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Comparative Study

MODIFIED ORAL HYGIENE PROTOCOLS TO PREVENT PERIODONTAL DISEASES. ROLE OF LASERS AND PHASE CONTRAST MICROSCOPES IN PERIODONTAL MAINTENANCE THERAPY

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ABSTRACT

The purpose of this research was to assess how the application of the oral irrigator modifies the bacterial flora using a phase contrast microscope in patients affected by chronic periodontitis and treated with laser and strictly home care protocol.

60 patients were included in periodontal maintenance therapy (PMT), with specific home oral hygiene instructions (sonic toothbrush plus oral irrigators, at least twice a day). Phase contrast-phase microscopic examinations displayed the existence of non-mobile (i.e., not pathologic) bacterial flora in all patients. They were then randomly divided into two groups, A and B. After professional oral hygiene, group A stops using an oral irrigator at home. After the professional oral hygiene, patients of group B were motivated to continue their oral hygiene protocol at home. After three months, the patients underwent a second microscopic analysis of bacterial plaque.

In the Group A patients, 90% of cases had a pathogenic bacterial flora change. 100% of the patients in group B showed non-mobile bacteria on phase contrast microscopic analysis, whereas group A showed mobile bacteria.

This research demonstrates that oral irrigator in home hygiene protocol plays a role in the long-term maintenance of non-pathogenic bacterial flora in periodontal patients.

KEYWORDS: *bacterial flora, oral irrigator, diode laser, hydrogen peroxide, oral hygiene, oral irrigator, phase contrast microscopy, photodynamic therapy, sonic toothbrush.*

INTRODUCTION

Maintaining periodontal health over time is crucial in guaranteeing the success of dental treatments. Periodontal maintenance therapy (PMT) and home oral hygiene protocols are the keys to success. We know from the literature and clinical experiences that without meticulously organized and performed PMT, patients with a predisposition to periodontal disease are at high risk of reinfection and progression of periodontal lesions. A large amount of failures after non-surgical

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or surgical periodontal therapies (1) is due to incomplete cleaning of the root superficies from bacteria belonging to red and orange Socransky's complexes, such as *Porphyromonas gingivalis*, *Treponema denticola*, *Bacteroides forsythus*, *Fusobacterium nucleatum,* and *Peptostreptococcus micros*. It has been shown (2) that new contamination of the periodontal pockets is total after a one-year follow-up, even with frequent dentist appointments, either with non-surgical or surgical therapies.

Since the 90s, reports have shown that decontamination by laser radiation is possible (3). Lasers can be applied alone or in association with a photosensitizer. Photodynamic therapy (PDT) is the association of light with a chromophore and oxygenated tissues (4). This technique aims to bring singlet oxygen to all tissues affected by pathogenic bacteria. However, if chromophores absorb the wavelengths, laser rays cannot break through tissues very deep. In addition, if the power density is too low, the decontaminating effects are insufficient. It happens because PDT protocols use LLLT (low-level laser therapy) energy to avoid thermal damage.

Recent research (5) proposed a combination between high power and high frequency of diode laser 980 nm (using high peak power combined with low average power to reduce thermal effects) and hydrogen peroxide 10 volume 3%, or modified hydrogen peroxide 10 volumes 3%. This treatment protocol has been named "photodynamic therapy without dye" or Oxygen High Level Laser Therapy (6). Several in vitro and in vivo studies showed the bactericidal activity of laser irradiation combined with hydrogen peroxide (7).

The aim of this research was to evaluate how much homecare with an oral irrigator has a role in maintaining a non-pathogenic bacterial flora in the oral cavity. The diagnostic tool is the phase contrast microscope (8) which provides qualitative data on the bacterial flora monitored during the six-monthly recalls of professional hygiene.

MATERIALS AND METHODS

The present study was carried out in conformity with the Declaration of Helsinki. Sixty patients from a private dental clinic in Bergamo, Italy, were recruited and were diagnosticated moderate generalized periodontitis in 2016, 2017, and 2018. In addition, they had laser-assisted non-surgical therapy and taught in-homecare protocol with a sonic toothbrush, interdental brush and oral irrigator. Subsequently, they underwent a second clinical evaluation in order to be sure that periodontal disease was stabilized. Afterwards, they were recruited in a 6-months recall protocol of professional oral hygiene.

For the periodontal maintenance therapy, the following maintenance home hygiene protocol was delivered: sonic brush with vertical movement (Broxo OraBrush, Santé Parodonte, Geneva, Switzerland), interdental brushes, and oral irrigators (Broxo OraJets, Santé Parodonte, Geneva, Switzerland) at least two times every day (Fig. 1-2-3).

Fig. 1. *Oral hygiene devices. Seven devices for optimal domiciliary hygiene procedures. The most important are: 1: manual toothbrush; 1: sonic brush with vertical movement; 2: interdental brushes; 3: oral irrigators.*

An evaluation of the subgingival plaque sample was performed on each patient by using contrast-phase microscopy. It was performed during PMT appointments every 6 months in a close recall scheme (Fig. 4-5).

Based on contrast phase microscopy results, two protocols were applied. In the case of non-pathogenic bacterial flora (i.e. static flora, Gram-positive bacteria) detection, standard periodontal therapy with appointments every 6 months was delivered. In the case of pathogenic bacterial flora (spirochete, moving flora) detection, immediate treatment with supra-gingival and sub-gingival ultrasonic instrumentation, air flow with bicarbonate powder, and a single-session of photodynamic therapy without dye (OHLLT) in the entire mouth was delivered. The last protocol has also been applied without signs of inflammation (i.e. pain, bleeding).

The single-stage session of photodynamic therapy without dye (OHLLT) is the irrigation of periodontal pockets with a Sioxyl solution. First, the operator aspires a Sioxyl solution from the gingival sulcus, leaving the remaining solution inside the pocket for 2 min. After that, an HF Diode Laser 980 nm, Fiber 400 microns (Wiser Doctor Smile, Lambda, Vicenza, Italy) is introduced in the periodontal pocket to reach the bottom. Then, radiation of subgingival tissues is carried out through a back-and-forth movement, using a specific program, 60 s for each side (2.5 W peak power, high frequency, 10 kHz, power average 0.5 W, Fluency 25000/cm2).

At the end of the professional oral hygiene procedure, patients were trained for homecare protocol with a sonic brush, interdental brushes, and oral irrigator at least twice daily.

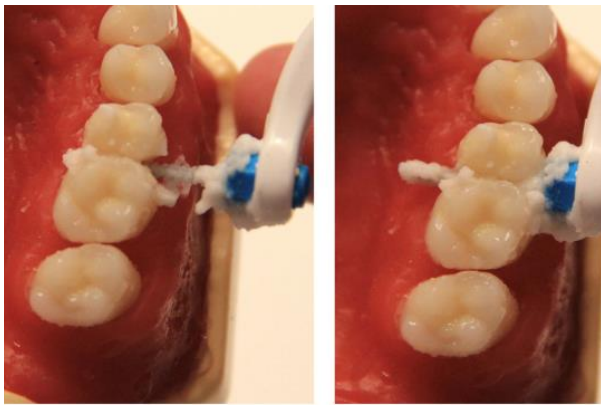

Fig. 2. *Interdental brushes.*

Fig. 3. *Oral irrigator during subgingival biofilm removal.*

After 1 month, patients were checked for periodontal disease with a contrast-phase microscope. If they have only non-pathogenic bacterial flora, patients returned to standard 6-month controls. If they have pathogenic bacterial flora, patients are trained again for oral hygiene associated with a single-stage session of laser-assisted PDT without dye.

Dental hygienists, therefore, are responsible for organizing this specific prevention and maintenance program, personalized and flexible, using a continuous and progressive educational process that can support the results obtained by the dentist.

All 60 patients recruited in periodontal support therapy had the following inclusion criteria:

- age from 35 to 65;
- no systemic diseases;
- periodontal clinical attachment loss of less than 5mm in at least two teeth;
- no tooth mobility;
- bleeding index under 30%;
- plaque index under 30%.

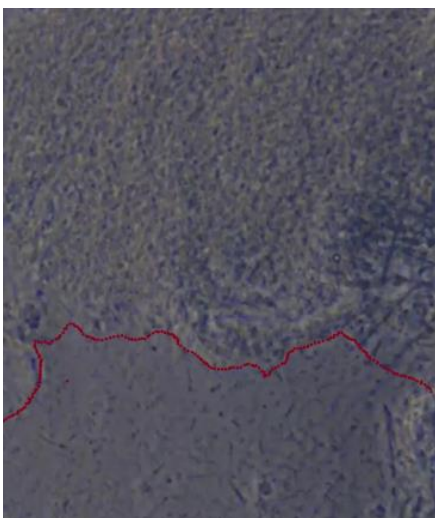

Fig. 4. *Contrast phase microscope with dividing line between non-pathogenic bacterial flora (above) and pathogenic bacterial flora (below).*

All 60 patients were evaluated for subgingival plaque check with a phase contrast microscope by the same operator. For examination, a periodontal curette is used. It collected bacterial plaque in the gingival sulcus. First, plaque is placed on a slide, then it is irrigated with a drop of physiological solution, fixed with a counterslide, and a drop of oil is placed on the counterslide to concentrate the light of the microscope. A 40x objective is recommended with an eyepiece of 15x (600 magnification).

Then it is possible to visualize which kind of bacteria are present in the plaque, as well as epithelial cells and polymorphonuclear cells. Contrast-phase microscope distinguishes between a non-pathogenic bacterial flora (Fig. 6) that is non-mobile and a pathogenic bacterial flora (Fig. 7) that is primarily mobile and composed of spirochetes such as Treponema Denticola.

The plaque was collected from the gingival sulcus in each patient. Microscopic analysis showed non-pathogenic bacterial flora for each sample (T0). All patients were then randomly divided into two groups, A and B. After a professional oral hygiene session, group A was asked to suspend the use of an oral irrigator and continue the home hygiene protocol only with a sonic toothbrush and interdental brushes. After the professional oral hygiene session, Group B was requested to continue the complete homecare protocol. Three months later, the patients underwent a second bacterial plaque analysis (T1), and the data were obtained and accessed through the Statistical Package for Social Sciences (SPSS).

Fig. 5. *Contrast-phase microscope.*

RESULTS

Patients belonging to Group A (Table I) (i.e. did not use the oral irrigator) had mobile bacterial flora in 90% of cases. Instead, 100% of patients belonging to group B (i.e. using the standard protocol with a sonic toothbrush, interdental brushes and oral irrigator for 3 months) showed non-mobile plaque on phase contrast microscopic evaluation.

Recruited patients had a plaque index of less than 30% (Table II). At the time of re-evaluation, group A showed a slight worsening of the plaque index: seven out of 30 patients had a plaque index greater than 30%. In group B, there was no worsening of the plaque index. The control group maintained the plaque index below 30% in all cases.

Table III and Table IV report the difference between periodontal and microbial parameters of groups A and B at T0 and T1, demonstrating that oral irrigator usage at home effectively maintains a non-mobile flora in the periodontal sulcus.

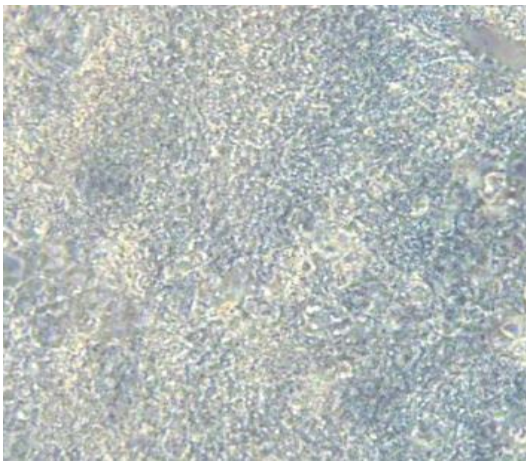

Fig. 6. *Non-pathogenic bacterial flora.*

DISCUSSION

The phase contrast microscope uses two principles (wavelength and amplitude) to create an image of cells (8, 9). Methodologically, contamination, sampling technique and sample preparation are important as they strongly influence the analysis result. The reproducibility of what is seen using contrast phase microscopy is high when the procedure is standardized. Sample analysis gives us some clinically relevant information (8, 10). Direct examination of the sub-gingival dental plaque under a phase contrast microscope allows one to characterize bacterial morphotypes without inflammatory signs. It also allows for evaluating the microbial density associated, or not, with active periodontal pockets.

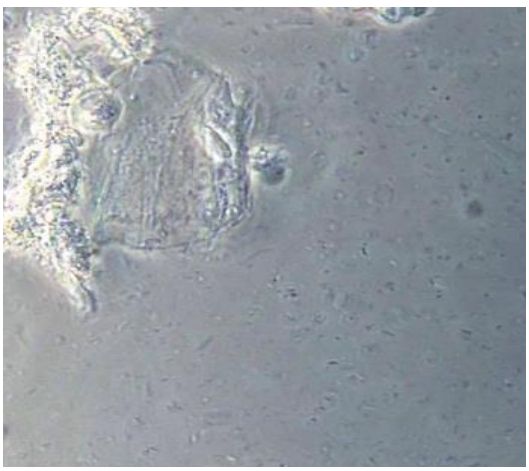

Fig. 7. *Pathogenic bacterial flora.*

Phase contrast microscopy provides qualitative data of bacterial flora that integrates the standard periodontal parameters such as plaque index, bleeding index and loss of clinical attachment collected and monitored during the six-monthly recalls of professional hygiene. Quirynen et al. (11) based the results of their research on the phase contrast microscope. Bollen, et al.,

(12) published a pilot study examining the long-term microbiological effects of a "full mouth" disinfection controlled with phase contrast microscopy. Yeom et al. (13) used the phase contrast microscope to evaluate the clinical and microbiological effects of the subgingival deposition of bioabsorbable microcapsules loaded with 10% of minocycline in 15 adult patients with periodontitis. Quirynen et al. (14) used a phase contrast microscope to evaluate the bacterial plaque around the implant surfaces. Acharya et al. (15) compared the efficacy of three motivational techniques to maintain good oral hygiene during fixed appliance orthodontic treatment. Phase contrast microscopy and the conventional plaque detection method were used to demonstrate that vertical brushing is the best; this reduces the need for frequent strengthening sessions of plaque control programs.

Analysis of the literature demonstrated that homecare periodontal support therapy could preferably include a sonic electric toothbrush (16-18) for the shock wave generated by the movement of the bristles combined with the oral irrigator (19-21). This last induces the solution of subgingival plaque through the disorganization of the salivary biofilm, which can potentially spread microorganisms in the gingival sulcus (1, 2, 22).

The present study demonstrated that using a specific home oral hygiene protocol is essential in periodontal maintenance therapy. Oral irrigators are important to maintain a non-pathogenic bacterial flora in subgingival plaque. If patients in

Table I. *Patients with not-mobile flora at first (T0) and second (T1) evaluation.*

Compatible flora	T_0	T_1
Group A	30	3
Group B	30	30

Table II. *Patients with plaque index less than 30% at first (**T0**) and second evaluation (**T1**).*

Plaque index < 30%	T_0	T_1
Group A	30	23
Group B	30	30

Table III. *Periodontal and microbial parameters among the A group and B group at T0.*

	Group A (*n* = 30)	Group B (*n* = 30)	*p* Value
	Mean ± sd	**Mean ± sd**	
BoP	0.14 ± 0.09	0.11 ± 0.10	<0.018
P.I.	0.23 ± 0.12	0.21 ± 0.11	0.006
P. D.	2.24 ± 0.23	2.25 ± 0.21	0.267

***BoP**: bleeding on probing; **P.I.**: plaque index; and **P.D.**: periodontal depth. *p value <0.001 is statistically significant.*

Table IV. *Periodontal and microbial parameters among the A group and B group at T1.*

	Group A (*n* = 30)	Group B (*n* = 30)	*p* Value
	Mean ± sd	**Mean ± sd**	
BoP	0.12 ± 0.05	1.12 ± 0.21	<0.0005 *
P.I.	0.12 ± 0.15	0.93 ± 0.21	<0.001 *
P.D.	2.16 ± 0.18	2.41 ± 0.24	0.135

***BoP**: bleeding on probing; **P.I.**: plaque index; and **P.D.**: periodontal depth. *p value <0.001 is statistically significant.*

PMT avoid using oral irrigators for 3 months, periodontal conditions deteriorate with an increase of pathogenic bacterial flora detected with phase contrast microscopy and worst periodontal parameters (PI, BOP, PD).
The patient must be constantly monitored and continuously remotivated to maintain excellent oral hygiene levels. Scientific literature proves that our protocol is a suitable method to stop the onset and advancement of periodontal infection. It also avoids relapses and stabilizes the results obtained in patients predisposed to periodontal disease.

CONCLUSION

The present study shows that phase contrast microscopy detects early sub-gingival plaque modifications due to poor home oral hygiene protocols application by patients and how the use of the oral irrigator is of paramount importance in the long-term maintenance of a non-pathogenic bacterial flora in the periodontal sulcus. It is clear that, although it is possible to maintain an acceptable plaque index only with a sonic toothbrush and pipe cleaners, a water jet allows the maintenance of non-pathogenic bacterial flora in the oral cavity of patients who have not stopped using it.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

Author Contributions

GLC and PC designed the research study; PE and GC performed the research; GLC and PC wrote the manuscript. All authors contributed to editorial changes in the manuscript and read and approved the final version. The authors declare no conflict of interest.

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