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Plum-blossom needle assisted photodynamic therapy for the treatment of oral potentially malignant disorder in the elderly

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ABSTRACT

Oral leukoplakia (OLK) is one of the most common oral potentially-malignant disorders (OPMD) with complex causes, a long disease course and a high tendency for recrudescence. Although a variety of methods exist for treating this disease, canceration rates remain high. Herein, we described a case of 72-year-old male patient with OLK of the palatine mucous membrane who had achieved complete remission after being treated with five sessions of plum-blossom needle (PBN) assisted 5-aminolevulinic acid-photodynamic therapy (ALA-PDT). The patient had since been subsequently placed under close observation (> 12 mo). To date, there has been no recurrence. PBN assisted PDT might be suitable for the treatment of OPMDs in patients presenting with epithelial hyperkeratosis.

1. Introduction

The mechanisms by which oral cancers arise depend on change in a number of different factors, genes and clinicopathological stage of the tumors [1,2]. OPMDs are chronic conditions that precede oral squamous cell carcinoma (OSCC), which may be a key factor in oral cancer malignant transformation [3,4]. Though surgery is still the main method to remove the lesions, there are a lot of important factors to understand and consider when choosing a therapy. Cases with oral potentially malignant disorders are often complicated by cardiovascular diseases in the elderly [5–7]. Thus, in an aging population, the old paradigms of treatment may not be suitable for all patients.

PDT is a minimally invasive targeted therapy based on the photodynamic effect. Photosensitizers can be used to drive the process of splitting oxygen into singlet oxygen and free radicals, causing localized photo damage and cell death [8]. Many clinical trials conducted over the course of the past decade have highlighted the feasibility of PDT in OPMDs [9–11]. Compared to systemic PDT, topical application avoids systemic photosensitization and is therefore favoured in oral disease. However, the effect of topical PDT remains limited due to the depth of the lesion and the degree of keratosis [12]. Yu et al [13] found that oral verrucous hyperplasia lesions with diameters no greater than 1.5 cm, epithelial dysplasia, or with a thin keratin layer ($\leq 40\mu$) have shorter treatment courses of PDT to achieve a complete remission than other types. According to previous reports, photodynamic effects can be enhanced by surface pretreatment of mucocutaneous lesions [14,15].

To date, reports of patients with PBN assisted PDT remain rare [16,17]. In addition, such cases have not been previously involved in oral disease. To address this, we discussed the feasibility of this application to OPMDs. This method seems to be a viable option for elderly patients, since it has minimal side effects, low risk of mutilation, and probably lower rates of recurrence than those following surgical treatments.

2. Case report

A 72-year-old man with a 50-year history of smoking was seen at the Peking University School and Hospital of Stomatology's Department of Oral Medicine on referral from the Department of Oromaxillofacial Surgery. He complained of having two thick white plaques on his hard palate for almost 5 years. In addition, he has had a 1-year history of mild pain and an uncomfortable sensation while eating. His medical history included long-term hypertension and coronary heart disease. Physical examination revealed the presence of irregularly shaped thick white lesions located bilaterally on the hard palate (Fig. 1A, B). A computed tomography (CT) scan failed to reveal any maxillary bone destruction. The patient first experienced a biopsy of his right hard palate at the Department of Oromaxillofacial Surgery, after which complete surgical resection was suggested. The final pathological diagnosis was of mild epithelial dysplasia with high hyperkeratosis and

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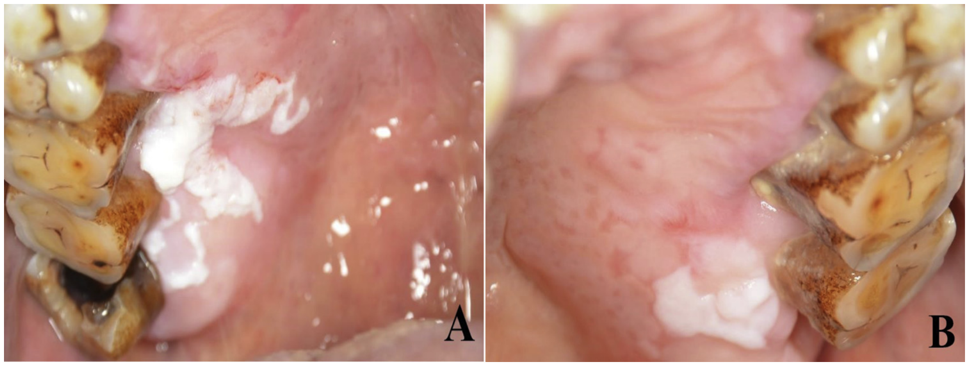

Fig. 1. Clinical photographs of the patient with oral leukoplakia before biopsy. (A) The lesions at the right hard palate and (B) the left side.

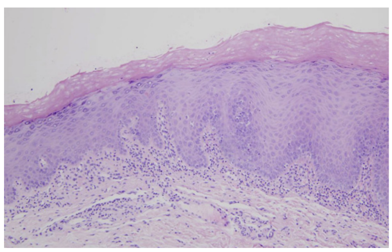

Fig. 2. Photomicrograph of the lesion showing hyperkeratotic epithelium and dysplasia with hyperchromatic cells (×10).

hypertrophy hyperplasia (Fig. 2). However, due to poor general condition and the location of the OLK, the patient refused surgical treatment and hoped to be treated conservatively.

In our department, a series of non-invasive screening were administered as diagnostic aids, including tissue autofluorescence imaging, toluidine blue staining and genetic cytology screening. All lesions analyzed by Visually Enhanced Lesion Scope (VELScope) fluorescence imaging showed no loss of autofluorescence (Fig. 3A, B). However, areas staining darkly with toluidine blue, indicating dysplastic changes, should be focused on and defined as high-risk areas which require treatment (Fig. 4). Moreover, quantitative DNA analysis showed two aneuploid cells. Nuclear extraction was carried out according to the Hedley method [18].

The patient was instructed to quit smoking and told to control dental plaque by chlorhexidine mouthwash. We first treated him with ALA-PDT alone, specifically as follows: The 10% ALA cream was prepared as a fresh thermosensitive hydrogel. A piece of medical absorbent cotton was applied to the lesion, which contain the above mentioned hydrogel because of its excellent absorption and hydrophilicity. Then the cotton sheet was also incubated with plastic wrap covering for 2 h. Next, a 635-nm LED light (100 J/cm^2, 100 mw/cm^2) is used to irradiate the lesions, as per previous reports [11,19]. The patient was treated once every 2 weeks. However, since there was no significant improvement to the lesions after 2 sessions of treatment, we next used PBN vertical tapping to penetrate the hyperkeratosis lesions until hemodiapedesis (Fig. 5). Before applying ALA-PDT, we cleaned up the surface of the lesions to avoid contamination. Subsequently, the patient underwent five sessions of PBN assisted PDT, with four on the left palate and one on the right. The treatment efficacy was evaluated by response of the lesion to the treatment. The plaques got gradually thinner and ultimately disappeared (Fig. 6). After 3 months of treatment,

Fig. 4. Toluidine blue staining obtained before treatment. The images depict the high-risk areas.

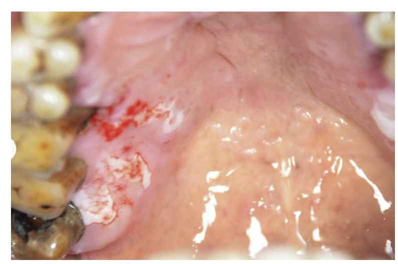

Fig. 5. Plum-blossom needle created tiny holes on the surface of the lesions.

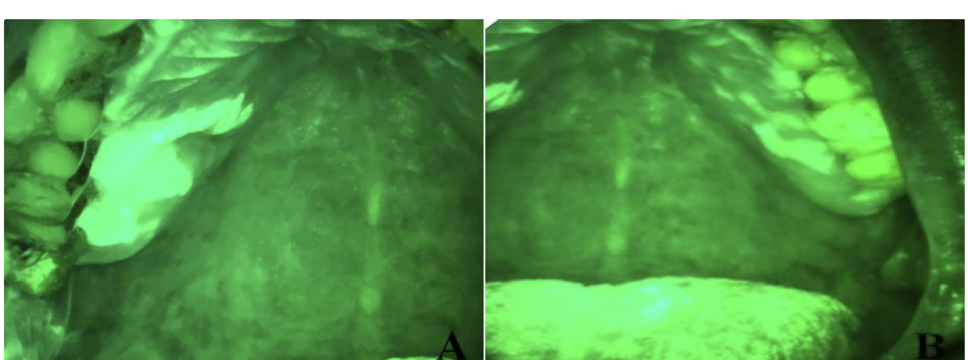

Fig. 3. Visually Enhanced Lesion Scope fluorescence imaging showed no loss of autofluorescence in the right hard palate (A) and the left side (B).

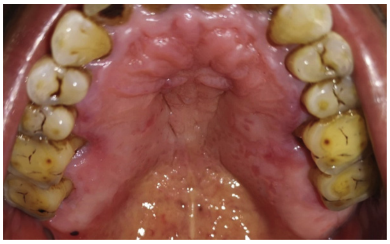

Fig. 6. Clinical photographs of the patient with oral leukoplakia after five sessions of plum-blossom needle with PDT.

Table 1
Results of ploidy status before and after treatment.

	Aiploid N (%)	Polyploid N (%)	Aneuploid N
Pre-treatment	1852 (92.0)	158 (7.9)	2
Post-treatment	2040 (91.1)	183 (8.9)	0

quantitative DNA analysis was repeated and aneuploidy was not observed. The differences in DNA content expressed as the ratios of DNA fluorescence are summarized in Table 1. There was no recurrence in the patient at the 12-month follow up.

3. Discussion

Active screening and earlier diagnosis and intervention are necessary for reducing cancer incidence and mortality [20]. Sharp increases in cancer rates have been noticed: According to 29 Cancer Groups reports, the global incidence of cancer has increased by about 10% between 1990 and 2016 [21]. In 2012, estimates are that 160,000 new OSCC cases have emerged, making this a common malignancy in Asia [22]. However, this unfortunately remains a poor OSCC prognosis. Warnakulasuriya et al [23] reported a higher risk of OMPDs transforming into oral cancer, a transformation which was also associated with increasing age. OMPDs are currently treated *via* topical surgical excision, oral retinoid drugs, laser, freezing, and other treatments [24]. This acts as a reminder that while current treatments are effective, they can always be improved.

PDT has been indicated for the management of oral inflammatory conditions such as OPMDs and OSCC. Aminolevulinic-acid (ALA) is the metabolic precursor of the photosensitizer-protoporphyrin IX (PpIX) in the heme biosynthesis pathway. As an endogenous metabolite ubiquitous in mammalian cells, ALA can reduce the synthesis time of PpIX, therefore allowing it to accumulate in certain tissues and cells [25]. Compared to other photosensitizing agents, ALA can cause mild phototoxic side effects to the normal tissues and their biochemical properties. Compared to other photosensitizing agents, due to rapid metabolism, ALA can cause mild phototoxic side effects to the normal tissues. In addition, the major advantage of ALA is the tumor selectivity, which is most likely dependent on the decreased activity of ferrochelatase [26]. hence its wide-scale application in clinical practice. Not only can PpIX be selective for dysplasic and malignant tissues [27,28], but it also has effects on both the innate and adaptive arms of the immune system. A study published by Reginato et al in 2014 demonstrated the stimulation of anti-tumor memory immunity following PDT *in vivo* [29]. Based on its immunological effects, ALA- PDT can potentially prevent the recurrence of oral precancer and cancer, which highlights its advantage over other treatments. Previous and current data from the literature on the efficacy of ALA-PDT in OMPDs highlight its therapeutic utility for the prevention of OLK carcinogenesis. A few clinical studies have demonstrated the efficacy of ALA-PDT in patients with OLK who had not received any previous treatment. The complete response rate was 83.3% and the recurrence rate was less than 10% [30]. In other trials, reports also discuss the feasibility and safety of ALA-PDT, as well as its optimal light dose *in vivo* [11,31,32].

Although previous research revealed a surprising outcome, results also showed that the tissue penetration depth of the photosensitizer influenced the effect, especially in patients with large affected areas and/or hyperkeratosis of the lesion [33]. Therefore, treatment including surgery, medicine and PDT, a combination of multiple modalities has become the standard for refractory cases. In this case, 2 sessions of ALA-PDT alone only made the lesions mildly thinner. However, these results had an upside, and we adjusted our treatment accordingly. The most common pretreatment for PDT is the use of a CO_2 laser to remove superficial oral mucosal lesions. A randomized clinical trial from Denmark evaluated the efficacy and safety of ablative laser resurfacing-assisted PDT compared with conventional PDT [34]. After laser combined with PDT treatment, 88% of patients with grade II-III keratosis showed complete recovery at follow up. However, the thermal coagulation zone (TCZ) which produced by laser irradiation, because of the heat, may inhibit the diffusion of photosensitizer from the laser hole into surrounding tissue [16].

It has already been confirmed that PBN can be served an effective new method for transdermal drug delivery in the field of PDT. PBN, or shallow puncture, is a conveniently modified modern acupuncture method. The plum-blossom needle consists of a hammer-like tool head and seven 4 mm long needles that are equal distances from each other (supplement Fig. 1). In the present case, the hyperkeratosis lesions were tapped at vertical distance of 2 cm until spot bleeding, which required 5–6 hits. A large study was published on this subject last year. Thirty two actinic keratosis patients were examined in a randomized, single-blinded, split-face controlled study to assess the effects of PDT with microneedle pretreatment. The article concludes that microneedle use can shorten ALA incubation time and improve the clearance of lesions [35].

In this study, we assessed the efficacy of PBN assisted PDT for the management of different skin problems. We reviewed all relevant literature, including only original studies, clinical studies, and interventions published in English [16,17,36,37]. In their studies, Wu et al [36] showed that PBN can enhance ALA delivery for Bowen's disease treatment. On one hand, complete response rates of the PBN-PDT and PDT groups achieved 77.78% and 40%, respectively. On the other hand however, it has definitely compromised the healing course as compared with traditional PDT. They further suggested that these holes could subsequently be deepened in an animal model created by PBN [16]. In this case, the patient received five sessions of ALA-PDT after PBN taping. Bilateral hard palate lesions got gradually thinner, eventually disappearing completely, and no anesthesia was necessary over the course of treatment.

We analyze the reasons for which there was only slight pain in the acupuncture process of PBN. The fact that the patient can still feel mild pain when the needles were inserted into the areas without hyperkeratosis. Based on the above phenomenon, we note that the overlying hyperkeratosis of lesions made the patient far less sensitive to pain over the duration of treatment. In fact, previous research has shown that substantial regional variation in pain sensitivity of the human oral mucosa. Pain thresholds were highest on the hard palate [38]. We also evaluate the affinity between the degree of the keratosis of lesions and touch. using clinical manifestation and quantitative DNA analysis as criteria. Following treatment, the ratio of aneuploid cells had dropped and the patient prognosis was likely changed [39].

To the best of our knowledge, this is the first time PBN assisted PDT is reported in the treatment of oral mucosal disease. Our findings suggest that this regimen could be an effective therapeutic strategy for OMPD.

Conflict of interest

The authors report no conflicts of interest related to this work.

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Our study received approval from the ethics committee of Peking University School and Hospital of Stomatology. The patient and family members also provided written informed consent to publish the case report details.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pdpdt.2019.01.011.

References

[1] S.S. Napier, P.M. Speight, Natural history of potentially malignant oral lesions and conditions: an overview of the literature, J. Oral Pathol. Med. 37 (2008) 1–10.
[2] F.W. Mello, A.F.P. Miguel, K.L. Dutra, A.L. Porporatti, S. Warnakulasuriya, E.N.S. Guerra, E.R.C. Rivero, Prevalence of oral potentially malignant disorders: a systematic review and meta-analysis, J. Oral Pathol. Med. 47 (2018) 633–640.
[3] K.J. Voltzke, Y.A. Lee, Z.F. Zhang, J.P. Zevallos, Winn D.M. Yu GP, T.L. Vaughan, E.M. Sturgis, E. Smith, S.M. Schwartz, S. Schantz, J. Muscat, H. Morgenstern, M. McClean, G. Li, P. Lazarus, K. Kelsey, M. Gillison, C. Chen, P. Boffetta, M. Hashibe, A.F. Olshan, Racial differences in the relationship between tobacco, alcohol, and the risk of head and neck cancer: pooled analysis of US studies in the INHANCE Consortium, Cancer Causes Control 29 (2018) 619–630.
[4] N. Azhar, M. Sohail, F. Ahmad, S. Fareeha, S. Jamil, N. Mughal, H. Salam, Risk factors of Oral cancer- A hospital based case control study, J. Clin. Exp. Dent. 10 (2018) e396–e401.
[5] G. Tirelli, S. Zacchigna, F. Boscolo Nata, E. Quatela, R. Di Lenarda, M. Piovesana, Will the mininvasive approach challenge the old paradigms in oral cancer surgery? Eur. Arch. Otorhinolaryngol. 274 (2017) 1279–1289.
[6] F. Chiesa, L. Sala, L. Costa, D. Moglia, M. Mauri, S. Podrecca, S. Andreola, R. Marchesini, G. Bandieramonte, C. Bartoli, et al., Excision of oral leukoplakias by CO2 laser on an out-patient basis: a useful procedure for prevention and early detection of oral carcinomas, Tumori 2 (1986) 307–312.
[7] S. Ballivet de Régloix, N. Badois, C. Bernardeschi, T. Jouffroy, C. Hofmann, Risk factors of cancer occurrence after surgery of oral intraepithelial neoplasia: a long-term retrospective study, Laryngoscope (2018).
[8] H. Gursoy, C. Ozcakir-Tomruk, J. Tanalp, S. Yilmaz, Photodynamic therapy in dentistry: a literature review, Clin. Oral Investig. 17 (2013) 1113–1125.
[9] N.P. Selvam, J. Sadaksharam, G. Singaravelu, R. Ramu, Treatment of oral leukoplakia with photodynamic therapy: a pilot study, J. Cancer Res. Ther. 11 (2015) 464–467.
[10] W. Zheng, M. Olivo, K.C. Soo, The use of digitized endoscopic imaging of 5-ALA-induced PPIX fluorescence to detect and diagnose oral premalignant and malignant lesions in vivo, Int. J. Cancer 110 (2004) 295–300.
[11] S.J. Wong, B. Campbell, B. Massey, D.P. Lynch, E.E.W. Cohen, E. Blair, R. Selle, J. Shklovskaya, B.D. Jovanovic, S. Skripkauskas, A. Dew, P. Kulesza, V. Parimi, R.C. Bergan, E. Szabo, A phase I trial of aminolevulinic acid-photodynamic therapy for treatment of oral leukoplakia, Oral Oncol. 49 (2013) 970–976.
[12] K.F. Fan, C. Hopper, P.M. Speight, G. Buonaccorsi, A.J. MacRobert, S.G. Bown, Photodynamic therapy using 5-aminolevulinic acid for premalignant and malignant lesions of the oral cavity, Cancer 78 (2015) 1374–1383.
[13] C.H. Yu, H.M. Chen, H.Y. Hung, S.J. Cheng, T. Tsai, C.P. Chiang, Photodynamic therapy outcome for oral verrucous hyperplasia depends on the clinical appearance, size, color, epithelial dysplasia, and surface keratin thickness of the lesion, Oral Oncol. 44 (2008) 595–600.
[14] H. Osman-Ponchet, A. Gaborit, K. Sevin, C. Bianchi, J.M. Linget, C.E. Wilson, G. Bouvier, Pretreatment of skin using an abrasive skin preparation pad, a microneedling device or iontophoresis improves absorption of methyl aminolevulinate in ex vivo human skin, Photodiagnosis Photodyn. Ther. 20 (2017) 130–136.
[15] M. Haedersdal, J. Katsnelson, F.H. Sakamoto, W.A. Farinelli, A.G. Doukas, J. Tam, R.R. Anderson, Enhanced uptake and photoactivation of topical methyl aminolevulinate after fractional CO2 laser pretreatment, Lasers Surg. Med. 43 (2011) 804–813.
[16] J. Chen, Y. Zhang, P. Wang, B. Wang, G. Zhang, X. Wang, Plum-blossom needling promoted PpIX fluorescence intensity from 5-aminolevulinic acid in porcine skin model and patients with actnic keratosis, Photodiagnosis Photodyn. Ther. 15 (2016) 182–190.
[17] P. Wang, L. Zhang, G. Zhang, Z. Zhou, H. Zhang, Z. Zhao, L. Shi, X. Wang, Successful treatment of giant invasive cutaneous squamous cell carcinoma by plum-blossom needle assisted photodynamic therapy sequential with imiquimod: case experience, Photodiagnosis Photodyn. Ther. 21 (2018) 393–395.
[18] D.W. Hedley, DNA analysis from paraffin-embedded blocks, Methods Cell Biol. 33 (1990) 139–147.
[19] N.P. Selvam, J. Sadaksharam, G. Singaravelu, R. Ramu, Treatment of oral leukoplakia with photodynamic therapy: a pilot study, J. Cancer Res. Ther. 11 (2015) 464–467.
[20] J. Polesel, C. Furlan, S. Birri, V. Giacomarra, E. Vaccher, G. Grando, C. Gobitti, F. Navarria, O. Schioppa, E. Minatel, E. Bidoli, L. Barzan, G. Franchin, The impact of time to treatment initiation on survival from head and neck cancer in north-eastern Italy, Oral Oncol. 67 (2017) 175–182.
[21] Global Burden of Disease Cancer Collaboration, C. Fitzmaurice, T.F. Akinyemiju, F.H. Al Lami, T. Alam, R. Alizadeh-Navaei, C. Allen, U. Alsharif, N. Alvis-Guzman, E. Amini, B.O. Anderson, O. Aremu, A. Artaman, S.W. Asgedom, R. Assadi, T.M. Atey, L. Avila-Burgos, A. Awasthi, H.O. Ba Saleem, A. Barac, J.R. Bennett, I.M. Bensenor, N. Bhakta, H. Brenner, L. Cahuana-Hurtado, C.A. Castañeda-Orjuela, F. Catalá-López, J.J. Choi, D.J. Christopher, S.C. Chung, M.P. Curado, L. Dandona, R. Dandona, J. das Neves, S. Dey, S.D. Dharmaratne, D.T. Doku, T.R. Driscoll, M. Dubey, H. Ebrahimi, D. Edessa, Z. El-Khatib, A.Y. Endries, F. Fischer, L.M. Force, K.J. Foreman, S.W. Gebrehiwot, S.V. Gopalani, G. Grosso, R. Gupta, B. Gyawali, R.R. Hamadeh, S. Hamidi, J. Harvey, H.Y. Hassen, R.J. Hay, S.I. Hay, B. Heibati, M.K. Hiluf, N. Horita, H.D. Hosgood, O.S. Ilesanmi, K. Innos, F. Islami, M.B. Jakovljevic, S.C. Johnson, J.B. Jonas, A. Kasaeian, T.D. Kassa, Y.S. Khader, E.A. Khan, G. Khan, Y.H. Khang, M.H. Khosravi, J. Khubchandani, J.A. Kopec, G.A. Kumar, M. Kutz, D.P. Lad, A. Lafranconi, Q. Lan, Y. Legesse, J. Leigh, S. Linn, R. Lunevicius, A. Majeed, R. Malekzadeh, D.C. Malta, L.G. Mantovani, B.J. McMahon, T. Meier, Y.A. Melaku, M. Melku, P. Memiah, W. Mendoza, T.J. Meretoja, H.B. Mezgebe, T.R. Miller, S. Mohammed, A.H. Mokdad, M. Moosazadeh, P. Moraga, S.M. Mousavi, V. Nangia, C.T. Nguyen, V.M. Nong, F.A. Ogbo, A.T. Olagunju, M. Pa, E.K. Park, T. Patel, D.M. Pereira, F. Pishgar, M.J. Postma, F. Pourmalek, M. Qorbani, A. Rafay, S. Rawaf, D.L. Rawaf, G. Roshandel, S. Safiri, H. Salimzadeh, J.R. Sanabria, M.M. Santric Milicevic, B. Sartorius, M. Satpathy, S.G. Sepanlou, K.A. Shackelford, M.A. Shaikh, M. Sharif-Alhoseini, J. She, M.J. Shin, I. Shiue, M.G. Shrime, A.H. Sinke, M. Sisay, A. Sligar, M.B. Sufiyan, B.L. Sykes, R. Tabarés-Seisdedos, G.A. Tessema, R. Topor-Madry, T.T. Tran, B.X. Tran, K.N. Ukwaja, V.V. Vlassov, S.E. Vollset, E. Weiderpass, H.C. Williams, N.B. Yimer, N. Yonemoto, M.Z. Younis, C.J.L. Murray, M. Naghavi, Global burden of disease cancer collaboration, global, regional, and national Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 Cancer groups, 1990 to 2016 a systematic analysis for the global burden of disease study, JAMA Oncol. 4 (2018) 1553–1568.
[22] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (2015) E359–386.
[23] S. Warnakulasuriya, A. Ariyawardana, Malignant transformation of oral leukoplakia: a systematic review of observational studies, J. Oral Pathol. Med. 45 (2016) 155–166.
[24] G. Lodi, A. Sardella, C. Bez, F. Demarosi, A. Carrassi, Interventions for treating oral leukoplakia, Cochrane Database Syst. Rev. 18 (2006) CD001829.
[25] A. Juzeniene, V. Iani, J. Moan, Clearance mechanism of protoporphyrin IX from mouse skin after application of 5-aminolevulinic acid, Photodiagnosis Photodyn. Ther. 10 (2013) 538–545.
[26] N. Schoenfeld, O. Epstein, M. Lahav, R. Mamet, M. Shaklai, A. Atsmon, The heme biosynthetic pathway in lymphocytes of patients with malignant lymphoproliferative disorders, Cancer Lett. 43 (1988) 43–48.
[27] T.J. Dougherty, Photodynamic therapy (PDT) of malignant tumors, Crit. Rev. Oncol. Hematol. 2 (1984) 83–116.
[28] B.J. Quirk, G. Brandal, S. Donlon, J.C. Vera, T.S. Mang, A.B. Foy, S.M. Lew, A.W. Girotti, S. Jogal, P.S. LaViolette, J.M. Connelly, H.T. Whelan, Photodynamic therapy (PDT) for malignant brain tumors–where do we stand? Photodiagnosis Photodyn. Ther. 12 (2015) 530–544.
[29] E. Reginato, P. Wolf, M.R. Hamblin, Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects, World J. Immunol. 4 (2014) 1–11.
[30] A. Sieroń, M. Adamek, A. Kawczyk-Krupka, S. Mazur, L. Ilewicz, Photodynamic therapy (PDT) using topically applied delta-aminolevulinic acid (ALA) for the treatment of oral leukoplakia, J. Oral Pathol. Med. 32 (2003) 330–336.
[31] G. Shafirstein, A. Friedman, E. Siegel, M. Moreno, W. Bäumler, C.Y. Fan, K. Morehead, E. Vural, B.C. Stack Jr, J.Y. Suen, Using 5-aminolevulinic acid and pulsed dye laser for photodynamic treatment of oral leukoplakia, Arch. Otolaryngol. Head Neck Surg. 137 (2011) 1117–1123.
[32] H.M. Chen, Tsai T. Yu CH, Y.C. Hsu, R.C. Kuo, C.P. Chiang, Topical 5-aminolevulinic acid-mediated photodynamic therapy for oral verrucous hyperplasia, oral leukoplakia and oral erythroleukoplakia, Photodiagnosis Photodyn. Ther. 4 (2007) 44–52.
[33] Y. Zhang, L. Zhang, D. Yang, G. Zhang, X. Wang, Treatment of oral refractory large area mucosal leukoplakia with CO2 laser combined with photodynamic therapy: case report, Photodiagnosis Photodyn. Ther. 20 (2017) 193–195.
[34] K. Togsverd-Bo, C.S. Haak, D. Thaysen-Petersen, H.C. Wulf, R.R. Anderson, M. Hædersdal, Intensified photodynamic therapy of actinic keratoses with fractional CO2 laser: a randomized clinical trial, Br. J. Dermatol. 166 (2012) 1262–1269.
[35] T.A. Petukhova, L.A. Hassoun, N. Foolad, M. Barath, R.K. Sivamani, Effect of expedited microneedle-assisted photodynamic therapy for field treatment of actinic keratoses: a randomized clinical trial, JAMA Dermatol. 153 (2017) 637–643.
[36] Y. Wu, P. Wang, L. Zhang, B. Wang, X. Wang, Enhancement of photodynamic therapy for Bowen's disease using plum-blossom needling to augment drug delivery, Dermatol. Surg. 44 (2018) 1516–1524.
[37] X. Mu, L. Wang, L. Wang, R. Ge, H. Dang, K. Mou, Plum-blossom needling enhanced the effect of photodynamic therapy on basal cell carcinoma, Photodiagnosis Photodyn. Ther. (2018) 339–341.
[38] P. Svensson, P. Bjerring, L. Arendt-Nielsen, S. Kaaber, Quantitative determinations of sensory and pain thresholds on human oral mucosa by argon laser stimulation, Pain 49 (1992) 233–239.
[39] S.Y. Kao, L. Mao, X.C. Jian, G. Rajan, G.Y. Yu, Expert consensus on the detection and screening of oral Cancer and precancer, Chin. J. Dent. Res. 18 (2015) 79–83.