Photodiagnosis and Photodynamic Therapy 26 (2019) 73–78

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

What is the most relevent factor causing pain during ALA-PDT? A multi-center, open clinical pain score research trial of actinic keratosis, acne and condylomata acuminata

Zhe Zheng[a,b,1], Ling-Lin Zhang[a,1], Lei Shi[a], Yun-Feng Zhang[a], Bo Wang[c], Qian-Qian Wu[a], Fang Fang[d], Wu-Qing Wang[e], Ronald Sroka[f,g], Xiu-Li Wang[a,*]

[a] Institute of Photomedicine, Shanghai Skin Disease Hospital, Tongji University, School of Medicine, Shanghai, China
[b] Dermatology, Clinical Medical of Yangzhou University, Yangzhou, Jiangsu, China
[c] Department of Dermatology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China
[d] Department of Dermatology, Jinshan Hospital of Fudan University, Shanghai, China
[e] Department of Dermatology, Minhang Hospital of Fudan University, Shanghai, China
[f] Laser-Forschungslabor, LIFE Center, University Hospital of Munich, Munich, Germany
[g] Department of Urology, University Hospital of Munich, Munich, Germany

ARTICLE INFO

Keywords:
Pain
Photodynamic therapy
Actinic keratosis
Acne
Condylomata
Acuminate

ABSTRACT

Background: To date, it has been reported that the intrinsic factors(lesions location, lesions area, disease tynpes) and extrinsic factors(fluence rate) contribute to the pain during 5-aminolevulinic acid photodynamic therapy (ALA-PDT). But there are few studies on pain during ALA-PDT and lack of sufficient clinical evidence related to the pain intensity.

Objective: To investigate pain intensity and its relative factors during ALA-PDT and to provide clinical implication.

Methods: The pain numeric rating scale (PNRS) score was used to evaluate the patients' pain intensity at different times during ALA-PDT irradiation from 0 to 10 min during treatment. Gender, age, lesions location, lesions area, ALA concentration and fluence rate were recored.

Results: The trial enrolled 274 patients in total, including 118 acne patients (in face), 30 actinic keratosis(AK) patients(in face), 126 Condylomatata acuminate patients(in genitalia). The average pain score in PDT was highest in the patients with actinic keratosis(7.3 ± 0.7), and that of condylomata acuminata was the lowest (4.5 ± 1.1) ($p < 0.05$). The highest pain score in patients with AK, acne and condylomata acuminata was 8, 6 and 6 respectively which occurred at 4 min, 4 min and 6 min respectively. The pain score of males was higher compared with females in all of the three diseases ($p < 0.05$). The pain score of facial diseases (5.6 ± 1.2) was higher than that of the genitalia (4.5 ± 1.1) ($p < 0.05$). The lesions area was positively correlated with the pain score ($p < 0.05$). In facial diseases, the pain score of patients with high fluence rate (7.3 ± 0.7) was higher than patients with low fluence rate (5.1 ± 0.9) ($p < 0.05$).

Conclusions: Intrinsic and extrinsic factors both correlate with pain during PDT. Intrinsic factors are difficult to change, so extrinsic factors are the key point to control. We can reduce the fluence rate and extend the treatment time, relieving pain intensity while still ensuring equivalent efficacy.

1. Introduction

5-Aminolevulinic acid photodynamic therapy (ALA-PDT) is widely used in dermatology for the treatment of actinic keratosis (AK), acne and condylomata acuminata in China [1–4]. It employs ALA as a photosensitizer (PS) precursor and visible light in the presence of oxygen, leading to the production of cytotoxic reactive oxygen species(ROS), which can damage the cellular organelles and cause cell death [5]. Compared with other conventional treatment modalities, ALA-PDT has numerous advantages, such as non-invasive technique conservation of the original structure and function of tissues or organs, large area of disease treatment, effective for invisible or metastatic tumor and good

* Corresponding author at: Institute of Photomedicine, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, 200443, China.
E-mail address: wangxiuli_1400023@tongji.edu.cn (X.-L. Wang).
[1] These authors have contributed equally to this work

https://doi.org/10.1016/j.pdpdt.2019.03.001
Received 31 January 2019; Received in revised form 18 February 2019; Accepted 1 March 2019
Available online 02 March 2019

cosmetic effect [6].

However, ALA-PDT sessions have to be paused because of pain [7]. At present, there are few studies on pain during ALA-PDT, and even lack of sufficient evidence of the risk factors for pain.

It has been reported that the lesion location, lesion area and type of diseases contribute to variations of pain during ALA-PDT [3,5,8]. However, there is no comprehensive analysis of the factors that influence pain. Therefore, this study is a prospective clinical study intended to explore the relationship between clinical relative factors and pain during ALA-PDT.

2. Patients and methods

2.1. Patients

Patients in Shanghai Skin Disease Hospital, Jinshan Hospital and Minhang Hospital

from October 2015 to October 2017 were included in the prospective study.

2.2. Inclusion standard

(1) Patients with moderate to severe facial acnes were selected and the diagnosis and classification of acne were referring to the Cunliffe classification method [9,10]. (2) Condylomata acuminata was diagnosed through histological examination or polymerase chain reaction test of HPV [5]. (3) The AK patients were diagnosed through dermoscopy and histopathology. (4) All patients were above 18 years old [5]. (5) Based on the approval of ethics committee specify, all patients signed informed consent and agreed to participate in this study.

2.3. Exclusion standard

(1) Patients with psychological problems, photosensitive disease, allergic to porphyrins or intolerable of treatment, pregnancy and lactation, taking oral contraceptives and systemic glucocorticoids were excluded [10,11]. (2) Before participating in the study, the patients who systematically took retinoic acid within 1 year,systemically took antibiotics within 1 month, and used topical medications for acne treatment within 2 weeks [5]. (3) Patients who had gonorrhea and non-gonococcal urethritis/cervicitis, autoimmune disease or immunosuppression (such as systemic lupus erythematosus, dermatomyositis, human immunodeficiency virus infection, transplantation or corticosteroid therapy, etc.) were excluded from the study [4]. (4) Patients who had taken painkillers or long-term painkillers during the past week were excluded. (5) Patients with hypertension and heart disease were excluded.

2.4. ALA-PDT treatment

In this study, freshly prepared 5%, 10% and 10% ALA cream (5-aminolevulinic acid, product name: ALA, a product of Shanghai Fudan Zhangjiang Bio-Pharmaceutical Co., Ltd., Shanghai, China) were used for the treatment of patients of acne, AK and condylomata acuminata respectively. The prepared cream was evenly applied to the skin lesions and 2 cm around it. After wrapping it with plastic film and protecting from light for 3 h, the acne patients were irradiated with a red LED device (630 ± 5 nm, Led 635, wuhan yage Co., Ltd., Wuhan, China.) by the fluence rate was 40 mw/cm^2 [12], energy 36–50 J/cm^2. The AK patients were irradiated with a red LED device by the fluence rate was 80 mw/cm^2 [10], energy 80–100 J/cm^2. The condylomata acuminata patients were treated with a red LED by the fluence rate was 80 mw/cm^2 [4], energy 80–100 J/cm^2. During this study, the patients were asked to report any adverse effect to the medical staff. The treatment was repeated in every 7–10 days, and 3–5 treatments were provided in total [10].

2.5. Pain evaluation

For each patient, the pain score was evaluated by using the pain numeric rating scale (PNRS). For a score of 0, there is no pain, and a score of 10 indicates the most intolerable degree of pain [13]. The scores when the patients experienced the most server pain (take integers) were used as the final result of assessment. During the treatment with the 7 times (0, 1, 2, 4, 6, 8, 10 min), the patients' pain scores were recorded by the same doctor. For patients who can't afford the pain, we stopped recording their pain scores and gave them cooling to relieve pain. Present study only selected the first treatment of each patient for analysis.

2.6. Statistical methods

SPSS 17.0 statistical software (SPSS, version 17.0 for Windows; SPSS, Chicago, III, USA) was used for statistical analysis. One-way analysis of variance was used to compare the components of the disease types. Gender, skin lesions, ALA concentrations, and fluence rate were compared using two independent samples *t*-test. The age and lesion area were used linear regression equation. In addition, a multivariate linear regression analysis of gender, disease type, fluence rate, age, and lesion area were performed. $p < 0.05$ indicates statistical significance.

3. Results

3.1. General information of patients included

As shown in Table 1, their average age of 274 patients was 34.3 ± 17.8 years old. Among them, 190 patients were male with

Table 1
Patients information.

Risk factors	AK	Age Mean ± SEM	Condylomata acuminata	Age Mean ± SEM	Acne	Age Mean ± SEM
Gender						
Male	11	72.5 ± 10.5	101	36.1 ± 12.3	78	22.6 ± 5.6
Female	19	72.5 ± 10.4	25	36.7 ± 12.1	40	34.3 ± 5.7
Localization						
Face	30	72.5 ± 10.4	0	36.1 ± 12.3	118	22.6 ± 5.6
Genitalia	0		126		0	
ALA concentration						
5%	0	72.5 ± 10.4	0	36.1 ± 12.3	118	22.6 ± 5.6
10%	30		126		0	
Fluence rate						
40mw/cm^2	0		0		118	22.6 ± 5.6
80mw/cm^2	30	72.5 ± 10.4	126	36.1 ± 12.3	0	

average age of 34.0 ± 17.5, and 84 patients were female with average age 34.5 ± 18.0. There were 118 (43.07%) acne patients (78 male patients and 40 female patients) with more than 12 inflammatory acne lesions on their face, and the course of them was between 6 months to 10 years. There were 30 (10.95%) AK patients (11 male patients and 19 female patients) and the course of disease was between 1 year to 10 years. There were 126 (45.99%) cases of condylomata acuminata (101 male patients and 25 female patients), located on mons pubis, labia majora, labia minora and crissum and the course of disease was between 1 month to 48 months. The symptoms included light red papillary or filiform neoplasm, soft and brittle, which tends to bleed when touched [9]. All patients completed the treatment, and no one withdrew during the halfway. All patients signed informed consent and agreed to participate in the present study based on the approval of ethics committee specify. All the patients participated in this photodynamic therapy had burning sensation, but all patients completed the treatment of all patient (Table 1).

3.2. Pain score during different time points in ALA-PDT

The pain scores of the patients with AK, acne and condylomata acuminata were all increased with time. The changes are all shown in Fig.1. At 4th minute, the pain score in acne and AK groups reached the maximum mean score, 5 points and 7 points respectively. At 6th minute, the maximum mean pain score was 4 points in condylomata acuminata. One-way analysis of variance was used to analysis disease types at 0 min, 4 min, 6 min. The score of pain in patients of AK, acne and condylomata acuminata had a significant statistical difference ($p < 0.05$). Among them, two independent samples *t*-test was used to analysis disease type in two groups respectively. It was shown that at the pain score had a significant statistical difference when the pain score of AK paients compared with the pain score of acne paients ($p < 0.05$), the pain score of AK paients compared with the pain score of Condylomata acuminate paients ($p < 0.05$), and the pain score of acne paients compared with the pain score of condylomata acuminata paients ($p < 0.05$). At 1 min, the pain score of acne paients VS pain score of condylomata acuminata patients have a significant statistical difference ($p < 0.05$). At 2 min, 8min, 10min, the pain score of AK paients VS pain score of acne patients have a significant statistical difference ($p < 0.05$), the pain score of AK paients VS pain score of condylomata acuminata patients have a significant statistical difference ($p < 0.05$).

3.3. Analysis the effects of relative pain factors of PDT

The disease type and lesion location were the single factor associated with the intensity of pain ($p < 0.05$). The pain score of AK was the highest and the condylomata acuminata was lowest, and the pain score of the facial diseases was higher than that of the genitals (Table 2).

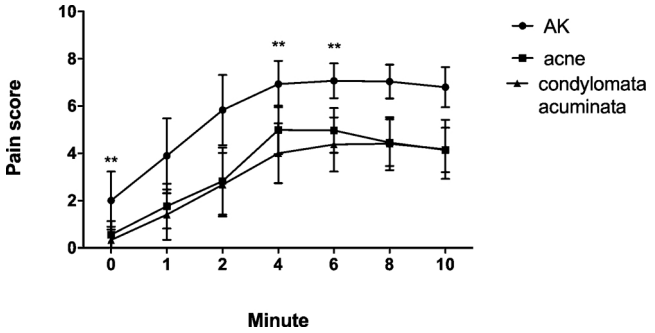

Fig. 1. Pain visual analogue scale at different time points in ALA-PDT for acne (10 min), AK (10 min), Condylomata acuminate (10 min).

Table 2
Single factor analysis of pain intensity.

Risk factors	n	Mean ± SEM	F	t	p value
Gender			8.974	1.523	0.130
Male	190	5.1 ± 1.2			
Female	84	4.9 ± 1.5			
Localization			3.457	−7.865	< 0.001
Face	148	5.6 ± 1.2			
Genitalia	126	4.5 ± 1.1			
Diagnosis			68.337		< 0.001
AK	30	7.3 ± 0.7			
Acne	118	5.1 ± 0.9			
Condylomata acuminata	126	4.5 ± 1.1			
ALA concentration			15.699	0 . 904	0.367
5%	118	5.1 ± 0.9			
10%	156	5.0 ± 1.5			
Fluence rate			15.699	0 . 904	0.367
40mw/cm^2	118	5.1 ± 0.9			
80mw/cm^2	156	5.0 ± 1.5			

SEM:standard error of the mean p < 0.05 is regarded as significant.

Linear regression analysis showed that there was a linear relationship between lesion area and pain score ($p < 0.05$). As shown in Fig. 2, when lesion area is less than 14 cm^2, the pain score is increased with lesion area. When the lesion area is more than 14 cm^2, even if the skin lesion area continues to increase, the pain score remains unchanged. As shown in Fig. 3, there was also a linear relationship between age and pain ($p < 0.05$). Among them, age under 40 years old patients had a positive correlation with the pain score (Table 3).

3.3.1. Multivariate linear regression analysis

According to the gender, disease type, fluence rate, age and lesion area, the linear regression model had statistical significance ($p < 0.001$, and adjust $R^2 = 0.515$). The gender, disease type, fluence rate and lesion lcation were statistically significant ($p < 0.05$) (Table 4).

3.4. Further analysis of the pain relative factors of PDT in the three diseases respectively

Gender has an effect on the pain scores of the three diseases ($p < 0.05$), and the pain scores of male are higher than women (Table 5).

As shown in Fig. 4, there was also a linear relationship between lesion area (not age) and pain score in condylomata acuminata, and there was a positive correlation (Table 6).

3.5. Analysis of the pain scores and relative facors of PDT in patients with facial diseases

Analysis of the pain in patients with acne and AK on gender, disease type, ALA concentration and fluence rate (Table 7). The average pain score of AK patients was higher than acne patients ($p < 0.05$). The pain score of patients with high ALA concentration was higher than patients with low ALA concentration ($p < 0.05$). The pain score of patients with high fluence rate was higher than patients with low fluence rate ($p < 0.05$). The pain scores of male are higher than women ($p < 0.05$).

Analysis of the effects of age and lesion area on the pain score in facial diseases by linear analysis. The results in Fig. 5 showed that there was a linear relationship between age and pain score, and there was a positive correlation ($p < 0.05$) (Table 8).

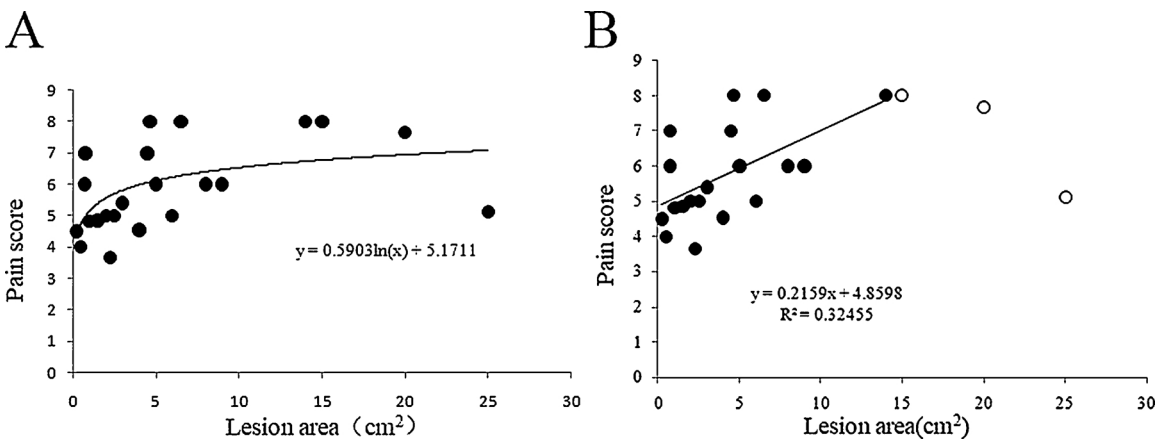

Fig. 2. Linear analysis of lesion area and pain score in the three diseases.

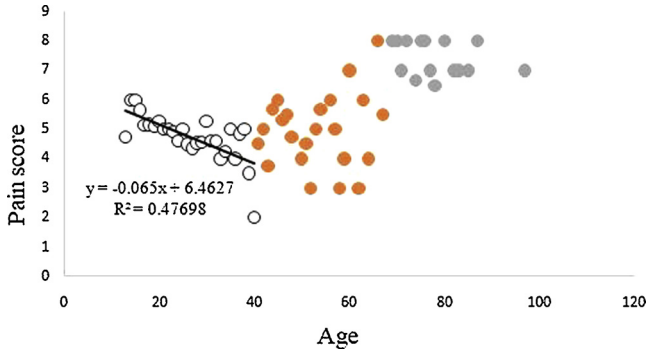

Fig. 3. Linear analysis of age and pain score in the three diseases.

Table 3
Linear regression analysis between age and pain score.

Risk factors	N	B	p value
Age			
≤40	208	−0.643	< 0.001
Lesion area	274	0.52	0.013

B: regression coefficient p < 0.05 is regarded as significant.

Table 4
Multivariate linear regression analysis results.

Risk factors	B	std	Beta	p value
Gender	−0.838	0.125	−0.301	< 0.0001
Diagnosis	1.449	0.119	0.766	< 0.0001
Fluence rate	0.045	0.688	0.688	0.001
Age	0.074	0.025	0.025	0.674
lesions area	0.049	0.439	0.439	0.043

B: regression coefficient.
Std: standard error.
Beta: standardized coefficients p < 0.05 is regarded as significant.

4. Discussion

ALA-PDT is widely used in the dermatology department currently. Pain is still a challenge during the treatment with complicated etiopathogenesis as well as unknown pathogenesis [14–17].

So far, the influencing factors of pain are still unclear. Some studies reported that during the treatment of lesions on face, the average pain score was associated with lesion area, gender and types of dieases [18,19]. However, the factors affecting pain in acne and condylomata acuminata patients and the pain intensity at multiple time points during treatment were not involved. This study was aimed to help analysing the specific factors that affect pain score, determining patients tolerance degree of pain during treatment, formulating intervention measures for adverse symptoms in advance and reducing the incidence of pain and alleviate the intensity of pain further.

Table 5
Gender analysis of pain score.

Diagnosis	n	Mean ± SEM	F	t	p value
AK			0.437	4.151	< 0.0001
Male	11	7.8 ± 0.4			
Female	19	6.9 ± 0.6			
Acne			1.230	6.873	< 0.0001
Male	78	5.5 ± 0.8			
Female	40	4.4 ± 0.9			
Condylomata acuminata			0.698	2.649	0.009
Male	101	4.6 ± 1.0			
Female	25	4.0 ± 1.2			

SEM:standard error of the mean p < 0.05 is regarded as significant.

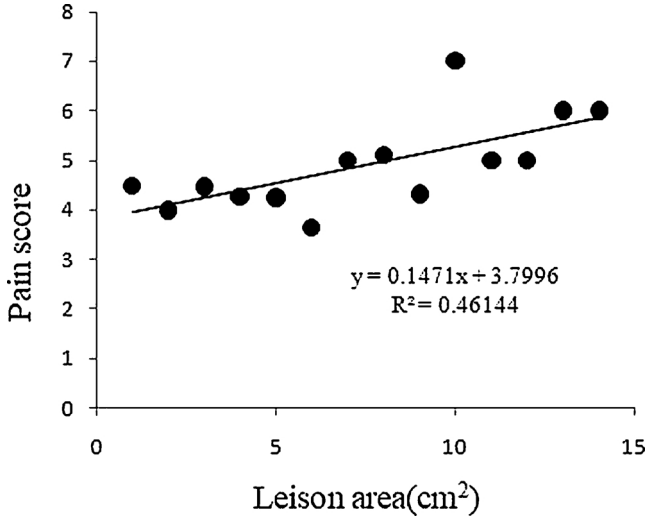

Fig. 4. Linear analysis of lesion area and pain score in Condylomata acuminate.

In our study, our results show that different diseases have different pain scores. The patients of AK have the highest pain score and the patients of condylomata acuminata lowest. This might be related to the lesion location of disease as different parts have different pain scores during ALA-PDT. The pain score of facial diseases patients are the highest because there are many nerves distributed on face that make

Table 6
Analysis of the effect about lesion area and age on pain score.

Diagnosis	N	B	p value
AK			
Lesion area	30	–	0.759
Age	30	–	0.753
Acne			
Age	118	–	0.392
Condylomata acuminata			
Lesion area	126	0.679	0.007
Age	126	–	0.987

B: regression coefficient $p < 0.05$ is regarded as significant.

Table 7
Effect of age, ALA concentration, fluence rate on pain scores.

Risk factors	n	Mean ± SEM	F	t	p value
ALA concentration			1.883	−11.691	< 0.0001
5%	118	5.1 ± 0.9			
10%	30	7.3 ± 0.7			
Fluence rate			1.883	−11.691	< 0.0001
40 mw/cm^2	118	5.1 ± 0.9			
80 mw/cm^2	30	7.3 ± 0.7			
Diagnosis			1.883	11.691	< 0.0001
AK	30	7.3 ± 0.7			
Acne	118	5.1 ± 0.9			
Gender			12.635	2.478	0.015
Male	89	5.8 ± 1.1			
Female	59	5.2 ± 1.4			

SEM:standard error of the mean $p < 0.05$ is regarded as significant.

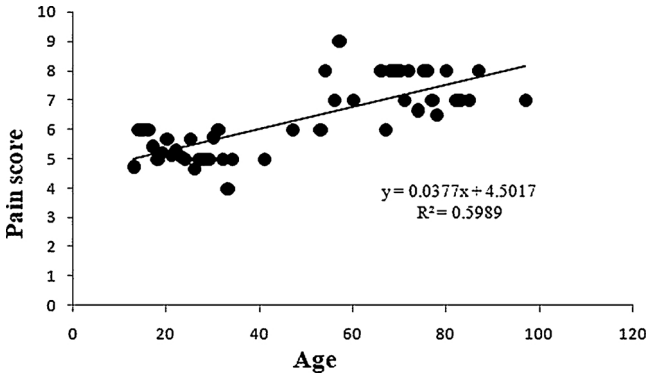

Fig. 5. Linear analysis of age and pain score in facial diseases.

Table 8
Effect of lesion area and age on pain score.

Risk factors	n	B	p value
Age	148	0.773	< 0.001
Lesion area	148	–	0.674

B: regression coefficient $p < 0.05$ is regarded as significant.

them more sensitive [20], which explain why the pain score of genitalia is lower than that of the facial diseases.

Although the pain scores of female patients were higher than those of males in the respective studies of the three diseases, there was no statistically significant comparison between the three diseases.

Interestingly, there is a negative correlation between age and pain score of patients under 40 years old. This may be due to the decline in people's the neuroepidermal density and sensitivity as they age [21], which reduces their tolenrance to pain.

It was showed that the larger lesion area is, the higher pain score is, which has a linear relationship. However in our study, after the lesion area reaches a certain value, the pain score will no longer increase. Through further analysis, the lesion area is positively correlated with the pain score in patients of condylomata acuminata.

In our analysis of the three diseases, the above therapeutic factors didn't show significant statistical difference. Interestingly, when we used low concentration and low fluence rate ALA-PDT to treat acne patients, the average pain score was still significantly higher than the pain score of patients with condylomata acuminata. The possible reason is that facial nerve intensive has a great impact on pain scores. It was reported that PDT can increase the sensitivity of sensory neurons to capsaicin, produced by prostaglandins [3]. Our studies show that when the patients with ficial diseases were treated with high fluence rate and high ALA concentration, the pain scores of patients were significantly higher than low ones with statistical difference. Additionally, it was observed that low concentration and low fluence rate treatment had the same effects compared with high treatment. And it can make acne patients with less pain and more comfortable [22]. Some articles show that daylight PDT in comparison with conventional PDT was as effective, better tolerated and nearly painless with high patient satisfaction, and may be considered a treatment of choice to meet needs of patients with mild or moderate facial AK patients [23]. And in our multivariate linear model, we found that in the three diseases, gender, disease type, lesion area, and fluence rate have an impact on the pain score. Among them, gender, disease types, lesion area, these three factors can not be changed by patients, but we can adjust the fluence rate to reduce the local ROS concentration in the patient's diseases lesions, thereby reducing the pain of patients, the local concentration of ROS is also a possible causes of pain [17,24]. So maybe we can give paitents low fluence rate and long time to complete ALA-PDT.

In summary, we believe that disease types, lesion location, gender, lesion area,age and fluence rate are all factors related to pain intensity during the ALA-PDT. Before clinical ALA-PDT, comprehensive evaluation of patients should be done based on the above factors. For patients with potential severe pain, appropriate analgesic measures can be provided to the patients. Through this study, we find that among the six influencing factors, disease types, lesion location, gender, lesion area, age are the intrinsic factors. So we can extend the treatment time and reduc the fluence rate, maybe it can relieve pain while ensuring efficacy at the same time. Nevertheless, further larger studies are needed to support the results of this promising report.

Funding sources

This work was supported by National Natural Science Foundation of China (81472538), Shanghai Shenkang Hospital Development Center (SHDC12015123), Shanghai Municipal Commission of Health and Family Planning (201540273, 2013SY007).

Conflicts of interest

No medical companies participated in funding this trial. The authors have no conflicts of interest to declare.

What's already known about this topic?

The average pain score was associated with lesion area, gender and types of dieases during the ALA-PDT.

What does this study add?

A detailed analysis about the factors affecting pain in acne, AK and condylomatata acuminata patients. And the pain intensity at multiple time points during treatment were involved in this study.

Acknowledgements

We would like to thank the professor Xiangming Li at Yangzhou University for his statistical guidance to this study.This work was

supported by National Natural Science Foundation of China (81472538), Shanghai Shenkang Hospital Development Center (SHDC12015123), Shanghai Municipal Commission of Health and Family Planning (201540273, 2013SY007).

References

[1] C.A. Morton, H.C. Wulf, R.M. Szeimies, Y. Gilaberte, N. Basset-Seguin, E. Sotiriou, S. Piaserico, R.E. Hunger, S. Baharlou, A. Sidoroff, L.R. Braathen, Practical approach to the use of daylight photodynamic therapy with topical methyl aminolevulinate for actinic keratosis: a European consensus, J. Eur. Acad. Dermatol. 29 (9) (2015) 1718–1723.

[2] S.R. Wiegell, H.C. Wulf, R.M. Szeimies, N. Basset-Seguin, R. Bissonnette, M.J.P. Gerritsen, Y. Gilaberte, P. Calzavara-Pinton, C.A. Morton, A. Sidoroff, L.R. Braathen, Daylight photodynamic therapy for actinic keratosis: an international consensus, J. Eur. Acad. Dermatol. 26 (6) (2012) 673–679.

[3] B. Wang, L. Shi, Y.F. Zhang, Q. Zhou, J. Zheng, R.M. Szeimies, X.L. Wang, Gain with no pain? Pain management in dermatological photodynamic therapy, Br. J. Dermatol. 177 (3) (2017) 656–665.

[4] X.L. Wang, H.W. Wang, H.S. Wang, S.Z. Xu, K.H. Liao, P. Hillemanns, Topical 5-aminolaevulinic acid-photodynamic therapy for the treatment of urethral condylomatata acuminata, Br. J. Dermatol. 151 (4) (2004) 880–885.

[5] S.R. Wiegell, H.C. Wulf, Photodynamic therapy of acne vulgaris using methyl aminolaevulinate: a blinded, randomized, controlled trial, Br. J. Dermatol. 154 (5) (2006) 969–976.

[6] A. Klein, P. Babilas, S. Karrer, M. Landthaler, R.M. Szeimies, Photodynamic therapy in dermatology - an update 2008, J. Dermatol. Ges. 6 (10) (2008) 839–845.

[7] C. Fink, A. Enk, P. Gholam, Photodynamic therapy - aspects of pain management, J. Dermatol. Ges. 13 (1) (2015) 15–22.

[8] C.B. Halldin, M. Gillstedt, J. Paoli, A.M. Wennberg, H. Gonzalez, Predictors of pain associated with photodynamic therapy: a retrospective study of 658 treatments, Acta Derm.-Venereol. 91 (5) (2011) 545–551.

[9] W.J. Cuncliffe, V. Goulden, Phototherapy and acne vulgaris, Br. J. Dermatol. 142 (5) (2000) 855–856.

[10] A. Ganemo, M. Virtanen, A. Vahlquist, Improved topical treatment of lamellar ichthyosis: a double-blind study of four different cream formulations, Br. J. Dermatol. 141 (6) (1999) 1027–1032.

[11] P. Mikolajewska, V. Iani, A. Juzeniene, J. Moan, Topical aminolaevulinic acid- and aminolaevulinic acid methyl ester-based photodynamic therapy with red and violet light: influence of wavelength on pain and erythema, Br. J. Dermatol. 161 (5) (2009) 1173–1179.

[12] L. Ma, L.H. Xiang, B. Yu, R. Yin, L. Chen, Y. Wu, Z.J. Tan, Y.B. Liu, H.Q. Tian, H.Z. Li, T. Lin, X.L. Wang, Y.H. Li, W.Z. Wang, H.L. Yang, W. Lai, Low-dose topical 5-aminolevulinic acid photodynamic therapy in the treatment of different severity of acne vulgaris, Photodiagn. Photodyn. 10 (4) (2013) 583–590.

[13] M.A. Ferreira-Valente, J.L. Pais-Ribeiro, M.P. Jensen, Validity of four pain intensity rating scales, Pain 152 (10) (2011) 2399–2404.

[14] R.C.W. Jones, L.J. Xu, G.F. Gebhart, The mechanosensitivity of mouse colon afferent fibers and their sensitization by inflammatory mediators require transient receptor potential vanilloid 1 and acid-sensing ion channel 3, J. Neurosci. 25 (47) (2005) 10981–10989.

[15] D.M. Bautista, J. Siemens, J.M. Glazer, P.R. Tsuruda, A.I. Basbaum, C.L. Stucky, S.E. Jordt, D. Julius, The menthol receptor TRPM8 is the principal detector of environmental cold, Nature 448 (7150) (2007) 204–208.

[16] J.T. Bell, A.K. Loomis, L.M. Butcher, F. Gao, B. Zhang, C.L. Hyde, J. Sun, H. Wu, K. Ward, J. Harris, S. Scollen, M.N. Davies, L.C. Schalkwyk, J. Mill, F.M.K. Williams, N. Li, P. Deloukas, S. Beck, S.B. McMahon, J. Wang, S.L. John, T.D. Spector, M. Consortium, Differential methylation of the TRPA1 promoter in pain sensitivity, Nat. Commun. 5 (2014).

[17] N.Y. Huang, J. Zeng, J. Liang, H.X. Qiu, Y. Wang, Y. Gu, A randomized, double-blind, placebo-controlled study of oral oxycodone plus acetaminophen for the treatment of pain in photodynamic therapy on port wine stains, Photodiagn. Photodyn. 11 (2) (2014) 134–140.

[18] S. Grapengiesser, F. Gudmundsson, O. Larko, M. Ericson, A. Rosen, A.M. Wennberg, Pain caused by photodynamic therapy of skin cancer, Clin. Exp. Dermatol. 27 (6) (2002) 493–497.

[19] P. Gholam, K. Denk, T. Sehr, A. Enk, M. Hartmann, Factors influencing pain intensity during topical photodynamic therapy of complete cosmetic units for actinic keratoses, J. Am. Acad. Dermatol. 63 (2) (2010) 213–218.

[20] T.A. Middelburg, T. Nijsten, M.H.A. Neumann, E.R.M. de Haas, D.J. Robinson, Red light ALA-PDT for large areas of actinic keratosis is limited by severe pain and patient dissatisfaction, Photodermatol. Photoimmunol. 29 (5) (2013) 276–278.

[21] I. Besne, C. Descombes, L. Breton, Effect of age and anatomical site on density of sensory innervation in human epidermis, Arch. Dermatol. 138 (11) (2002) 1445–1450.

[22] Y. Ma, Y. Liu, Q.Q. Wang, J. Ren, L.H. Xiang, Prospective study of topical 5-aminolevulinic acid photodynamic therapy for the treatment of severe adolescent acne in Chinese patients, J. Dermatol. 42 (5) (2015) 504–507.

[23] J.P. Lacour, C. Ulrich, Y. Gilaberte, V. Von Felbert, N. Basset-Seguin, B. Dreno, C. Girard, P. Redondo, C. Serra-Guillen, I. Synnerstad, M. Tarstedt, A. Tsianakas, A.W. Venema, N. Kelleners-Smeets, H. Adamski, B. Perez-Garcia, M.J. Gerritsen, S. Leclerc, N. Kerrouche, R.M. Szeimies, Daylight photodynamic therapy with methyl aminolevulinate cream is effective and nearly painless in treating actinic keratoses: a randomised, investigator-blinded, controlled, phase III study throughout Europe, J. Eur. Acad. Dermatol. 29 (12) (2015) 2342–2348.

[24] J. Barge, T. Glanzmann, M. Zellweger, D. Salomon, H. van den Bergh, G. Wagnieres, Correlations between photoactivable porphyrins' fluorescence, erythema and the pain induced by PDT on normal skin using ALA-derivatives, Photodiagn. Photodyn. 10 (4) (2013) 683–693.