The Use of Photoactivated Blue-O Toluidine for Periimplantitis Treatment in Patients with Periodontal Disease

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The tissue inflammatory changes in the patient with dental implants can compromise the treatment; therefore, various methods of peri-implantitis treatment were proposed. The scope of our study was to assess the changes in the clinical parameters in patients treated with photo-activated toluidine blue O as an adjunctive method to the standard scaling and root planing.

Keywords: photo-activated, toluidine blue 0, therapy peri-implantitis, periodontal disease

The introduction of dental implants as a procedure to replace natural missing teeth has been a major advance in the management of edentulous and partially edentulous individuals. When an implant is inserted into the oral cavity, it provides a new and physically different surface for the colonization of microorganisms that might already be part of the oral microflora. The early development of biofilms on implant surfaces is similar to the one observed on natural teeth and other restorative materials places in the oral cavity. The pathogenic activity generates host inflammatory responses which determine peri-implantary lesions, progressively leading to peri-implantitis. The development of peri-implantitis appears to be accompanied in large part by an increase in bacterial species that have been found to increase also in periodontitis. These include periodontal pathogens, such as *P.gingivalis*, *T.forsythia* and *A.actinomycetemcomitans*, as well as additional taxa including staphylococci and enteric rods.

Therefore, an active fight against the pathogens becomes a gold-standard. The treatment of peri-implantitis includes an etiologic step, of mechanical debridement, a surgical step and a maintenance period of treatment. An efficient etiologic treatment minimizes the need for further surgical maneouvres. Various methods for cleaning of implant surfaces have been described in order to treat failing implants. Among other things, citric acid, air-powder abrasive treatment, mechanical cleaning with curettes or scalers or the application of plastic-coated ultrasonic scalers are used.

Photodynamic therapy was discovered accidentally at the beginning of the 20th century [1] and was then applied in the medical field for the light-induced inactivation of cells, microorganisms or molecules [2,3]. Photodynamic therapy basically involves three nontoxic ingredients: visible harmless light; a nontoxic photosensitizer; and oxygen. It is based on the principle that a photosensitizer (i.e. a photo-activatable substance) binds to the target cells and can be activated by light of a suitable wavelength. Following activation of the photosensitizer through the application of light of a certain wavelength, singlet oxygen and other very reactive agents are produced that are extremely toxic to certain cells and bacteria [4].

The photosensitizer can be applied in the targeted area by topical application, aerosol delivery or interstitial injection. The light that activates the photosensitizer must be of a specific wavelength with a relatively high intensity. Although it has been reported that antimicrobial photodynamic therapy can lead to DNA damage, it seems that bacterial killing by the photochemical reaction is mainly caused by damage to the bacterial cytoplasmic membrane [5].

In antimicrobial photodynamic therapy, the particular photosensitizers employed are toluidine blue O [tolonium chloride: (7-amino-8-methyl-phenothiazin-3-ylidene)-dimethyl-ammonium ($C_{15}H_{16}N_3S^+$)], methylene blue [3,7-bis(dimethyl-amino)phenazathionium chloride tetramethylthionine chloride ($C_{16}H_{18}N_3ClS$) or phenothiazine-5-ium, 3,7-*bis*(dimethylamino)-chloride], erythrosine, chlorine e6 and hematoporphyrin, which have been shown to be safe when employed in the medical field. Toluidine blue O is a solution that is blue–violet in color. It can stain granules within mast cells, and proteoglycans and glycosaminoglycans within connective tissues. The aim of the study was to assess the effects of the photodynamic therapy on the periodontal clinical parameters in patients with peri-implantitis, as an adjunctive method to the classical mechanical treatment.

Experimental Part

Materials and Methods

The present study was conducted in the Faculty of Medicine of the "Lucian Blaga" University of Sibiu, in association with the Periodontology Clinic of "Gr.T.Popa" University of Medicine and Pharmacy, Ia°i, between February 2013 and March 2014.

The methodology of the present study respected the international standards and all experiments were conducted according to the ethical directives of the Helsinki Declaration. The information and confirmation principles for research purposes were strictly respected; the signed informed consent for study inclusion was obtained from each patient.

We recruited a number of 44 patients, with dental implants and peri-implantitis, randomly assigned to two groups: the study group and the control group. The inclusion

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Parameter	Median (Min-Max, IQR)		*p*-value
	Study group	Control group	
GI	2.2 (1.7-3.2, 0.5)	2.4 (1.4-3.0, 0.5)	0.904
PI	2.2 (0.7-3.2, 0.8)	1.4 (0.7-3.2, 1.0)	0.001
GBI	100 (50.0-100.0, 25.0)	75 (50.0-100.0, 25.0)	0.232
Pocket depth (mm)	5.8 (5.0-6.0, 1.0)	5.6 (4.4-6.0, 1.0)	0.363
CAL (mm)	6.7 (5.2-8.0, 1.4)	6.2 (4.4-8.0, 1.7)	0.455
GI: Gingival Index; PI: Plaque Index; GBI: Gingival Bleeding Index; CAL: clinical attachment level			

Table 1
CLINICAL PARAMETERS FOR THE STUDY AND THE CONTROL GROUP AT BASELINE

Parameter	Baseline Median (Min-Max, IQR)			At 3 months Median (Min-Max, IQR)			*p*-value (baseline-3 months)	
	Study	Control	*p*-value	Study	Control	*p*-value	Study	Control
PPD (mm)	5.8 (5.0-6.0, 1.0)	5.6 (4.4-6.0, 1.0)	0.363	3.0 (2.0-6.0, 1.0)	4.0 (2.0-6.01.0)	0.016	<0.01	<0.01
CAL (mm)	6.7 (5.2-8.0, 1.4)	6.2 (4.4-8.0, 1.7)	0.455	4.0 (2.6-7.0, 2.0)	4.5 (2.0-7.0, 2.0)	0.021	<0.01	<0.01
GI	2.2 (1.7-3.2, 0.5)	2.4 (1.4-3.0, 0.5)	0.904	1.0 (0.0-3.0, 0.5)	1.5 0.5-2.8, 1.0)	<0.01	<0.01	<0.01
PI	2.2 (0.7-3.2, 0.8)	1.4 (0.7-3.2, 1.0)	0.001	0.5 (0.0-2.5. 1.0)	0.5 (0.0-1.5, 0.7)	0.426	<0.01	<0.01
GBI	100 (50.0-100.0, 25.0)	75 (50.0-100.0, 25.0)	0.232	25 (0.0-100.0, 50.0)	50.0 (0.0-100.0, 75.0)	<0.01	<0.01	<0.01
Min-Max, IQR: minimum, maximum and inter-quartile range; PPD: pocket probing depth; CAL: clinical attachment level; GI: Gingival Index; PI: Plaque Index; GBI: Gingival Bleeding Index								

Table 2
DIFFERENCES IN CLINICAL PARAMETERS BETWEEN GROUPS AND WITHIN THE GROUPS, WHEN COMPARED AT BASELINE, AFTER 3 MONTHS

criteria were represented by the presence of probing pocket depths (PPD) between 4 and 6mm. the exclusion criteria comprised any systemic disease which could influence the peri-implant tissues (inflammatory and infectious diseases), smoking, use of antibiotics/anti-inflammatory drug in the past 6 months, periodontal treatment in the last 6 months, pregnancy/lactation, allergy to toluidine blue.

Each patient was clinically examined and the following parameters were registered at baseline and at 3 months after the treatment protocol:

- probing pocket depth (PPD) was assessed as the primary outcome following intervention using a William's graduated periodontal probe at six inter-dental sites (mesio-buccal, buccal, disto-buccal, mesio-oral, oral and disto-oral);

- clinical attachment levels (CAL), gingival index (GI) [6], gingival bleeding index (GBI) [7] and plaque index (PI) [8] were assessed before and after treatment as secondary outcomes.

All the 44 subjects were treated by an experienced specialist and clinical outcomes were measured by another specialist who was blinded to patient selection. The control group was administered scaling and root planing (SRP) by carbon fiber hand scalers and Gracey curettes (Hu-Friedy). No other treatment was given to this group. Full-mouth supragingival and subgingival scaling was performed at all sites within 24 h. This group included 22 subjects (19 women and 5 men; mean age: 38.4 ± 9.6 years).

The test group included 22 subjects (11 women and 11 men; mean age: 40.8 ± 8.3 years) and was managed by a photo-activated disinfection treatment (PDT) in addition to SRP. The LED source used in this study was in the red spectrum (wavelenght of 635nm, Denfotex UK) and a viscous solution of tolonium chloride 0.01mg/mL provided by the manufacturer served as a photo-sensitizer (Denfotex UK). We followed all the steps from the operatory protocol, according to the manufacturer's recommendations. After the isolation of the site, the photo-sensitizer was meticulously placed in the peri-implantary pockets, followed by a LED irradiation for 60 s. For the pockets with a depth higher than 5 mm we used a special Perio-tip for the light source.

A subject-level analysis was performed statistically for each of the parameters using SPSS software for Windows, Version 16.0. Median (minimum to maximum; inter-quartile range) for the clinical variables were calculated for each treatment. Significant difference between the test and control groups with respect to categorical data was assessed using Chi-square test, whereas Mann-Whitney U-test was used for continuous variable. Likewise, Wilcoxon's Signed Rank Test was used for finding significant changes from baseline to various intervals within the test and control groups.

Results and disscutions

We examined and treated a number of 44 subjects, divided in two groups: the study group (n=22, 19 women and 5 men; mean age: 38.4 ± 9.6 years) and the control group (n=22, 11 women and 11 men; mean age: 40.8 ± 8.3 years).

No significant differences were found between the test and control group of patients with regard to the baseline values of clinical parameters ($p > 0.05$), except for the plaque index ($p < 0.01$) (table 1).

The changes in PPD, CAL, GI, PI and GBI for test and control groups are presented in table 2, expressed as median with minimum, maximum and inter-quartile range (IQR).

As compared to control group, PPD and CAL showed statistically significant reduction in the test group at 3 months ($p < 0.05$).

A statistically significant improvement in gingival index and gingival bleeding index was seen for the study group ($p < 0.01$) after 3 months of PDT, whereas the difference in plaque index was above the significance level ($p > 0.05$).

In our study, SRP was given to both the two groups as it would be unethical to deny the conventional treatment to anyone. When compared with baseline data, PPD showed higher improvement in the test group than control group at recall visits of 3 months. Statistically significant reduction in GI was observed in the test group as compared to the control group after 3 months of PDT ($p < 0.01$). PI also showed significant reduction for the study group ($p < 0.05$).

Another notable effect was the statistically significant reduction in GBI in the test group after 3 months ($p < 0.01$).

It can be seen in this study that PDT has a positive effect on patient care, mainly due to the considerably fast resolution of overt inflammation in the gingival tissues, which is supported by the significant reduction in GI, PI and GBI. A plausible explanation for improvement in GI and GBI in test group patients could be due to bacterial load reduction and inactivation of bacterial virulence factors and cytokines when the toluidine blue is irradiated.

In a clinical case-series study, Haas et al. [9] investigated the clinical effects of treatment of antimicrobial photodynamic therapy (toludine blue O + diode laser) in combination with guided bone regeneration using autogenous bone grafts on 24 implants diagnosed with peri-implantitis in 17 patients. They reported that 21 implants out of 24 showed improvements in the bone defect after a mean observation period of 9.5 months.

In a case report Schuckert et al. [10] demonstrated effective bone regeneration within bone defects around implants affected by peri-implantitis following surgical therapy using photodynamic therapy (tolonium chlorine + 100 mW diode laser) to decontaminate the implant surface and the application of recombinant human bone morphogenetic protein-2.

Non-laser photo-activation sources such as light emitting diodes (LED) are more compact and less expensive than lasers [11]; furthermore, LED radiation is less harmful to the human cornea [12].

Conclusions

The LED photo-activated adjunctive therapy with toluidine blue O as a photo-sensitizer exerted an important role in the improvement of the clinical parameters in patients with peri-implantitis, when compared to the simple usage of conventional SRP therapy, with significant reduction of the local inflammation and of the pocket depths. Moreover, LED therapy is less expensive than laser as a light source, making this method a more accessible one to the large usage. Our results support the clinical usage of LED photo-activated therapy as an effective option in the etiological treatment of compromised implants.

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