layer had better PDT outcomes than those corresponding lesions, respectively. Pink to red and dysplastic oral OVH and OEL lesions usually had thinner surface keratin layer, leading to diffusion of more ALA into the lesions. Furthermore, dysplastic OVH and OEL lesions usually had more permeable epithelium (due to wide intercellular spaces of the dysplastic epithelium); this also resulted in diffusion of more ALA into the lesions. In addition, the dysplastic epithelium may retain more ALA than the hyperplastic epithelium, and the thinner keratin layer may only have a minimal effect on the reduction of the light intensity. In addition, there are more epithelial cells in the cell division cycle in dysplastic OVH and OEL lesions than in non-dysplastic OVH lesions (17). Dysplastic epithelial cells in the cell division cycle are more susceptible to the destruction by PDT-generated singlet oxygen molecules and free radicals than those epithelial cells not in the cell division cycle. The sufficient photosensitizers and light dose finally resulted in a better clinical outcome for those OVH and OEL lesions with pink to red color, epithelial dysplasia, and thinner surface keratin layer.

This study showed that the laser light-mediated topical ALA-PDT was as effective as the LED light-mediated topical ALA-PDT for treatment of OVH and OEL lesions (11–16). This finding suggests that the total light dose rather than the light source is more important for a successful PDT outcome. LED light device has the advantages of being a simpler, smaller, lighter, less expensive, and more portable light source than the laser machine. However, the LED chips are very sensitive to the heat generated during the PDT treatment and may deteriorate rather quickly after repeated use. This results in the need of frequent changes of the LED chips every 6–12 months. In contrast, the laser system is well-designed, more stable, and power-adjustable. It can be used for a long period of several years without the need of changing the laser light source. However, it is heavier and more expensive. Therefore, the choice of using either the LED or laser light source depends on the budget of the institute.

For oral precancerous lesions, ALA-PDT is one of the best treatments of choice. Topical ALA-PDT is even superior to systemic ALA-PDT because the former uses a small amount of ALA (20–200 mg) per treatment and has no systemic side effects and skin photosensitivity even within initial 48 h after PDT (11–16, 18). Topical ALA-PDT is conservative and noninvasive, is easily accepted by the patients, and usually results in little or no scar formation. In addition, our previous studies and this study proved that the topical ALA-PDT using either the LED or laser light is very effective for treatment of OVH and OEL lesions (11–16). Therefore, we suggest the use of topical ALA-PDT as the first-line treatment of choice for both OVH and OEL lesions in the near future.

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Conflict of Interest

None.