International Journal of Dentistry and Oral Health

Volume 8 Issue 5,
July 2022

Citation
Liudmila Korkina et al. (2022). Post-COVID Periodontitis: Clinical Efficacy of Fermented Carica Papaya L. and Possible Mechanisms
Int J Dent & Ora Hea. 8:5, 43-61

ISSN 2471-657X

Published by
Biocore Group |
www.biocoreopen.org/ijdoh/archive.php

Research Article

Post-COVID Periodontitis: Clinical Efficacy of Fermented *Carica Papaya L.* and Possible Mechanisms

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Article History: **Received:** July 01, 2022;
Accepted: July 08, 2022;
Published: July 18, 2022.

Abstract

Periodontitis is a risk factor for infection by COVID-SARS-2 virus, increased morbidity of COVID-19 disease, and aggravation of post-COVID syndrome. The incidence and severity of periodontitis after the infection are greatly increased. There is a need for affordable and clinically efficient non-surgical means to treat COVID-associated periodontitis. Fermented papaya gel (FPG) with antibacterial, immunomodulating, and redox balancing properties was clinically efficient towards post-COVID syndrome. An open randomised case control clinical trial on the efficacy of FPG applications into gingival pockets of patients with periodontitis diagnosed after COVID was performed. The study population consisted of patients recovered from severe/moderate COVID-19 disease 3-6 months ago. The patients with mild periodontitis (n = 92) were recruited. Dynamics of clinical symptoms was assessed by dental indexes CPITN and PMA at the entrance, on the 14th, 30th, and 60th days after standard therapy (control groups) or its combination with 7g FPG a day/14 days (experimental group). The control groups were formed of post-COVID and NO COVID periodontitis patients. Gingival crevicular fluid (GCF) was analysed to quantify periodontitis pathogens, NO2/NO3, antioxidant capacity, inflammatory cytokines, and MMP-8. At the entrance, 4 out of 6 periodontitis pathogens were present in larger quantities in both post-COVID groups versus NO COVID group. The standard therapy alone was more efficient in the NO COVID group. Addition of FPG to standard protocol resulted in more evident clinical, microbiological, immunological, and biochemical improvements as compare to the control post-COVID patients. COVID-associated periodontitis showed an alteration of periodontitis-related microbiota. Standard approach is less effective for post-COVID versus NO COVID-19 periodontitis. FPG addition led to increased clinical efficacy due to restoration of microbiota pattern and balance between pro-/anti-inflammatory cytokines, normalisation of redox balance, and MMP-8.

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research,authorship, and/or publication of this article.

Introduction:

The COVID-19 pandemic has affected millions of people around the world and is considered as a major health threat in many countries. This infectious disease caused by severe acute respiratory syndrome coronavirus (SARS-2) affects not only the lungs, but many other vital organs as well, resulting in a high-risk of severe morbidity and mortality [1,2]. COVID-19 has been associated with periodontitis [3-5], which is regarded, mainly, in hypothetic with a question mark in the title publication though, as one of the most important and sometimes even leading risk factors for this viral infection [6-9]. At present, although several effective vaccines had been developed and applied for public vaccination that have reduced the spread and severity of the disease, the problem of long "tail" of post-COVID complications negatively affecting quality of life persists [10]. Periodontal health and oral health in general have been in focus and reviewed in a number of publications as related to serious health problems in the post-COVID period [11-15]. COVID-19 disease involves the lungs and other organs primarily through cytokine storm and oxidative stress, which have been implicated in many other inflammatory disorders, including periodontal diseases [2,15,16]. The post-COVID syndrome consists of a variety of symptoms expressed beyond the acute phase of COVID-19 disease and its duration could extend as far as 200 days after the COVID-19 disease was diagnosed [17]. According to leading views, practically all adverse effects of COVID-19 disease and post-COVID syndrome, including periodontitis, are secondary to pre-exiting immune distress before the viral infection and/or aggravated in the course of the disease [18], to hyper-inflammation due to overload of pro-inflammatory cytokines (cytokine storm) [2,7] and hyper-activation of neutrophils [19], cell death by the neutrophil extracellular trapping (NETosis) [20,21], and aggressive oxidative stress [16]. Of great importance, major risk factors for COVID-19 disease and periodontitis, such as diabetes, obesity, cardio-vascular pathologies, etc., are overlapping [22-24].

Periodontitis is a chronic inflammatory infectious pathology caused by dental plaque bacteria. The infection-induced inflammatory process leads to progressive destruction of the tissues supporting the teeth, such as the gum, periodontal ligament, cementum, and alveolar bone. Periodontitis is currently regarded as a dysbiotic inflammatory disease with a negative impact on both oral and extra-oral sites [25,26]. High throughput methods, such as proteomics and genomics, facilitated discriminative and quantitative analyses of oral microbiota associated with periodontitis. The chronic inflammation of periodontal tissues is induced by poly-microbial "complexes"of Gram-negative, mainly anaerobic micro organisms [27-29], i.e., associations forming biofilms in gingival pockets and supra-gingival plaque [30]. In such biofilms, microbes are more pathogenic and less sensitive to traditional antibiotic therapies [31] owing to their innate or/and acquired capacity of adaptation to both anti-microbial drugs and host [32-35]. A few of the numerous microbes residing in the oral cavity, mainly anaerobic Gram-negative and Gram-positive bacteria, may cause periodontal pathology. For example, a group of periodontal pathogens of high risk consists of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola,* while pathogens of moderate risk in concentrations higher than the threshold are *Porphyromonas endodontalis, Fusobacterium nucleata, and Prevotella intermedia* [36-38].

Fermented Papaya *(Carica papaya L.)* preparations have been used in the folk medicine of South East Asia for hundreds of years. They are considered in the Asian pharmacopoeias as treatment for sore throats and wound healing, anti-malarial and anti-bacterial remedies. Numerous phytochemical, pharmacological, and clinical discoveries of the mechanisms underlying their health properties have become a basis for their popularity in Western medicine. Now fermented papaya products in a form of food supplements, actives for oral care products, and cosmetics are commercialised around the world. Fermented papaya possesses anti-inflammatory, antioxidant, and immune-modulatory properties [39-42] and has been shown to have anti-diabetic effects in the animal and clinical studies [43,44]. Recently, our group has published data on clinical efficacy of fermented papaya and noni towards post-COVID syndrome, first of all, against long lasting impairment of cardio-vascular and respiratory systems as well as altered immune system functions and redox imbalance [19]. Anti-microbial action, anti-gingivitis, and anti-periodontitis effects of fermented papaya has been shown in the clinical trial of oral administration of fermented papaya gel [42]. The intracellular bacterial killing, initially compromised in periodontitis glanulocytes and macrophages, was augmented due to unusual "antibiotic-like" mechanism, when suppression of bacterial catalase, an inducible enzyme protecting bacteria from the oxidative burst of the host phagocytes, occurred [41]. Another mechanism of anti-bacterial effects of fermented papaya has been attributed to the induction of NADPH-oxidase, a key "oxidative burst" enzyme fighting bacterial infections [45]. The pronounced redox balancing properties and the stimulation of phagocytosis by fermented papaya have been demonstrated as well [40,46,47].

Keeping in mind that causative reasons for post-COVID complications, such as a greatly altered immune response shifting from over-reaction (cytokine storm, increased immunoglobulins, and acute generalised inflammation) to immune suppression (lymphopenia, secondary bacterial/viral infections, and chronic inflammation) and peculiar redox changes could be balanced by the fermented papaya at affordable costs and absolute safety (see references above).

In the present study, an open randomised case control clinical trial on the clinical efficacy of topical application of fermented papaya gel (FPG) to gingival pockets of patients with mild periodontitis

induced/followed by previous severe COVID-19 disease was carried out. We also tried to evaluate possible mechanisms underlying this efficacy by assessing quantitative pattern of periodontitis pathogens, local balance of cytokines related to chronic inflammation, content of matrix metalloprotease 8 (MMP-8), a key enzyme related to the periodontal tissue loss, and redox status in gingival crevicular fluid (GCF).

Keywords :

clinical trial; COVID-19 disease; fermented papaya; gingival crevicular fluid; inflammatory cytokines; MMP-8; periodontal pathogens; periodontitis; post-COVID; redox balance

Materials and Methods

Product for Examination

Standardised fermented papaya gel (FPG, manufacturer - Carica Ltd., Manila, The Philippines) is a food grade product commercialised as a food supplement. FPG is produced by a long-term (more than 6 months) fermentation of wild non-cultivated species of mountain papaya fruit parts (skin and seeds) driven mainly by *Lactobaccilus casei* and Koji yeasts. After the fermentation was completed, the final gel was filtered and sterilised by autoclave. Seven grams of FPG were packed into sterile plastic syringes with an application nozzle. The FPG has been widely studied for its health effects, such as antioxidant, anti-inflammatory, metal chelating, anti-diabetic, anti-bacterial, and anti-post-COVID syndrome [42,43,45,46].

Patients and Study Design

Totally, 198 subjects who experienced COVID-19 infection/disease 3-6 months ago were questioned on the state of their oral health during the disease and in the post-COVID period using specially developed Questionnaire at Dentistry and Maxillofacial Surgery Department of the Kabardino-Balkar Berbekov's State Medical University (Nal'chik, Russia).

Those who were diagnosed with a mild form of periodontitis and corresponded to Inclusion Criteria, were asked to participate in the clinical study on the efficacy of FPG. The study enrolled a group of 92 patients of both sexes (age range 35–55 years). The recruited patients suffered severe or moderate COVID-19 disease 3-6 months before the recruitment. All patients were informed about the goals and possible side effects of treatment in accord with the study protocol, which was scrutinised and approved by the local Ethical Committee (Protocol MD-005-2021). The patients were randomly assigned to experimental or control groups. All subjects consented to personal and anamnestic data collection and biological material sampling.

Inclusion Criteria

Patients, who were infected by SARS-2-COVID virus 3-6 months prior to entering the trial; both sexes; 30-65 years; mild periodontitis.

Exclusion Criteria

Out of the age range of 30-65 years; moderate and severe periodontitis; post-COVID period out of the range of 3-6 months; periodontitis prior to SARS-2-COVID infection; hepatitis B, C, D, F, and G; HIV infection; allergy in acute phase; acute infectious diseases; chronic diseases in acute phase.

Healthy donors matched by sex and age (n = 25, age range 33-57 years) were recruited from the Medical Department staff and trainees, who donated gingival crevicular fluid (GCF). The normal ranges of different markers in GCF derived from the measurements performed on this biological material.

No patients or controls entering the study had taken any drugs or nutraceutical supplements known to interfere with the bacterial insemination, redox status, or inflammation for at least six weeks. No alcohol- or drug-abusers were present in any of the recruited patients. All the patients were treated by traditional hygienic and therapeutic protocols, if needed. Traditional treatment protocols included education to oral hygiene, plaque removal, teeth enamel polishing, and elimination of tartar, if needed. In addition, local instillations of 0.06% chlorohexidine gluconate into periodontal pockets were regularly performed as antimicrobial and anti-inflammatory procedures.

The patients of the post-COVID experimental group (n = 24, age range = 35-60 years) additionally received intra-gingival pocket applications (7g of fermented papaya gel (FPG) per patient per day) for 14 consecutive days. The FPG was kept inside the pocket for 15 min and then washed out by physiological solution.

The patients of the post-COVID control group (n = 18, age range = 36-59 years) received standard therapy described above.

The patients of NO COVID control group (n = 25, age range = 36-59 years) received standard therapy.

All recruited periodontitis patients were visited 4 times for clinical assessment and biological material collection. The clinical trial features are collected in Table 1.

Table 1. Demographic distribution, clinical assessment, laboratory tests, and treatment of patients with mild form of periodontitis in the experimental and control groups.

Group	Diagnosis	Number Number	Age, years	Treatment	Clinical assessment	Laboratory tests	Visits for clinical assessment and biological material collection
Post-COVID, periodontitis fermented papaya (FPG)	Mild periodontitis, COVID-19, 3-6 months ago	24	35-60	Fermented papaya gel (FPG) application daily for 14 days + standard	Dental indexes CPITN, PMA +Questionnaire	Cytokines (IL-1β, IL-6, IL-17A, IL-10), NO2/NO3, MMP-8, AOA in crevicular fluid, RT-PCR for periodontal pathogens	**Visit 1 -** before **Visit 2 -** 14 days after the trial beginning **Visit 3 -** 30 days after the trial cessation **Visit 4 -** 60 days after the trial cessation
Post-COVID, periodontitis	mild periodontitis, COVID-19, 3-6 months ago	18	36-59	standard	Dental indexes CPITN, PMA +Questionnaire	Cytokines (IL-1β, IL-6, IL-17A, IL-10), NO2/NO3, MMP-8, AOA in crevicular fluid, RT-PCR for periodontal pathogens	**Visit 1 -** before **Visit 2 -** 14 days after the trial beginning **Visit 3 -** 30 days after the trial cessation **Visit 4 -** 60 days after the trial cessation
Periodontitis control	mild periodontitis, NO COVID	25	36-59	standard	Dental indexes CPITN, PMA +Questionnaire	Cytokines (IL-1β, IL-6, IL-17A, IL-10), NO2/NO3, MMP-8, AOA in crevicular fluid, RT-PCR for periodontal pathogens	**Visit 1 -** before **Visit 2 -** 14 days after the trial beginning **Visit 3 -** 30 days after the trial cessation **Visit 4 -** 60 days after the trial cessation
Healthy controls	NO periodontitis NO COVID	25	33-57	–	Dental indexes CPITN, PMA	cytokines (IL-1β, IL-6, IL-17A, IL-10), NO2/NO3, MMP-8, AOA in crevicular fluid,	

Clinical Assessment

A Questionnaire on the oral health status of post-COVID patients (n = 198) was composed and the patients were asked to answer the questions.

Clinical efficacy of FPG was assessed by objective clinical indices of mild periodontitis [42,48,49]. These indices included the gingival and plaque indexes, for example, the state of gingival inflammation was evaluated by Parma's papillae-gum margin-alveolar (PMA) index and the International CPITN test. All the indexes were determined 4 times at days 0, 14, 30, and 60 of the clinical study.

Biological Material Collection and Processing

The gingival crevicular fluid (GCF) was collected after 12 hours of complete fasting and limitation/abstinence from water consumption. The oral site of biological material collection was determined by a dentist. In the case of gum inflammation, the GCF was collected from the inflamed points. At least two samples from different points were taken from a patient. In brief, a sterile Watmann's paper pin of 3mm width was carefully introduced into the tooth pocket or into the gingival sulcus and kept in place for 2 min. The filter paper pin was then transferred either into vials containing 2 mL phosphate buffer solution for

biochemical tests or into DNA test tubes for RT-PCR analyses for differential microbial counts. Plaque was collected from six teeth by a sterile dental probe and mixed with GCF of the same patient to be further examined by RT-PCR. Vials and DNA test tubes were immediately placed into a thermo container and analysed within 4 hours.

Reagents and Assay Kits

The majority of chemical reagents and solvents, H2O2 standard, and mediums for human and bacterial cell cultivation were purchased from Sigma Chemical Co. (St. Louis, MO, USA); kits for enzyme activities and nitrite/nitrate assays were from Cayman Chem. Co. (Ann Arbor, MI, USA); monoclonal antibodies for enzyme-linked immunosorbent assay (ELISA) interleukin and total human MMP-8 kits were from R&D Systems (Minneapolis, MN, USA); kits for protein determination were from Bio-Rad Laboratories (Bio-Rad Inc., Hercules, CA, USA).

Differential Bacterial Concentrations in GCF and Plaque Determined by a Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qrPCR) Method

DNA was isolated from samples of GCF and plaque and kept on ice for no more than 12 h. DNA was amplified with iQTM Supermix using the MiniOpticon Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). All real-time assays were carried out under the following conditions: 35 cycles of denaturation at 95 °C for 15 s; annealing and extension at 60 °C for 60 s. Melt curve analysis was performed to confirm the specificity of the amplified products. All samples were run in triplicate, and relative expression was determined by normalising samples to housekeeping genes. The primers corresponding to six periodontal pathogens of high and medium risk as well as periodontitis-associated bacteria [50] were used *(Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Tanerella forsythia, Prevotella intermedia, and Fusobacterium nucleatum).* The results were expressed as a sum of lg (∑lg) of bacterial number in the mixture of GCF and plaque sample of 10 randomly selected patients of each group. These calculations were done for each pathogen.

Reduction-Oxidation (Redox) Assays

The GCF levels of nitrites/nitrates ($NO2^-$ /$NO3^-$, expressed as μmoles/L or μM) were measured spectrophotometrically by Griess reagent Kit, following the manufacturer's instructions. The total antioxidant activity (total AOA) of GCF was measured by the method described elsewhere. In brief, 100 μL of egg yolk was mixed with 10 μL GCF, collected from gingival sulcus or a periodontal pocket. Then, 100 μL of FeSO4 was added and the volume was adjusted to 1 mL by a physiological solution. The mixture was incubated at room temperature for 30 min, and 0.5 mL of 20% trichloroacetic acid plus 0.1 mL of 0.01 M butyl hydroxy toluol (ionol) in ethyl alcohol were added. The tubes were centrifuged at 1500× g for 10 min and supernatant was collected. The mixture of 0.7 mL of supernatant and 0.6 mL of 0.5% thiobarbituric acid (TBA) was heated at 100 °C for 30 min, cooled down, and an absorbance at a wave length of 532 nm was determined. Antioxidant activity was expressed in % of the control samples without biological material.

Cytokine and matrix metalloproteinase 8 (MMP-8) Assays

The GCF levels of pro-inflammatory interleukins 1beta (IL-1ß), IL-6, IL-17A, and anti-inflammatory interleukin 10 (IL-10) were measured by enzyme-linked immunosorbent assay (ELISA) purchased from R&D Systems (Minneapolis, MN, USA), following the manufacturer's instructions. Cytokine concentrations were expressed in pg/mL of GCF, and each protein factor was quantified in the linear range of its calibration curve. The total MMP-8 protein content in GCF was determined by a sandwich ELISA assay (R&D Systems) following the manufacturer's instructions.

Statistical Analysis

All biochemical measurements were done in triplicate, and data were statistically evaluated. Statistical analysis of clinical and laboratory data was performed using the STATISTICA 6.0 program (StatSoft Inc., Tulsa, OK, USA). Reported data were treated as continuous. Normality of data was checked using the Shapiro–Wilk test. Since the distribution of the data in the groups was significantly different from normal, non-parametric statistics was used. Values were presented as mean ± standard error of the mean of triplicate analyses. The Mann–Whitney U-test for independent samples was employed for comparison between placebo and experimental groups. Significance was assumed at a p-value of <0.05.

Results

Oral health of patients during COVID and post-COVID period

During visits to dentistry unit, 198 post-COVID patients of both sexes (age range 35-60 years) were questioned on their oral problems during and after COVID disease (Table 2). The Questionnaire contained several most common complaints connected to COVID described in the literature [12,13]. The

answers were collected for patients survived severe form of COVID disease (n = 25), moderate (n = 60), and mild, mainly, asymptomatic form (n = 113). As expected, people after mild asymptomatic COVID have less frequent complaints to gum bleeding, gum pain at chewing, and halitosis during COVID and in the post-COVID than people during/after severe or moderate form of the disease. The duration of the oral problems was shorter than 2 weeks for the great majority of these patients and at the moment of questioning (3-6 months post-COVID) none of them experienced oral health problems. On contrast, about 60% of responders from severe and moderate post-COVID groups complained gum bleeding started during COVID disease, which was aggravated post-COVID in 40% of them. The frequency of gum pain ranged from 60% to 70% for both groups. Complaints to halitosis during COVID expressed 75% of post moderate COVID versus 44% of post severe COVID subjects. In the post-COVID period, this problem persisted in 64% of post severe COVID and in 49% of post moderate COVID patients. Majority of patients of the former group (68%) kept complaining for more than 3 months after COVID disease while the latter group patients mainly stopped complaining after 2-4 weeks.

Table 2. Complaints of post-COVID patients on oral health problems.

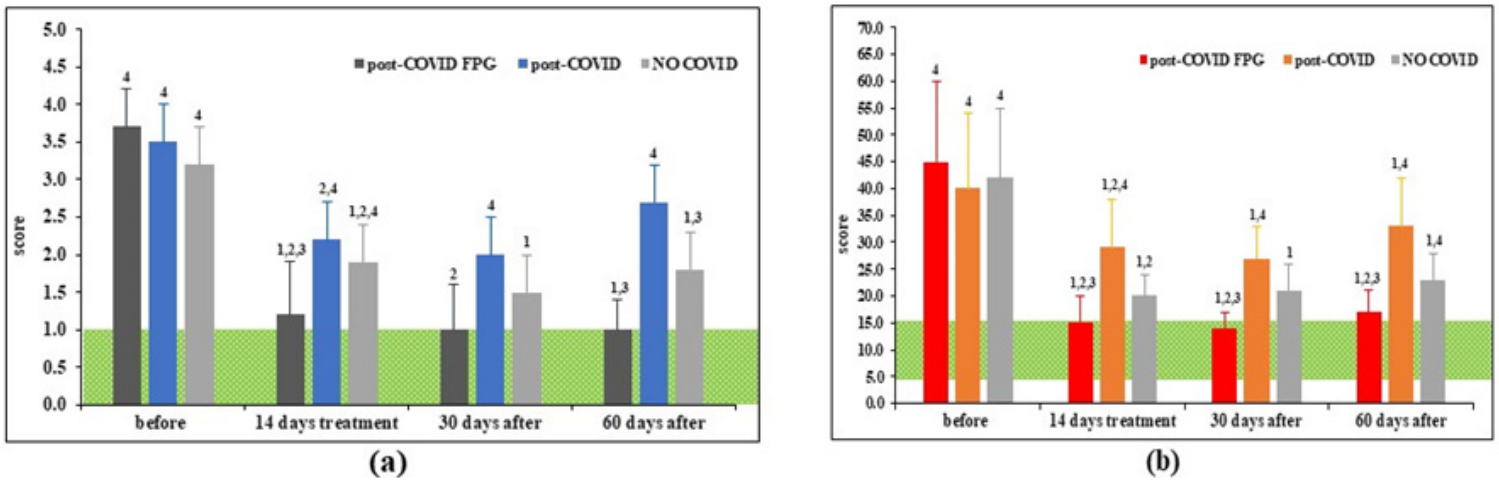
	Groups (n = 198; age range - 35-60 years; F/M = 101/96), Answers		
Question	***Group 1, post-severe COVID (n = 25) Answers, number (%)***	***Group 2, post-moderate COVID (n = 60) Answers, number (%)***	***Group 3, post-mild/ asymptomatic COVID (n=113) Answers, number (%)***
Gum bleeding during COVID disease	YES - 15 (60%) NO - 10 (40%)	YES - 35 (58,3%) NO - 25 (41,7%)	YES - 12 (10,6%) NO - 101 (89,4%)
Gum bleeding post-COVID	Aggravated - 10 (40%) diminished - 10 (40%) no change - 5 (20%)	Aggravated - 25 (41,7%) diminished - 10 (16,6%) no change - 25 (41,6%)	Aggravated - 0 diminished - 12 (10,6%) no change - 101 (89,4%)
Gum pain during chewing	YES - 15 (60%) NO - 10 (40%)	YES - 35 (58,3%) NO - 25 (41,7%)	YES - 12 (10,6%) NO - 101 (89,4%)
Itching, burning, pain post-COVID	YES - 18 (72%) NO - 7 (28%)	YES - 35 (58,3%) NO - 25 (41,7%)	YES - 12 (10,6%) NO - 101 (89,4%)
Halitosis during COVID disease	YES - 11 (44%) NO - 14 (56%)	YES - 45 (75%) NO - 15 (25%)	YES - 32 (28,3%) NO - 81 (71,7%)
Halitosis in post-COVID	YES - 16 (64%) NO - 9 (26%)	YES - 29 (49,3%) NO - 31 (51,7%)	YES - 5 (4,4%) NO - 107 (95,6%)
How long were pain, bleeding, and halitosis after COVID disease	> 3 months - 17 (68%) 2 weeks - 4 (16%) 1 month - 1 (4%) 2-3 months - 3 (12%) no complaints - 0	> 3 months - 5 (8,3%) 2 weeks - 15 (25%) 1 month - 20 (33,4%) 2-3 months - 5 (8,3%) no complaints - 15 (25%)	> 3 months - 2 (1,7%) 2 weeks - 10 (8,8%) 1 month - 10(8,8%) 2-3 months - 0 no complaints - 91 (80,5%)

Clinical assessment of periodontitis in post-COVID and NO-COVID Patients

Visual observation by dentists, macro photos, and dental indexes served as a clinical confirmation of mild periodontitis diagnosis. Upon diagnosis, all periodontitis patients received standard therapeutic protocols for 2 weeks. For 24 post-COVID patients diagnosed with periodontitis, in addition to standard therapy, the intra gum pocket administration of 7g FPG for 14 consecutive days was recommended. The changes in dental indexes scores during the therapy and 30 and 60 days after it are shown in **Figure 1.** Both indexes, CPITN and PMA were highly increased over the normal ranges before therapy but there was no statistically significant difference between the control and experimental groups. Immediately after the

therapy completion (the 14th day), indexes in the experimental group were normalised and remained at the upper level of normality for 60 days after therapy cessation. As for the 1st control group (post-COVID, mild periodontitis, standard treatment), both indexes diminished versus background values but remained much greater than normal values for the whole period of observation. Periodontitis NO-COVID patients (the 2nd control group) responded to standard therapy in a more satisfactory way than the post-COVID controls but still were out of the normality range for 60 days of observation.

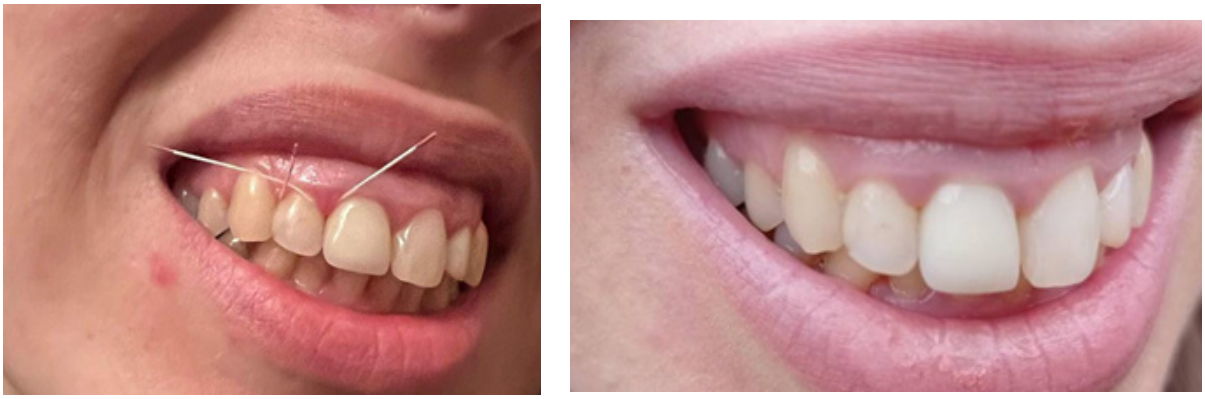
Figure 1. Dynamics of dental indexes in periodontitis patients: effects of fermented papaya gel



(FPG).

(a) index CPITN, score, (b) index PMA, score. 1 - p <0,05 versus before the trial; 2 –p <0,05 versus previous visit; 3 –p <0,05 versus periodontitis NO COVID; 4 - p <0,05 versus healthy donors.

Photo 1: illustrates effects of therapy: standard protocol + FPG for 14 days.



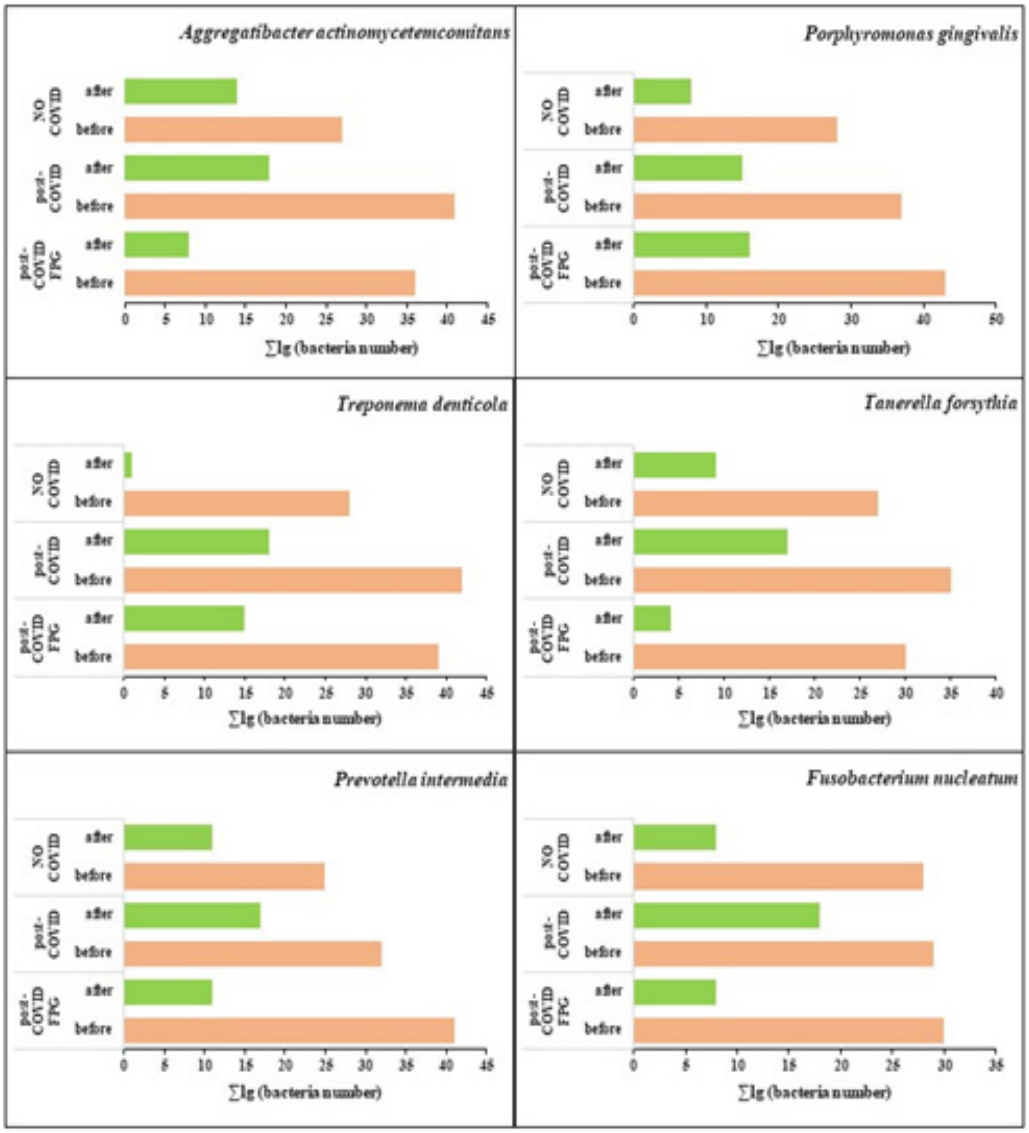
(a) (b)

Photo 1: Post-COVID periodontitis patient before (a) and after (b) the treatment by standard therapy plus fermented papaya gel administration (intra gum pocket, 7g, 14 days).

Periodontitis Pathogen load in the mixture of gingival crevicular fluid (GCF) and plaque of post-COVID and NO-COVID patients with Periodontitis

Quantitive determination of periodontitis pathogens (**Figure 2**) in the mixture of GCT and plaque by RT-PCR showed that high and medium risk bacteria *(Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, and Prevotella intermedia)* load was initially significantly heavier for 2 post-COVID groups than for NO-COVID group while the background load with *Tanerella forsythia* and *Fusobacterium nucleatum* did not differ for all groups studied. Both therapies resulted in statistically significant unload of all periodontal pathogens studied (P<0.05 for the control post-COVID group and P<0.01 for the experimental post-COVID and NO COVID groups)

Figure 2: Differential bacterial load in the mixture of plaque and gingival crevicular fluid (n = 10 in each group) before and after the trial



X axis: ∑lg of bacterial number was calculated for 10 patients/samples. Y axis: post-COVID FPG corresponds to post-COVID group before (orange colour) and after (green colour) a 14-day-long standard therapy + FPG applications; post-COVID group before (orange colour) and after (green colour) a 14-day-long standard therapy; NO COVID group before (orange colour) and after (green colour) a 14-day-long standard therapy.

When the efficacy of anti-bacterial therapies (standard protocol and standard protocol+FPG) was calculated in the percentage to initial bacterial load for a single patient (Table 3), the anti-bacterial action of standard therapy towards all but *A. actinomycetemcomitans* periodontal pathogens was always higher in the NO COVID than in the post-COVID group, while standard+FPG therapy was always more effective as compare to standard therapy alone in the post-COVID groups. Experimental therapy versus NO COVID standard therapy was more effective against *A. actinomycetemcomitans, T. forsythia, and P. intermedia,* equally effective against *F. nucleatum*, and less effective against *P. gingivalis and T. denticola.*

Table 3: Efficacy of anti-bacterial therapy (% of initial load) on periodontal pathogens in post-COVID groups and NO COVID group of patients with mild periodontitis.

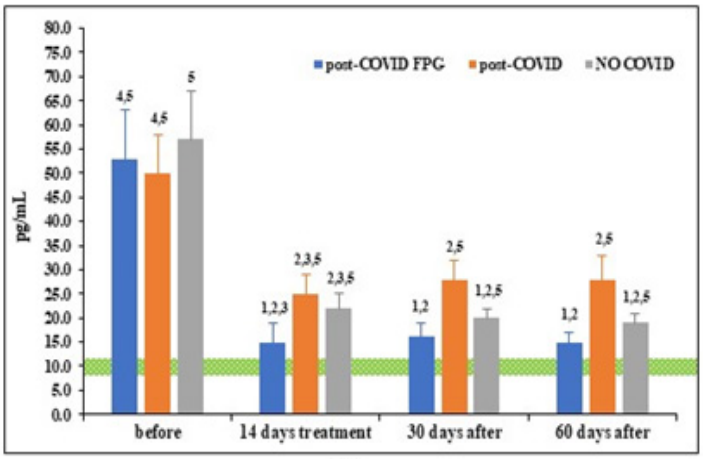
Periodontitis pathogen	Groups (n =10) and treatment					
	post-COVID, standard + FPG treatment, bacteria number (average lg/person)		post-COVID, standard treatment, bacteria number (average lg/person)		NO COVID, standard treatment, bacteria number (average lg/person)	
	Before	After (% of initial load)	Before	After (% of initial load)	Before	After (% of initial load)
Aggregatibacter actinomycetemcomitans	3,6	0,8 (25%)	4,1	1,8 (44%)	2,7	1,4 (52%)
Porphyromonas gingivalis	4,3	1,6 (37%)	3,7	1,5 (40%)	2,8	0,8 (28%)
Treponema denticola	3,9	1,5 (38%)	4,2	1,8 (43%)	2,8	0,1 (3%)
Tanerella forsythia	3,0	0,4 (13%)	3,5	1,7 (48%)	2,7	0,9 (33%)
Prevotella intermedia	4,1	1,1 (27%)	3,2	1,7 (53%)	2,5	1,1 (44%)
Fusobacterium nucleatum	3,0	0,8 (27%)	2,9	1,8 (62%)	2,8	0,8 (28%)

Pro-inflammatory and anti-inflammatory cytokines in the gingival crevicular fluid (GCF) of post-COVID patients and NO-COVID patients with periodontitis

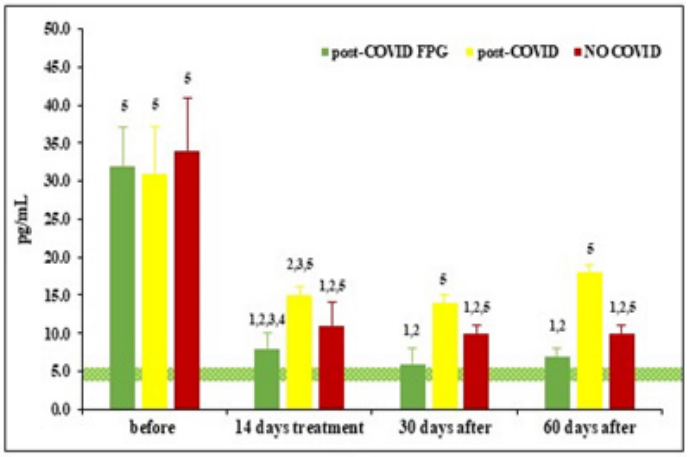
There was an evident and expected imbalance of pro- and anti-inflammatory cytokines characteristic for both COVID-19 disease and periodontitis (Figure 3). At the entrance to the trial, levels of pro-inflammatory cytokines IL-1ß (Figure 3a), IL-6 (Figure 3b), and IL-17A (Figure 3c) were highly increased over the normality range while anti-inflammatory IL-10 content was much lower than the normal values (Figure 3d). Of note, the initial degree of over expression and suppression of all inflammatory cytokines studied did not statistically differ for the three groups.

Both therapeutic protocols applied (standard and standard+FPG for 14 days) resulted in amelioration of the initial alterations from the normality. However, standard therapy alone in the control post-COVID group was less efficient as compare to the control NO-COVID group. The difference was statistically significant (Figures 3a-3d). FPG added to standard treatment practically normalised levels of pro-inflammatory IL-1ß, IL-6, and IL-17A proteins. This normalising effect persisted for at least 60 days after the therapy was terminated in accord with the clinical trial protocol (Figures 3a-3c). There was a clear-cut statistical difference between the effects of standard protocol+FPG and standard protocol alone. Regarding anti-inflammatory IL-10, FPG was again much more efficient against standard therapy in the post-COVID groups and slightly more efficient

Figure 3: Effects of fermented papaya gel (FPG) intra-gum pocket administration (7g, 14 days) on the content (pg/mL) of inflammation-related cytokines in the GCF.



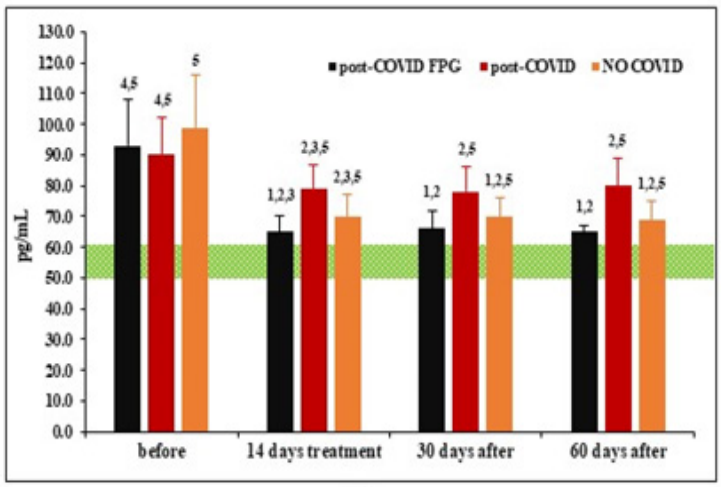
(a)



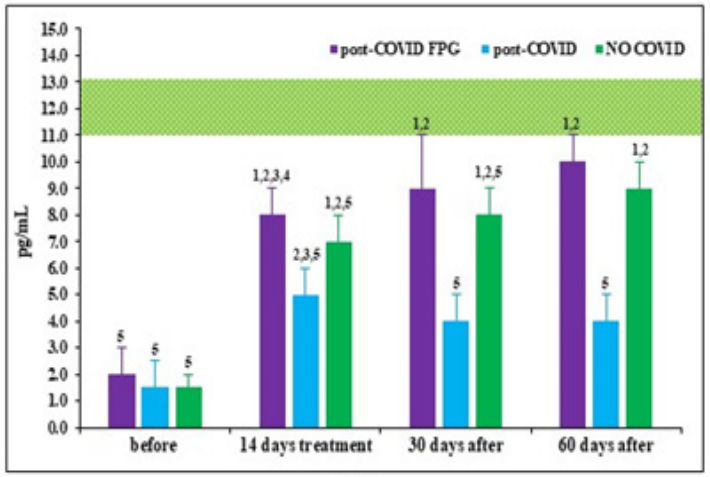
(b)

Versus NO COVID control group (Figure 3d). The positive effect became gradually more visible after 14 days of the therapy. Collectively, standard therapy was able of restoring the balance pro-/anti-inflammatory cytokines in close vicinity to pathological site (GCF) mainly in periodontitis patients who did not experienced COVID-19 disease. At the same time, its efficacy decreased in the post-COVID patients. Addition of FPG to standard protocol of treatment greatly improved its immuno-modulating action in post-COVID periodontitis patients.

(a) IL-1ß; (b) IL-6; (c) IL-17A; (d) IL-10. Green areas correspond to the normal range of values in GCF of donors without periodontitis. 1 –p <0,05 versus control post-COVID group; 2 - p <0,05 versus the same group before the trial; 3 –p <0,05 versus previous visit; 4 –p <0,05 versus periodontitis NO COVID group ; 5 - p <0,05 versus healthy donor



(c)



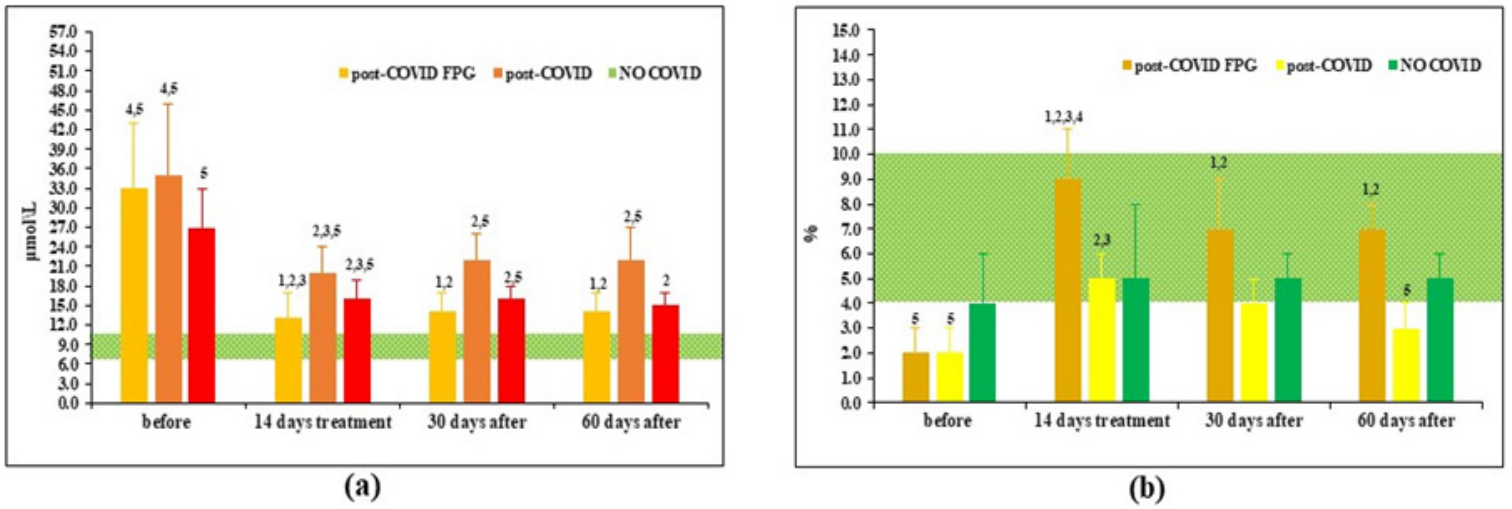
(d)

Markers of oxidative stress in gingival crevicular fluid (GCF) of post-COVID and NO-COVID periodontitis patients

The following markers of redox balance shift in favour of oxidation (oxidative stress) in GCF were monitored: the content of nitrites and nitrates (expressed as a ratio NO2/NO3), the products of nitric oxide and superoxide anion-radicals interaction, and the total antioxidant activity (AOA), which reflects the capacity of GCF to inhibit lipid peroxidation induced by ferrous ions (Fe+2). The content of oxidative agents nitrites/ nitrates was significantly increased over the normal values at the entrance in the trial while AOA was

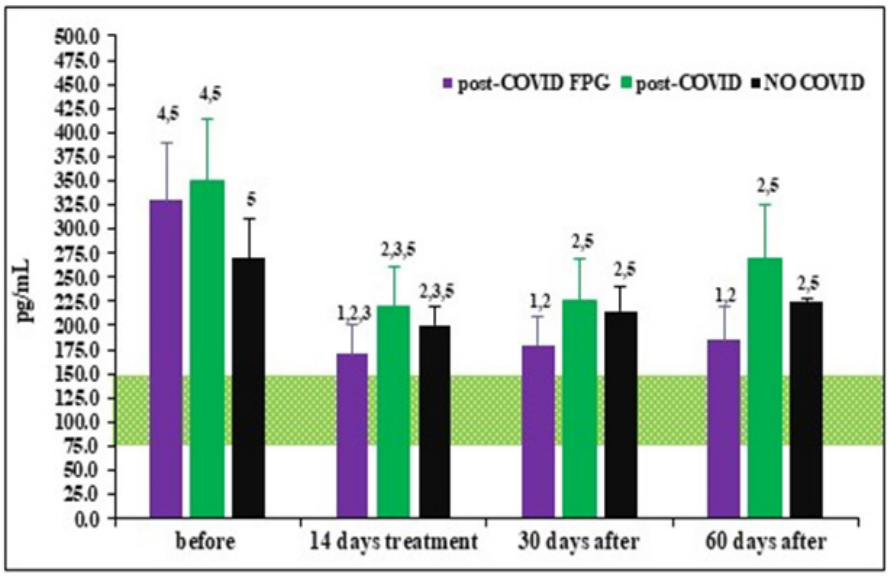
suppressed. Here, the degree of oxidative stress was higher in post-COVID groups versus NO COVID periodontitis group (Figures 4a and 4b). After a-14 day-long treatment, the content of oxidants in the experimental group reached the upper border of normal range and remained as low up to the end of observation (60 days). In the control (post-COVID, no FPG) group, NO2/NO3 ratio remained higher-than-normal at all points of the observation while AOA was low and gradually decreasing with the time. There were statistically significant differences between the experimental and post-COVID control groups at all points of measurements. Statistical differences were also confirmed between the two control groups and between the experimental and NO COVID control groups.

Figure 4: Effects of fermented papaya gel (FPG) intra-gum pocket administration (7g, 14 days) on



the nitrites/nitrates content in and antioxidant capacity (AOA) of the GCF.

Figure 5: Dynamics of MMP-8 protein content (pg/mL) in GCF: effect of FPG local administration to post-COVID periodontitis patients.



1 –p <0,05 versus control post-COVID periodontitis; 2 - p <0,05 versus before the trial; 3 –p <0,05 versus previous visit; 4 –p <0,05 versus periodontitis no COVID; 5 - p <0,05 versus healthy donors.

(a) Dynamics of nitrites/nitrates content (μM) in GCF. Green area corresponds to a range of normal values; (b) Dynamics of AOA (%) of the GCF. Green area corresponds to a range of normal values. 1 –p <0,05 versus control post-COVID periodontitis; 2 - p <0,05 versus the same group before the trial; 3 –p <0,05 versus previous visit; 4 –p <0,05 versus periodontitis NO COVID; 5 - p <0,05 versus healthy donors.

Matrix metalloprotease 8 (MMP-8) protein in the gingival crevicular fluid of post-COVID and NO-COVID periodontitis patients

Indeed, MMP-8 protein levels were over-expressed in the beginning of the study, especially, in the two post-COVID groups **(Figure 5)**. They sharply dropped by the end of the treatment period and remained practically at the normal range of values for the experimental FPG group while higher-than-normal for the both control groups. There was no statistical difference between the two control groups.

Discussion

A preliminary questioning of post-COVID patients (n = 198) on the state of their oral health during and after the COVID-19 disease showed that majority of patients (>50%), who survived severe disease and a moderate form of COVID-19 complained for gum bleeding, pain, itching, and burning that lasted for more than 3 months after the infection **(Table 2)**. Halitosis, which appeared during COVID disease was aggravated afterwards in post severe COVID patients (44% versus 64% complaining patients) and diminished in post moderate COVID patients (75% versus 50% complaining patients). Patients after mild or asymptomatic COVID-19 expressed less complaints to major oral problems (approx. 10% complaining patients) moreover the problems disappeared rapidly in the post-COVID period (after 3 months only 1,7% complaining subjects remained). Our data correspond to previously published observations on the oral health problems connected to COVID-19 [11-15]. Furthermore, it has been noticed that gingival bleeding and pain preceded first symptoms of COVID infection and were coincidental with positive PCR testing, fever, and other clinical signs of the COVID-19 disease [50]. Here, we showed for the first time dependance of the incidence of oral health problems with severity of COVID-19 disease. It seems that long-lasting gingivitis after severe and moderate forms of COVID is a major risk factor for the development of COVID-associated periodontitis.

Actually, 92 post-COVID patients (mild periodontitis diagnosed for the first time after COVID-19 disease) recruited to the clinical study, experienced either severe or moderate COVID-19 disease 3-6 months before entering the trial. Topical administration of FPG was well tolerated by the participants so they all (100%, no drop-outs) completed the study without adverse effects. Satisfactory and durable clinical efficacy was confirmed by repeated measurements of periodontitis severity indices (Figures 1a and 1b) and macro photographs **(Photo 1)**. Of note, both indices, CPITN and PMA, were indistinguishable for 3 groups at the entrance point of the study. These indices corresponded to mild periodontitis by definition so presence or absence of previous COVID-19 disease could not influence them. Both indices were normalised in the experimental group by the 14th day of treatment and remained at the upper normality level for 60 days of post-treatment observation. As for control groups of NO COVID and post-COVID, standard therapy was efficient and statistically significantly diminished CPITN and PAM, however, efficacy was more evident in NO COVID than in post-COVID group. At the same time, standard treatment alone has never brought indices to normality. On this basis, we concluded that standard therapy (mechanical removal of subgingival plaque and local antibiotic) is not sufficient to treat COVID-associated periodontitis and might require additional therapeutic approaches specifically targeting post-COVID aetiology and pathogenic mechanisms of periodontitis. Addition of FPG could be also recommended to NO COVID periodontitis patients to facilitate desired clinical results.

The key pathogenic feature of periodontitis involves the interplay of microbiota present in the plaque and GCF with the innate and adaptive immune host responses. Inflammation and destruction of periodontal tissues occur due to inadequate host responses to a microbial biofilm containing gram-negative pathogens. In the present study, we measured the presence and quantity of 5 well established periodontitis pathogens and 1 bacteria numerically dominant in dental plaque biofilms, and greatly increased in number at sites of periodontitis although not responsible for destructive periodontal disease *(F. nucleatum)* [51]. All 6 microorganisms belong to Gram-negative pathogens being mainly obligatory anaerobic. Only *A. actinomycetemcomitans* is a facultative anaerobic microbe with a good tolerance to oxygen. These microbial species are thought to be implicated into aggressive and chronic periodontal disease as well as in rheumatoid arthritis through citrullination of neutrophil proteins that are autoantibodies in both pathologies [52]. An autoimmunity develops also during COVID-19 disease and persists in the post-COVID period that hypothetically connects the viral disease to chronic poly-microbial periodontitis [53,54]. The differential microbial quantitation was determined in a mixture of plaque and GCF, biological material taken from the closest vicinity to a pathological site and reflecting changes in the quantity and specificity of periodontal microbiota. Initially, the bacterial load was heavier in the 2 post-COVID groups than in the control NO COVID group excluding for the load with *F. nucleatum* and T. forsythia, which were found similar **(Figure 2)**. On these grounds, one can conclude that during quite

long post-COVID period (3-6 months) previous or still persisting in the gum pockets viral infection [6] has significant negative impact on the local periodontal microbiota thus aggravating risk/severity of COVID-associated periodontitis.

Since poly-microbial infection is a major etiological factor in periodontitis, the primary lines of standard treatment included mechanical removal of microbial biofilms and topical antibiotics. The need for repeated painful cleaning procedures and the acquired resistance of dental bacteria to antibiotics prompted an extensive search for alternative, nontoxic, clinically and cost-effective remedies to decrease local bacterial overload. Among the most effective disrupters of microbial biofilms, flavonoids, 2-aminoimidazole alkaloids, and halogenated furanones of plant origin have been identified [47,55]. FPG is a concentrate of plant-derived secondary metabolites with anti-bacterial properties [42]. Indeed, additional application of FPG to intra gum pockets was more effective than standard therapy alone in the GCF unload from pathogens in post-COVID groups **(Table 3)**. Of great interest, standard therapy was extremely effective against high risk periodontal pathogens *P. gingivalis* and *T. denticola* while the same therapy was much less effective in the post-COVID group. The observed difference could not be explained merely by the heavier microbial load in the control post-COVID group. Since bacteria killing potential of local antibiotic used for standard therapy is equal for both groups, viral infection could suppress somehow constitutive and induced anti-microbial defence mechanisms in the periodontal tissue.

These mechanisms are numerous and include chemotaxis of immune cells, first of all granulocytes, to infected cite, their stimulation to produce cytokine and reactive oxygen species, activation of defensins, anti-microbial proteins, neutrophil extracellular trapping (NET), over-expression of Toll-like receptors, and many others [16,54]. In response to this massive anti-microbial attack, microbes evolved their own defence, like mutations, inhibition of NET components, microbial anti-oxidant enzymes/systems induction, matrix metalloproteases induction, defensins' destruction, etc. [42,54]. The exact mechanisms of interplay between COVID infection and periodontal microbiota remained unknown. From our previous publications [42,54], we could assume that FPG could have an additional to standard therapy impact to unload GCF from *A. actinomycetemcomitans* by suppression of microbial catalase in these partly aerobic microbes induced by H2O2 produced by stimulated granulocytes in response to microbial infection. Furthermore, FPG is an activator of phagocytosis and intracellular microbial killing [42,54,56]. Even not being direct antibiotic, FPG suppresses microbial survival by different mechanisms. Therefore, it could be considered as an adjuvant to standard antibiotic therapy.

Normal reaction of human organism to infection is inflammation and oxidative burst of granulocytes, then, of other immune cells. Both events are the first line defence against microbial/viral invasions. Local and generalised oxidative stress since long time have been considered as molecular hallmark of periodontitis. Redox imbalance (reductive/oxidative shift in favour of oxidation) has been first hypothesised and then, found in COVID and post-COVID patients [16,19]. An increased lipid peroxidation and decreased antioxidant activity have been found in saliva, GCF, and blood plasma of patients with periodontitis [57,58]. Periodontitis severity and bone resorption markers, such as osteoprotegerin levels and receptor activator of nuclear factor kappaB (NF B) ligand, were closely associated with total oxidant/antioxidant balance in (GCF) [59].

Our findings that initial levels of nitrites and nitrates in GCF, reflecting nitric oxide overproduction and an elevated risk of peroxynitrite formation, were greatly increased compared to normal values in all groups studied, especially, in post-COVID groups **(Figure 4a)**, that well correlated with the previous publications on periodontitis [42] and post-COVID-19-connected nitrosative stress [42]. NO COVID group was characterised by lower-than-COVID-connected NO2/NO3 levels in GCF. We assumed that previous COVID-19 disease contributed to the local production of nitrogen reactive species. As expected, addition of FPG to therapeutic protocol practically normalised NO2/NO3 levels for a long period of time thus, diminishing the risk of nitrosative damage of periodontal tissue and following inflammation-promoting effects [40]. The dynamics of AOA in GCF was completely inverse as compare to that for NO2/NO3 **(Figure 4b).** While background AOA of NO COVID group was at normal levels, GCF AOA of post-COVID groups was significantly suppressed. FPG administration significantly increased compromised capacity of GCF to attenuate ferrous ions-driven lipid peroxidation mainly due to high metal chelating potential of the fermented product [46]. It has also been reported [47] that saliva and its major components, such as albumin and mucin, substantially increase antioxidant capacity of fermented papaya by solubilisation of plant-derived polyphenols.

Collectively, redox balance in GCF was partly or completely restored after both standard and standard+FPG therapies although the latter protocol was more effective.

Chronic local inflammation in periodontitis was confirmed by the remarkably increased initial levels of major pro-inflammatory cytokines (IL-1 , IL-6, and IL-17A) and suppressed level of anti-inflammatory cytokine IL-10 involved in the development of periodontitis and its severity in GCF (Figures 3a-3d). Highly increased circulating levels of these inflammation-related cytokines while suppressed content of anti-inflammatory IL-10 have been recently shown in COVID-19 patients [60-62]. These cytokines are essential components of cytokine storm, a pathogenetic marker of life threatening viral disease. Their levels gradually lowered in the post-COVID period but persisted in patients with post-COVID syndrome [19].

The therapeutic course of FPG led to complete and durable normalisation of cytokine status locally in a close vicinity to pathological site. Previous publications have shown that the marked reduction of clinical parameters corresponded to decreased levels of IL-1β and thiobarbituric acid reactive substances in GCF after successful periodontal treatments [63,64]. Higher-than-normal amount of IL-1β before therapy correlated with disease severity, while an inverse relationship between IL-1β and IL-10 has been shown [65]. Similar conclusions regarding salivary IL-1β dynamics have been drawn in a case-control clinical study enrolling the patients before and after successful periodontal therapy [66].

Matrix metalloprotease 8 (MMP-8) or collagenase 2 belongs to a family of metalloproteases. MMP-8 is the only mammalian enzyme to degrade collagens, triple helical proteins, which are the major components of bones, cartilage, and dentin [67]. The MMP-8 enzyme is secreted from neutrophil granules in response to inflammatory signal. The MMP-8 is also thought to be a key factor regulating 27 IL-6 and IL-8 expressions in the innate immune response [68]. The enzyme is one of the most studied biomarkers of periodontitis, its severity, and efficacy of a periodontitis treatments [69,70]. Systematic reviews have shown MMP-8 to be a reliable tool for diagnosing periodontal diseases [21,71]. We found **(Figure 5)** that initial MMP-8 levels in GCF of all three groups of periodontitis patients were remarkably higher-than-normal that correlated with the previously published data. We also noticed that the enzyme protein content was statistically significantly lower in the control NO COVID group. It might mean that previous or/and persisting exposure to viral infection further induced MMP-8 synthesis and release from neutrophils, phagocytosing functions and energy (ATP) storage of which were significantly altered by COVID infection [19]. Complex therapy with FPG applications practically normalised MMP-8 content by the 14th day, the effect lasted until the end of the study. Mono therapy with topical antibiotic was less effective in post-COVID than in NO COVID subjects. The dynamics of GCF MMP-8 may be explained in obvious terms of elimination of infectious agents, attenuation signals to granulocytes to release MMP-8-containing granules, followed by the inhibition of MMP-8-regulated production of inflammatory cytokines.

Conclusions

COVID-associated periodontitis is characterised by an alteration of periodontitis-related microbiota, redox balance, and MMP-8 content in gingival crevicular fluid. Standard therapeutic protocols (mechanical removal of plaque and topical antibiotic) are less effective for post-COVID versus NO COVID-19 periodontitis. FPG addition to standard therapy led to substantially increased clinical efficacy, mainly, due to restoration of microbiota pattern and balance between pro-/anti-inflammatory cytokines, normalisation of redox balance, and MMP-8 content. Topical administration of FPG in combination with conventional protocols could be feasible, safe, and cost-effective mean to prevent and treat both COVID-associated and not associated periodontitis at initial stages.

References

[1] Libby, P.; Luscher, T. COVID-19 is, in the end, an endothelial disease. Eur. Heart J. 2020, 41, 3038–3044.

[2] Fodor, A.; Tiperciuc, B.; Login, C.; Orasan, O.H.; Lazar, A.L.; Buchman, C.; Hanghicel, P.; Sitar-Taut, A.; Suharoschi, R.; Vulturar, R.; et al. Endothelial dysfunction, inflammation, and oxidative stress in COVID-19-mechanisms and therapeutic targets. Oxid. Med. Cell. Longev. 2021, 2021, 8671713. https://doi.org/10.1155/2021/8671713.

[3] Campisi, G.; Bizzoca, M.E.; Lo Muzio, L. COVID-19 and periodontitis: reflecting on a possible association. Head & Face Medicine 2021, 17, 16. https://doi.org/10.1186/s13005-021-00267-1.

[4] Molayem, S.; Pontes, C.C. The Mouth-COVID Connection: Il-6 Levels in Periodontal Disease-Potential Role in COVID-19-Related Respiratory Complications. J. Calif. Dent. Assoc. 2020, 40, 68–80.

[5] Marouf, N.; Cai, W.; Said, K.N.; Daas, H.; Diab, H.; Chinta, V.R.; Hssain, A.A.; Nicolau, B.; Sanz, M.; Tamimi, F. Association between periodontitis and severity of COVID-19 infection: A case–control study. J. Clin. Periodontol. 2021, 48, 483–491.

[6] Badran, Z.; Gaudin, A.; Struillou, X.; Amador, G.; Soueidan, A. Periodontal pockets: a potential reservoir for SARS-CoV-2? Med Hypothesis 2020, 143, 109907. https://doi.org/10.1016/j.mehy.2020.109907.

[7] Silvestre, F.J.; Marcuez-Arrico, C.F. COVID-19 and periodontitis: a dangerous association? Front Pharmacol 2021, 12, 789681. doi: 10.3389/fphar.2021.789681.

[8] Anand, P.S.; Jadhav, P.; Kamath, K.P.; Kumar, S.R.; Vijayalaxmi, S.; Anil, S. A case-control study on the association between periodontitis and coronavirus disease (COVID-19). J Periodotol 2022, 93, 584-590. doi: 10.1002/JPER.21-0272.

[9] Pitones-Rubio, V.; Chavez-Cortez, E.G.; Hurtado-Caramena, A.; Gonsalez-Rascon, A.; Serafin-Higuera N. Is periodontal disease a risk factor for severe COVID-19 illness? Medical Hypothesis 2020, 144, 109969. https://doi.org/10.1016/j.mehy.2020.109969.

[10] Anaya, J.-M.; Rojas, M.; Salinas, M.L.; Rodriguez, Y.; Roa, G.; Lozano, M.; Rodriguez-Jimenez, M.; Montoya, N.; Zapata, E.; Post-COVID study group; Monsalve, D.M.; et al. Post-COVID syndrome. A case series and comprehensive review. Autoimm. Rev. 2021, 20, 102947. https://doi.org/10.1016/j.autrev.2021.102947.

[11] Wu, Y.; Cheng, X.; Jiang, G.; Tang, H.; Ming, S.; Tang, L.; Lu, J. Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in COVID-19 patients during hospitalization. Biofilms and Microbiomes 2021, 7, 61. https://doi.org/10.1038/s41522-021-00232-5.

[12] Tsuchiya, H. Oral symptoms associated with COVID-19 and their pathogenic mechanisms: a literature review. Dent J 2021, 9, 32. https://doi.org/10.3390/dj9030032.

[13] Rusu, L.-C.; Ardelean, L.C.; Tigmeanu, C.V.; Matichescu, A.; Sauciur, I.; Bratu E.A. COVID-19 and its repercussions on oral health: a review. Medicina 2021, 57, 1189. https://doi.org/10.3390/medicina57111189.

[14] Coke, C.; Davison, B.; Fields, N.; Fletcher, J.; Rollings, J.; Roberson, L.; Challagundla, K.; Sampath, C.; Cade, J.; Farmer-Dixon, C.; Gangula, P.N. SARS-CoV-2 Infection and Oral Health: Therapeutic Opportunities and Challenges. J. Clin. Med. 2021, 10, 156.

[15] Darestani, M.N.; Akbari, A.; Yaghobee, S.; Taheri, M.; Akbari, S. COVID-19 Pandemic and Periodontal Practice: The Immunological, Clinical, and Economic Points of View. Biomed Res Int 2022, 2022, 3918980. doi: 10.1155/2022/3918980.

[16] Slominski, R.M.; Stefan, J.; Athar, M.; Holick, M.F.; Jetten, A.M.; Raman, C.; Slominski, A.T. COVID-19 and vitamin D: A lesson from the skin. Exp. Dermatol. 2020, 29, 885–890.

[17] Kamal, M.; Abo Omirah, M.; Hussein, A.; Saeed, H. Assessment and characterisation of post-COVID-19 manifestations. Int. J. Clin. Pract. 2021, 75, 13746. https://doi.org/10.1111/ijcp.13746.

[18] Takahashi, Y.; Watanabe, N.; Kamio, N.; Kobayashi, R.; Iinuma, T.; Imai, K. Aspiration of periodontopathic bacteria due to poor oral hygiene potentially contributes to the aggravation of COVID-19. J Oral Sci 2020, 23, 1–3. doi:10.2334/josnusd.20-0388.

[19] Kharaeva, Z.; Shokarova, A.; Shomakhova, Z.; Ibragimova, G.; Trakhtman, P.; Trakhtman, I.; Chung, J.; Mayer, W.; De Luca, C.; Korkina, L. Fermented Carica papaya and Morinda citrifolia as Perspective Food Supplements for the Treatment of Post-COVID Symptoms: Randomized Placebo-Controlled Clinical Laboratory Study. Nutrients 2022, 14, 2203. https://doi.org/10.3390/nu14112203

[20] Gupta ,S.; Chhina, S.; Arora, S.A. A systematic review of biomarkers of gingival crevicular fluid: Their predictive role in diagnosis of periodontal disease status. J Oral Biol Craniofac Res 2018, 8, 98-104.

[21] Gupta, S., and Sahni, V. The Intriguing Commonality of NETosis between COVID-19 & Periodontal Disease. Med Hypotheses 2020, 144, 109968. doi:10.1016/j.mehy.2020.109968.

[22] Preshaw, P.M.; Alba, A.L.; Herrera, D.; Jepsen, S.; Konstantinidis, A.; Makrilakis, K.; Taylor, R. Periodontitis and diabetes: A two-way relationship. Diabetologia 2011, 55, 21–31.

[23] Paul, O.; Arora, P.; Mayer, M.; Chatterjee, S. Inflammation in Periodontal Disease: Possible Link to Vascular Disease. Front. Physiol. 2021, 11, 609614.

[24] Larvin, H.; Wilmott, S.; Kang, J.; Aggarwal, V. R.; Pavitt, S.; Wu, J. Additive Effect of Periodontal Disease and Obesity on COVID-19 Outcomes. J Dent Res 2021, 100, 1228–1235. doi:10.1177/00220345211029638.

[25] Hajishengallis, G.; Chavakis, T.; Hajishengallis, E.; Lambris, J.D. Neutrophil homeostasis and inflammation: Novel paradigms from studying periodontitis. J Leukoc Biol 2015, 98, 539–548.

[26] Hajishengallis, G. Periodontitis: From microbial immune subversion to systemic inflammation. Nat Rev Immunol 2014, 15, 30–44.

[27] Darveau, R.P. Periodontitis: A polymicrobial disruption of host homeostasis. Nat. Rev. Microbiol. 2010, 8, 481–490.

[28] Dosseva-Panova, V.T.; Popova, C.L.; Panov, V.E. Subgingival microbial profile and production of pro inflammatory cytokines in chronic periodontitis. Folia Med. 2014, 56, 152–160.

[29] Zhu, Z.; Chen, W.; Hao, L.; Zhu, G.; Lu, Y.; Li, S.; Wang, L.; Li, Y.P. Ac45 silencing mediated by AAV-sh-Ac45-RNAi prevents both bone loss and inflammation caused by periodontitis. J. Clin. Periodontol. 2015, 42, 599–608.

[30] Haffajee, A.D.; Socransky, S.S.; Patel, M.R.; Song, X. Microbial complexes in supragingival plaque. Oral Microbiol. Immunol. 2008, 23, 196–205.

[31] Socransky, S.S.; Haffajee, A.D.; Cugini, M.A.; Smith, C.; Kent, R.L. Microbial complexes in subgingival plaque. J. Clin. Periodontol. 1998, 25, 134–144.

[32] Giuffrè, A.; Borisov, V.B.; Arese, M.; Sarti, P.; Forti, E. Cytochrome bd oxidase and bacterial tolerance to oxidative and nitrosative stress. Biochim. Biophys. Acta 2014, 1837, 1178–1187.

[33] Maisonneuve, E.; Gerdes, K. Molecular mechanisms underlying bacterial persisters. Cell 2014, 157, 539–548.

[34] Douglas, C.W.; Naylor, K.; Phansopa, C.; Frey, A.M.; Farmilo, T.; Stafford, G.P. Physiological adaptations of key oral bacteria. Adv. Microb. Physiol. 2014, 65, 257–335.

[35] Heindorf, M.; Kadari, M.; Heider, C.; Skiebe, E.; Wilharm, G. Impact of Acinetobacter baumannii superoxide dismutase on motility, virulence, oxidative stress resistance and susceptibility to antibiotics. PLoS ONE 2014, 9, e101033, doi:10.1371/journal.pone.0101033.

[36] Ezzo, P.J.; Culter, C.W. Microorganisms as risk indicators for periodontal disease. Periodontology 2003, 32, 24–35.

[37] Nonnenmacher, C.; Dalpke, A.; Mutters, R.; Heeg, K. Quantitative detection of periodontopathogens by real-time PCR. J. Microbiol. Methods 2004, 59, 117–125.

[38] Lee, H.J.; Kim, J.K.; Cho, J.Y.; Lee, J.M.; Hong, S.H. Quantification of subgingival bacterial pathogens at different stages of periodontal diseases. Curr. Microbiol. 2012, 65, 22–27.

[39] Sy, J.B.A.; Hsu, T.-C.; Limaye, A.; Liu, J.-R. Oral administration with a traditional fermented multi-fruit beverage modulates non-specific and antigen-specific immune responses in BALB/c mice. PLoS ONE 2020, 15, e0233047. https://doi.org/10.1371/journal.pone.0233047.

[40] Rimbach, G.; Park, Y.C.; Guo, Q.; Moini, H.; Qureshi, N.; Saliou, C.; Takayama, K.; Virgili, F.; Packer, L. Nitric oxide synthesis and TNF-alpha secretion in RAW 264.7 macrophages: Mode of action of a fermented papaya preparation. Life Sci. 2000, 67, 679–694.

[41] Collard, E.; Roy, S. Improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of a fermented papaya preparation. Antioxid. Redox Signal. 2010, 13, 599–606.

[42] Kharaeva, Z.F.; Zhanimova, R.L.; Mustafaev, M.S.; De Luca, C.; Mayer, W.; Thai, J.C.S.; Tuan, R.T.S.; Korkina, L. Effects of standardised fermented papaya gel on clinical symptoms, inflammatory cytokines, and nitric oxide metabolites in patients with chronic periodontitis: An open randomised clinical study. Med. Inflamm. 2016, 2016, 9379840. https://doi.org/10.1155/2016/9379840.

[43] Das, A.; Dickerson, R.; Das Ghatak, P.; Gordillo, G.M.; Chaffe, S.; Saha, A.; Khanna, S.; Roy, S. May dietary supplementation augment respiratory burst in wound-site inflammatory cells? Antioxid. Redox Signal. 2018, 28, 401–405.

[44] Dickerson, R.; Deshapande, B.; Gnyawali, U.; Lynch, D.; Gordillo, G.M.; Schuster, D.; Osei, K.; Roy, S. Correction of aberrant NADPH oxidase activity in blood derived mononuclear cells from type II diabetes mellitus patients by a naturally fermented papaya preparation. Antioxid. Redox Signal. 2012, 17, 485–491.

[45] Dickerson, R.; Banejee, J.; Rauckhorst, A.; Pfeiffer, D.R.; Gordillo, G.M.; Khanna, S.; Osei, K.; Roy, S. Does oral supplementation of a fermented papaya preparation correct respiratory burst function of innate immune cells

in type 2 diabetes mellitus patients? Antioxid. Redox Signal. 2015, 22, 339–345.

[46] Osato, J.A.; Korkina, L.G.; Santiago, L.A.; Afanas'ev, I.B. Effects of bio-normalizer (a food supplementation) on free radical production by human blood neutrophils, erythrocytes, and rat peritoneal macrophages. Nutrition 1995, 11, 568–572.

[47] Fibach, E.; Ginsburg, I. The antioxidant effect of fermented papaya preparation in the oral cavity. Phytother. Res. 2015, 29, 1317–1322. https://doi.org/10.1002/ptr.5381.

[48] Barnett, M. L. Suitability of gingival indices for use in therapeutic trials. Is bleeding a sine qua non?" J Clin Periodontol 1996, 23, 582–586.

[49] Marks, R.G.; Magnusson, I.; Taylor, M.; Clouser, B.; Maruniak, J.; Clark, W.B. Evaluation of reliability and reproducibility of dental indices. J Clin Periodontol 1993, 20, 54–58.

[50] Manzalawi, R.; Alhmamey, K.; Abdelrasoul, M. Gingival bleeding associated with COVID-19 infection. Clin Case Rep 2020, 9, 294–297. doi: 10.1002/ccr3.3519.

[51] Signat, B.; Roques, C.; Poulety, P.; Duffaut, D. Role of Fusobacterium nucleatum in periodontal health and disease. Curr Issues Mol Biol 2011, 13, 25-36. http://www.cimb.org.

[52] Konig, M.F.; Abusleme, L.; Reinholdt, J.; Palmer, R.J.; Teles, R.P.; Sampson, K.; Rosen, A.; Nigrovic, P.A.; Sokolove, J.; Giles, J.T.; Moutsopoulos, N.M.; Andrade, F. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Sci Transl Med 2016, 8 , 369ra176. doi:10.1126/scitranslmed.aaj1921.

[53] Klok, F.A.; Kruip, M.J.H.A.; van der Meer, N.J.M.; Arbous, M.S.; Gommers, D.A.M.P.J.; Kant, K.M.; Kaptein, F.H.J.; van Paassen, J.; Stals, M.A.M.; Huisman, M.V.; et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020, 191, 145–147.

[54] Magán-Fernández, A.; Rasheed Al-Bakri, S.M.; O'Valle, F.; Benavides-Reyes, C.; Abadía-Molina, F.; Mesa, F. (2020). Neutrophil Extracellular Traps in Periodontitis. Cells 2020, 9, 1494. doi:10.3390/cells9061494.

[55] Buommino, E.; Scognamiglio, M.; Donnarumma, G.; Fiorentino, A. ; D'Abrosca, B. Recent advances in natural product-based anti-biofilm approaches to control infections. Mini-Rev Med Chem 2015, 14, 1169–1182.

[56] Kharaeva, Z.; Mustafaev, M.S.; Khazhmetov, A.V.; Gazaev, I.H.; Blieva, L.Z.; Steiner, L.; Mayer, W.; De Luca, C.; Korkina, L.G. Anti-bacterial and anti-inflammatory effects of toothpaste with Swiss medicinal herbs towards patients suffering from gingivitis and initial stage of periodontitis: from clinical efficacy to mechanisms. Dent J 2020, 8, 10. doi:10.3390/dj8010010.

[57] Chapple, I.L.C.; Mason, G. I.; Garner I.; Mattews J.B.; Thorpe, G.H.; Maxwell, S.R.; Whitehead, T.P. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. Ann Clin Biochem 1997, 34, 412–421.

[58] Esen, C.; Alkan, B. A.; Kirnap, M.; Akgul, O.; Isıkoglu, S.; Erel, O. The effects of chronic periodontitis and rheumatoid arthritis on serum and gingival crevicular fluid total antioxidant/oxidant status and oxidative stress index. J Periodontol 2012, 83, 773–779.

[59] Baltacioglu, E.; Kehribar, M.A.; Yuva, P.; Alver, A.; Atagun, O.S.; Karabulut, E.; Akalin, F.A. Total oxidant status and bone resorption biomarkers in serum and gingival crevicular fluid of patients with periodontitis. J Periodontol 2014, 85, 317–326.

[60] Hasichaolu; Zhang, X.; Li, X.; Li, X.; Li, D. Circulating cytokines and lymphocyte subsets in patients who have recovered from COVID-19. Bio Med. Res. Int. 2020, 2020, 7570981. doi.org/10.1155/2020/7570981.

[61] Li, Q.; Xu, W.; Li, W.-X.; Huang, C.-L.; Chen, L. Dynamics of cytokines and lymphocyte subsets associated with the poor prognosis of severe COVID-19. Eur. Rev. Med. Pharmacol. Sci. 2020, 24, 12536–12544.

[62] Han, H.; Ma, Q.; Li, C.; Liu, R.; Zhao, L.; Wang, W.; Zhang, P.; Liu, X.; Gao, G.; Liu, F.; et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity. Emerg. Microbes Infect. 2020, 9, 1123–1130. https://doi.org/10.1080/22221751.2020.1770129.

[63] Tuter, G.; Kurtis, B.; Serdar, M. Interleukin-1□ and thio-barbituric acid reactive substance (TBARS) levels after phase I periodontal therapy in patients with chronic periodontitis. J Periodontol 2001, 72, 883–888.

[64] Shrestha, D.; Choi, Y.H.; Zhang, J.; Hazlett, L.J.; Merchant, A.T. Relationship between serologic markers of periodontal bacteria and metabolic syndrome and its components. J Periodontol 2015, 86, 418–430.

[65] Goutoudi, P.; Diza, E.; Arvanitidou, M. Effect of periodontal therapy on crevicular fluid interleukin-1□ and interleukin-10 levels in chronic periodontitis. J Dentistry 2004, 32, 511–520.

[66] Kaushik, R.; Yeltiwar, R. K.; Pushpanshu, K. Salivary interleukin-1□ levels in patients with chronic periodontitis before and after periodontal phase i therapy and healthy controls: a case-control study. J Periodontol 2011, 82, 1353–1359.

[67] Gul, S.; Zardawi, F.; Abdulkareem, A.; Shaikh, M.; Al-Rawi, N.; Zafar, M. Efficacy of baseline MMP-8 level in gingival crevicular fluid to predict the outcome of nonsurgical periododntal treatment in periodontitis patients: a systematic review. Int J Environ Res Public Health 2022, 19,x. https://doi.org/10.3390/xxxxx.

[68] Thirkettle, S.; Decock, J.; Arnold, H.; Pennington, C. J.; Jaworski, D.M.; Edwards, D.R. Matrix metalloproteinase 8 (collagenase 2) induces the expression of interleukins 6 and 8 in breast cancer cells. J Biol Chem 2013, 288, 16282–16294.

[69] Sorsa, T.; Alassiri, S.; Grigoriadis, A.; Räisänen, I.T.; Pärnänen, P.; Nwhator, S.O.; Gieselmann, D.R.; Sakellari, D. Active MMP-8 (aMMP-8) as a Grading and Staging Biomarker in the Periodontitis Classification. Diagnostics (Basel) 2020, 10, 61.

[70] Sorsa, T.; Gursoy, U.K.; Nwhator, S.; Hernandez, M.; Tervahartiala, T.; Leppilahti, J.; Gursoy, M.; Kononen, E.; Emingil, G.; Pussinen, P.J.; Mantila, P. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontol 2000 2016, 70, 142-163.

[71] de Morais, E.F.; Pinheiro, J.C.; Leite, R.B.; Santos, P.P.A.; Barboza, C.A.G.; Freitas, R.A. Matrix metalloproteinase-8 levels in periodontal disease patients: a systematic review. J Periodontal Res 2018, 53, 156-163.

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Conceptualization, Z.K. and L.K.; methodology, Z.K., A.S., E.B., I.T., R.T., and G.I.; software, W.M., J.C., and I.T.; validation, Z.K., A.S., and G.I.; formal analysis, Z.K., J.C., and C.D.; investigation, A.S., E.B., I.T., R.T., and G.I.; resources, Z.K., W.M., I.T., and L.K.; data curation Z.K., J.C., and C.D.; writing—original draft preparation, L.K.; writing—review and editing, W.M., R.T., and C.D.; visualization, W.M., C.D., and L.K.; supervision, L.K. and W.M.; project administration, Z.K. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding. The APC was funded by Fame Team International Ltd, Honk Kong.

Institutional Review Board Statement

The Protocol of the clinical study was scrutinized and approved by the Ethic Committee of Kabardino-Balkar Berbekov's State Medical University (Nal'chik, Russia), Protocol number MD-005-2021.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent to publish the data was not applicable for this study.

Data Availability Statement: Not applicable.

Acknowledgments

We gratefully acknowledge Fame Team International Ltd, Honk Kong for shouldering the costs of the publication.

Conflicts of Interest

W.M. is the Head of the R&D department of MEDENA AG, involved in the research, development, and production of oral care products. C.D is the Head of the Regulatory Affairs Department of MEDENA AG. Drs. De Luca and Mayer did not influence the process of evaluation and presentation of major results or the conclusions drawn from the results obtained. All other co-authors declare absence of the conflict of interest.