

Impact of periodontal therapy on systemic markers of inflammation in patients with metabolic syndrome: a randomized clinical trial

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Abstract

Background

Although there is evidence of positive effect of periodontal therapy on systemic inflammation, this response is highly variable among subjects. It was the aim of this clinical investigation to determine the impact of periodontal treatment on systemic markers of inflammation in patients with metabolic syndrome (MetS) and periodontitis.

Methods

In this parallel-arm, double blind, randomized controlled clinical trial, 63 patients with MetS and severe periodontitis were randomly assigned to receive intensive periodontal treatment (IPT; scaling and root planing plus azithromycin 500 mg, q.d., for three days) or minimal periodontal treatment (MPT; supragingival professional mechanical plaque removal plus a placebo). The primary outcome was the impact of the tested interventions on hs-CRP serum levels at 6 months. As secondary outcomes, differences in the levels of cytokines, markers of prothrombotic states, carbohydrate and lipids metabolism, as well as blood pressure, were measured at 3 and 6 months after therapy.

Results

The ITT population consisted on 63 subjects randomly assigned to either MPT (n = 31) or IPT (n = 32) groups. At baseline, mean hs-CRP was 3.9 mg/L (standard deviation, SD = 2.9) and 3.9 mg/L (SD = 3.4), respectively, and no significant differences in their cardiometabolic risk profiles were detected between groups. After 6 months, unadjusted mean hs-CRP were 2.9 mg/L (standard error, SE = 0.4) and 4.0 (SE = 0.8), respectively. Adjusting for baseline hs-CRP, sex, age, smoking status and body mass index, hs-CRP was 1.2 mg/L (95% confidence interval, [CI 0.4; 2.0]; p = 0.004) lower in the IPT group than in the MPT group. In the secondary outcomes, significant reductions in IL-1 β , TNF- α , HbA1c and blood pressure were observed in the IPT group at 3 months, when compared to the MPT group.

Conclusion

Effective periodontal treatment significantly reduced hs-CRP after 6 months in patients with MetS and severe periodontitis. Periodontal therapy might be useful to reduce cardiovascular risk in these patients.

Trial registration:

ClinicalTrials.gov Registration Number: NCT03960216.

Background

Metabolic syndrome (MetS) is a cluster of medical conditions associated with the risk of developing atherosclerotic cardiovascular disease (CVD) [1]. It is characterized by central obesity (defined by waist circumference), impaired glucose regulation, high blood pressure and dyslipidaemia (i.e. low high-density lipoproteins [HDL] and/or elevated triglycerides) (IDF Consensus Report, 2015). Although in certain individuals these conditions may occur in isolation, when they cluster as MetS, there is a significant increase in the risk for CVD, diabetes and overall mortality, even after adjusting for the traditional risk factors [2, 3]. MetS is not a disease in itself, but it is a serious health condition and a major global public health concern that affects about 10–40% of adults in developed countries, in parallel with the rise in prevalence of obesity [4, 5]. In the United States of America, its prevalence has increased from 28%, in 1988–1994, to 34%, in 1994–2004, according to the National Health and Nutrition Examination Surveys (NHANES) [6].

Periodontitis is a highly prevalent chronic inflammatory disease characterized by the destruction of tooth supporting tissues, eventually leading to tooth loss and significantly impairing the patient's quality of life. In fact, severe periodontitis is one of the most prevalent diseases worldwide, with an age standardized prevalence of 9.8% and with approximately 796 millions of cases [7]. Several epidemiological studies and systematic reviews have consistently shown an association between periodontitis and an increased risk for future atherosclerotic cardiovascular events, independently of traditional risk factors [8]. This association has been explained by the systemic effect of this infectious disease, evidenced by a significant increase in C-reactive protein [hs-CRP]) and other biomarkers of systemic inflammation, which may contribute to the overall inflammatory burden and to an increased risk of atherogenesis and hyperglycaemia [9–11].

A recent systematic review with meta-analysis has shown evidence for a potential association between periodontitis and MetS [11–14]. This association may be explained by the shared chronic state of systemic inflammation in both conditions [15], resulting in oxidative stress and insulin resistance [16–18]. This bi-directional association may be explained by the increase of systemic inflammation as a consequence of periodontitis, that may act as a contributor to the development of MetS [11]; while the low-grade systemic inflammation characteristic of obesity and impaired glucose regulation may negatively affect the periodontal status [19].

The possible effect of periodontal treatment on systemic inflammation, has been reported in several studies assessing the levels of inflammatory markers after treatment, showing significant reductions in interleukin (IL)-6 and hs-CRP [20–22]. However, this systemic impact has not been consistent and the variability in the reported outcomes has been attributed to inclusion of patients without clear measures of exposure, based on clinical or radiographic surrogates, rather than on the actual infectious burden, what may not reflect the degree of chronic inflammatory exposure [23, 24]. Also, the selection of patients may have not included those with relevant risk for CVD [25, 26].

It was, therefore, the purpose of this parallel-arm, double blind, randomized controlled clinical trial, to determine whether the treatment of periodontitis in patients with MetS could reduce the cardiometabolic risk, measured by means of: i) markers of systemic inflammation and prothrombotic states (hs-CRP, α -1 antitrypsin and fibrinogen levels); ii) pro-inflammatory cytokine profiles; and, iii) markers of carbohydrate and lipid metabolism.

Methods

Study design and participants

This study was designed as a parallel-group, double-blind, single-centre, randomized clinical trial (RCT), with a 6-month follow-up. Patients were recruited among those diagnosed of MetS and included in an active program of cardiovascular risk prevention at the Fuenlabrada University Hospital (Madrid Regional Hospital Network, Fuenlabrada, Madrid, Spain). The study protocol was approved by the institutional ethic committee (Internal Code 12/206, Hospital Clínico de San Carlos, Madrid) and registered at ClinicalTrials.gov (NCT03960216). This RCT was conducted between November 2012 and June 2018.

Participants were consecutively enrolled into the study if they fulfilled the following criteria:

- age 35 to 65 years,
- diagnosis of MetS according to the criteria established by Alberti et al. (2009) [27], based on the presence of, at least, three of the following risk factors:
 - elevated waist circumference (WC) (≥ 94 cm in men, ≥ 80 cm in women),
 - elevated triglycerides (≥ 150 mg/dL),
 - reduced high density lipoprotein (HDL) cholesterol (< 40 mg/dL in males; < 50 mg/dL in females),
 - elevated blood pressure (systolic ≥ 130 and/or diastolic ≥ 85 mm Hg),
 - elevated fasting plasma glucose (FPG) (≥ 100 mg/dL),
- presence of, at least, 16 teeth,
- diagnosis of stages III-IV generalised periodontitis, according to the criteria of the 2018 EFP-AAP classification [28],
- presence of, at least, eight sites with probing pocket depth (PPD) ≥ 6 mm and four sites with clinical attachment level (CAL) ≥ 5 mm, distributed in, at least, two different quadrants [29]; with the objective of recruiting participants with severe periodontal destruction (defined by CAL) and a high level of inflammation (defined by PPD).

Participants were excluded in presence of:

- uncontrolled systemic diseases other than diabetes or hypertension (e.g. chronic kidney disease, chronic lung disease, acute pulmonary disease during the previous 3 months, history of stroke, myocardial infarction, angor pectoris or revascularization during the previous 6 months, neoplasms, etc.),
- history of any surgical treatment in the previous 3 months,
- alcoholism or psychiatric disorders,
- history of systemic antibiotic usage over the previous 3 months,
- non-surgical periodontal treatment during the previous 6 months, or surgical periodontal treatment over the previous 12 months.

All the patients recruited according to these criteria and willing to participate in the study received verbal and written information. Upon signing the approved consent form, they were referred for periodontal treatment to the Postgraduate Clinic of Periodontology, at the Faculty of Odontology (University Complutense of Madrid, Spain).

Study interventions

All patients received standardised oral hygiene instructions (consisting on the use of a manual toothbrush and specific inter-dental brushes) and when indicated, extraction of hopeless teeth. Then, patients were randomized to:

- an intensive periodontal treatment (IPT; test group), which consisted on two sessions (one week apart) of non-surgical subgingival instrumentation (scaling and root planing, SRP), under local anaesthesia, with a combination of ultrasonic scalers (Minipiezon Electromedical Systems EMS, Nyon, Switzerland) and hand cures, together with adjunctive administration of a systemic antibiotic (azithromycin 500 mg, q.d., for three days), administered at the last session of SRP.
- A minimal periodontal treatment (MPT; control group), which consisted on two sessions of supragingival professional mechanical plaque removal, eliminating supragingival plaque and calculus with an ultrasonic scaler, without subgingival instrumentation, together with the adjunctive administration of placebo medication (identical capsules containing lactose 500 mg, q.d, for 3 days) administered at the last session of SRP.

Both treatment groups received, after the first treatment session, an antiseptic mouth rinse, containing 0.12% chlorhexidine and 0.05% cetylpyridinium chloride (Perio-Aid 0.12%; Dentaaid, Barcelona, Spain), to be used b.i.d., for 14 days. Oral hygiene instructions were reinforced in both groups at the 3- and 6-month visits, but no additional periodontal treatment was provided during the study, unless a subject demonstrated periodontitis progression, defined as presence of interproximal attachment loss ≥ 3 mm in ≥ 2 teeth [30]. These patients were withdrawn from the study and immediately received the appropriate periodontal treatment.

Randomization and masking

Patients were randomly assigned to receive IPT or MPT (1:1) using a computer-generated list, with a random-block size of 20. Allocation concealment was ensured with the use of opaque envelopes, opened at the time of the start of periodontal treatment, by the assigned clinicians (HV and EM). Patients were unaware of the assigned treatment group and the provided adjunctive medication (azithromycin or placebo) were externally identical capsules, numbered according to the randomization list. All other investigators [clinical examiners, laboratory staff, staff involved in data collection (MM)] were masked to the group allocation.

Study flow

Two trained and calibrated clinical examiners (EM and ML) collected medical histories and carried out a comprehensive oral and periodontal examination, at baseline, and at each follow-up visit (3 and 6 months after therapy). Periodontal parameters included full-mouth measurements of PPD, CAL, bleeding on probing (BOP), plaque index (PII) [31] and gingival index (GI) [32], at 6 sites per tooth, excluding third molars, using a UNC-15 probe (Hu-Friedy; Chicago, Illinois, USA). Full-mouth periapical radiographs and/or a panoramic radiograph were also taken at baseline. Before the start of the study, clinical examiners were calibrated. The calibration exercise consisted on repeated measurements of five volunteer patients. The achieved inter-examiner intraclass correlation coefficient for CAL was 0.98 (95% confidence interval -CI: [0.97; 0.98]) and for PPD 0.98 (95% CI: [0.98; 0.98]). Exposure to tobacco smoking was registered as current (number of cigarettes and years of exposure were noted), former (those who had stopped smoking at least 1 year previously) or never smoker. Measurements of weight, height, waist circumference and blood pressure were carried out at baseline and at the 3- and 6-month examinations. History of any other used medication was collected at baseline and updated at each study visit.

Fasting blood samples were collected at baseline, and at each study visit, into three 7 mL ethylenediaminetetraacetic acid (EDTA)-containing tubes. One EDTA-containing tube was stored at the Hospital at -80°C, for later analysis of hs-CRP, α -1 antitrypsin, fibrinogen, white blood cell counts (WBC), glycated haemoglobin (HbA1c; using high-performance liquid chromatography), FPG, insulin, creatinine and standard lipid fractions [total cholesterol, HDL cholesterol, LDL (low-density lipoprotein) cholesterol and triglycerides]. The other two tubes were stored at -80°C for later determination of cytokine profiles [IL-1 β , IL-6, IL-18 and tumour necrosis factor (TNF)- α] at the Molecular Biology Laboratory (Faculty of Odontology, University Complutense of Madrid), by means of multiplex assays.

At baseline, and 3 and 6 months after treatment, microbiological samples were collected before the periodontal examination. In brief, after removing the supra-gingival biofilm with sterile cotton rolls and gently air-dried to eliminate any contamination with saliva, two consecutive sterile medium paper points (#30, Maillefer Ballaigues, Switzerland) were inserted in the four deepest pockets showing BOP, one per quadrant, and left in place for 10 seconds. Paper points were transferred into one common vial, containing 1.5 mL of reduced transport fluid (RTF) [33]. Detailed information on laboratory procedures for the microbiological analyses is provided in Supplementary Materials.

Outcomes

To assess the efficacy of the tested interventions on periodontal health, mean periodontal clinical parameters (PPD, CAL, BOP, PII, GI), and mean counts, proportions and frequency of detection of target periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Parvimonas micra*, *Campylobacter rectus* and *Fusobacterium nucleatum*) were compared at 3 and 6 months.

Compliance with antibiotic intake and occurrence of adverse events (any change in anatomical, physiological or metabolic functions occurring in any phase of the clinical study, whether or not associated with it, were also registered. Patients were exited from the study, in presence of serious adverse effects under the criteria of the medical principal investigator (AZ) or when the criteria for periodontitis recurrence were met.

The primary outcome was the evaluation of the differences between the test and control groups in the mean hs-CRP serum levels at 6 months after the periodontal intervention. As secondary outcomes, the mean levels at 6 months of α -1 antitrypsin, fibrinogen, inflammatory markers (IL-1 β , IL-6, IL-8 and TNF- α), HbA1c, FPG, insulin, lipid fractions and blood pressure were compared.

Homeostasis Model Assessment scores (HOMA2-IR, HOMA2- β and HOMA2-IS) were calculated with a HOMA2 calculator released by the Diabetes Trial Unit, University of Oxford: HOMA Calculator (<https://www.dtu.ox.ac.uk/homacalculator/>). The concentrations of IL-1 β , IL-6, IL-8 and TNF- α were measured using high-sensitivity multiplex map human immunoassays (Millipore® corporation, Cat. #HSTCMAG-28SK, Billerica, MA, USA) using a Luminex-200 System Unit with the XY platform (Luminex® Corporation, Oosterhout, Netherlands). Detailed information on laboratory procedures for the immunoassays is provided in Supplementary Materials.

Data analysis

The sample size calculation was based on detecting a mean difference between groups of 2 mg/L in serum hs-CRP levels with an estimated standard deviation (SD)=2.5 mg/L. To detect statistically significant differences (α =0.05) with an 80% power, a minimum of 25 subjects per group were needed, which including an expected 20% drop-out rate, resulted in a final sample of 30 participants per group.

Data were reported as mean and SD, unless otherwise specified [e.g. n (%)]. All analyses were performed using the intention-to-treat population and the last observation carried forward (LOCF) approach for missing values. Analyses were repeated for all primary and secondary outcomes using post-hoc missing-value analyses. In addition, per-protocol analyses were conducted for all outcomes; the estimates derived from these analyses were reported for the primary outcome (hs-CRP) and any secondary or post-hoc outcomes, if they differed from estimates obtained in the intention-to-treat population.

The primary and secondary outcomes were modelled using multilevel linear regression. The models included the respective baseline measurement, treatment group, study visit (i.e. baseline, 3 months and 6

months), and a treatment-time interaction term as explanatory variables (covariates). Additional covariates included the main risk determinants for CVDs and periodontitis (i.e. age and sex) and the main risk factors also linked to a worse clinical periodontal outcome after treatment [i.e. smoking status and body mass index (BMI)]. Natural logarithmic transformation of the data, where appropriate, was applied. Differences between study groups for primary and secondary outcomes at all study follow-up visits (3 and 6 months) were adjusted for the respective baseline values, whereas the absolute values for each treatment group at a given time-point were not. For all analyses, a two-sided p value of less than 0.05 was considered as significant. The analyses were carried out using STATA version 13.1 with SVY package (StataCorp College Station, TX, USA).

Results

Patient characteristics

Between November 28, 2012, and December 11, 2017, 854 subjects with MetS were screened, being 765 excluded since they did not meet the study criteria and 63 (70.8%) out of 89 subjects were finally enrolled and randomly assigned to either the test-IPT (n = 32) or the control-MPT (n = 31) groups (Fig. 1). Three patients (5%) were lost in both groups over the 6-month period. These patients did not significantly differ from the included participants in regard to any of the outcome variables considered in the study. All included subjects were Caucasians. Two major adverse events occurred during the course of the study, one in a patient in the test (hospitalization for urine infection), and the other in the control group (acute myocardial infarction). These events were not considered to be related to the study, and they did not prevent the patients from attending the follow-up visits. Occurrence of minor adverse events were similar between the study groups (Table S1).

The cardiometabolic risk profiles at baseline (Table 1) did not show any significant difference between groups. Participants were predominantly men (around 70% in both groups), aged between 50 and 65 years and mostly either never or former smokers (75.9% in the IPT group and 89.3% in the MPT group). Subjects in both groups presented a mean BMI close to class 3 obesity (39.1 kg/m² for the IPT group and 38.0 kg/m² in the MPT group; p = 0.618) and a mean WC ≈ 120 cm, indicative of prominent visceral fat storage. High hs-CRP levels were observed in both groups, with 53.1% and 54.8% of patients in IPT and MPT groups, respectively, presenting values ≥ 3 mg/L. The percentage of subjects with HbA1c levels ≥ 7% were 31.25% and 25.8%, respectively. No differences between groups were observed in the medication use at baseline (Table S2) and changes in medication throughout the study were negligible [n = 3 (4.8%); corresponding to an increase in insulin dosage]. Nine patients (two in the test and seven in the control group) received systemic antimicrobials during the study (apart from the one administered in the IPT group), but differences between groups were not statistically significant (p = 0.070).

Clinical and microbiological periodontal outcomes

Periodontal clinical parameters were similar at baseline in both groups (Table 2. Figure 2). The mean number of teeth at baseline in the IPT and MPT groups were 22.6 (SD = 3.8) and 22.0 (SD = 4.2) ($p = 0.611$), respectively; with a similar number of tooth extractions performed in both groups [0.7 (SD = 1.5) and 0.2 (SD = 0.5), respectively ($p = 0.130$)].

As compared with the MPT group, the IPT had lower values for PII at 6 months (absolute difference, 0.4; 95% CI: [0.1; 0.7]; $p = 0.003$). Patients in the IPT group had lower scores for BOP (absolute difference, 31.1%; 95% CI: [22.3; 40.0]; $p < 0.001$) than those in the MPT group, 6 months after therapy. Similarly, patients in the IPT group had significantly less sites with PPD ≥ 4 mm (32.6%; 95% CI: [23; 42]; $p < 0.001$) and experienced a significantly higher CAL gain, than the control group, 6 months after therapy (1.0, SD = 0.8 mm, versus 0.3, SD = 0.6 mm, respectively ($p < 0.001$)).

The microbiological outcomes, expressed as counts and proportions of each target bacterial species, at each time point, are presented in Tables S3 and S4. At baseline, both test and control groups harboured similar proportions of periodontal pathogens (*P. gingivalis* in 15.6% and 20.5%, respectively, and *P. intermedia* in 5.5% and 6.3%, respectively). Total anaerobic counts were not significantly different between groups throughout the study, although significant reductions occurred in the IPT group, 3- and 6 months post-treatment. Similarly, the counts of *P. gingivalis* were significantly lower in the IPT group, to the MPT group, at 3- (6.9 ln CFUs; 95% CI: [4.0; 9.9]; $p < 0.001$) and 6 months (6.6 ln CFUs; 95% CI: [3.5; 9.6]; $p < 0.001$). Significantly reduced counts were also found for *P. intermedia* (5.7 ln CFUs; 95% CI: [2.9; 8.5]; $p < 0.001$; and 3.1 ln CFUs; 95% CI: [0.3; 6.0]; $p = 0.031$) and *T. forsythia* (5.7 ln CFUs; 95% CI: [3.2; 8.2]; $p < 0.001$ and 3.1 ln CFUs; 95% CI: [0.2; 6.0]; $p = 0.035$), in the IPT group, at 3- and 6 months post-therapy.

Impact of the interventions in the systemic inflammatory status

The primary outcome, mean hs-CRP concentration, was reduced after 3 and 6 months in the patients in the test group (IPT), but not in the control group (MPT) (Fig. 3). Differences between treatment groups (adjusted for age, sex, smoking status, BMI and baseline hs-CRP) was 1.4 mg/L (95% CI [0.5; 2.2]; $p = 0.001$) at 3 months and 1.2 mg/L (95% CI [0.4; 2.0]; $p = 0.004$) at 6 months (Table 3). Post-hoc analyses showed that the proportion of IPT patients with hs-CRP levels ≥ 3 mg/L decreased significantly from 53.1% at baseline to 40.6% (3 months) and to 43.8% (6 months) with p values < 0.001 for both time points. The proportion of MPT patients with hs-CRP levels ≥ 3 mg/L increased from 54.8% at baseline to 58.1% at 3 and 6 months ($p < 0.05$). The odds ratio for a patient to move from a hs-CRP value ≥ 3 mg/L to a hs-CRP value < 3 mg/L, if assigned to the IPT group versus the MPT group, was 5.4 (95% CI [1.0; 31.6]; $p = 0.040$). At 6 months, the percentage of patients in the IPT group experiencing a reduction in hs-CRP levels was 68.8%, while this percentage was 29.0% in the MPT group ($p < 0.001$).

Among the variables included in the multilevel linear regression (treatment group, baseline hs-CRP values, age, sex, smoking habit and BMI), only belonging to the IPT group ($p = 0.004$), baseline hs-CRP levels ($p < 0.001$) and being a smoker ($p = 0.014$), significantly and independently predicted the variance of hs-CRP decline over 6 months. Further analyses indicated a significant interaction between smoking status and treatment group ($p < 0.001$) with post-hoc testing demonstrating that the differences in hs-CRP reduction

were statistically significant between groups only in never-smokers ($p = 0.022$) and former-smokers ($p = 0.001$). However, this finding may be associated with the small number of current smokers in the MPT group ($n = 3$).

In the secondary outcomes, significant reductions in IL-1 β and TNF- α at 3 months were observed in the IPT group, when compared to the MPT group (Table 3). However, differences between groups were observed for these biomarkers at 6 months, neither for the other inflammatory markers (α -1 antitrypsin, fibrinogen, white blood cell count, IL-6 and IL-8) at any time point after therapy. No differences in BMI or waist circumference between patients in the IPT or MPT groups were observed throughout the study.

Metabolic, vascular and renal outcomes

HbA1c was reduced, after 3 months, in patients in the IPT group when compared to the MPT group (Table 4, Fig. 4), with a difference, after adjustment for covariates, of 0.3% (95% CI: [0.1; 0.6]; $p = 0.013$). Post-hoc analyses showed that the proportion of patients with HbA1c $\geq 7\%$ decreased significantly in the IPT group, from 31.25% at baseline to 18.8% at 3 months ($p = 0.028$), with no changes in the MPT group. No differences between groups were observed at 6 months. FPG, fasting insulin concentrations, HOMA2 scores and lipid fractions did not significantly differ between groups or visits. The multilevel linear regression determined that the variance in HbA1c, observed at 3 months, was only significantly predicted by being in the IPT group ($p = 0.013$) and by the baseline HbA1 percentage ($p < 0.001$), without any significant additional effect in the model for age, sex, BMI or smoking status.

IPT significantly reduced blood pressure at 3 months (Table 4, Fig. 4). Systolic blood pressure was significantly reduced at 3 months in the IPT group, when compared to the MPT group, after adjustment for covariates (7.3 mm Hg; 95% CI: [1.9; 12.6]; $p = 0.008$). The reduction for diastolic blood pressure in the IPT group, when compared to the MPT group, lasted for 6 months (at 3 months, 7.8 mm Hg; 95% CI: [1.3; 14.4]; $p = 0.019$; at 6 months, 11.0 mm Hg; 95% CI 2.9, 19.1; $p = 0.009$). No differences in creatinine between patients in the IPT or MPT groups were observed throughout the study.

Discussion

The present study has demonstrated that an effective periodontal treatment in patients with severe periodontitis and MetS results in significant reductions of systemic inflammation and atherosclerotic risk biomarkers. The tested periodontal treatment, consisting on non-surgical subgingival instrumentation with adjunctive systemic antibiotic administration (azithromycin 500 mg, q.d., for three days), when compared to a control group with community periodontal care (supragingival plaque control), resulted in statistically significant reductions of hs-CRP, IL-1 β and TNF- α at 3 months, and hs-CRP at 6 months. Furthermore, significant improvements in vascular function and significant reductions in HbA1c also occurred in the test group.

The impact of this tested periodontal intervention on hs-CRP was a 30.8% reduction from baseline values and a difference of 1.2 mg/L at 6 months when compared with the control treatment. These results show

that the improvements in periodontal health in these MetS patients, in spite of being actively following strict cardiovascular risk reduction protocols (including specific cardioprotective drug therapies), significantly improved their hs-CRP levels, and hence their cardiovascular risk, since it has been recently reported that hs-CRP reductions significantly reduces cardiovascular events, cardiovascular mortality and all-cause mortality [34].

These results are coincident with a previous RCT evaluating the effect of periodontal treatment in patients with MetS, also reporting significant reductions of hs-CRP levels at 12 months, although in that trial no significant differences among the treatment groups were detected, when comparing the additional effect of adjunctive use of systemic antibiotics [35].

Since MetS is a cluster of medical conditions associated with cardiovascular risk, these results would, therefore, apply to any condition with increased cardiovascular risk. Indeed clinical trials and systematic reviews with meta-analyses, evaluating the impact of periodontal treatment in CVD patients undergoing secondary prevention, have shown similar outcomes [36–41]. Bokhari et al., assessing the impact of periodontal treatment in patients with coronary heart disease (CHD) and periodontitis, reported a 30% decrease in mean hs-CRP levels [37]. In contrast, the results from the *Periodontitis and Vascular Events (PAVE)* study carried out in CHD patients with periodontitis, reported that periodontal therapy was not able to maintain the reduction of serum hs-CRP levels at 6 months. However, this study population did not have severe periodontitis and the periodontal therapy rendered was not able to demonstrate a significant effect on the periodontal parameters, when compared with the control group, with community periodontal care. In the present investigation, however, all patients had severe periodontitis, and there was a clear and significant improvement in all periodontal outcomes in the test group, when compared with the control group. These facts should clearly explain the reported differences between the studies

In spite of the fact that periodontitis is defined as a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms [28], most of the previous reports evaluating the impact of periodontal treatment on cardiovascular risk have relied on surrogate markers of exposure, either clinical (probing depths) or radiographic (bone loss), but they did not assess the exposure by measuring the bacterial burden or the presence of putative pathogens. In the present investigation we have identified high counts of anaerobic bacteria and high proportions and counts of *P. gingivalis*, a keystone periodontal pathogen strongly associated with periodontitis, in all patients at baseline. The tested intervention significantly reduced, both the counts of anaerobic bacteria and *P. gingivalis*. Although previous cross-sectional studies have shown an independent significant association between presence of a significant subgingival bacterial burden and levels of periodontal pathogens with surrogate measurements of atherosclerotic risk as intima-media thickness (IMT) or hs-CRP [42, 43], this is the first RCT demonstrating that effective periodontal treatment significantly reduced these microbiological exposure measurements and that this microbiological impact was associated with significant reductions in hs-CRP.

The impact of periodontal therapy on inflammatory cytokines (IL-1 β , IL-6, IL-8 and TNF- α) was also evaluated at 3 and 6 months. IL-1 β and TNF- α showed significant reductions at 3 months, but not at 6 months. These results are also in agreement with a recent RCT in periodontitis patients with type 2 diabetes, where significant differences between groups were noted in TNF- α after treatment [40], and corroborate the referred impact of an effective periodontal treatment on cardiovascular risk, since both IL-1 β and TNF- α have a pro-atherogenic effect and have been associated as significant and independent predictors of cardiovascular events and overall mortality [44].

Other significant improvements associated to the effective periodontal treatment were the significant reductions (0.3%) in HbA1c, at 3 months, and a significant reduction in arterial blood pressure, thus suggesting that this effect was not limited to the improvement in systemic inflammation, but also on the vascular function and metabolic control. In fact, periodontal inflammation has been associated with both insulin resistance and endothelial dysfunction [39, 45, 46]. The fact that the significance in HbA1c reduction in the treatment group was lost at 6 months, may be due to the lack of repeated periodontal interventions during the course of the study. Effective periodontal maintenance care has shown in other studies to maintain the positive effect in the glycaemic control [29, 38, 40].

Similarly, recent studies have shown a significant association between periodontitis and hypertension and have suggested that periodontal treatment could reduce arterial blood pressure [47, 48]. In this RCT, we have reported an adjusted statistically significant reduction of systolic and diastolic blood pressure (7.8 and 7.3 mmHg, respectively), three months after effective periodontal treatment. These findings may be meaningful, since a 10 mmHg reduction in systolic blood pressure or a 5 mmHg reduction in diastolic blood pressure has been associated with a 25–30% reduction of cardiovascular events [49], and half of the hypertensive adults, remain poorly controlled in spite of effective medications [50].

The present clinical trial, however, may have relevant limitations. One is that the tested intervention consisted on a combination of subgingival debridement and the adjunctive administration of azithromycin. This antibiotic has shown significant reductions in a global inflammatory score comprised by hs-CRP, IL-1 β , IL-6 and TNF- α , at 6 months, but not on CRP alone [51], and hence, the cardiovascular effect shown in this study could be attributed to the antibiotic rather than the periodontal therapy. However, the adjunctive use of systemic antimicrobials to subgingival instrumentation is clearly justified by the periodontitis severity in the selected sample, since this modality of therapy has demonstrated a significant added effect on clinical and microbiological outcomes when compared to subgingival debridement alone in severe periodontitis patients [52], as well as in randomized clinical trials evaluating the impact of intensive periodontal therapy in cardiovascular risk outcomes [40, 46].

Another possible limitation could be the selection of the main outcome variable (hs-CRP) as a surrogate for cardiovascular risk, since its predictive value may be limited [53], although this is the biomarker most frequently reported in screening and cardiovascular risk reclassification [54–56]. Further investigations should also focus on other surrogate measures of subclinical atherosclerosis, like the coronary artery calcium (CAC) or, when possible, on true endpoints, such as major cardiovascular events.

Conclusions

In conclusion, the results of the present study provide evidence that effective periodontal therapy reduced the cardiovascular risk in patients with MetS and severe periodontitis by significantly reducing hs-CRP levels, pro-inflammatory mediators, blood pressure and HbA1c levels. Larger studies with longer follow-up are needed to determine whether these shown short-term benefits can be sustained long-term and result in reductions in the cardiovascular morbidity and mortality in these patient population.

Abbreviations

MetS, metabolic syndrome; IPT, intensive periodontal treatment; MPT, minimal periodontal treatment; hs-CRP, high sensitivity C reactive protein; ITT, intention-to-treat; PP, per protocol; IL, interleukin; TNF, tumor necrosis factor, HbA1c, glycated haemoglobin, CVD, cardiovascular disease; HOMA, homeostasis model assessment; WC, waist circumference; BMI, body mass index; FPG, fasting plasma glucose; HDL, high density lipoprotein; LDL, low density lipoprotein; PII, plaque index; CAL, Clinical attachment level; PPD, probing pocket depth, BOP, bleeding on probing; SRP, scaling and root planning; RTF, reduced transport fluid; EDTA, ethylenediaminetetraacetic acid; LOCF, last observation carried forward.

Declarations

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Not applicable.

Authors' contributions

All authors contributed significantly to this work. MS, DH and AZ conceived the study. EM, ML, HV, MM, JM and LV contributed to data collection. Statistical analysis was carried out by EM. EM drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All participants gave their informed consent. The protocol of this investigation was reviewed and approved by the institutional ethic committee (Internal Code 12/206, Hospital Clínico de San Carlos, Madrid).

Consent for publication

Not applicable

Competing interests

All authors declare that they have no conflict of interests.

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Tables

Table 1. Baseline characteristics of the intention-to-treat population with metabolic syndrome (MetS).

Variable ^a	Intensive Periodontal Therapy (n=32)	Minimal Periodontal Therapy (n=31)	p value
	56.7 (6.5)	58.3 (5.8)	0.319
			0.839
Male (%)	22 (68.8%)	22 (70.9%)	
Female (%)	10 (31.2%)	9 (29.1%)	
			0.112
Never smoker (%)	15 (46.9%)	11 (35.5%)	
Former smoker (%)	9 (28.1%)	17 (54.8%)	
Current Smoker (%)	8 (25.0%)	3 (9.7%)	
Index (BMI; kg/m ²)	39.1 (5.6)	38.0 (4.7)	0.618
Waist circumference (WC) (cm)	120.1 (18.4)	119.0 (9.1)	0.848
Blood pressure (BP; mm Hg)			
Systolic BP (mm Hg)	148.1 (21.5)	138.6 (18.3)	0.112
Diastolic BP (mm Hg)	91.3 (18.2)	84.1 (11.2)	0.143
Serum C-reactive protein (CRP; mg/L)	3.9 (2.9)	3.9 (3.4)	0.831
	2.8 (IQR 1.5 - 4.9)	2.4 (IQR 1.4-4.9)	
High CRP levels ≥3 mg/L (%)	17 (53.1%)	17 (54.8%)	0.975
Glucose (mg/dL)	145.6 (29.7)	138.5 (28.1)	0.369
Insulin (mIU/L)	419.7 (108.7)	398.5 (89.1)	0.259
White blood cell count (WBC; K/ μ L)	7.8 (1.9)	7.5 (1.7)	0.445
Pro-inflammatory mediators in serum (pg/mL)			
IL-1 β	1.5 (0.9)	1.9 (1.2)	0.161
IL-6	2.2 (1.8)	2.8 (1.9)	0.178
	1.9 (IQR 1.3-2.3)	2.8 (IQR 1.4-3.9)	
IL-8	6.9 (9.7)	5.4 (3.0)	0.105
	3.3 (IQR 2.3-5.7)	4.7 (IQR 3.5-6.3)	
TNF- α	7.9 (6.2)	8.7 (8.6)	0.957
	5.7 (IQR 4.8-10.2)	6.3 (IQR 4.1-8.9)	
	6.3 (1.2)	6.0 (1.0)	0.328
	6.1 (IQR 5.5-6.9)	5.7 (IQR 5.2-6.5)	
Subjects with HbA1c \geq 7 % (%)	10 (31.25%)	8 (25.8%)	0.774
Glucose (FPG; mg/dL)	128.6 (30.3)	133.0 (51.7)	0.500
	125 (IQR 107-154)	112 (IQR 97-159)	
Insulin (mIU/L)	19.3 (10.8)	14.5 (9.3)	0.033
	18.7 (IQR 12.3-27.6)	11.1 (IQR 9.4-15.8)	
Insulin resistance	104.8 (69.0)	92.7 (50.6)	0.519
Insulin sensitivity	59.0 (55.3)	62.6 (28.0)	0.061
	38.3 (IQR 30.0-62.3)	66.5 (IQR 43.3-75.1)	
Insulin resistance	2.6 (1.4)	2.0 (1.2)	0.063
	2.6 (IQR 1.6-3.3)	1.5 (IQR 1.3-2.3)	
Urea nitrogen (mg/dL)	174.8 (34.7)	189.4 (48.4)	0.203
Urea nitrogen (mg/dL)	46.1 (13.3)	46.9 (12.4)	0.858
Urea nitrogen (mg/dL)	114.3 (34.7)	105.7 (44.9)	0.535
Urea nitrogen (mg/dL)	129.5 (52.3)	136.6 (42.5)	0.582
Urea nitrogen (mg/dL)	0.9 (0.5)	0.9 (0.3)	0.461
	0.8 (IQR 0.7-0.9)	0.9 (IQR 0.8-1.0)	

^a Data are mean (standard deviation, SD), n (%), or median (interquartile range, IQR).

HOMA2, Homeostasis Model Assessment 2.

Table 2. Periodontal parameters in both groups over time.

Variable	Baseline (BL)		3 months (3M)		ΔBL-3M		6 months (6M)		ΔBL-6M	
	Mean (SD)	p value	Mean (SD)	p value	Mean (SD)	p value	Mean (SD)	p value	Mean (SD)	p value
(0-3)										
IPT Group	1.8 (0.4)	0.444	0.8 (0.3)*	0.002	1.0 (0.5)	0.012	0.8 (0.3)*	0.003	1.0 (0.4)	0.013
MPT Group	1.9 (0.5)		1.3 (0.7)*		0.6 (0.6)		1.2 (0.6)*		0.7 (0.5)	
κ (0-3)										
IPT Group	1.8 (0.4)	0.091	0.6 (0.2)*	<0.001	1.2 (0.5)	<0.001	0.6 (0.3)*	<0.001	1.1 (0.5)	<0.001
MPT Group	2.0 (0.4)		1.4 (0.7)*		0.6 (0.6)		1.4 (0.6)*		0.5 (0.5)	
Bleeding Score (%)										
IPT Group	59.8% (20)	0.139	24.9% (17)*	<0.001	32.8% (21)	0.025	20.5% (11)*	<0.001	37.6% (17)	<0.001
MPT Group	67.8% (20)		48.4% (21)*		19.4% (20)		51.6% (18)		16.1% (21)	
Intental Pocket Depth										
IPT Group	3.7 (0.9)	0.747	2.7 (0.3)*	<0.001	1.1 (0.6)	<0.001	2.6 (0.3)*	<0.001	1.2 (0.6)	<0.001
MPT Group	3.8 (0.9)		3.5 (0.7)		0.3 (0.8)		3.5 (0.6)		0.3 (0.8)	
Inf Periodontal Pockets										
IPT Group	55.3% (22)	0.427	13.9% (12)*	<0.001	39.4% (20)	<0.001	11.2% (10)*	<0.001	43.7% (20)	<0.001
MPT Group	59.7% (19)		41.2% (23)*		18.5% (18)		43.9% (21)*		15.9% (19)	
Inf Periodontal Pockets										
IPT Group	3.5% (6)	0.739	0.1% (0)*	0.013	4.0% (6)	0.007	0.3% (1)*	0.016	3.8% (6)	0.018
MPT Group	4.1% (7)		3.6% (6)		0.6% (2)		3.3% (6)		0.8% (2)	
Int Attachment Level										
IPT Group	4.9 (1.0)	0.293	3.9 (1.0)*	0.010	0.9 (0.7)	0.001	3.8 (0.9)*	0.001	1.0 (0.8)	<0.001
MPT Group	5.2 (1.3)		4.8 (1.4)		0.3 (0.5)		4.9 (1.3)		0.3 (0.6)	
eth										
IPT Group	22.6 (3.8)	0.611	21.9 (4.1)	0.944	0.7 (1.5)	0.130	21.9 (4.1)	0.944	0.7 (1.5)	0.130
MPT Group	22.0 (4.2)		21.9 (4.2)		0.2 (0.5)		21.9 (4.2)		0.2 (0.5)	

* Intra-group significant difference when compared to baseline (p<0.05)

IPT, Intense Periodontal Therapy; MPT, Minimal Periodontal Therapy; SD, standard deviation.

Table 3. Inflammatory biomarkers and clinical variables 3 and 6 months after periodontal treatment, by study group.

Variable	3 months (3M)			6 months (6M)		
	Mean (SE) or n (%)	Δ 3M (95% CI)	p value	Mean (SE) or n (%)	Δ 6M (95% CI)	p value
CRP (mg/L)						
IPT Group	2.7 (0.4)*	1.4 (0.5, 2.2)	0.001	2.9 (0.4)*	1.2 (0.4, 2.0)	0.004
MPT Group	3.9 (0.6)			4.0 (0.8)		
CRP levels \geq 3 mg/L (%)						
IPT Group	13 (40.6%)*	5.4 (1.0, 31.6)	0.040	14 (43.8%)*	2.6 (0.4, 15.9)	0.312
MPT Group	18 (58.1%)			18 (58.1%)		
IL (pg/dL)						
IPT Group	138.4 (6.2)	-4.9 (-15.6, 5.8)	0.368	137.5 (5.7)	-6.9 (-20.3, 6.4)	0.308
MPT Group	130.0 (5.0)			127.6 (5.2)		
TNF (pg/dL)						
IPT Group	421.8 (20.4)	-27.4 (-64.3, 9.4)	0.144	419.6 (21.8)	-25.0 (-66.6, 16.5)	0.238
MPT Group	398.3 (17.9)			400.5 (16.1)		
WBC (K/ μ L)						
IPT Group	7.5 (0.4)	-0.1 (-0.7, 0.5)	0.768	7.9 (0.7)	-0.4 (-1.5, 0.7)	0.515
MPT Group	7.8 (0.3)			7.6 (0.2)		
IL-6 (pg/mL)						
IPT Group	0.9 (0.1)*	1.2 (0.0, 2.4)	0.046	1.5 (0.2)	-0.1 (-0.5, 0.3)	0.601
MPT Group	2.3 (0.5)			1.5 (0.2)		
IL-1 β (pg/mL)						
IPT Group	1.9 (0.4)	0.3 (-0.8, 1.3)	0.584	2.0 (0.4)	0.1 (-0.9, 1.2)	0.806
MPT Group	2.6 (0.4)			2.5 (0.4)		
IL-18 (pg/mL)						
IPT Group	4.6 (1.1)*	1.0 (-0.9, 2.8)	0.307	5.0 (1.2)	0.8 (-2.0, 3.5)	0.584
MPT Group	5.4 (0.8)			6.0 (1.2)		
IL-17 (pg/mL)						
IPT Group	6.4 (0.8)*	3.2 (0.2, 6.2)	0.037	6.3 (0.8)*	1.6 (-1.6, 4.8)	0.333
MPT Group	10.0 (2.3)			8.2 (1.4)		
BMI (kg/m ²)						
IPT Group	39.1 (1.6)	0.1 (-0.2, 0.5)	0.503	39.2 (1.6)	0.0 (-0.1, 0.2)	0.611
MPT Group	38.0 (1.6)			38.0 (1.6)		
Waist circumference (WC; cm)						
IPT Group	120.1 (4.5)	0.2 (-0.5, 1.0)	0.527	120.1 (4.6)	0.4 (-0.1, 1.0)	0.115
MPT Group	119.2 (2.7)			119.2 (2.7)		

Data are unadjusted mean and standard error (SE). p values are for the adjusted Δ values (Δ 3M and Δ 6M), that are adjusted for baseline values, age, sex, smoking habit and body mass index. In case of the percentage of subjects with CRP levels \geq 3 mg/L, the adjusted Δ values are expressed as odds ratios and 95% confidence intervals (CI). Adjustment for BMI was made for baseline values, age, sex and smoking habit. * Intra-group significant difference when compared to baseline (p<0.05). IPT, Intense Periodontal Therapy; MPT, Minimal Periodontal Therapy; SIS, Summary Inflammatory Score; IL, interleukin; TNF, tumor necrosis factor

Table 4. Metabolic, vascular and renal measures at 3 and 6 months after periodontal treatment by study group.

Variable	3 months (3M)			6 months (6M)		
	Mean (SE) or n(%)	Δ 3M (95% CI)	p value	Mean (SE) or n(%)	Δ 6M (95% CI)	p value
IPT Group	5.9 (0.1)*	0.3 (0.1, 0.6)	0.013	6.0 (0.1)*	0.2 (0.0, 0.5)	0.110
MPT Group	6.1 (0.2)			6.1 (0.2)		
Δ IC \geq 7 % (%)						
IPT Group	6 (18.8%)*	21.7 (0.8-623.3)	0.073	9 (28.1%)	0.7 (0.1-4.9)	0.739
MPT Group	8 (25.8%)			7 (22.6%)		
Glucose (FPG; mg/dL)						
IPT Group	123.3 (7.9)	12.8 (-3.0, 28.6)	0.112	121.0 (6.3)	8.9 (-9.2, 26.9)	0.336
MPT Group	130.0 (8.8)			130.5 (9.7)		
U/L)						
IPT Group	17.2 (2.9)	-1.4 (-6.5, 3.6)	0.583	14.3 (2.1)	1.3 (-3.0, 5.7)	0.548
MPT Group	14.1 (1.4)			14.4 (1.7)		
Protein						
IPT Group	106.0 (14.8)	-13.6 (-37.2, 10.1)	0.260	106.1 (14.0)	2.2 (-21.6, 26.0)	0.856
MPT Group	87.2 (10.6)			100.4 (11.9)		
Sensitivity						
IPT Group	67.0 (13.6)	-6.1 (-27.3, 15.1)	0.573	65.8 (11.6)	-8.2 (-24.7, 8.3)	0.330
MPT Group	57.9 (5.4)			59.7 (5.3)		
Resistance						
IPT Group	2.3 (0.4)	-0.2 (-0.8, 0.5)	0.666	2.2 (0.3)	0.0 (-0.6, 0.6)	0.940
MPT Group	2.0 (0.2)			2.0 (0.2)		
(mg/dL)						
IPT Group	184.0 (8.4)	-9.7 (-20.9, 1.5)	0.090	183.5 (7.5)	-5.2 (-17.3, 6.9)	0.397
MPT Group	180.6 (8.1)			189.9 (9.2)		
(mg/dL)						
IPT Group	46.2 (3.8)	0.8 (-8.1, 9.7)	0.858	47.2 (2.7)	-4.0 (-8.8, 0.7)	0.097
MPT Group	47.1 (3.1)			48.4 (2.7)		
(mg/dL)						
IPT Group	109.6 (8.5)	-5.7 (-16.9, 5.6)	0.327	107.6 (6.6)	-2.2 (-17.9, 13.4)	0.779
MPT Group	103.5 (7.0)			107.5 (8.3)		
(mg/dL)						
IPT Group	136.5 (9.7)	-0.2 (-18.5, 18.1)	0.984	125.6 (9.7)	1.2 (-16.5, 18.9)	0.895
MPT Group	155.4 (17.5)			131.7 (8.3)		
Blood pressure (mm Hg)						
IPT Group	136.4 (3.0)*	7.3 (1.9, 12.6)	0.008	136.4 (2.8)	4.7 (-11.7, 21.1)	0.574
MPT Group	139.4 (2.7)			144.4 (4.1)		
Blood pressure (mm Hg)						
IPT Group	84.8 (3.8)	7.8 (1.3, 14.4)	0.019	81.8 (2.8)	11.0 (2.8, 19.1)	0.009
MPT Group	89.6 (5.4)			86.9 (1.8)		
(mmHg)						
IPT Group	1.0 (0.1)	0.0 (0.0, 0.1)	0.353	1.0 (0.1)	0.0 (-0.1, 0.1)	0.924
MPT Group	0.9 (0.1)			1.0 (0.1)		

Data are unadjusted mean and standard error (SE). p values are for the adjusted Δ values (Δ 3M and Δ 6M), that are adjusted for baseline values, age, sex, smoking habit and body mass index. In case of the proportions (%), the adjusted Δ values are expressed as odds ratios and 95% confidence intervals (CI). * Intra-group significant difference when compared to baseline (p<0.05). IPT, Intense Periodontal Therapy; MPT, Minimal Periodontal Therapy; MetS, metabolic syndrome; HDL, high-density lipoproteins; LDL, low-density lipoproteins

Figures

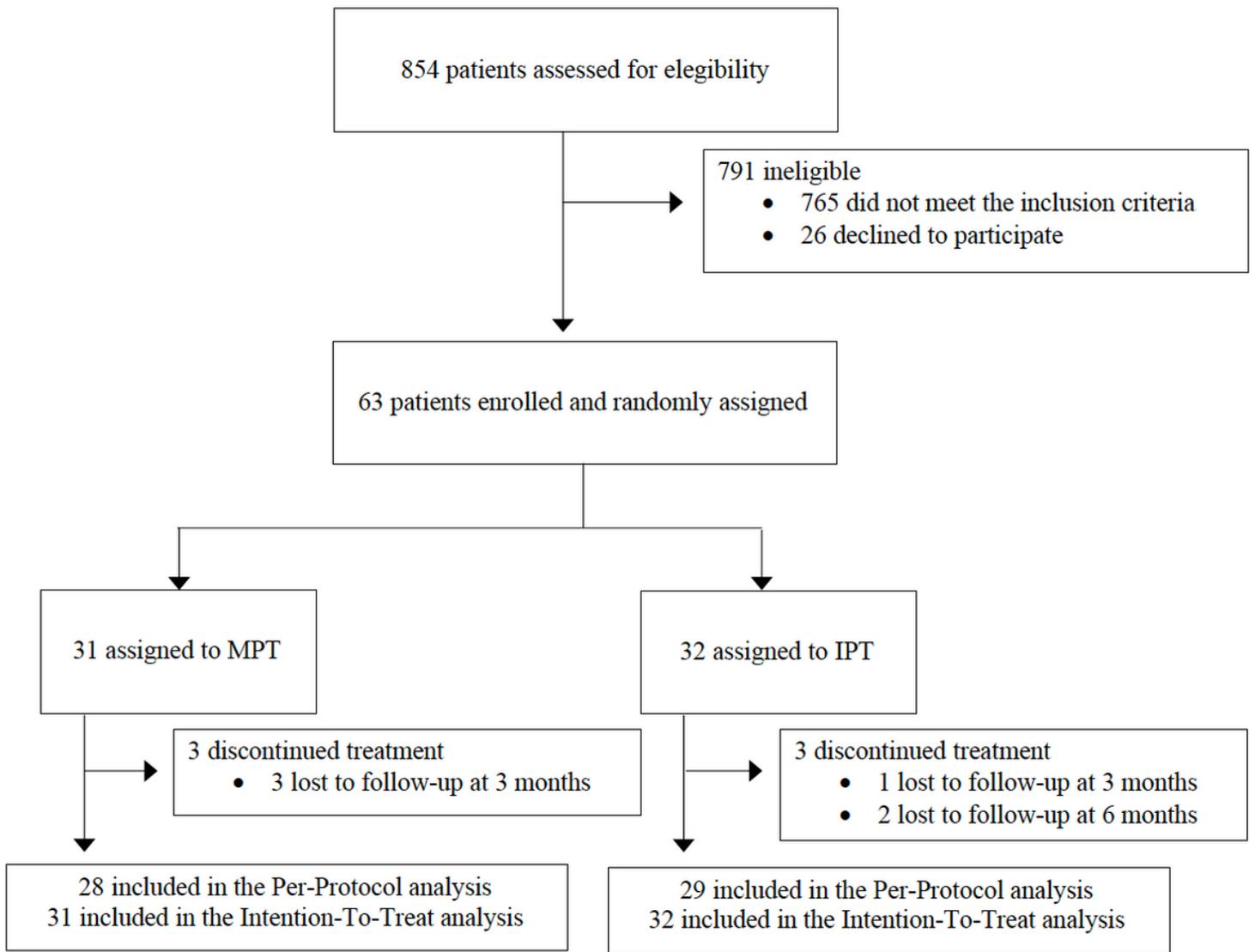


Figure 1

Flow-Chart of the Trial

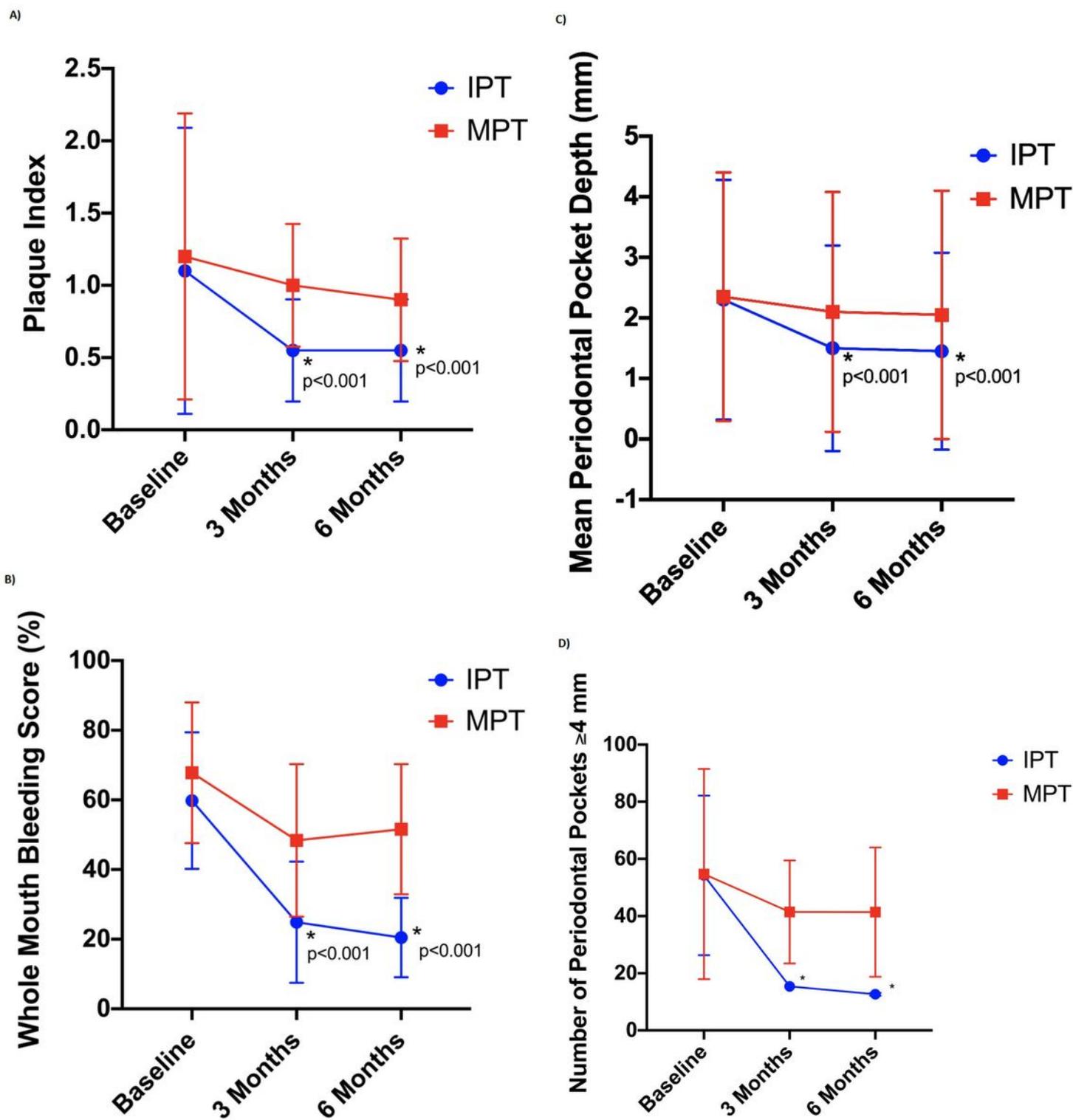


Figure 2

Periodontal measures at baseline and follow-up visits at 3 and 6 months. Data are unadjusted means and standard deviations (SDs). IPT, Intense Periodontal Therapy; MPT, Minimal Periodontal Therapy. * Between-groups differences were significant for all periodontal measures at 3 and 6 months ($p < 0.01$)

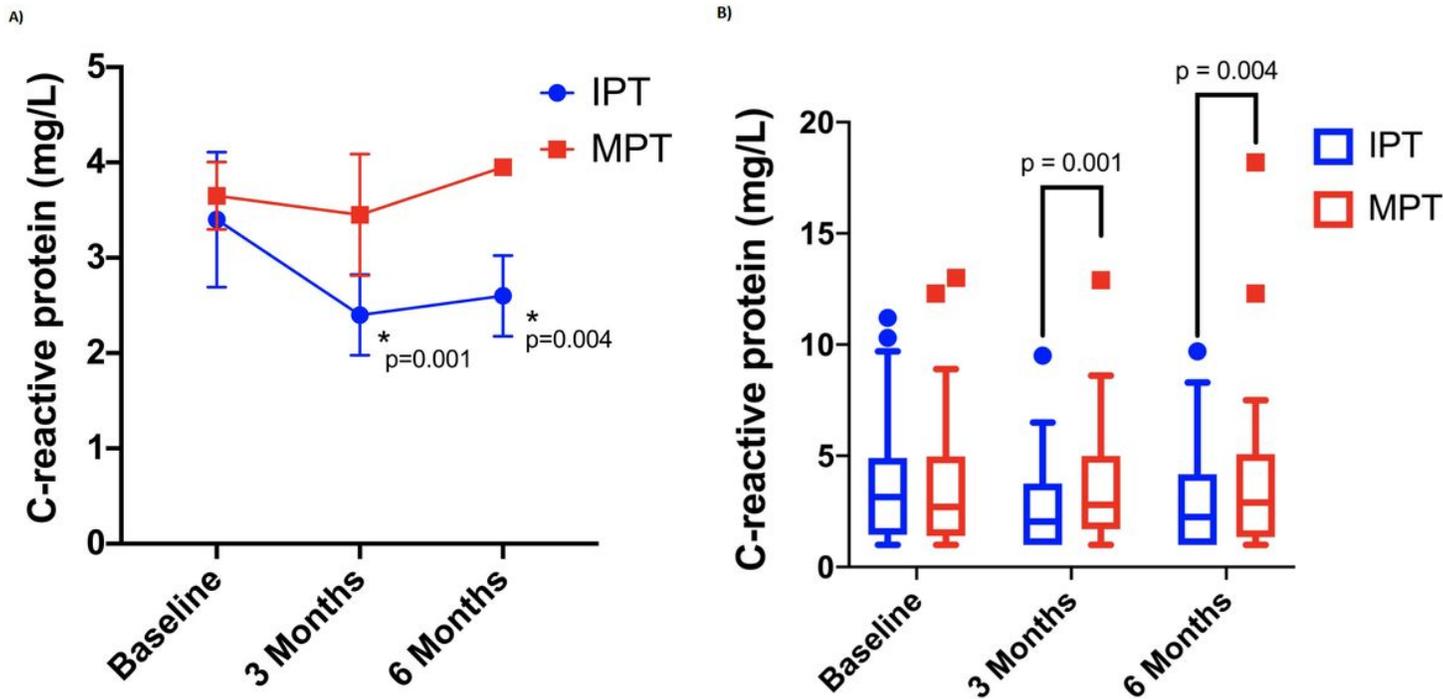


Figure 3

C-reactive Protein (CRP) during the 6-month study period. A) Data are unadjusted means and standard deviations (SDs). Adjusted p-values (for age, sex, smoking, baseline values and body mass index) for between-group difference at 3 and 6 months were $p=0.001$ and $p=0.004$, respectively. B) Boxplot showing CRP concentrations over visits by study group. Values are presented as median, 25% and 75% quantiles, whiskers corresponding to minimum and maximum values within the interval [25% quantile – 1.5 x interquartile range, 75% + 1.5 x interquartile range], and outliers. IPT, Intense Periodontal Therapy; MPT, Minimal Periodontal Therapy.

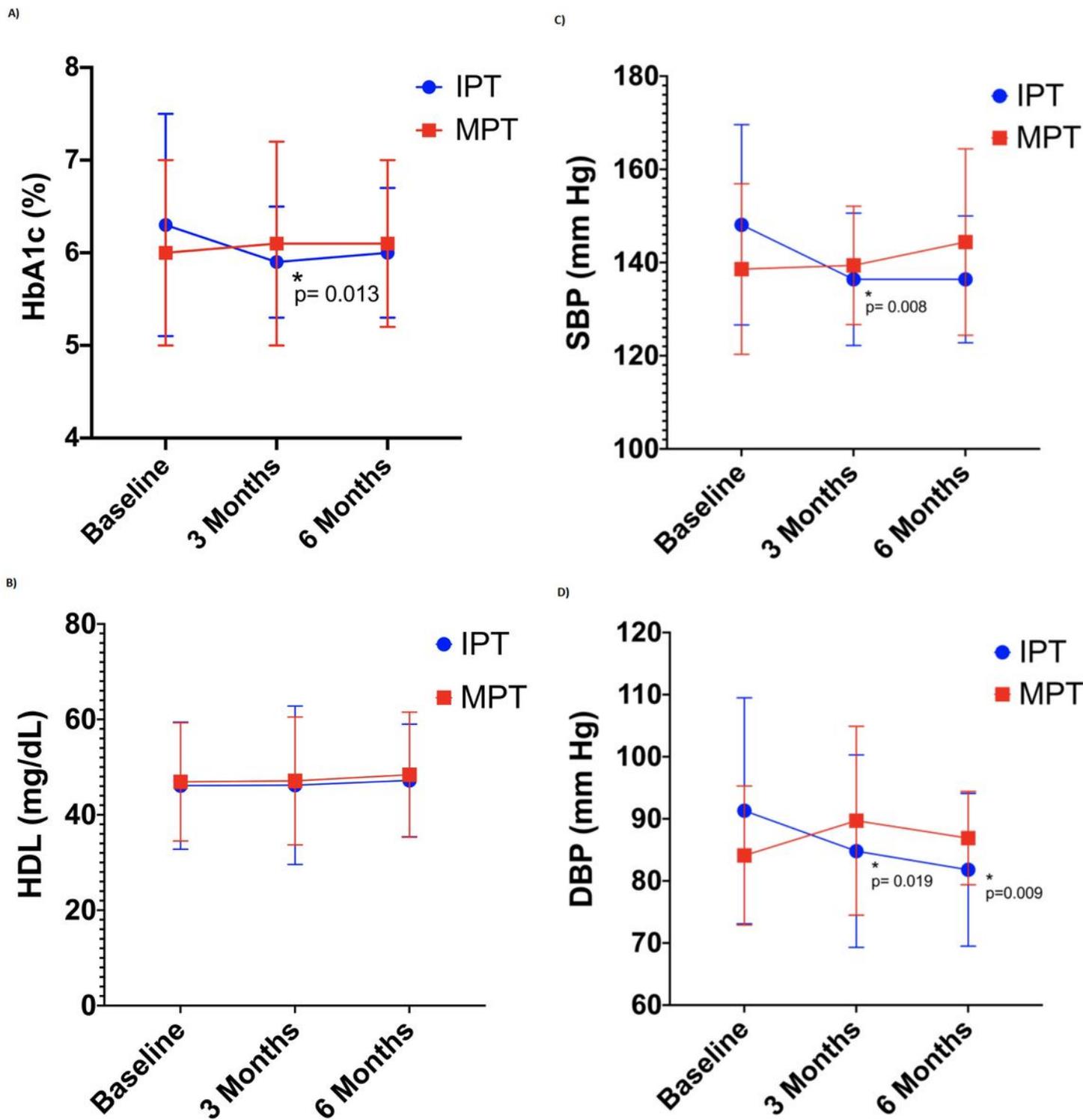


Figure 4

Metabolic and vascular measures at baseline and follow-up visits at 3 and 6 months. Data are unadjusted means and standard deviations (SDs); *p*-values are adjusted for age, sex, smoking, baseline values and body mass index. A) Glycated haemoglobin (HbA1c, %) during the 6-month study period. B) High-density lipoproteins (HDL) during the 6-month study period. C) Systolic blood pressure (SBP) during the 6-month study period. D) Diastolic blood pressure (DBP) during the 6-month study period.

Supplementary Files

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