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Clinical and microbiological evaluation of one-stage full-mouth disinfection: a short-term study

Avaliação clínica e microbiológica do protocolo one-stage full-mouth disinfection: estudo de curto prazo

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Resumo

Introdução: Os pacientes parecem aderir melhor ao tratamento periodontal de curto prazo. Além disso, tratamentos de tempo curto possuem melhor custo-benefício. No entanto, os benefícios associados a este tipo de tratamento ainda requerem investigações adicionais. **Objetivo**: O presente estudo avaliou longitudinalmente indivíduos com periodontite crônica clínica e microbiologicamente tratados pelo protocolo *one-stage full-mouth disinfection*. **Material e método**: Dezesseis indivíduos (49,87 ± 8,22), que se enquadraram nos critérios de inclusão/exclusão foram incluídos. Um examinador calibrado, avaliou índice de placa e gengival, profundidade de bolsa e nível clínico de inserção pré e pós terapia. Amostras subgengivais coletadas dos cinco sítios periodontais mais doentes estabeleceram a carga bacteriana total e níveis de *P. gingivalis* e *S. oralis* por qPCR. *One-stage full-mouth disinfection* foi realizado com instrumentos manuais associado a gel de clorexidina e solução. Após, os indivíduos utilizaram clorexidina 0,12% para bochecho, duas vezes ao dia, durante os dois meses. Dados foram comparados pelo teste pareado t de Student (p<0,05). **Resultado**: A análise estatística revelou que o tratamento proporcionou melhorias clínicas e microbianas significativas em três meses. A carga bacteriana total evidenciou reduções mais pronunciadas do pré para o pós-tratamento (p=0,0001). Similarmente, *P. gingivalis* e *S. oralis* mostraram redução no pós-tratamento. **Conclusão**: Após 3 meses de monitoramento, indivíduos com periodontite crônica apresentaram melhora clínica e microbiana com o protocolo *one stage full-mouth disinfection.*

Descritores: Periodontite crônica; microbiologia; terapia; *Porphyromonas gingivalis*; *Streptococcus oralis.*

Abstract

Introduction: Patients seem to adhere better to short-term periodontal treatment schemes. Besides, time-reduced treatments are more cost-effective. However, the degree of benefits related to this type of treatment still requires additional investigations. **Aim**: The present short-term study evaluated clinical and microbiological outcomes, from baseline to 3-months, of chronic periodontitis subjects treated by the one-stage full-mouth disinfection protocol. **Material and method**: Sixteen chronic periodontitis subjects (mean-age 49.87 ± 8.22) who met inclusion/exclusion criteria were included. A calibrated examiner measured whole-mouth plaque and gingival indices, periodontal pocket depth and clinical attachment level at baseline and at 3-months. Subgingival samples were also collected from the 5 most diseased periodontal sites to determine total bacterial load and levels of *P. gingivalis* and *S. oralis* by real time qPCR. Periodontal treatment consisted of full-mouth manual debridement plus wide intraoral use of chlorhexidine in gel and solution. Additionally, after debridement, individuals rinsed 0.12% chlorhexidine at home twice a day for the following 2 months. Data monitored were compared by paired Student-t test (p<0.05). **Result**: Statistical analysis revealed that, in general, one-stage full-mouth disinfection treatment provided significant clinical and microbiological improvements at 3-months. Total bacterial load showed one of the most pronounced reductions from baseline to 3-months (p=0.0001). Also, subgingival levels *P. gingivalis* and *S. oralis* reduced overtime. **Conclusion**: After a short period of monitoring, chronic periodontitis subjects showed clinical and microbial improvements following one-stage full-mouth disinfection treatment.

Descriptors: Chronic periodontitis; microbiology; therapy; *Porphyromonas gingivalis*; *Streptococcus oralis.*

INTRODUCTION

Periodontal diseases are one of the most common in the oral cavity and have an infectious nature triggered by pathogenic microorganisms that first lead to gingivitis and may develop to periodontitis and, if not treated, to tooth loss[1,2]. Gingivitis is clinically verified by spontaneous or provoked (depending on its severity) bleeding. Periodontitis is characterized by loss of attachment in the conjunctive tissues followed by alveolar bone destruction and consequent formation of periodontal pockets[1,2].

Among several bacterial species capable of colonizing the human oral cavity, *Porphyromonas gingivalis*, a Gram-negative anaerobe colonizes distinct niches and is described as one of the major pathogens associated with periodontal breakdown[3]. In relation to the total bacterial counts, levels of *P. gingivalis* are percentually higher in periodontally diseased sites than in healthy sites[4] while *Streptococcus oralis* is known as the predominant colonizer in the early stage of dental plaque biofilm formation[5].

Because of the microbial nature of periodontal disease its prevention requires reducing biofilm[6] and educating individuals to a good level of oral hygiene[7]. In despite of periodontal disease can be treated successfully by means of both mechanical non-surgical and surgical therapy, periodontal maintenance therapy is a crucial factor for the success of periodontal treatment[8].

After a long time of quadrant treatment, the concept of one-stage full mouth disinfection[9] emerged to avoid transmission of pathogenic microorganisms from not treated periodontal pockets to those recently debrided and thus in the healing process. The original protocol proposed by Quirynen's group adopted combined mechanical and chemical procedures to eliminate plaque and/or calculus from teeth and other oral microbial reservoirs such as tongue and tonsils, thus promoting full-mouth disinfection within a 24-hour period. Periodontal pockets were irrigated by chlorhexidine rinse which was also used daily at home as an additional preventive measure to control biofilm formation. Some of the benefits of this protocol were related to a potential stimulus of the immunological response and a better cost-benefit relation[9].

Therefore, this present short-term study evaluated clinical and microbiological effectiveness of the one-stage full-mouth disinfection protocol in a sample of chronic periodontitis subjects using a three-month longitudinal design.

MATERIAL AND METHOD

The subjects included in the current study signed an informed consent form, which had been previously approved by the Institutional Committee on Research Involving Human Subjects of the University of Taubate (Protocol 521/10).

The study population was composed of sixteen generalized chronic periodontitis individuals registered in the screening periodontal program of the University of Taubate, school of dentistry in the year of 2012. All participants both genders were 18 or older, had at least 20 natural teeth and had chronic periodontitis according to the criteria established[10].

1. *Clinical Periodontal Examinations*

Participants were submitted to a complete periodontal examination, in three times, [1] first to determine a periodontal diagnosis, [2] second to establish baseline data and [3] third, 90 days after the one stage full-mouth disinfection protocol intervention to assess treatment effectiveness. Periapical and or panoramic radiographs were taken only in the first periodontal examination [1].

Probing depth (PD), clinical attachment level (CAL), plaque index[11] (PI) and gingival index[12] (GI) were taken from all teeth, except for third molars, with the help of a manual periodontal probe (PCPUNC 15 Hu-friedy Mfg Co Inc. Chigago IL). One trained and calibrated examiner, blinded to type of treatment, conducted all clinical measurements. Baseline data analysis was performed to determine if the intraexaminer reliability was calibrated. Using kappa statistics (*K*) for the categorical clinical measurement variables, such as periodontal probing depth and clinical attachment level, the standard error of these measurements was monitored. The examiner's clinical measurement technique was considered calibrated if the standard error for the measurements was ≤ 0.8 and a *K* value ranged between 0.8 and 0.95. The reproducibility of the intraexaminer measurements was recalculated prior to the final clinical exams.

Debridement procedures were carried in a single stage (within 24 hours) with Gracey and McCall curettes and Hirschfield files. The process was divided in two appointments (60 minutes each) in two consecutive days. In the beginning of each session subjects rinsed 20 ml 0.12% chlorhexidine (CHX) solution for 30 seconds (the last 10 seconds were of gargling), and in the end of each session there was tongue brushing for 1 minute with a 1% CHX gel and, again, rinsing for 30 seconds. Additionally, individuals were instructed to rinse 20 ml 0.12% CHX for 30 seconds twice a day for 60 consecutive days after the single stage debridement.

2. *Sampling for Microbial Analysis and qPCR Reaction*

Subgingival samples were collected based on the methodology previously used by our group[13]. More specifically, subgingival samples were collected from mesio-buccal aspect of the five teeth showing more evidence of periodontal disease (preferably showing higher values of probing depth, clinical attachment loss and gingival inflammation) using sterile paper points, number 30, inserted into the depth of the pocket after the removal of supragingival plaque using sterile curettes. Sixty seconds after placement in the pocket, paper points were immediately inserted in a microtube and kept on ice. The bacterial cells in the microtubes were dispersed using a vortex mixer at maximum speed for one minute, and the resulting bacterial suspension was saved in a freezer at –80 °C until laboratorial processing.

Genomic DNA (gDNA) was extracted and purified from the pellet using PureLink® Genomic DNA Mini Kit (Life Technologies, Carlsbad, CA, USA) according to manufacturer's specifications.

The quantification of total number of bacterial cells, *P. gingivalis* and *S. oralis* was carried out by qPCR using TaqMan assay (TaqMan® Universal PCR Master Mix II, Life Technologies) with specific set of primers/probes (Table 1) in an ABI 7500 Fast Real Time PCR System® (Life Technologies) following manufacturer's instructions in 20ul reactions. The qPCR conditions were: 50 °C for 2 minutes, 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

The absolute quantification of the target organisms were determined by the plotting of the cycle threshold (Ct) value obtained from each clinical sample against a standard curve generated with known concentration of gDNA of reference bacterial strains (Table 1) in 10-fold serial dilutions (10^2-10^7 cells). Negative control (purified PCR-grade water instead of the DNA template) was included in all PCR reactions.

3. *Statistical Analysis*

A statistical analysis was performed comparing data from baseline to 3-months. Clinical and microbiological data was statistically analyzed using paired-Student t test. The level of statistical significance adopted was 95% (α=0.05) and the software was SPSS 13.0 (IBM® SPSS® Statistics).

RESULT

The present study included 16 individuals: 8 women and 8 men (49.87 ± 8.22 mean age). The evaluation of clinical PD, CAL, PI, and GI showed that all parameters significantly improved after treatment (Table 2). In addition, it was observed that total bacterial load (universal), *P. gingivalis* and *S. oralis* showed a statistically significant ($p<0.05$) reduction after periodontal treatment (Figure 1).

DISCUSSION

Traditionally, periodontal debridement procedures are made in quadrants or sextants with regular intervals of one or two weeks. It is known that the clinical success of this type of treatment happens mainly because of the reduction of periodontal pathogens generally accompanied by the increase of beneficial bacteria[16] and the subsequent establishment of a healthy microbiota. However, these microbial changes could be impaired in individuals with more severe periodontal disease, since they are more susceptible to intra-oral cross contamination involving the variety of aerobic

Table 1. Primers/Probes and reference stains used for microbial quantification by qPCR

Primers/Probe	Reference	Reference stain
Universal		
Forward: 5'-TGGAGCATGTGGTTTAATTCGA-3'	Nonnenmacher et al.[14] (2004)	*Escherichia coli* (ATCC 25922)
Reverse: 5'-TGCGGGACTTAACCCAACA-3'	Nonnenmacher et al.[14] (2004)	
Probe: 5'-CACGAGCTGACGACA(AG)CCATGCA-3'	Nonnenmacher et al.[14] (2004)	
P. gingivalis		
Forward: 5'-ACCTTACCCGGGATTGAAATG-3'	Saito et al.[15] (2011)	*P. gingivalis* (W83)
Reverse: 5'-CAACCATGCAGCACCTACATAGAA-3'	Saito et al.[15] (2011)	
Probe: 5'-ATGACTGATGGTGAAAACCGTCTTCCCTTC-3'	Saito et al.[15] (2011)	
S. oralis		
Forward: 5'-TTGGCTCAATTCCCTTTGAC-3'	Designed for this study*	*S. oralis* (ATCC 10557)
Reverse: 5'-GTCCAAACAAGCCACCACTT-3'	Designed for this study*	
Probe: 5'-ACAACATATCAACAGGCGCA-3'	Designed for this study*	

*Designed using Primer3 software online version (v. 0.4.0). NCBI Blast database was used to check Primer/Probe specificity.

Table 2. Distribution of clinical periodontal parameters before (baseline) and 90 days after periodontal treatment

	PD	CAL	PI	GI
Baseline	3.72 ± 0.95	5.01 ± 1.92	0.43 ± 0.23	0.35 ± 0.34
90 days	2.13 ± 0.76	3.54 ± 1.82	0.24 ± 0.27	0.13 ± 0.17
p value	**0.0321**	**0.0138**	**0.0295**	**0.0341**

Paired Student-t test, Wilcoxon ($p<0.05$).

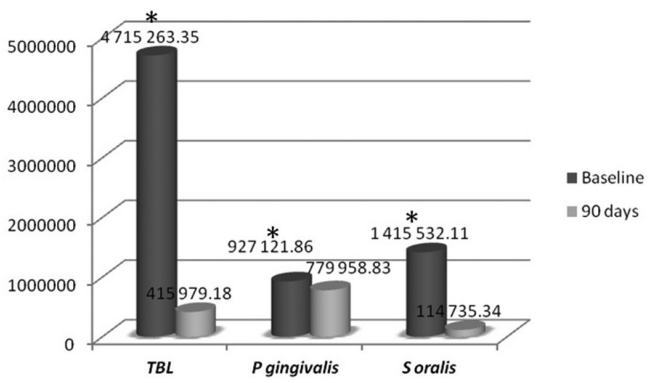

Figure 1. Distribution of the final number of copies before and after treatment for universal primer, *P. gingivalis* and S.oralis

and anaerobic bacteria[17] which can translocate from not treated sites to those recently treated. In this context, the concept of one-stage full mouth disinfection emerges and aims mainly at minimizing transmission of pathogenic microorganisms from the periodontal pockets still not treated to those already treated[9].

Chlorhexidine is an antimicrobial agent widely used in Dentistry. Although in this protocol CHX had demonstrated good results, authors felt like it was necessary to check the degree of benefit brought by the product[18,19]. Therefore, we decided that would be interesting to contribute to clarify doubts left by other studies such as that of which concluded that the greatest benefit derived from intense mechanical therapy carried within 24 hours[20]. In a posterior study, however, the same group reported controversial results after observing that the prolonged use of CHX brought beneficial effects to the protocol. During explanation of potential risks and benefits of this study, subjects were informed about possible side effects related to CHX. Patients did not complain about side effects although examiner was able to identify teeth staining.

Individuals included in this study showed a microbial profile with high copies of bacterial DNA (see Figure 1) verified by q-PCR in baseline, so the adoption of the 24 hour treatment seemed convenient and beneficial, since it could minimize the chance of intra-oral transmissibility. Such hypothesis was verified when a drastic bacterial reduction was observed by the final reduced total bacterial load after therapy. This finding corroborates the study which affirmed that one-stage full mouth protocol immediately decrease the number of bacteria in mild to advanced periodontitis. This reduction will positively affect the recolonization process due to the slowing of supragingival and consequently subgingival biofilm formation[21].

P. gingivalis, is one of the many bacteria colonizing the mouth, found in different sites such as periodontal pockets, oral mucosa and tongue dorsum[22,23]. It is a Gram-negative microorganism and an important etiologic agent of periodontal disease. Colonization by this pathogen results in tissue lesion due to the exacerbated production of a variety of virulence factors. Besides, this bacterium seems to have a significant role in the progression of chronic periodontitis[24,25] and, in high levels, is usually related to deep periodontal pockets[26]. Our study showed that, after therapy, there was a statistically significant reduction in the levels of *P. gingivalis* in comparison to baseline. Total bacterial reduction associated with the decrease in the levels of *P. gingivalis* may easily be connected to the improvement of clinical variables observed in this study. All parameters – PD, CAL, PI, PG – showed statistically significant improvement after therapy. It is known that some clinical periodontal parameters respond more quickly following treatment while others only manifest improvement or worsening later.

S. oralis is known as the predominant colonizer in the early stage of dental plaque biofilm formation[27]. Primary colonizers alter the surface not only by their physical presence but also they are likely to represent a new "surface-attached" phenotype with distinct metabolic activity and surface properties, thus altering their surroundings and creating new niches for other bacteria to colonize28.This specie seems to be less pathogenic for periodontitis, and our results showed that the prevalence was markedly low in periodontitis patients, like P.gingivalis. We assumed that the considerable amount of *S. oralis* could be eliminated from the periodontal pockets by initial treatments, and its prevalence in subgingival plaque would decrease in the present study.

After a short-term period of monitoring results obtained in this study have lead us to conclude that, one-stage full mouth disinfection protocol can be a treatment option to chronic periodontitis subjects. In addition, our study demonstrated that the adjunctive daily use of an antimicrobial agent to mechanical plaque control could result in a retarded subgingival recolonization by periodontal pathogens in examined population.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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