

THE USE OF OZONE THERAPY FOR TREATMENT OF PERIODONTAL DISEASE: A SPLIT-MOUTH, RANDOMIZED, CONTROLLED CLINICAL TRIAL

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Periodontal treatment has the aim to reduce oral infection and prevent the progression of the disease. The potential benefits of new therapy with Ozonline® for periodontal treatment, include improved patient compliance and an easier access to periodontal pocket. The objective of this study was to explore the efficacy of Ozonline® in the treatment of chronic periodontitis in adult patients. A randomized controlled split-mouth study was carried out in ten patients (5 men and 5 women age 42-73 mean 55 ± 7) with a diagnosis of chronic periodontitis. None of these patients received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler with ozonated water (Ozonline®). 10 microbiological samples were collected from upper left quadrants and 10 from upper right quadrants from each patient. Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. 20 localized chronic periodontitis sites were selected (10 in left quadrants and 10 in right quadrants). After the treatment with Ozonline®, a remarkable decrease in bacteria amount, both for some species and for the total count was observed in the left quadrants respect to right ones. Specifically, *T. forsythia* and *T. denticola* were eradicated whereas Total Bacteria Loading and *Fusobacterium Nucleatum* showed a reduction of 38% and 55%, respect to right quadrants. Our study demonstrated the efficacy of the Ozonline® in the management of moderate to severe chronic periodontitis.

The term periodontal disease (PD) is generally used to describe diseases affecting the gums and tooth support tissues, causing damage to the connective tissue and alveolar bone (1). Specific bacteria of the biofilm formed by plaque cause PD. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites (1). PD affects about the 50% of adults, or more, while the severe form of periodontitis affects around 10% (range: 5-20%) of adults and moderate periodontitis around

30%. In elderly people, the prevalence of PD is estimated about 70-90% of individual's aged 60-74 years (1). Periodontal therapy is important to reduce local inflammation and bacteremia. Recently it has been stated that periodontal disease appears to increase the risk of cardiovascular disease, pulmonary disease, preterm and low birth weight (2).

The oral microbiota is constituted by many bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. Periodontal treatment has the aim to reduce oral infection and prevent the progression of the

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disease. Periodontal non-surgical therapy with scaling and root planning associated with a good level of domestic oral hygiene can prevent the onset of the disease and allow for proper maintenance of oral health (3, 4).

Good oral hygiene has the aim to control bacterial plaque, but when the patient's attention to oral hygiene decreases, it is possible to experience a recurrence of the disease. In addition, periodontal disease presents a characteristic trend with periods of remission and exacerbation of the disease. Scaling and root planning are efficacy, but a better microbiological control of oral microbiota can be reached by the administration of ozonated water in association.

The aim of this study was to investigate the efficacy of ultrasonic scaling with ozonated water, acting as bactericidal in the management of moderate to severe chronic periodontitis.

Ozon therapy

The main use of ozone in dentistry relies on its antimicrobial properties. It is proved to be effective against both Gram-positive and Gram-negative bacteria, viruses and fungi (5). Nagayoshi et al (5) examined the effect of ozonated water on oral microorganisms and dental plaque. Dental plaque samples were treated with 4mL of ozonated water for 10 seconds. They observed that ozonated water was effective for killing gram-positive and gram-negative oral microorganisms and oral *Candida albicans* in pure culture as well as bacteria in plaque biofilm and therefore might be useful to control oral infectious microorganisms in dental plaque.

MATERIALS AND METHODS

Ten patients (5 men and 5 women) were randomly selected with a diagnosis of chronic periodontitis. Patients who qualified for the study were in the age group of age 42-73 (mean 55 ± 7) and did not receive any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they met any of the following criteria: pregnancy; a history of taking antibiotics or using anti-bacterial mouth rinses for the past 6 months; has teeth with furcation involvement; patients with a history

of smoking, and drug or alcohol abuse. The subjects that volunteered to participate in the study, signed consent forms and followed a detailed verbal description of the procedure. This study was conducted in accordance with the Declaration of Helsinki and independently approved and reviewed by the local Institution's Ethical Committee.

Clinical methods

Twenty sites (distal pockets of the left and right first upper molar) were selected from the 10 patients. Each selected site was subjected to microbial analysis. At the first visit, after recording the clinical parameters at each site in selected patients at the baseline, microbiological samples were collected from pre-selected sites. The mouth has been divided into upper right and left quadrants. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler plus ozonated water (4 mg/ml in distilled water) (Ozonline®). Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. For the collection of subgingival samples, the sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of sub-gingival samples and were immediately transferred to microbiological laboratory for processing. The microorganisms processed were the three bacterial species more involved in periodontitis that constitute the red complex group: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*.

Several methods have been used for microbiological testing in periodontitis (7). However, many techniques have not been fully accepted due to low sensitivity or specificity, moreover sometimes they are slow, expensive and laborious. In this study we have used the (LAB-test®) developed by the LAB corporation (Ferrara, Italy), which is rapid and sensitive, allowing to detect and quantify the three bacterial species more involved in periodontitis that constituted the red complex group: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*. Both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction. They appear closely 'linked' topologically in the developing biofilm and are considered the first pathogens involved in the clinical destruction of periodontal tissues.

Real-Time Polymerase Chain Reaction

Primers and probes oligonucleotides have been

designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequences. For each sample, two real-time polymerase chain reaction (PCR) runs were performed. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe, matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria in a multiplex PCR. The reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed by using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15s at 95°C and 60 s at 57°C for 40 cycles. All the experiments were performed including non-template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as a standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same number of plasmids, in serial dilutions ranging from 10¹ to 10⁷ copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions. The copy numbers for individual plasmid preparations was estimated using a Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of the relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling were performed in a separate laboratory with dedicated pipettes.

Statistical analysis

Descriptive statistics was registered using Microsoft Excel spreadsheets. Paired T-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

RESULTS

After the treatment with ultrasonic scaler plus ozonated water with Ozonline®, a remarkable decrease in total bacteria amount and in the individual microbic species was observed in the left periodontal pockets respect to right pockets. Specifically, *T. forsythia* and *T. denticola* were eradicated whereas the total bacteria loading and *Porphyromonas gingivalis* showed a percentage reduction of 38% and 55%, respect to right pockets. Ozonated water with Ozonline® did not show any side effects and was not observed to cause discomfort or to produce adverse reactions in time. No patient reported pain, burning, tingling sensation or numbness. Despite the spread of the data, preventing a correct statistical evaluation, the results obtained clearly demonstrated a reduction of microbial concentration after treatment with Ozonline®.

DISCUSSION

The oral microbiota is constituted by many bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic microorganisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. The oral cavity is suitable for invasion of many microorganisms. Mechanical debridement is effective in both breaking the biofilm and reducing the bacterial load.

However, the effects of mechanical instrumentation may be improved with the association of Ozonline®, providing an additional benefit in controlling the progress of disease. Nagayoshi et al (5) tested the efficacy of three different concentrations of ozone water (0.5, 2, and 4 mg/ml in distilled water) on the time-dependent inactivation of cariogenic, periodontopathogenic and endodontopathogenic microbes (*Streptococcus*, *Porphyromonas gingivalis* and *endodontalis*, *Actinomyces actinomycetemcomitans*, *Candida albicans*) in culture and in biofilms. They confirm

that ozonated water was highly effective in killing of both gram positive and gram-negative microorganisms.

Depending on the dosage, the oral microbes were inactivated after 10 seconds. Gram negative anaerobes, such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis* were substantially more sensitive to ozonated water than gram positive oral streptococci and *Candida albicans* in pure culture. Furthermore, ozonated water had strong bactericidal activity against bacteria in plaque biofilm. In addition, ozonated water inhibited the accumulation of experimental dental plaque *in vitro*.

Ramzy et al (6) irrigated the periodontal pockets by ozonized water in 22 patients suffering from aggressive periodontitis (age range from 13 to 25 years). Periodontal pockets were irrigated with 150 ml of ozonated water over 5 to 10 minutes once weekly, for a clinical four weeks study, using a blunt tipped sterile plastic syringe. High significant improvement regarding pocket depth, plaque index, gingival index and bacterial count was recorded related to quadrants treated by scaling and root planning together with ozone application. They also reported significant reduction in bacterial count in sites treated with ozonized water.

In the study by Karapetian et al, (8) peri-implantitis treatment with conventional, surgical and ozone therapy methods was investigated, and it was found that the most effective bacteria reduction was in the ozone-treated patient group. The authors concluded that the main challenge seems to be the decontamination of the implant surface, its surrounding tissue and the prevention of recolonization with periodontal pathogenic bacteria.

Kshitish and Laxman (9) conducted a randomized, double-blind, crossover split-mouth study on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time-intervals, i.e. baseline (0 days) to 7th day, with a washout period of 4 days followed by a second time interval of 7 days. Subgingival irrigation of each half of the mouth with either ozone or chlorhexidine was done at different time intervals. They observed a higher percentage of reduction in plaque index (12%), gingival index (29%) and

bleeding index (26%) using ozone irrigation as compared to chlorhexidine. The percentile reduction of Aa (25%) using ozone was appreciable as compared to no change in Aa occurrence using chlorhexidine. The antifungal effect of ozone from baseline (37%) to 7th day (12.5%) was pronounced during the study period, unlike chlorhexidine did not demonstrate any antifungal effect. No antiviral property of ozone was observed. The antiviral efficacy of chlorhexidine was better than that of ozone. They concluded that despite the substantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms.

Periodontal disease and peri-implantitis is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (9-16, 51-53). Periodontal disease and peri-implantitis diagnosis and therapy has been increased a great interest in the last years both in basic (17-28) and advanced research (29-35). Besides the treatment of periodontal disease and peri-implantitis requires a multidisciplinary approach (36-50, 54-70).

Our study evaluated the efficacy of ozonated water Ozonline® in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall decreasing in bacterial loading. A new dimension was added to this investigation by microbiological examination of bacterial morphotypes using periodontal tests. It was thought appropriate to evaluate the effect of Ozonline® on subgingival microbial population, the primary etiological factor for periodontitis. The results demonstrated a marked reduction in total number of organisms in each patient from baseline to 7 days.

REFERENCES

1. Sagar A. Full mouth versus quadrant treatment in chronic periodontitis. *Prim Dent J* 2014; 3(3):66-9.
2. Feres M, Faveri M, Figueiredo LC, Teles R, Flemmig T, Williams R, Lang NP. Group B. Initiator paper. Non-surgical periodontal therapy: mechanical debridement, antimicrobial agents and other modalities. *J Int Acad Periodontol* 2015; 17(1S):21-30.
3. Ciantar M. Time to shift from scaling and root planing to root surface debridement. *Prim Dent J*

- 2014; 3(3):38-42.
4. Hammerle CH, Giannobile WV, Working Group 1 of the European Workshop on P. Biology of soft tissue wound healing and regeneration--consensus report of Group 1 of the 10th European Workshop on Periodontology. *J Clin Periodontol* 2014; 41(S15):S1-5.
 5. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiology & Immunology*. 2004; 19:240.
 6. Ramzy MI, Gomaa HE, Mostafa MI, Zaki BM. Management of Aggressive Periodontitis Using Ozonized Water. *Egypt. Med. J. N R C*. 2005; 6(1),229-45.
 7. Carinci, F., Girardi, A., Palmieri, A., Martinelli, M., Scapoli, L., Avantaggiato, A., Nardp, G.M., Lauritano, D. LAB-Æ-test 2: Microflora and periodontal disease *European Journal of Inflammation*, 2012; 10 (1)95-8.
 8. Karapetian VE, Neugebauer J, Clausnitzer CE, Zoller JE. Comparison of Different Periimplantitis Treatment Methods. Available from: http://www.helbo.at/datasheets/post_er_karapetian_0304.pdf
 9. Jezi G, Mangano C, Barone A, Tirone F, Baggi L, Tromba G, Piattelli A, Giuliani A. Jawbone remodeling: a conceptual study based on Synchrotron High-resolution Tomography. *Sci Rep* 2020; 10(3):3777.
 10. Ruggiero F, Carbone D, Mugavero, R, Palmieri A, Lauritano D, Baggi L, Nardone M, Martinelli M, Carinci F. Human polyomavirus in tonsillar microbiota of an Afghan population group. *J Biol Regul Homeost Agents* 2018; 32(2 Suppl 1):185-90.
 11. Ottria L, Candotto V, Cura F, et al. Human papilloma virus associated with oral cancer and preventive strategies: The role of vaccines. *J Biol Regul Homeost Agents* 2018; 33(2 Suppl 1):61-5.
 12. Gallusi G, Avantaggiato P, Baggi L, Marchetti E. Why dentists and dental hygienists should improve programs for inducing smoking cessation? The effect of tobacco smoking on periodontal health. *J Biol Regul Homeost Agents* 2020; 34(1 Suppl 1):133-8.
 13. Mummolo S, Severino M, Campanella V, Barlattani A Jr, Quinzi V, Marchetti E. Chlorhexidine gel used as antiseptic in periodontal pockets. *J Biol Regul Homeost Agents* 2019; 33(3 Suppl. 1):83-8.
 14. Mummolo S, Tieri M, Nota A, et al. Salivary concentrations of *Streptococcus mutans* and *Lactobacilli* during an orthodontic treatment. An observational study comparing fixed and removable orthodontic appliances. *Clin Exp Dent Res* 2020; 6(2):181-7.
 15. Marchetti E, Tecco S, Santonico M, Vernile C, Ciciarelli D, Tarantino E, Marzo G, Pennazza G. Multi-Sensor Approach for the Monitoring of Halitosis Treatment via *Lactobacillus brevis* (CD2)-Containing Lozenges--A Randomized, Double-Blind Placebo-Controlled Clinical Trial. *Sensors (Basel)* 2015; 15(8):19583-96.
 16. Lauritano D, Lucchese A, Gabrione F, et al. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. *Int J Environ Res Public Health* 2019; 16(2): E210.
 17. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, et al. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. *Int J Mol Sci* 2019; 20(16).E3897.
 18. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. *Int J Mol Sci* 2019; 20(8):E2047.
 19. Lauritano D, Oberti L, Gabrione F, et al. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. *Oral Oncol* 2019; 97:7-17.
 20. Lauritano D, Moreo G, Carinci F, et al. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. *Int J Environ Res Public Health*. 2019; 16(19):E3763.
 21. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. *Mini Rev Med Chem*. 2019; (13)(Q2)
 22. Lauritano D, Moreo G, Della Vella F, et al. Oral Health Status and Need for Oral Care in an Aging Population: A Systematic Review. *Int J Environ Res Public Health*. 2019; 16(22).
 23. Petruzzi M, Della Vella F, Cassandro A, et al. Dorsal tongue porphyrin autofluorescence and *Candida* saprophytism: A prospective observational study. *PLoS One*. 2019; 14(9):e0223072.
 24. Lauritano D, Arrica M, Lucchese A, et al. Oral lichen

- planus clinical characteristics in Italian patients: A retrospective analysis. *Head and Face Medicine*, 2016; 12 (1):18.
25. Lauritano D, Attuati S, Besana M, et al. Oral and craniofacial manifestations of Ellis-Van Creveld syndrome: a systematic review. *Eur J Paediatr Dent* 2019; 20(4): 306-10.
 26. Lorenzi C, Arcuri L, Lio F, Dolci A, Arcuri C. Radiosurgery in dentistry: a review. *Clin Ter* 2019; 170(1):e48-e54.
 27. Arcuri L, Contaldo M, Santoro R, et al. Radiosurgery-assisted gingival displacement: a proof of concept technique to enhance the efficiency of intraoral digital impression. *J Biol Regul Homeost Agents* 2018; 32(2 Suppl 1):197-201.
 28. Di Girolamo M, Barlattani A Jr, Grazzini F, et al. Healing of the post extractive socket: Technique for conservation of alveolar crest by a coronal seal. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 1):125-35.
 29. Di Girolamo M, Baggi L, Pirelli P, et al. 3D X-ray microscopic analysis on a prosthetically loaded implant with platform-switching and conical connection: A case report. *Oral Implantol (Rome)* 2017; 10(3):241-6.
 30. Andreasi Bassi M, Bedini R, Pecci R, Ioppolo P, Lauritano D, Carinci F. Mechanical Properties of Abutments: Resin-Bonded Glass Fiber-Reinforced Versus Titanium. *Int J Prosthodont* 2016; 29(1):77-9.
 31. Arcuri L, Lorenzi C, Vanni A, Bianchi N, Dolci A, Arcuri C. Comparison of the accuracy of intraoral scanning and conventional impression techniques on implants: a review. *J Biol Regul Homeost Agents* 2020; 34(1 Suppl 1):89-97.
 32. Andreasi Bassi M, Andrisani C, Lico S, Silvestre F, Gargari M, Arcuri C. Increasing volume of vestibular soft tissues in flapless implant surgery through a modified connective punch technique: a controlled clinical trial. *Oral Implantol (Rome)* 2016; 9(3):143-50.
 33. Arcuri C, Barlattani A Jr, Mazzetti V, Carosi P, Ferrigno N, Laureti M. Immediate restoration of a single upper central integrating digital workflow and a novel dental implant: a case report. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 2):171-7.
 34. Ferro R, Pasini M, Fortini A, Arrighi A, Carli E, Giuca MR. Evaluation of maxillary and mandibular arch forms in an Italian adolescents sample with normocclusion. *Eur J Paediatr Dent*. 2017; 18(3):193-8.
 35. Giuca MR, Giannotti L, Saggese R, Vanni A, Pasini M. Evaluation of cephalometric, hormonal and enzymatic parameters in young obese subjects. *Eur J Paediatr Dent*. 2013; 14(3):175-80
 36. Quinzi V, Marchetti E, Guerriero L, Bosco F, Marzo G, Mummolo S. Dentoskeletal Class II Malocclusion: Maxillary Molar Distalization with No-Compliance Fixed Orthodontic Equipment. *Dent J (Basel)*. 2020; 8(1):E26.
 37. Quinzi V, Rossi O, Paglia L, Marzo G, Caprioglio A. Paediatric Orthodontics Part 2: Periodontal effects of maxillary expansion. *Eur J Paediatr Dent* 2019; 20(2):164-6.
 38. D'Apuzzo F, Grassia V, Quinzi V, Vitale M, Marzo G, Perillo L. Paediatric Orthodontics. Part 4: SEC III protocol in Class III malocclusion. *Eur J Paediatr Dent* 2019; 20(4):330-4.
 39. Piancino MG, Di Benedetto L, Matakacena G, Deregibus A, Marzo G, Quinzi V. Paediatric Orthodontics Part 3: Masticatory function during development. *Eur J Paediatr Dent*. 2019; 20(3):247-9.
 40. Mummolo S, Nota A, Marchetti E, Padricelli G, Marzo G. The 3D Tele Motion Tracking for the Orthodontic Facial Analysis. *Biomed Res Int* 2016; 2016:4932136.
 41. Quinzi V, Ronsivalle V, Campanella V, Mancini L, Torrisi S, Lo Giudice A. New Technologies in Orthodontics: A Digital Workflow to Enhance Treatment Plan and Photobiomodulation to Expedite Clinical Outcomes. *Appl Sci* 2020; 10,1495.
 42. Campanella V, Gianni L, Libonati A, Gallusi G. Shaping Ability of Reciproc R25 File and Mtwo System Used in Continuous and Reciprocating Motion. *J Contemporary Dent Practice* 2020; 21(2):171-7.
 43. Libonati A, Di Taranto V, Gallusi G, Montemurro E, Campanella V. CAD/CAM customized glass fiber post and core with digital intraoral impression: a case report. *Clin Cosmet Investig Dent* 2020; 12:17-24.
 44. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. *Indian J Dent Res* 2010; 21:341-8.
 45. Lauritano D, Lucchese A, Di Stasio D, et al. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and

- Gingival Fibroblasts. *Int J Mol Sci.* 2019; 20(8).
46. Lauritano D, Lucchese A, Gabrione F, et al. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. *Int. J. Environ. Res. Public Health* 2019; 16,210.
 47. Lauritano D, Oberti L, Gabrione F, et al. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. *Oral Oncol.* 2019; 97:7-17.
 48. Lauritano D, Moreo G, Carinci F, et al. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. *Int J Environ Res Public Health.* 2019; 16(19).
 49. Carinci F, Lauritano D, Bignozzi CA, et al. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. *Int J Mol Sci.* 2019; 20(16).E3897.
 50. Andreasi Bassi M, Bedini R, Pecci R, Ioppolo P, Lauritano D, Carinci F. Mechanical Properties of Abutments: Resin-Bonded Glass Fiber-Reinforced Versus Titanium. *International Journal of Prosthodontics* 2016; 29(1):77-9.
 51. Mummolo S, Severino M, Campanella V, Barlatta A, Quinzi V, Marchetti E. Periodontal disease in subjects suffering from coronary heart disease *Journal of Biological Regulators and Homeostatic Agents*, 2019; 33(3),73-82
 52. Quinzi V, Scibetta ET, Marchetti E, et al. Analyze my face *Journal of Biological Regulators and Homeostatic Agents*, 2018; 32(2)149-58.
 53. Quinzi V, Ferro R, Rizzo FA. The Two by Four appliance: A nationwide cross-sectional survey *European Journal of Paediatric Dentistry*, 2018; 19(2),145-50.
 54. Giuca MR, Lardani L, Pasini M, Beretta M, Gallusi G, Campanella V. State of the art on MIH. Part 1 Definition and aepidemiology. *Eur J Paediatr Dent.* 2020; 21(1):80-2.
 55. Peroni D, Pasini M, Iurato C, Cappelli S, Giuca G, Giuca MR. Allergic manifestations to local anaesthetic agents for dental anaesthesia in children: a review and proposal of a new algorithm. *Eur J Paediatr Dent.* 2019; 20(1):48-52.
 56. Giuca MR, Cappè M, Carli E, Lardani L, Pasini M. Investigation of Clinical Characteristics and Etiological Factors in Children with Molar Incisor Hypomineralization. *Int J Dent.* 2018; 2018:7584736.
 57. Tripodi D, Martinelli D, Pasini M, Giuca MR, D'Ercole S. Black Stains: a microbiological analysis and a view on familiarity and susceptibility to tooth decay of patients in childhood. *Eur J Paediatr Dent.* 2016; 17(4):261-6.
 58. Mummolo S, Sapio S, Falco A, Vittorini OL, Quinzi V. Management of pedodontic patients in moderate sedation in clinical dentistry: evaluation of behaviour before and after treatment. *J Biol Regul Homeost Agents.* 2020; 34(1 Suppl. 1):55-62.
 59. Campanella V, Gallusi G, Nardi R, et al. Dentinal substrate variability and bonding effectiveness: SEM investigation. *J Biol Regul Homeost Agents* 2020; 34(1 Suppl 1):49-54.
 60. Campanella V, Gallusi G, Di Taranto V, et al. Effect of pressure and light curing of composite micro hardness. *J Biol Regul Homeost Agents* 2020; 34(1 Suppl 1):39-47.
 61. Di Murro B, Gallusi G, Nardi R, Libonati A, Angotti V, Campanella V. The relationship of tooth shade and skin tone and its influence on the smile attractiveness. *J Esthet Restor Dent* 2020; 32(1):57-63.
 62. Arcuri L, Lorenzi C, Bianchi N, Marchetti E, Barlattani A. Fit evaluation of cad/cam fabricated all-ceramic restorations based on direct and indirect digitalization in vivo: a systematic review. *J Biol Regul Homeost Agents* 2019; 33(3 Suppl 1):103-11.
 63. Campanella V, Gianni L, Libonati A, Gallusi G. Shaping Ability of Reciproc R25 File and Mtwo System Used in Continuous and Reciprocating Motion. *J Contemporary Dent Practice* 2020; 21(2):171-7.
 64. Giuca MR, Pasini M, Tecco S, Marchetti E, Giannotti L, Marzo G. Skeletal maturation in obese patients. *Am J Orthod Dentofacial Orthop* 2012 Dec;142(6):774-9.
 65. Mummolo S, Tieri M, Tecco S, Mattei A, Albani F, Giuca MR, Marzo G. Clinical evaluation of salivary indices and levels of *Streptococcus mutans* and *Lactobacillus* in patients treated with Occlus-o-Guide. *Eur J Paediatr Dent* 2014 Dec;15(4):367-70.
 66. Ballesio I, Angotti V, Gallusi G, Libonati A, Tecco S, Marzo G, Campanella V. Durability of adhesion

- between an adhesive and post-space dentin: Push-out evaluation at one and six months. *Int J Adhes Adhes* 2012; 38: 75-78.
67. Ferro R, Besostri A, Olivieri A, Quinzi V, Scibetta D. Prevalence of cross-bite in a sample of Italian preschoolers. *Eur J Paediatr Dent*. 2016; 17(4):307-309.
68. Campanella V, Libonati A, Nardi R, Angotti V, Gallusi G, Montemurro E, D'Amario M, Marzo G. Single tooth anesthesia versus conventional anesthesia: a cross-over study. *Clin Oral Investig*. 2018 Dec;22(9):3205-3213.
69. Daniele V, Macera L, Taglieri G, Di Giambattista A, Spagnoli G, Massaria A, Messori M, Quagliarini E, Chiappini G, Campanella V, Mummolo S, Marchetti E, Marzo G, Quinzi V. Thermoplastic Disks Used for Commercial Orthodontic Aligners: Complete Physicochemical and Mechanical Characterization. *Materials (Basel)* 2020;13(10):E2386.
70. Mummolo S, Nota A, Albani F, Marchetti E, Gatto R, Marzo G, Quinzi V, Tecco S. Salivary levels of *Streptococcus mutans* and *Lactobacilli* and other salivary indices in patients wearing clear aligners versus fixed orthodontic appliances: An observational study. *PLoS One* 2020;15(4):e0228798.