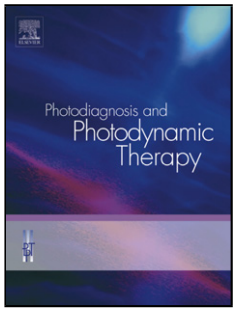

Title: Efficacy of photodynamic therapy in the inactivation of oral fungal colonization among cigarette smokers and non-smokers with denture stomatitis

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Efficacy of photodynamic therapy in the inactivation of oral fungal colonization among cigarette smokers and non-smokers with denture stomatitis

1. Introduction

Oral rehabilitation of partially and completely edentulous individuals is usually done using partial and complete dentures, respectively [1]. Dentures bases are commonly fabricated from polymethyl methacrylate resin [2]; however, fungal colonization (such as *Candida* species, predominantly *Candida albicans* [*C. albicans*]) on the porous surface of the acrylic resin is a major cause of oral mucosal inflammatory conditions, such as denture stomatitis (DS) [3]. DS has been reported in up to 67% of complete denture wearers [4]; and is commonly seen on the palatal mucosa [4]. DS is characterized by the presence of small yellowish areas in the hard palate, which discharge a whitish creamy material on gentle pressure [5]. The surrounding mucosa may also be erythematous. Another risk factor that has been associated with an increased oral candida carriage is cigarette smoking [6, 7]. The precise mechanism by which cigarette smoking enhances oral *Candida* carriage remains unclear; however, it has been suggested that the aromatic hydrocarbons contained in cigarette smoke act as nutrients for the growth and proliferation of fungi [8]. Moreover, according to Arendorf and Walker [9], cigarette smoke induces alterations in epithelial tissues thereby facilitating fungal carriage.

Various techniques that have been used for the disinfection of denture surfaces include the use of (a) disinfectant solutions (such as alkaline glutaraldehyde, sodium hypochlorite and povidine-iodine), (b) antiseptic mouthwashes (such as chlorhexidine) and microwave irradiation [10-14]. however, these techniques have been associated with denture staining and compromised mechanical properties (such as linear stability and elastic modulus) and soft tissues irritation [10, 13,

15-18]. Antimicrobial photodynamic therapy (aPDT) involves the activation of a specific dye (photosensitizers) by a light source (400-700 nm) [19, 20]. aPDT has been used for the control of microbes associated with oral inflammatory conditions, such as periodontitis and oral premalignant and malignant lesions [21-24]. aPDT The resulting reaction produces reactive oxygen species (ROS) which are lethal to microbes [25]. In a recent systematic review, Varela Kellesarian et al. [26] assessed the efficacy of aPDT in the disinfection of acrylic denture surfaces. The authors concluded that that the role of aPDT in the disinfection of denture surfaces is debatable [26]. One explanation is that most of the studies included in this systematic review were performed in-vitro [26]; which therefore could have discommoded the clinical implementation of the results reported. Since cigarette smoking is associated with an increased oral fungal carriage [27], it is hypothesized that aPDT is effective in the inactivation of oral fungal colonization among cigarette smokers and non-smokers with DS.

The aim of the present clinical study was to assess the efficacy of aPDT in the inactivation of oral fungal colonization among cigarette smokers and non-smokers with DS.

2. Material and methods

2.1. Ethical approval

The study was approved by the Research Ethics Review Committee of the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Volunteering individuals were requested to read and sign a consent form. The participants were given the freedom to withdraw their participation at any stage of the investigation without penalty.

2.2. Eligibility criteria

The inclusion criteria comprised of the following : (a) completely edentulous individuals; (b) individuals wearing complete dentures; (c) cigarette smokers (individuals who reported to have been smoking at least 1 cigarette daily since at least 12 months [28]; (d) non-smokers (individuals who reported to have never used any form of tobacco products [28]. The exclusion criteria were as follows: (a) refusal to sign the consent form; (b) habitual alcohol consumption; (c) patients with systemic diseases such as HIV or acquired immune deficiency syndrome, cardiovascular disorders, diabetes mellitus, hepatic disorders and renal disorders.

2.3. Questionnaire

A questionnaire was used to gather information regarding age, gender, number of cigarettes smoked daily, duration of smoking habit (in years), and duration of denture in function in years.

2.4. Clinical oral examination

Clinical oral examination was performed to determine location of denture in the jaws (maxillary and mandibular or both) and presence of oral mucosal lesions in the oral vestibule (particularly in the palate, dorsum of the tongue, floor of the mouth and buccal mucosae.

2.5. Diagnosis of denture stomatitis

The clinical presentation of erythema and edema on the oral mucosa covered by the denture base was used in the clinical diagnosis of DS. In addition, exfoliative cytology of mucosal surfaces was

performed using the periodic acid-Schiff (PAS) cytology to assess the absence or presence of *Candida hyphae [29]*. A BBL CultureSwab (Becton, Dickinson and Company) was used to swab the denture and the mucosal surface. All samples were cultured on Sabouraud's dextrose agar containing quemicetine succinate. The samples were spiral plated to Sabouraud's dextrose plates and a quantitative value of the colony forming units per milliliter (CFU/ml) was obtained [29]. Among smokers and non-smokers with DS, sampling from the denture and mucosal surfaces covering the denture was performed preoperatively and 3 months after aPDT.

2.6. Antimicrobial photodynamic therapy

In the present study, haematoporphyrin derivative (Photogem, Photogem LLC Co, Moscow, Russia) was used as a photosensitizer. Solutions of 500 mg/L (pH 6.6) of the PS were prepared by dissolving the powder in sterile saline. The prepared solution was stored kept in a dark room in a sterile spray bottle. Two light emitting diodes (LEDs) (LXHL-PR09; Luxeon III Emitter, Lumileds Lighting, San Jose, CA, USA) devices were designed by Instituto de Fı´sica de Sa˜o Carlos (University of Sa˜o Paulo, Sa˜o Carlos, SP, Brazil), which covered the wavelength range between 440 nm and 460 nm, were used. One device that contained 24 LEDs (24 mW/cm^2) uniformly distributed throughout the device was used to irradiate the denture. The second device comprised of 10 LEDs (260 mW) uniformly distributed on a circular platform, was used to irradiate the erythematous areas on the oral mucosae covered by the dentures [30]. The intensity of light delivered was 102 mW/cm2 , considering a distance of 2 cm from the platform inside the mouth to the deepest area of the palate. The denture and erythematous mucosae were sprayed with the PS and irradiated with their respective light emitting diode for 20 minutes (122 J/cm^2).

2.7. Postoperative instructions

Up to the 3-month follow-up period, all participants were advised to brush their dentures a toothpaste, after every meal and before going to sleep. They were also instructed to immerse their denture/s in filtered water overnight.

2.8. Statistical analysis

Statistical analysis was performed using a software program (SPSS, v.20.0 for Windows, IBM, Chicago, IL., USA). The CFU/mL values were logarithm transformed to achieve a normal distribution. Inter- and intra-group comparisons were performed using the Tukey's post hoc test. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Study participants

In total, 22 males with DS (12 smokers and 10 non-smokers) were included. The mean ages of smokers and non-smokers was 73.8±2.5 and 70.5±1.2 years, respectively. The duration and daily frequency of cigarette smoking was 20.6±4.5 years and 12.3±1.5 cigarettes daily, respectively. Smokers and non-smokers had been wearing complete dentures since 6.2±0.8 and 5.8±0.4 years, respectively. All patients were completely edentulous and were wearing complete dentures in both arches. Erythematous lesions were present on the palatal mucosa only in smokers and non-smokers with DS. Exfoliative cytology of erythematous palatal mucosal surfaces confirmed the presence of fungal hyphae in all individuals. There was no clinical evidence of lesions on the tongue, floor of the mouth and buccal mucosae among smokers and non-smokers with DS.

3.2. Mean±standard deviations of fungal colony forming units (CFU/ml) from the palate of smokers and non-smokers

The preoperative mean CFU/ml values of fungal carriage among smokers and non-smokers was 106.7±6.3 CFU/ml and 93.6±8.4 CFU/ml, respectively. At baseline, there was no statistically significant difference in the mean CFU/ml values of fungal carriage among smokers and non-smokers with DS. At 3-months follow-up, there was a statistically significant decrease in the mean CFU/ml among smokers (25.5±8.3 CFU/ml) compared with their respective baseline values (106.7±6.3 CFU/ml) ($P<0.01$). Among non-smokers, the mean fungal CFU/ml values were 12.7±0.8 CFU/ml compared with their respective baseline values (93.6±8.4 CFU/ml) ($P<0.01$). At 3-months follow-up, CFU/ml levels were statistically significantly higher among smokers (25.5±8.3 CFU/ml) compared with non-smokers (12.7±0.8 CFU/ml) ($P<0.05$). At 3-months follow-up, there was no clinical evidence of palatal erythematous mucosa among smokers and non-smokers.

4.Discussion

Studies have reported that aPDT has been effective in the management of oral infections, such as endodontic infections, peri-implantitis and periodontitis [31-36]. The present study was based on the hypothesis that aPDT is effective in the inactivation of fungal colonization among cigarette smokers and non-smokers with DS. However, to our knowledge from indexed literature, the present study is the first one to assess the efficacy of aPDT in the elimination of fungal species from the denture and oral soft tissues among cigarette smokers and non-smokers. The present results support this hypothesis since aPDT caused a statistically significant decrease in the fungal CFU at 3-months follow-up in smokers as well as non-smokers. One explanation for these results can be associated with the mode of action of aPDT. The mechanism of action of aPDT involves the excitation of

photosensitizer dye molecules by laser light or visible light of specific wavelength. This undergoes transition of dye molecule from ground singlet state to excited state triplet. The triplet state photosensitizer reacts with endogenous oxygen resulting in the formation of highly reactive singlet oxygen. These ROS are highly cytotoxic causing microbial cell death [37, 38]. However, an interesting finding in the present study was that although aPDT caused a significant reduction in the fungal CFU/ml among smokers as well as non-smokers, at 90 days of follow-up, fungal species were still isolated from the palatal mucosa and denture surfaces of smokers and non-smokers. This outcome was most likely expected as a result of progressive recolonization of dentures and oral mucosae among smokers as well as non-smokers. Moreover, the high affinity of fungi to adhere to and colonize acrylic surfaces may also have contributed towards recolonization since factors such as cell-surface hydrophobicity and ability to form biofilm augments fungal adherence to acrylic-based surfaces [39, 40]. It is noteworthy that despite the reduction in CFU/ml in both groups at 90 days of follow-up, Fungal carriage was statistically significantly higher among smokers than non-smokers. This outcome could be associated with the nutritive effect of nicotine (a major constituent in cigarettes smoke) on oral fungal proliferation. In their experimental study, Hsia et al. [8] proposed that the tobacco content (mainly nicotine) provide a nutritious medium to enhance proliferation of fungal species. It is therefore hypothesized that smokers are an increased risk of redeveloping DS even after aPDT in case they continue with their smoking habit. It is highly emphasized that community based tobacco awareness programs should routinely be conducted to increase the public awareness about the detrimental effect of smoking on health.

It is noteworthy that in the present study, smokers as well as non-smokers were advised to regularly clean their dentures particularly in between meals and place their dentures in filtered water before sleeping. The isolation of fungal species after 90 days of follow-up could be associated with

the lack of adherence to these instructions by the participants. Moreover, this outcome also suggests that aPDT is mainly an adjunct therapy and the outcomes of aPDT in terms of minimizing fungal carriage may not be significant enough without mechanical cleaning of the denture surfaces. Therefore, complete as well as partial denture wearers should be advised to regularly clean their denture surfaces to minimize denture related infections such as DS.

A limitation of the present study is the method used to isolate oral fungal species. An *in vitro* study showed that *C. albicans* was able to invade a reconstituted human oral epithelium over a period of 48 h through hyphal penetration into the superficial epithelium together with features of cellular internalization of yeasts [41]. Therefore, a delicate cotton swab used in this study for recovering yeast may underestimate the real burden of fungal species present on the palatal mucosa and denture surface. Hence, another sampling technique, such as an oral rinse method using saline or sterile water, would have been valuable to monitor the overall yeast burden in the oral cavity of the patients at baseline and at subsequent evaluations. Moreover, in the present study, patients with systemic diseases such as diabetes mellitus were excluded. It has been reported that oral fungal colonization is significantly higher among hyperglycemic patients compared with individuals with normal blood glucose levels [42]. It is therefore speculated that the outcomes of aPDT is compromised to a greater extent in edentulous diabetic smokers as compared to non-diabetic smokers in the same group. Further studies are needed to test this hypothesis.

5.Conclusion

aPDT is effective in the inactivation of oral fungal colonization among cigarette smokers and non-smokers with. The role of denture hygiene is also emphasized.

Conflict of interest

None declared.

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