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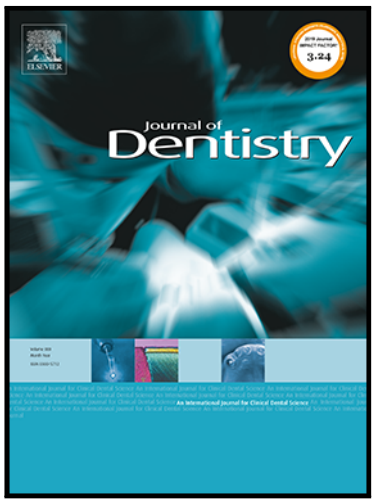

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The application of a Fasting-mimicking Diet in Periodontitis. A feasibility study

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KEYWORDS: Fasting; Periodontitis; Diet; Gingival Crevicular Fluid; Serum

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ABSTRACT

Objectives: The aim of this study was to test the feasibility of a fasting mimicking diet on the systemic and periodontal response following non-surgical periodontal therapy.

Methods: Twenty patients with periodontitis were randomized to receive steps 1 and 2 of periodontal treatment alone (following their normal diet) or with an adjunctive 5-day course of fasting-mimicking diet (FMD). Blood and gingival crevicular fluid (GCF) samples were collected to study the levels of inflammatory biomarkers, along with clinical parameters and patients reported outcome measurements (PROMs). All patients

were followed up at day-1, day-7 and 3 months post-treatment and food diaries were completed to assess their compliance.

Results: Nineteen patients completed the 3-months follow-up. Only minimal adverse events including nausea, fatigue, weakness and dizziness were reported in the test group, with no differences in PROMs between groups. Test patients exhibited a non-statistically significant 3-months serum hs-CRP reduction of 0.20 ± 0.30 mg/l compared with 0.11 ± 0.52 mg/l in controls (p=0.632) and a trend for lower GCF levels of MMP-8, IL-6 and IL-1B post-treatment compared with controls.

Conclusions: This study suggests that one cycle of adjunctive FMD is feasible and may modulate the inflammatory response post-non-surgical periodontal therapy. Larger studies are needed to test this hypothesis. (clinicaltrials.gov ID: NCT05684627).

CLINICAL RELEVANCE

For the first time, a 5-days cycle of FMD as an adjunct to non-surgical periodontal therapy was assessed in Turkish individuals with periodontitis stages III-IV. The findings showed that FMD is feasible and may reduce inflammatory markers one day post-treatment.

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INTRODUCTION

Although periodontitis is a microbially-driven inflammatory disease of the supporting apparatus of the teeth, its effects may be felt systemically and in other organs and tissues [1]. Periodontal treatment, consisting of supra- and sub-gingival professional mechanical plaque removal, is associated with a short-term acute phase inflammatory response, characterised by elevation of circulating acute-phase inflammatory markers, followed by a longer-term reduction in these molecules [2]. In the intermediate to longer term this is accompanied by changes in markers of peripheral vascular health such as C-reactive protein (CRP) [3-5].

While the inflammatory response can be beneficial to periodontal healing, strategies have been envisaged to limit its systemic impact [6]. Several studies have recently focused on the possible effect of dietary interventions to reduce inflammation and improvement of periodontal conditions [7-9]. Periodic fasting and caloric restriction have clearly emerged as beneficial regarding reduction of biological age and reduced risk of systemic diseases [10, 11]. A novel mode of fasting has been introduced to increase patient compliance, due to the fact that people are usually not able to follow fasting for a long period [12]. In particular, the fasting-mimicking diet (FMD), consisting of 30-50% of the normal caloric intake for 4-7 consecutive days followed by a refeeding ad libitum period once a month, showed very promising beneficious effects in terms of reduction of risk factors for aging, diabetes, autoimmunity, cardiovascular disease, neurodegeneration and cancer [13]. A recent systematic review from our group suggested that caloric restriction may have an effect on periodontal inflammation and outcomes of treatment [14]. Most studies in this field have been focused on animal models [15-18], with very few assessing the potential effect of a caloric restriction diet in periodontal conditions in humans [9, 19, 20].

No studies to our knowledge have yet tested the potential effect of FMD on periodontal inflammation and treatment outcomes. Furthermore, it is unclear whether compliance to such strict diet can be achieved in patients receiving periodontal therapy. Therefore, the aim of this study was to assess the feasibility of a fasting-mimicking diet as adjunct to non-surgical periodontal therapy and to generate initial data about its potential impact on local and systemic inflammation, as well as on clinical outcomes. The null hypothesis is that a cycle of FMD diet combined with non-surgical periodontal therapy does not affect CRP levels 90 days post-treatment.

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METHODS

Patient population

This was a feasibility randomised controlled trial. The CONSORT guidelines were followed. The study was registered on clinicaltrials.gov (NCT05684627). One hundred consecutive periodontitis patients referred by their general dental practitioners to the study therapists for periodontal treatment were included in this study. Ethics approval for the analysis was given by Hatay Mustafa Kemal University (Turkey) Tayfur Ata Sökmen Ethics Committee on October 24, 2022 (4298783/05046) (2022/91). Each patient gave written consent to take part

in the study. Patient visit took place from March 2023 to February 2024 at Akdeniz University, Turkey. The following inclusion criteria were considered for patient recruitment:

- Age 18-70 years
- Generalized periodontitis stage III-IV, grade B or C (Tonetti et al. 2018)
- Minimum of 24 teeth present
- Self-reported systemic health
- Body mass index: 18.5-30 kg/m^2 (normal weight to overweight, but not obese)
- Be willing and competent (verbally and cognitively) to give written informed consent and complete a medical history form.
- Be willing and physically able to carry out all study procedures

Exclusion Criteria were:

- Periodontal treatment in the last 12 months
- Presence of hopeless teeth, acute dental conditions, teeth with endo-perio lesions and necrotising periodontal diseases
- Smoking (defined as self-reported use of any cigarettes or electronic cigarettes for at least 5 years)
- Mental illness, including severe depression, dementia
- Drug dependency
- Hormone replacement therapy (DHEA, estrogen, thyroid, testosterone)
- Severe hypertension (systolic BP > 200 mm Hg and/or diastolic BP > 105 mm Hg).
- Currently taking part in other clinical trial
- Pregnant or breastfeeding women
- Taking medications (including systemic anti-inflammatory medication within 3 months of the study, corticosteroids, immunosuppressive agents, anticoagulants/antiplatelet drugs, antihypertensive medications (e.g., ACE inhibitors, beta-blockers, calcium channel blockers), antidiabetic drugs (e.g., insulin, metformin) and hormonal medications (oral contraceptives, Hormone Replacement Therapy)
- Systemic antibiotic intake within 3 months
- Current orthodontic treatment
- Alcohol intake greater than two drinks per day for women and three drinks per day for men
- Denture wearer/presence of dental implants
- Obvious signs of untreated caries and other significant oral diseases

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- Unable or unwilling to complete the dietary intervention
- Special dietary requirements incompatible with the study interventions
- Significant food allergies

Clinical examination

Following consent, at baseline, self-reported patient medical and smoking histories were checked. The following periodontal measurements were taken at six sites/tooth: dichotomous full mouth plaque scores (FMPS), full mouth probing pocket depth (PPD), recession (REC) of the gingival margin from the cemento-enamel junction (CEJ), bleeding on probing (FMBS) [21], tooth mobility [22] and furcation involvement [23]. Clinical attachment level (CAL) was calculated as PPD+REC. Clinical parameters were assessed by gentle probing using a UNC-15 periodontal probe and a Nabers probe for furcation involvement.

Randomisation and allocation concealment

Randomisation and allocation concealment were rigorously implemented to ensure the integrity and reliability of the results. The process involved the use of a randomisation sequence generated by sealedenvelope.com randomisation service. Treatment allocation for each patient was printed and placed in sealed envelopes by personnel not directly involved in the study. Subsequently, another member of staff not involved in the study opened these envelopes during the baseline visit and communicated allocation (test or control group) to patients. In brief, test patients received detailed instructions regarding the diet and its products intake, whereas controls were encouraged to continue with their normal diet as usual. This allocation concealment procedure effectively prevented any foreknowledge of group assignment, thereby maintaining the integrity of the randomisation process. Moreover, to minimize potential sources of bias, both the examiner and operator were blinded to the group assignment throughout the duration of the study.

Diet

Patients from the test group underwent a cycle of FMD (ProLon, L-Nutra Inc.) that started the same day of the non-surgical periodontal therapy (specifically, in the morning, at breakfast, before receiving the treatment). The FMD program is a plant-based diet program designed to attain fasting-like effects while providing both macro- and micronutrients to minimize the burden of fasting and adverse effects [24]. The FMD regime consists of 100% ingredients that are generally regarded as safe (GRAS) and comprises vegetable-based soups,

energy bars, energy drinks, cracker snacks, olives, herbal teas, and supplements. All items to be consumed per day were individually boxed to allow the subjects to choose when to eat while avoiding accidentally consuming components of the following day [24]. Water (suggested as 2 litres/day) was the only 'external' item test participants were allowed to ingest during the diet cycle. The FMD consisted of a 5-days regimen that provides approximately 1100 kilocalories for the first day; approximately 750 kilocalories per day for the second to the fifth day [24]. On the 6th day, known as the transition day, patients gradually resumed a normal diet. In details, they were suggested to begin with liquid and very soft foods, such as soups, fruit juices, smoothies, and centrifuges, followed by light foods such as rice, pasta, and small portions of legumes and/or fatty fish. A normal diet was, then, resumed starting from the 7th day. On the other hand, patients from the control group continued with their normal (ad libitum) diet for the whole study period. Furthermore, dietary anamnesis questionnaires were given at baseline and 3 months, and a food diary was completed during the first week by both test and control patients (more details can be found in Supplementary Material 1), in particular, to check the compliance of test patients.

GCF sampling

Gingival crevicular fluid (GCF) was collected using Periopaper (OraFlow Inc.) from the mesial sulcus of the first molars of each study participant. GCF sampling was performed prior to periodontal probing, to avoid blood contamination. Periopaper was placed at the entrance of the sulcus until slight resistance was felt and then left for 30s. Samples visually contaminated with blood or diluted with saliva during sampling were discarded. The strips were pooled in an Eppendorf tube and transferred to −80°C. The samples were sent to King's College London (United Kingdom) at the end of the study, where GCF was extracted using PBS with protease inhibitors 1X (1 tablet for 10 ml of PBS) (Complete ULTRA tablets, Mini; EDTA-free). Briefly, 25 µl of PBS/protease inhibitor cocktail was added and centrifuged at 11000 g for 15 min at 4°C. An additional 25 µl of PBS/protease inhibitor cocktail was then added and centrifuged at 11 000 g for 15 minutes at 4°C, to a total volume of 50 µl. The eluted GCF was stored at −80°C until analysis.

Blood sampling

Blood samples were collected from each participant using standard venepuncture techniques. Blood samples consisted of two tubes: one for blood and one for serum. A total of 13-15 mL of whole blood was drawn into sterile vacutainer tubes containing clot activator and gel

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separator. The tubes were gently inverted to ensure proper mixing of the anticoagulant and the blood sample. Blood was immediately aliquoted in Eppendorfs and stored at -80°C. Subsequently, the other samples were allowed to clot at room temperature for 30 minutes to one hour to facilitate serum separation. Following clot formation, the tubes were centrifuged at 4000 rpm for 5 minutes. This centrifugation step effectively separated the serum from the clot and cellular components. The resulting serum samples were then carefully transferred into labelled microcentrifuge tubes using a sterile pipette and stored at -80°C until analysis following transportation to King's College London.

Biomarkers analyses

Serum samples underwent ELISA in order to detect circulating levels of High-sensitive C Reactive Protein (Hs-CRP). GCF samples were analysed by performing high-sensitivity Multi-Analyte ELISAs (Ella™ Automated Immonoassay System; ProteinSimple) in order to assess levels of IL-1a, IL-1b, IL-6, IL-10, IL-17 and MMP-8, according to manufacturer instructions. Each sample was analysed in duplicate for both procedures.

Periodontal treatment and follow-ups

The study flowchart is presented in Figure 1. All patients received a comprehensive treatment plan, aimed at step 1 and 2 of periodontal therapy [25], including oral hygiene instructions, risk factor control and supra-gingival scaling. Steps 1 and 2 were carried out concomitantly in these cases, and a full-mouth treatment approach was employed. The treatment visit included oral hygiene instructions, as well as supra- and sub-gingival instrumentation of all four quadrants in one long appointment in the morning, using Gracey curettes (American Eagle & Hu Friedy) and ultrasonic instruments (Woodpecker & EMS). Patients were then reassessed at day 1, day 7 and day 90 post-treatment.

Patients reported outcome measurements (PROMs)

OHIP-14 questionnaires [26] were given at baseline and at 3 months to assess patient reported outcome measurements (PROMs). Details can be found in Supplementary Material 2.

Examiner calibration

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The single masked study examiner (author IO) underwent a training exercise and intra-examiner calibration before the start of the study by collecting 6-point pocket depths twice on five non-study patients. The K score for intra-agreement coefficient for PPD was 0.86.

Statistical analysis and sample size calculation

A convenience sample size of 20 patients was considered for this feasibility/pilot study [27, 28], as no previous studies had been published using this particular fasting-mimicking diet in patients with periodontitis. Data from all patients were entered into a spreadsheet and proofed for entry errors. Continuous variables are reported as means and standard deviations. Feasibility was assessed by number of patients who managed to complete the diet and reported adverse events in the test group, together with other progression criteria such as acceptance of blood samples and willingness to be randomised (details can be found in Supplementary Material 3). The main outcome of the study was circulating CRP levels at 3 months post-treatment. Paired t-test was used to detect significant changes between baseline and 3 months for the primary outcomes using the patient as unit of analysis. Data were used for a sample size calculation. The Shapiro-Wilk Test was applied to verify if serum and GCF biomarkers followed a normal distribution and, following it, they were log transformed for analysis. ANOVA was used to compare biomarker levels between test and control groups.

RESULTS

Baseline characteristics

A total of 20 patients were enrolled in the study. Table 1 reports baseline demographic and clinical characteristics of all patients. Patients allocated to the test group had a higher percentage of females (80% vs. 20%) and higher BMI ($p<0.05$) compared with controls. No statistically significant differences were detected between groups at baseline for full-mouth plaque and bleeding scores, average PPD and CAL and number of PPDs >4mm and >5mm. Total treatment time was 122 minutes in the test group and 132 minutes in the control group.

Feasibility, diet and adverse events

Nineteen of the patients attended all study visits up to the 3-months visit, while one test patient was lost to follow-up as they moved, making it impossible to attend the last visit.

All patients in the test group reported completing the 5-diet FMD cycle, as confirmed by the food diary completed by the patients for the whole duration of the FMD cycle. No patients

reported any serious adverse events, with 50% of subjects in test group referring minor side effects. In details, two patients reporting mild nausea, another two referring to mild weakness and one subject that had fatigue, nausea and dizziness. Feasibility criteria are reported in Supplementary Material 3. At 3 months, all patients (mainly from test group) appeared to have increased their water consumption, while dietary habits of test patients (and controls, as expected) did not appear to have changed compared to the beginning of the study.

Clinical outcomes at 3 months

Statistically significant reductions in number of PPDs >5mm and average CAL were detected between baseline and day 90 for all patients combined ($p=0.045$ and $p<0.001$ respectively). Table 2 reports clinical parameters of test and control patients at baseline and 3 months. No differences were detected for any of the clinical parameters between groups at 3 months. Intra-group differences between baseline and 3 months showed statistically significant reductions in average PPD ($p=0.019$), average CAL ($p<0.001$) and FMBS (p-0.031) only in the control group. 'Pocket closure' (reduction of PPD to < 5mm) was 83.9% and 80.4% at 3 months for control and test groups respectively. Endpoints of therapy (no PPD ≥ 6 mm and no PPD > 4 mm with no bleeding on probing) were reached in 3 test and 2 control patients (25% of the overall total).

Serum hs-CRP

Table 3 reports baseline serum hs-CRP levels and changes across the following three study timepoints for patients divided by treatment group. Average overall hs-CRP at baseline was 0.91 ± 0.47 mg/l, increasing at day-1 in both groups and then decreasing up to day-90. Figure 2 shows the trend for change in hs-CRP levels in tests and controls. Although hs-CRP levels were slightly higher in test compared with controls at baseline, an opposite trend was detected at day 7 and day 90. In detail, at 7 days a hs-CRP decrease of 0.05 ± 0.39 mg/l was observed in test patients versus 0.03 ± 0.42 mg/l in controls (not statistically significant between groups), and at 3 months a hs-CRP reduction of 0.20 ± 0.30 mg/l was observed in test patients compared with 0.11 ± 0.52 mg/l in controls (not statistically significant between groups).

GCF analysis

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Figure 3 shows GCF biomarker levels across the three study timepoints for patients divided by treatment group. No significant differences in any analyte values were found at baseline for test vs control groups. The majority of biomarkers increased at day-1 for both groups and then progressively decreased at day-7 and at day-90. However, at day-1 test patients exhibited lower values compared with controls for MMP-8 (p=0.011), IL-6 (p=0.036) and IL-1b (p=0.013), with a similar trend for IL-1a (p=0.082). These trends continued but were not statistically significant at subsequent timepoints. Further details are shown in Supplementary Material 4.

PROMs

No differences between tests and controls were observed in terms of patients reported outcome measurements. Details of these results can be found in Supplementary Material 2.

DISCUSSION

This study was the first to use a fast-mimicking diet (FMD) and to confirm its safety and feasibility as adjunct to non-surgical periodontal therapy. This plant-based diet replicates the physiological effects of fasting while still allowing the consumption of carefully controlled foods consisting of soups, bars, snacks, teas and supplements [24]. As this diet had not been previously employed in this application, its feasibility was demonstrated by complete self-reported adherence to the 5-day diet cycle, as observed from the food diary and from the fulfilled progression criteria.

The feasibility of the FMD was confirmed by the fact that all 10 test patients completed it with minor side effects and no major adverse events and the study's progression criteria to a larger trial were satisfied. In line with the existing literature in other settings [24], only fatigue, dizziness, nausea and weakness were reported. This means that conceivably this diet could be employed in patients with periodontitis, if proven to beneficially modulate inflammation. However, the correct protocol (i.e., timing and number of cycles and target patient groups) needs to be clarified. In addition, despite dietary habits seemed to not have been changed after the completion of the study for both groups, test subjects reported an increased daily consumption of water by the end of study compared with baseline.

Circulating hs-CRP and a panel of six GCF biomarkers were investigated in FMD patients compared with those continuing their normal diet, to investigate the potential effect of the

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FMD in modulating post-treatment inflammation. We performed full-mouth non-surgical therapy in order to better test the effect of FMD, following a previous model to test the acute-phase response post-periodontal treatment [29]. Serum hs-CRP dynamics following periodontal treatment were line with what is described in the literature, with an increase immediately after NSPT (day 1), followed by a decrease from day 7 to day 90 [30]. This transient surge in inflammation indicates that the period immediately following periodontal treatment is a sensitive window during which nutritional interventions might exert significant effects. Furthermore, emerging evidence from studies such as by Rasperini and co-workers suggests that dietary components, particularly those found in the Mediterranean diet like micronutrients and olive oil, can further modulate the inflammatory response post-treatment [31]. These findings collectively support the concept that integrating targeted dietary interventions with periodontal therapy may reduce systemic inflammation and improve clinical outcomes. The reductions in circulating CRP in controls in the present study appear to be lower (but starting from lower baseline values) than in previous studies on non-surgical therapy [32, 33] [34]. No statistically significant differences between test and control groups were detected at any timepoint. However, following treatment serum hs-CRP levels in test patients remained lower than those of control patients and the hs-CRP reduction at 3 months was almost double for test compared with control patients. Such a difference may potentially be clinically relevant for cardiovascular risk [35, 36], mainly in non-systemically healthy subjects that usually present higher baseline values of CRP [37] but probably not in the patients presented here. Based on these differences (0.20 ± 0.30 mg/l reduction at 3 months in test patients compared with 0.11 ± 0.52 mg/l in controls), a sample size calculation for a larger trial can be carried out. A total of 352 patients need to enter a two-treatment parallel-design study to have 80% probability that the study will detect a treatment difference at a two-sided 0.05 significance level, with the observed CRP inter-group difference and standard deviation at 3 months.

A very interesting picture emerged from the GCF cytokine analysis, where test patients, in line with serum CRP results, exhibited a less marked increase (or in some cases a lack of increase) immediately post-treatment. The fact that at day-1 MMP-8, IL-1b and IL-6 were lower in test compared with controls ($p<0.05$) means that the FMD may reduce the inflammatory biomarkers increase normally seen in the acute-phase response post-periodontal treatment [38-41]. Similar trends for a potential effect of the FMD on limiting the inflammation post-periodontal treatment were detected in serum and GCF, although at

different time points. A possible explanation is that the inflammatory response, which shows a marked increase one day after periodontal treatment [2, 29], may be particularly susceptible to modulation during this acute phase. It is likely that the FMD played a significant role in influencing inflammation when the pathway was most prone to change, as indicated by the alterations in biomarker levels observed in GCF samples one day after periodontal treatment. However, this cannot be stated due to the small number of individuals in this trial. As the inflammatory peak decreased after day-1, inter-group differences still existed but were no longer statistically significant. In contrast, the more marked serum hs-CRP reduction in the test group compared with controls (albeit not statistically significant) was evident in the slightly longer term (3 months). It is also important to highlight that there was a difference in terms of BMI between groups (25.3 ± 2.6 for tests compared with 27.8 ± 2.5 for controls). Studies revealed that FMD might work better in overweight/obese than normal weight patients [13, 42] thus being a possible impediment to the success of FMD in our trial.

In addition to modulating systemic inflammation, dietary interventions may influence the composition of the subgingival microbial community. For instance, one study demonstrated that [9] intermittent fasting can alter the subgingival microflora by reducing the abundance of key periodontopathogens and promoting a more balanced bacterial ecosystem. This observation is in line with emerging evidence that dietary patterns rich in plant-based foods are associated with increased oral microbial variety and reduced dysbiosis [43]. Together, these findings suggest that a nutritional strategy such as the periodic FMD used in this investigation might offer a novel approach to improving periodontal health through the modulation of the oral microflora.

In terms of clinical outcomes, an overall reduction of almost 2.0 mm for PPD was observed in this study, double the 1.0 mm that a recent systematic review has reported [44], whereas bleeding on probing reduced by almost 40%, against the 56% observed in the same review. ‘Pocket closure’ was slightly higher than the average expected benchmark following steps 1 and 2 of periodontal treatment (83.9% and 80.4% at 3 months for control and test groups respectively) [44]. No differences were detected for clinical outcome between test and controls, suggesting that the FMD, used in this single 5-day cycle, may not affect the clinical response to steps 1 and 2 of periodontal therapy.

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The main strength of this study is to prove the feasibility of a fasting-mimicking approach as adjunct to steps 1 and 2 periodontal therapy and to provide data for a sample size calculation for a larger study aiming to use the FMD to modulate the inflammatory response in periodontal therapy. Such modulation may be beneficial to achieve a faster and more effective resolution of inflammation in periodontitis [30] and, systemically, to reduce the risk of other comorbidities that have inflammation as a common denominator [45-47].

Limitations of this trial include (i) the study design (pilot with small sample size), (ii) the short-term follow-up, (iii) only one FMD cycle, not excluding that repeated cycles may have more pronounced effects, (iv) the imbalance between male and female patients between groups, (v) the start of the FMD on day 1 rather than earlier, vi) it is hard to disentangle the potential effect of the caloric restriction from that of the specific items in the diet provided to test patients.

CONCLUSIONS

Within its limitations, this study showed the feasibility of a fasting-mimicking diet in patients with advanced periodontitis and the potential direction of effect of FMD in modulating the inflammatory response. Further studies with larger sample size, more FMD cycles and longer follow-up are needed to confirm and build upon these findings and, hopefully, to explore in depth the potential benefits that FMD could provide to periodontal patients in the medium-long term.

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AUTHORS CONTRIBUTION: Giuseppe Mainas: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (lead); project administration (lead); writing original draft (lead). **Ipek Ozgu, Buse Bayraktar Ayakta, Kemal Ustun**; investigation (equal); writing—review and editing (equal). **Aysegul Sari, Manlio Vinciguerra, Mark Ide**: Formal analysis (equal); writing—review and editing (equal). **Luigi Nibali**: Conceptualization (lead); formal analysis (equal); investigation (equal); methodology (lead); project administration (lead); writing original draft (lead).

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Conceptualization; formal analysis; investigation; methodology; project administration; supervision; writing original draft; writing-review and editing.

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TABLES

		Frequency		Test vs Control p=
		Test	Control	
Age		Mean: 39.9 ± 8.8	Mean: 40.9 ± 7.8	0.791
BMI		Mean: 25.3 ± 2.6	Mean: 27.8 ± 2.5	0.038
Gender	**Male**	2	8	0.023
	Female	8	2	
Periodontal diagnosis- Stage	**III**	9	8	0.531
	IV	1	2	
Periodontal diagnosis- Grade	**C**	10	10	1.0
Periodontal diagnosis- Extent	**Localised**	3	3	1.0
	Generalised	7	7	

Table 1. Patient characteristics. Mean ± standard deviation is reported for continuous variables

	Baseline		**90 days post-NSPT**		**P value for difference test-control at 3 months (baseline)**
	Test	**Control**	**Test**	**Control**	
Full-mouth plaque score	78.7 ± 7.8	79.1 ± 8.9	39.1 ± 12.5	39.2 ± 9.2	0.981 (0.916)
Full-mouth bleeding score	77.6 ± 9.7	78.8 ± 9.6	43.0 ± 10.7	44.5 ± 6.5	0.711 (0.784)
Average PPD (mm)	5.1 ± 0.5	5.0 ± 0.6	3.1 ± 0.3	3.1 ± 0.4	0.795 (0.675)
Average CAL	5.3 ± 0.7	5.2 ± 0.8	3.3 ± 0.5	3.5 ± 0.5	0.364 (0.945)

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(mm)					
Number of PPDs > 4 mm	104.1 ± 25.3	93.0 ± 31.5	17.5 ± 12.0	19.3 ± 11.8	0.742 (0.396)
Number of PPDs > 5 mm	56.2 ± 27.3	50.9 ± 27.4	4.4 ± 0.6	4.1 ± 4.8	0.913 (0.670)

Table 2. Clinical characteristics of test and control patients at baseline and post-NSPT. Means and standard deviations are reported.

		Test (n=10)	**Control (n=10)**	**P value for difference test-control**
Average hs-CRP baseline	Baseline	0.95 ± 0.58	0.88 ± 0.35	0.762
	Day 1 change	0.64 ± 0.71	0.66 ± 0.62	0.953
	Day 7 change	-0.05 ± 0.39	0.03 ± 0.42	0.663
	Day 90 change	-0.20 ± 0.30*	-0.11 ± 0.52	0.632

Table 3. Serum hs-CRP levels in test and control patients at all study timepoints *= n is equal 9 for test and day 90, due 1 case lost to follow-up.

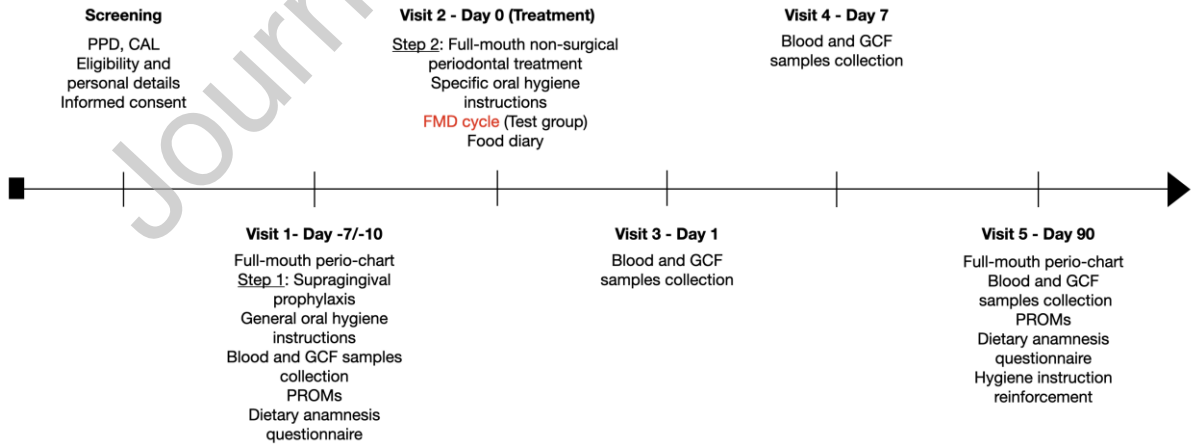

Figure 1

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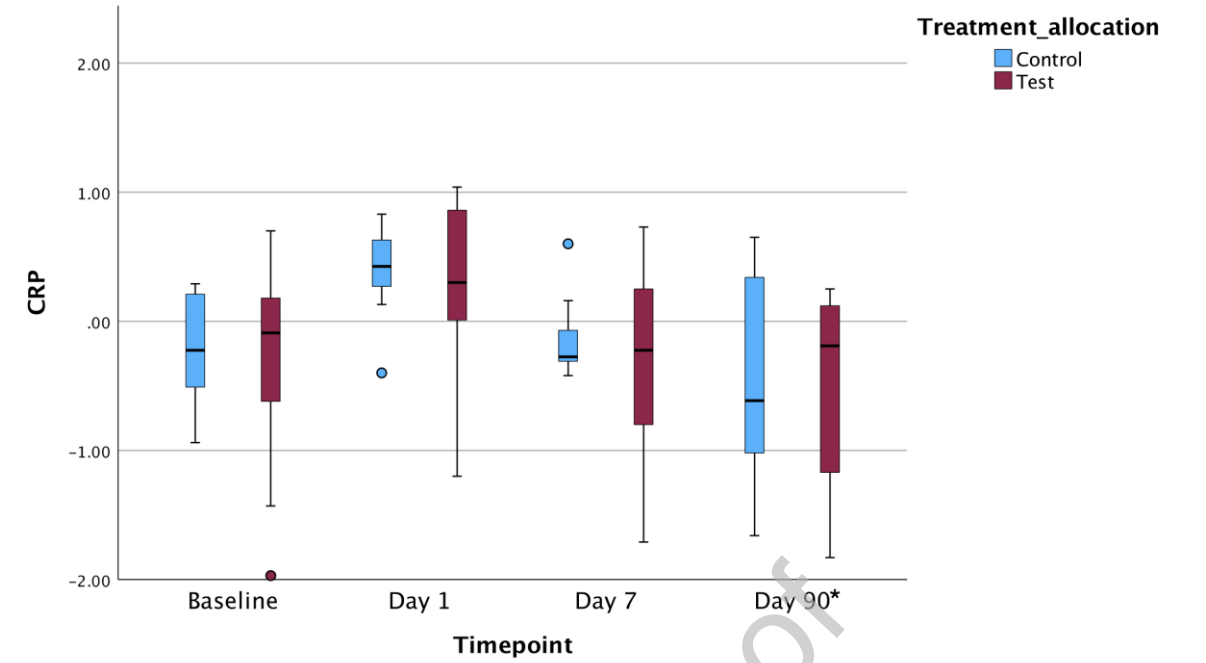

Figure 2

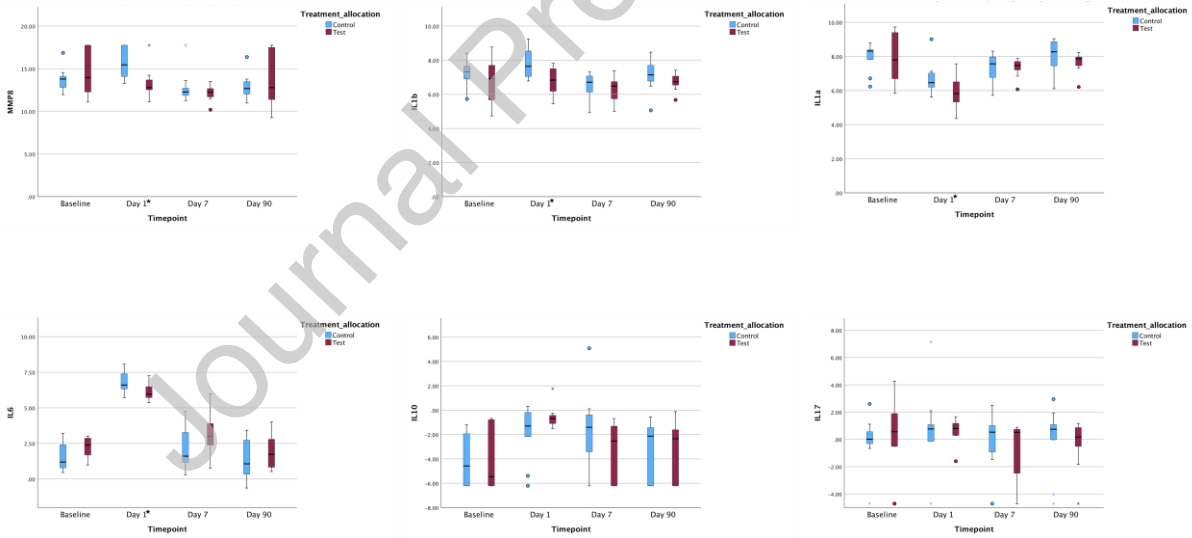

Figure 3

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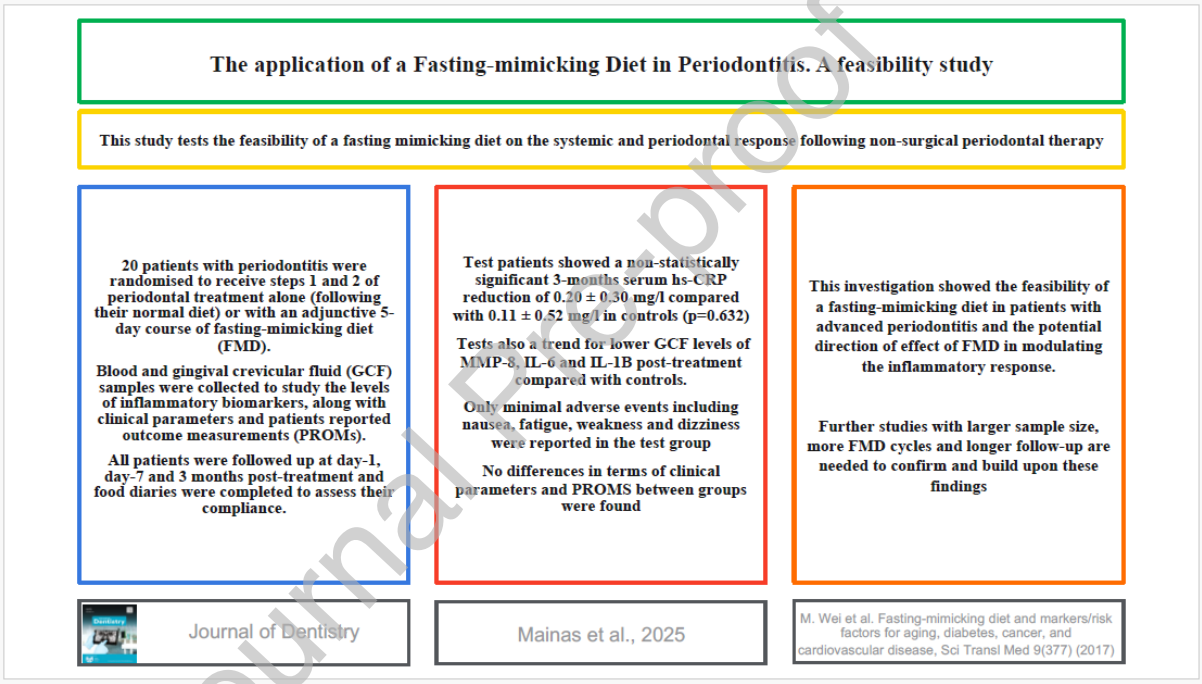
FIGURE LEGEND

Figure 1. Flow-chart of the study

Figure 2. Boxplot of serum levels of hs-CRP across all timepoints. *statistically significant difference between study groups

Figure 3. Boxplots of GCF biomarkers across all timepoints. *statistically significant difference between study groups

Graphical abstract



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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: