Acute-phase response following full-mouth versus quadrant non-surgical periodontal treatment in subjects with comorbid type 2 diabetes: A randomized clinical trial

By

Morena Petrini[1,2*], Stefano Gennai[1,3*], Crystal Marruganti[1,3,4], Marco Nisi[1,3], Rossana Izzetti[1,3], Marina Peric[1,3], Urska Marhl[1,3], Filippo Graziani [1,3]

1. Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy.
2. Department of Medical, Oral and Biotechnological Science, University of Chieti, Chieti, Italy.
3. Sub-Unit of Periodontology, Halitosis and Periodontal Medicine, University Hospital of Pisa, Pisa, Italy.
4. Unit of Periodontology, Endodontology and Restorative dentistry, Department of Medical Biotechnologies, University of Siena, Siena, Italy.

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Abstract

Aim: To compare the level of inflammatory markers and endothelial function 24 hours (Day1) and 90 days (Day90) after conventional quadrant-wise (Q-SRP) versus full-mouth scaling (FM-SRP) in patients affected by type 2 diabetes mellitus (T2DM).

Methods: 40 patients affected by periodontitis and T2DM were randomly allocated to receive FM-SRP or Q-SRP and followed up at Day1 and Day90. Serum samples, vital signs and flow-mediated dilation (FMD) parameters were collected at baseline, Day1 and Day90. Periodontal variables were collected at baseline and Day90.

Results: FM-SRP produced a significant increase in C-reactive Protein (CRP) and a significant reduction in FMD at Day1 compared to Q-SRP ($p<0.05$). The absolute change in HbA1c from baseline to Day90 was significantly improved in the Q-SRP (ΔHbA1c=-1.59±1.20) compared to the FM-SRP group (ΔHbA1c=-0.8±0.95) ($p=0.04$). The linear multivariate regression model showed that the higher the relative C-reactive Protein (CRP) increase at Day1, the lower the reduction in HbA1c levels (ΔHbA1c) at Day90 ($F=2.12$; $p=0.04$; adjusted $R^2=41.04\%$).

Conclusions: FM-SRP triggers a moderate acute-phase response at 24 hours after treatment compared to Q-SRP. Such systemic acute perturbations seem to offset the benefic systemic effects of periodontal treatment in terms of HbA1c reduction and improvement in endothelial function in T2DM subjects.

1 Introduction

Periodontitis is a chronic inflammatory disease that was found to be associated with several non-communicable diseases, including obesity, metabolic syndrome, cardiovascular diseases, and diabetes mellitus (Charupinijkul et al., 2022; Jepsen et al., 2020; Sanz et al., 2020a). A large body of evidence investigated the relationship between periodontitis and diabetes extensively. Subjects with periodontitis present higher serum levels of HbA1C and fasting blood glucose, as well as a higher prevalence of prediabetes/diabetes compared to healthy controls. Moreover, subjects with severe forms of periodontitis have a 29% increased risk for diabetes compared to healthy subjects (Graziani et al., 2018). In turn, poor glycemic control in subjects with diabetes is associated with an unhealthy periodontal status and treatment outcomes (Sanz et al., 2018).

Inflammation embodies the main biological mechanism underpinning the relationship between periodontitis and type 2 diabetes mellitus (T2DM). Hyperglycemic conditions can directly promote pro-inflammatory host response in the periodontal environment via the advanced glycation end product/receptor for the advanced glycation end-product axis. This environmental state increases the production of pro-inflammatory cytokines resulting in a bidirectional influence between diabetes and periodontitis (Polak et al., 2020). Consistent reports show that non-surgical periodontal therapy (NSPT) can positively affect glycemic control, improving serum HbA1c levels (Madianos & Koromantzos, 2018). So, it is recommended to treat periodontitis in T2DM patients in order to minimize the systemic consequences and thus restoring tissue homeostasis.

Although the systemic benefits of NSPT have been widely described in literature (Graziani et al., 2010; Madianos & Koromantzos, 2018), NSPT was shown to be associated with an acute inflammatory response characterized by a post-operative increase in systemic inflammatory

markers, such as C-reactive Protein (CRP) and Interleukin-6 (IL-6) (Graziani et al., 2015, 2019). Moreover, the duration of NSPT was linked with the magnitude of the systemic inflammatory response after treatment. Indeed, a quadrant scaling approach (Q-SRP) produces a significantly lower acute phase response compared to a full mouth approach (FM-SRP) in terms of inflammatory biomarkers (Graziani et al., 2015, 2019).

Such perturbations in both systemic inflammatory and vascular responses can increase the risk for vascular events in subjects with comorbidities; hence opting for a treatment approach characterized by a reduced post-operative acute phase response would be recommendable (Sanz et al., 2020).

Therefore, this study aims to compare quadrant-wise scaling and root planning (Q-SRP) *vs* FM-SRP in terms of acute-phase responses following NSPT in subjects with periodontitis and concomitant T2DM.

2 Materials and Methods

2.1 *Study design*

The current study was a single-center, parallel group, randomized controlled clinical trial with a 3-month follow up. The study protocol was approved by the University Hospital of Pisa Ethics committee (Pisa, Italy) (protocol number 3399) and it was registered in a clinical trials database (NCT03087266).

2.2 *Participant selection*

All individuals attending the Unit of Periodontology at the University Hospital of Pisa (Italy) were screened for eligibility. Subjects were eligible to participate if they met the following

inclusion criteria: i) age between 18 and 70 years; ii) diagnosis of type 2 diabetes mellitus (T2DM) ; iii) diagnosis of periodontitis defined as proximal attachment loss ≥3 mm in ≥2 non-adjacent teeth (Tonetti et al., 2005); iv) at least 20% of the entire dentition with Probing Depth (PD) ≥5 mm; v) Full Mouth Bleeding Score (FMBS) ≥20%. Subjects were excluded if: i) pregnant or lactating females; ii) had a diagnosis of any systemic disease other than diabetes; iii) prescribed with any pharmacological treatment within 3 months before the inclusion in the study except for diabetes medications; iv) they underwent periodontal treatment in the last 6 months. All participants were provided with the subject information sheet and gave written informed consent, which was followed by collection of medical and dental histories and comprehensive oral examination.

2.3 *Clinical Parameters*

At baseline examination, all participants received a full periodontal evaluation by a single masked calibrated examiner. Calibration was assessed on intra-examiner repeatability for Clinical Attachment Level (CAL) measurement. Repeatability was judged adequate when reaching a percentage of agreement within ±2 mm between repeated measurements of at least 98% (Graziani et al., 2010). Periodontal parameters such as PD and recession (REC) were assessed full-mouth and rounded to the nearest millimeter (UNC15 mm periodontal probe); CAL values were obtained by the sum of PD and REC. Plaque and Bleeding on Probing (BoP) were recorded dichotomously six sites per tooth in order to calculate the Full Mouth Plaque Score (FMPS) and the Full Mouth Bleeding Score (FMBS), respectively (Ainamo & Bay, 1975; O'Leary et al., 1972).

2.3.1 *Blood collection and serum markers*

Blood samples were collected from a venipuncture in the antecubital fossa before 8am after an overnight fast for all participants. All analyses were performed by the ISO-certified laboratory at the University Hospital of Pisa (Pisa, Italy) by an operator blinded to group allocation. High-sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides, as well as glycated hemoglobin (HbA1C), insulin and glucose levels, were measured following standard laboratory procedures.

Serum CRP was measured by immunoturbidometry (Cobas, Roche Diagnostic, Manheim, Germany) and a Multiplex array (Meso Scale Discovery, Rockville, Maryland, MD, USA) was used to assay interleukin 6 (IL-6) levels.

Intra-and inter-assay coefficient of variations were all <6%.

2.3.2 *Vital signs*

At baseline examination, vital signs were collected by an experienced operator and encompassed:

- Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured in triplicates using an automatic oscillometric device (OMRON-705IT, Omron, Kyoto, Japan); the mean blood pressure (BP) was then measured as the mean of the last two measurements;
- Heart rate (beats per minute-bpm) was calculated as the average of two measurements;
- Body temperature (Celsius degree-°C) was measured twice with tympanic reading (Genius TM 2, Covidien LLC, USA) and the average measurement was registered.

2.3.3 *Endothelial function*

The Flow-Mediated Dilation (FMD), together with other measures of endothelial function, were recorded by assessing the endothelium-dependent vasodilation of the brachial arteryby means of ultrasound imaging (Acuson XP 128/10, Siemens), as previously performed (Czesnikiewicz-Guzik et al., 2019; Tonetti et al., 2007). Measurements were performed by a single experienced examiner blinded to treatment assignment. FMD was calculated as the change (expressed as percentage) from baseline to the peak diameter 45 to 75 seconds after the release of the blood-pressure cuff of the sphygmomanometer. After 10 minutes of rest, FMD was measured also after sublingual administration of 25µg of nitroglycerin, following the same protocol.

2.3.4 *Health related quality of life*

The impact of periodontal treatment on quality of life in both groups was assessed using the the Oral Health Impact Profile-14 (OHIP-14) (Slade, 1997) questionnaire at baseline, Day 1 and Day 90. The questionnaire was fulfilled independently by the patient in a quiet, separate room.

2.4 *Randomization and allocation concealment*

Participants were randomly allocated in a 1:1 ratio to receive either FM-SRP or Q-SRP therapy by using a computer-generated random sequence. Allocation to treatment was concealed in consecutively numbered opaque envelopes and revealed to the therapist and patient on the day of the treatment.

2.5 *Periodontal treatment*

Before the day of the treatment, all patients received Oral Hygiene Instructions (OHI) and motivation sessions. Patients were instructed to use a rotating oscillating toothbrush (Oral B®) and interproximal brushes; OHIs were reinforced during each treatment session and follow up visits. Supra and subgingival mechanical instrumentation of the root surface was performed by a single periodontist, using both hand and ultrasonic instrumentation with fine tips (EMS, Nyon, Switzerland); local anesthesia was performed only when needed. During the clinical sessions, treatment time was measured with a chronometer by a research nurse. FM-SRP was performed within a single session. Q-SRP was performed one quadrant per session, with an interval on 1 week in between instrumentation sessions for a total of 3 weeks. The first session always comprised the instrumentation of the upper right quadrant; alternatively, the left maxillary quadrant was chosen if less than 6 teeth were present.

2.6 *Follow ups*

Figure 1 shows the study outline. All patients were re-examined at Day1 and Day90. Day1 in the FM-SRP group was defined as 24 hours upon completion of the instrumentation, while Day1 in the Q-SRP group was defined as 24 hours after the first session of treatment. On the other hand, Day90 in the FM-SRP was 90 days after the completion of the treatment, and Day90 in the Q-SRP was 90 days after the completion of the last session of treatment. Recorded variables at each timepoint are shown in Figure 1.

2.7 *Statistical analysis*

All analyses were performed through an *ad hoc* statistical software (STATA BE, version 17, StataCorp LP, Texas, USA) setting the level of significance at 5%. Sample size was estimated in order to detect an intergroup 3.5mg/l difference in serum CRP levels at Day1 (D'Aiuto et

al., 2004); considering a power of 90% and alpha=0.05 and in order to compensate for a 10% drop-out rate, a final sample of 20 participants per group was planned.

Continuous variables were expressed as mean and Standard Deviation (SD), while categorical variables were expressed as proportions. Variables were adjusted for baseline characteristics according to external knowledge. Whenever present, missing data were handled with the Last Observation Carried Forward approach. After verification of data distribution, intra- and intergroup comparisons were performed through parametric or corresponding non-parametric tests. Correlations were performed with Spearman rank analyses. A linear multivariate regression model was built to evaluate the impact of the relative CRP difference at Day1 (Day1 concentration minus baseline, divided by baseline and multiplied by 100) on the absolute change in HbA1c (ΔHbA1c) between Day90 and baseline. The best model was chosen according to the lowest value of Cp Mallow's coefficient. Results of the OHIP-14 were presented as severity, *i.e.* the sum of the response codes to all 14 items, and as extent, *i.e.* the number of items identified as 'Fairly often' or 'Very often' (Tsakos et al., 2012).

3 Results

3.1 *Participant characteristics*

Three-hundred and twenty-seven individuals were screened for eligibility; then, forty individuals were eventually enrolled in the current study. No participant was lost to follow up at Day90. The study sample was constituted mainly by males, obese, non-smoking subjects approximately in their sixth decade of life; no differences were present between the two groups at baseline (Table 1).

3.2 *Clinical parameters*

Both groups showed significant benefits in terms of periodontal parameters. Major reductions in the percentage and number of pockets, plaque accumulation and bleeding on probing were noted at Day90 (p<0.01), with no intergroup differences (p>0.05) (Table 2).

3.2.1 *Serum markers and vital signs*

Results of serum makers and vital signs are shown in Table 3. FM-SRP group showed significantly higher CRP values compared to Q-SRP group (p<0.05) (5.55±6.27 and 4.33±6.76, respectively) (Table 3); the relative CRP increase at Day1 was significantly higher in the FM-SRP group than in the Q-SRP group and it was positively associated with treatment time (R=0.53; p<0.01). FM-SRP group showed a significant increase in IL-6 concentration at Day1 (p<0.01).

At Day 90, HbA1c levels were significantly reduced by both treatment protocols (p<0.05) with no intergroup differences; the HbA1c reduction between baseline and Day90 (ΔHbA1c) was almost two times higher in the Q-SRP group (ΔHbA1c=-1.59±1.20) compared to the FM-SRP group (ΔHbA1c=-0.8±0.95) (p=0.04) (Table 4). Lipid fractions, SBP, DBP, heart rate and body temperature did not show any significant differences between study groups and across timepoints (p>0.05) (Table 3).

3.2.2 *Endothelial function*

The FM-SRP group demonstrated a significant worsening in the absolute difference in FMD between baseline and Day1 (ΔFMD=-0.86±0.92) compared to the Q-SRP group (ΔFMD=0.54±0.39) (p=0.04).

The absolute difference in FMD between Day90 and baseline was also improved, even though not significantly (Table 4). The absolute difference in FMD best across the same timepoints was almost three times higher in the Q-SRP (ΔFMDbest=2.39±2.26) than in the FM-SRP group (ΔFMDbest=0.69±1.97) (p=0.04).

3.2.3 *OHIP-14*

At 24 hours, the FM-SRP group demonstrated an increase in the OHIP-14 extent compared to baseline (p=0.04). Overall, both Q-SRP group and FM-SRP group showed a significant reduction in the OHIP-14 extent between baseline and Day90 (p=0.04), which was comparable across groups (p>0.05). Periodontal treatment significantly and comparably reduced the OHIP-14 severity at Day90 when performed with either the Q-SRP (p=0.03) or the FM-SRP protocol (p=0.04) (Table 2).

3.3 *Linear multivariate regression model*

Results of the linear multivariate regression model are shown in Table 5. The higher the relative CRP increase at Day1, the lower the reduction in HbA1c levels at Day90 (ΔHbA1c Day90-baseline) (p=0.02). Smoking status (smokers, p=0.01) and treatment group (FM-SRP, p=0.03) were significant predictors in this observation. The model was statistically significant (F=2.12; p=0.04) with an adjusted R^2 of 41.04%. Linear predictions of HbA1c levels at 3 months (ΔHbA1c Day 90-baseline) on the relative CRP increase at Day 1 are graphed in Figure 2.

4. Discussion

Full-mouth non-surgical periodontal treatment is associated with a higher acute inflammatory response compared to quadrant delivery in subjects affected by periodontitis and type 2 diabetes mellitus in the first 24 hours post-operatively. At 3 months, periodontal treatment was capable to ameliorate glycemic control and periodontal conditions in both groups with no differences among the two groups. However, in subjects where treatment determined a higher acute systemic perturbation a lower reduction of glycated hemoglobin was noted. Indeed, patients undergoing quadrant treatment showed a higher reduction of glycated hemoglobin levels and a significant improvement in endothelial function.

One day after treatment, patients undergoing full mouth instrumentation showed a significant perturbation of the inflammatory status characterized by a drastic enhancement of CRP and IL-6 values, as already been noted in systemically healthy subjects (D'Aiuto et al., 2013; Graziani et al., 2015). The reason for such findings may be related to both post-operative bacteremia and the local trauma derived from the sub-gingival instrumentation, triggering a higher production of local cytokines, which finally induce a liver-originated acute phase proteins release (Heinrich et al., 1990; Ide et al., 2004). Full-mouth deliveries may be associated with higher trauma as longer time of instrumentation is needed. Accordingly, treatment time was associated with higher post-operative increase of CRP, as noted in this and in our previous trial (Graziani et al., 2015).

Conversely, conventional quadrant scaling determined only a modest non-significant variations of acute phase proteins confirming the findings of our group comparing the same two protocols in systemically healthy subjects (Graziani et al., 2015). Plausibly, the extent of trauma, as measured by the reduced treatment time, and the bacteremia produced after

instrumentation of one quadrant may not be constitute a sufficient trigger to the liver-induced inflammation.

The acute inflammation noted after full-mouth treatment may determine some acute state of vascular dysfunction as it was noted by the significant reduction of FMD noted in this group in the first 24 hours after treatment and already noted in systemically healthy patients (Seinost et al., 2005; Tonetti et al., 2007). This might be of importance if considering that subjects with diabetes retain an increased risk for cardiovascular events (Sarwar et al., 2010). Cautiously, cardiovascular safety of the longer sessions of periodontal treatment in co-morbid patients has been questioned due to the higher level of systemic perturbation noted and the transient endothelial impairment (Sanz et al., 2020b).

Three months after treatment significant reductions of glycated hemoglobin was noted in both groups. This was expected and in line with the current knowledge (D'Aiuto et al., 2013; Madianos & Koromantzos, 2018). Nevertheless, a significantly higher reduction was noted in the quadrant group when compared to the full-mouth. Indeed, the multivariate regression analysis indicated that the higher increase of post-operative CRP noted in the full-mouth delivery is correlated with a lower reduction of glycated hemoglobin 3 months after treatment. Therefore, this finding suggests that acute inflammatory events taking place 24 hours after treatment could induce long-lasting systemic effects, finally resulting in a worse glycemic control. The biological mechanisms underpinning these results may relate to the ability of CRP to impair insulin signaling and insulin sensitivity through the phosphorylation of the insulin receptor, as demonstrated by preclinical evidence (Xi et al., 2011; Xu et al., 2007). Nonetheless, the exact molecular and kinetic mechanisms still have to be clarified.

Periodontal treatment was successfully conducted in both groups (Suvan et al., 2020). No differences among the two groups were also noted further confirming the available data indicating the comparable performance of both treatments (Eberhard et al., 2008; Suvan et al., 2020). Moreover, periodontal treatment determined a significant amelioration of oral health-related quality of life both in terms of severity and extent of the involved psychometric testing. The reduction noted is considered to a level that is clinically meaningful for the patient (Graziani & Tsakos, 2020). Once again, no differences were noted among the two groups with the exception of some alteration of quality of life noted one day after treatment in the full-mouth group.

To the best of the authors' knowledge there are no trials investigating the acute inflammatory effects of periodontal treatment in patients affected by type 2 diabetes mellitus. However, the reader should bear in mind some obvious limitations. The overall number of patients involved is modest, although a formal sample size estimation was performed. Moreover, the comparison is made between the inflammatory status after full-mouth instrumentation and the one recorded after instrumentation of the first quadrant, assuming that after the instrumentation of the other quadrants a similar inflammatory reaction might occur. However, these data was not collected.

Conclusions

In conclusion, both quadrant and full-mouth protocols are capable of determining important periodontal and glycaemic improvements in patients with periodontitis and diabetes type 2 three months after treatment. Nevertheless, full mouth treatment determines a higher transient acute systemic inflammation and endothelial dysfunction in the first day after

treatment to an extent that is approximately 7-times higher than the one noted after quadrant treatment. Moreover, an inferior reduction of glycated hemoglobin was noted in this group and further analysis indicated that subjects with high level of immediate post-operative inflammation are related with inferior benefits in terms of glycemic control. Hence, these findings possibly suggest that in subjects affected by periodontitis and type 2 diabetes mellitus a conventional treatment should be preferred in order to maximize the benefits of periodontal treatment.

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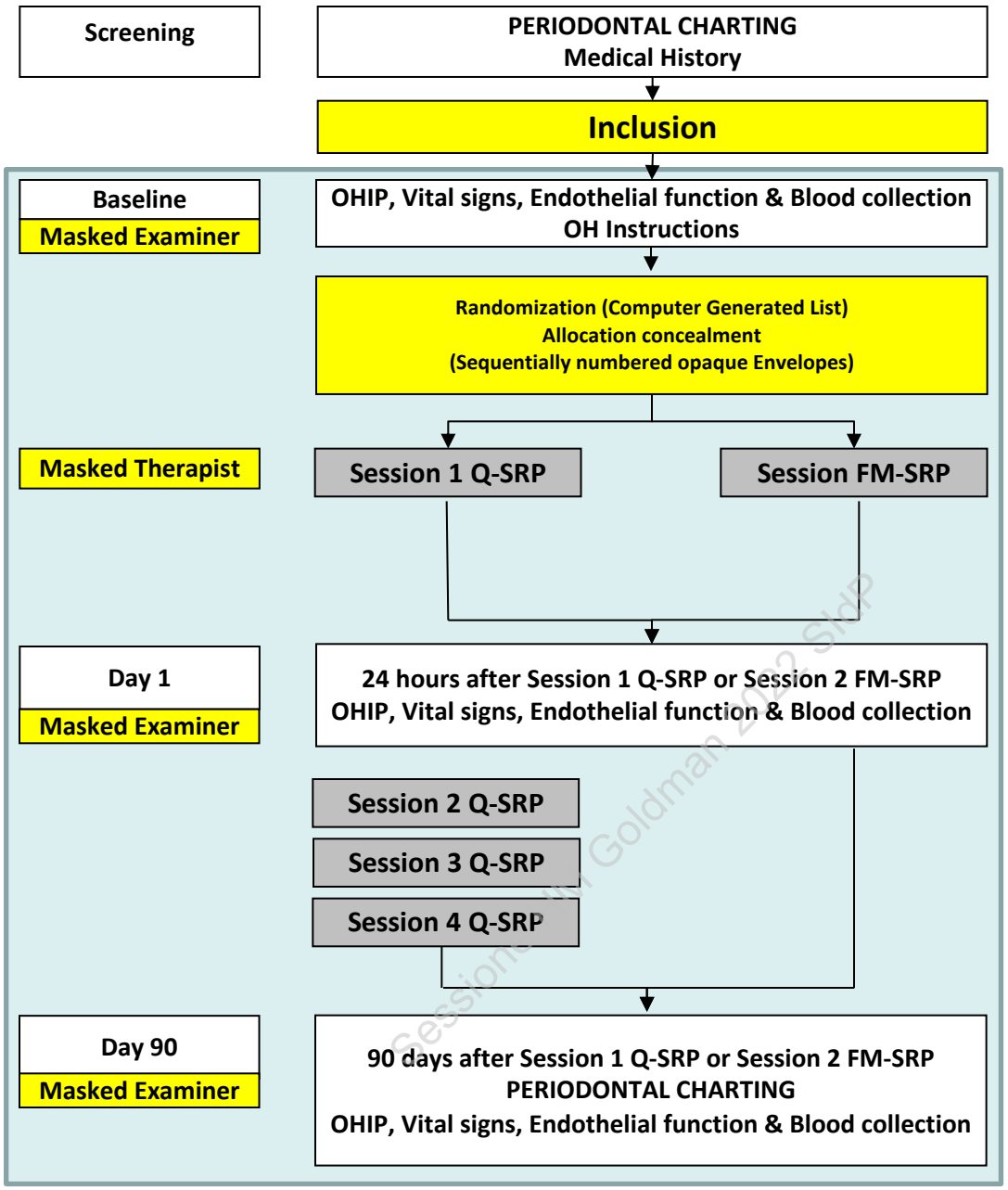
Screening
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Randomization (Computer Generated List)
Allocation concealment
(Sequentially numbered opaque Envelopes)
Masked Therapist
Session 1 Q-SRP
Session FM-SRP
Day 1
Masked Examiner
24 hours after Session 1 Q-SRP or Session 2 FM-SRP
OHIP, Vital signs, Endothelial function & Blood collection
Session 2 Q-SRP
Session 3 Q-SRP
Session 4 Q-SRP
Day 90
Masked Examiner
90 days after Session 1 Q-SRP or Session 2 FM-SRP
PERIODONTAL CHARTING
OHIP, Vital signs, Endothelial function & Blood collection

Figure 2: linear predictions of HbA1c levels at 3 months (Day 90) (ΔHbA1c Day 90-baseline) (y axis) on the relative C-reactive protein (CRP) increase at Day 1 (x axis). Covariates were dichotomized as follows: i) Group (0=Q-SRP; 1=FM-SRP); ii) Age (0 if age<55 years old; 1 if age≥55 years old); iii) Smoking Habit (SH) (0=non-smokers; 1=smokers); iv) BMI (0 if BMI<25; 1 if BMI≥25).

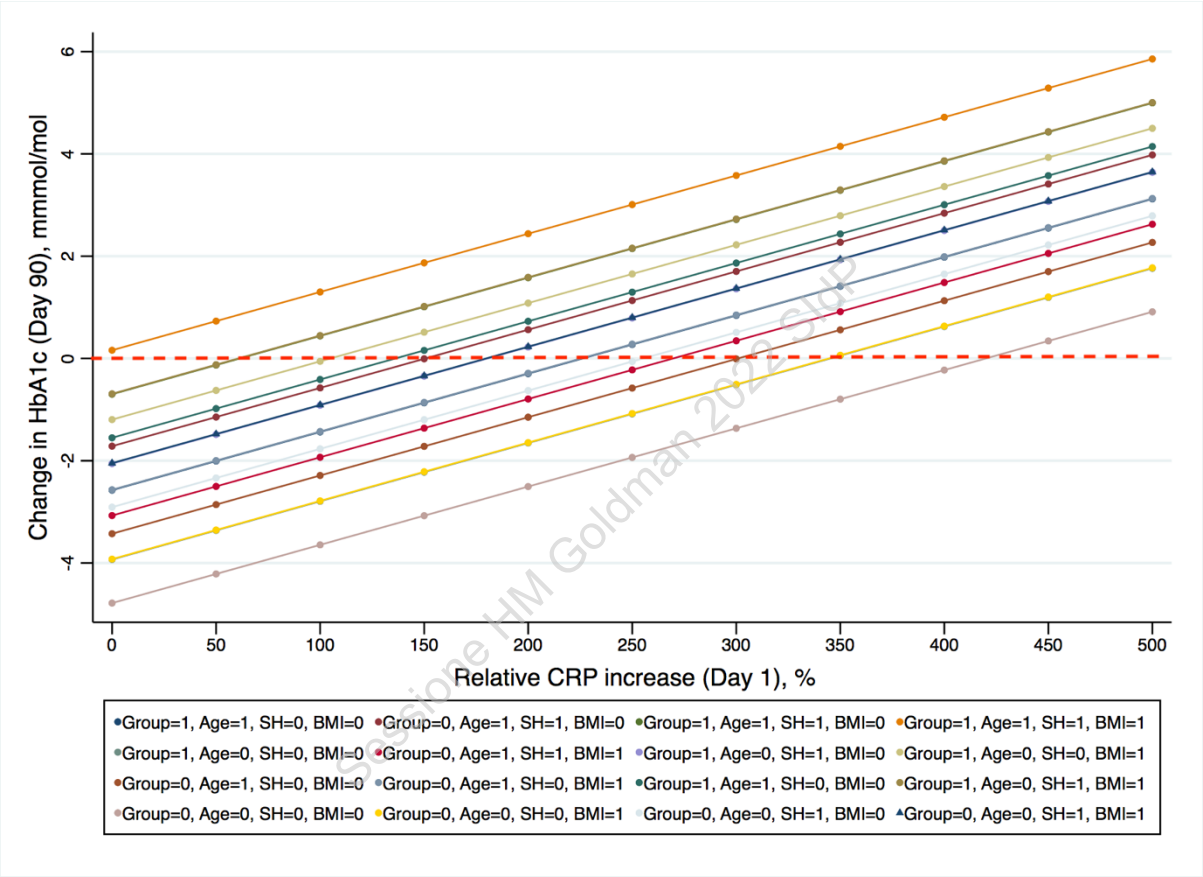

Table 1: patients characteristics by study group.

Variable		Quadrant Mean±SD/N(%)	Full-mouth Mean±SD/N(%)
Age (years)		62.45±9.60	56.89±10.37
Gender			
	Males	12 (60%)	12 (63.16%)
	Females	8 (40%)	7 (36.84%)
BMI		29.22±6.00	29.47±7.45
	Regular weight	2 (10%)	1 (5%)
	Overweight	4 (20%)	7 (35%)
	Obese	14 (70%)	12 (60%)
Smoking			
	Yes	5 (25%)	8 (42.11%)
	No	15 (75%)	11 (57.89%)
Smoking duration (years)		22.33±9.29	26.83±18.82
Number of cigarettes/day		21.25±6.29	12.38±6.55
Number of teeth		22.4±4.42	24.3±4.78
Treatment time per session (min)		27.25±7.39	57.59±13.22

Abbreviations: SD, Standard Deviation; BMI, Body Mass Index.

Table 2. periodontal parameters and Oral Health Impact Profile 14 between groups and at each timepoint. Values with different superscript letters are different at the 5% level.

Variable	Timepoint	Mean±SD		*p*-value between groups
		Q-SRP	FM-SRP	
N. pockets<4mm	*Baseline*	58.9±32.96	78.4±36.92	
	Day90	113.67±26.44	108.55±39.03	0.06
	p-value intragroup	***<0.01***	***<0.01***	
N. pockets≥4mm	*Baseline*	75.5±37.85	66.95±21.21	
	Day90	22.67±19.37	37.25±24.62	0.07
	p-value intragroup	***<0.01***	***<0.01***	
N. pockets≥5mm	*Baseline*	56.65±40.72	43.5±24.66	
	Day90	10.11±10.34	20.9±16.10	0.08
	p-value intragroup	***<0.01***	***<0.01***	
N. pockets≥6mm	*Baseline*	15.75±14.37	15.65±10.89	
	Day90	2.78±2.28	4.65±3.86	0.08
	p-value intragroup	***<0.01***	***<0.01***	
%PD<4mm	*Baseline*	44.21±23.92	51.64±18.39	
	Day90	83.31±13.31	73.06±18.53	0.09
	p-value intragroup	***<0.01***	***<0.01***	
%PD≥4mm	*Baseline*	55.78±23.92	47.94±17.60	
	Day90	16.58±13.36	26.94±18.53	0.06
	p-value intragroup	***<0.01***	***<0.01***	
%PD≥5mm	*Baseline*	41.61±27.39	32.14±19.96	
	Day90	7.52±7.46	15.11±12.06	0.10
	p-value intragroup	***0.01***	***<0.01***	
%PD≥6mm	*Baseline*	11.63±9.34	11.52±8.33	
	Day90	2.11±1.77	3.52±3.02	0.07
	p-value intragroup	***<0.01***	***<0.01***	
FMPS (%)	*Baseline*	83.58±30.54	79.51±26.39	
	Day90	33.36±23.13	37.25±26.89	0.28
	p-value intragroup	***<0.01***	***<0.01***	
FMBS (%)	*Baseline*	68.98±32.59	56.73±21.83	
	Day90	14.00±12.60	23.85±18.11	0.78
	p-value intragroup	***<0.01***	***<0.01***	
Mean PD (mm)	*Baseline*	3.96±0.69	3.74±0.64	
	Day90	2.78±0.34	3.04±0.55	0.06
	p-value intragroup	***<0.01***	***<0.01***	
Mean REC (mm)	*Baseline*	0.52±0.76	0.54±0.62	
	Day90	0.89±0.85	0.99±0.92	0.10
	p-value intragroup	***<0.001***	***0.001***	
Mean CAL (mm)	*Baseline*	4.48±1.08	4.29±1.01	
	Day90	3.68±0.91	4.03±1.26	0.07
	p-value intragroup	***<0.01***	***<0.01***	
OHIP-14 Severity†	*Baseline*	8.56±4.89[a]	7.14±4.48[a]	
	Day1	5.81±3.35[a]	6.54±4.28[a,b]	0.35
	Day90	4.81±2.14[b]	5.77±2.43[b]	0.65
	p-value intragroup	***0.03***	***0.04***	
OHIP-14 Extent†	*Baseline*	0.55±0.23[a]	0.60±2.68[a]	
	Day1	0.77±1.43[a]	1.15±2.49[b]	***0.04***
	Day90	0.38±1.41[b]	0.45±1.12[c]	0.39
	p-value intragroup	***0.04***	***0.04***	
Perceived Pain†	*Day1*	5.37±2.55	5.43±1.79	0.99

Abbreviations: SD, Standard Deviation; Q-SRP, Quadrant Scaling and Root Planing; FM-SRP, Full-Mouth Scaling and Root Planing; PD, Probing Depth; FMPS, Full Mouth Plaque Score, FMBS, Full Mouth Bleeding Score; REC, Recession; CAL, Clinical Attachment Level; OHIP, Oral Health Impact Profile.

Notes: All *p*-values refer to intra- and inter-group differences adjusted for baseline characteristics (age, BMI, gender, smoking and baseline body temperature), except when otherwise specified.

† Unadjusted *p*-values.

Table 3: vital signs and serum makers between study groups and at each timepoint. Values with different superscript letters are different at the 5% level.

Variable	Timepoint	Mean±SD		*p*-value between groups
		Q-SRP	FM-SRP	
Systolic blood pressure (mmHg)	*Baseline*	128.5±14.15	130.79±14.93	
	Day1	128.61±13.91	132.22±16.29	0.26
	Day90	125.29±27.35	129.75±18.88	0.12
	p-value intragroup	1.00	0.26	
Diastolic blood pressure (mmHg)	*Baseline*	78.75±8.72	75.11±11.09	
	Day1	77.78±9.43	72.22±9.11	0.15
	Day90	74.94±12.50	74.6±11.32	0.17
	p-value intragroup	0.43	0.45	
Heart rate (bpm)	*Baseline*	72.6±13.36	73.58±13.83	
	Day1	71.55±8.75	74.11±13.61	0.31
	Day90	71±12.28	70.65±11.66	0.08
	p-value intragroup	0.19	0.84	
Body temperature (°C)	*Baseline*	36.46±0.35	36.47±0.38	
	Day1	36.42±0.39	36.59±0.31	0.41
	Day90	36.38±0.37	36.47±0.45	0.97
	p-value intragroup	0.96	0.64	
Glucose (mg/dL)	*Baseline*	105.37±16.76	115.53±36.50	
	Day1	113.79±46.05	120.5±44.95	0.21
	Day90	108.94±18.98	114.1±40.53	0.12
	p-value intragroup	0.19	0.06	
Cholesterol (mg/dL)	*Baseline*	165±38.78	187.55±39.23	
	Day1	165.05±37.74	184.4±34.59	0.28
	Day90	162.66±40.69	182.15±33.95	0.08
	p-value intragroup	1.00	0.84	
HDL (mg/dL)	*Baseline*	48.6±10.97	50.5±12.90	
	Day1	47.95±9.55	50.85±13.01	0.69
	Day90	46.55±12.24	50.55±9.87	0.78
	p-value intragroup	0.80	0.50	
Triglycerides (mg/dL)	*Baseline*	136.8±90.13	156±46.70	
	Day1	128.35±67.42	142.45±96.41	0.48
	Day90	146.77±58.14	142.35±51.30	0.97
	p-value intragroup	0.43	0.59	
HbA1c, mmol/M	*Baseline*	48.2±9.01	51.4±15.20	
	Day1	-	-	
	Day90	46.61±6.52	50.6±11.42	0.74
	p-value intragroup	***0.02***	***0.04***	
Insulin (µU/mL)	*Baseline*	13.35±11.10	14.12±10.66	
	Day1	9.65±5.35	12.15±9.02	0.42
	Day90	12.44±7.98	12.63±8.62	0.15
	p-value intragroup	0.50	0.48	
CRP (mg/L)	*Baseline*	4.20±4.48	3.32±6.18[a]	
	Day1	4.33±6.76	5.55±6.27[b]	***0.04***
	Day90	3.98±6.16	3.67±5.73[a,b]	0.19

	p-value intragroup	0.06	***<0.01***	
Relative CRP increase (Day 1, %)	-	33.01±65.55	248.40±446.89	***0.03***
IL-6 (pg/mL)	*Baseline*	5.21±4.74	3.63±3.28[a]	
	Day1	5.45±6.37	4.69±5.34[b]	0.26
	Day90	4.38±4.30	4.52±7.75[a,b]	0.18
	p-value intragroup	0.92	***<0.01***	
Relative IL-6 increase (Day 1, %)	-	20.91±109.78	39.51±168.95	0.69

Abbreviations: SD, Standard Deviation; Q-SRP, Quadrant Scaling and Root Planing; FM-SRP, Full-Mouth Scaling and Root Planing; bpm, beats per minute.
Notes: All *p*-values refer to intra- and inter-group differences adjusted for baseline characteristics (age, BMI, gender, smoking and baseline body temperature).

Table 4: absolute changes of specific parameters between groups over timepoints.

Variables	Group	Mean±SD Day1-BL	*p*-value	Mean±SD Day90-Day1	*p*-value	Mean±SD Day90-BL	*p*-value
ΔHbA1c (mmol/M)	*Q-SRP*	-	-	-	-	-1.59±1.20	***0.04****
	FM-SRP	-		-		-0.8±0.95	
ΔCRP (mg/L)	*Q-SRP*	0.13±0.82	***0.02****	-0.35±0.82	0.09*	-0.22±1.41	0.22*
	FM-SRP	2.23±1.05		-1.88±1.74		0.35±1.26	
ΔIL-6 (pg/mL)	*Q-SRP*	0.24±1.22	0.61*	-1.07±2.02	0.25*	-0.83±2.79	0.40*
	FM-SRP	1.06±3.79		0.83±4.37		0.89±3.15	
ΔFMD (%)	*Q-SRP*	0.54±0.39	***0.04****	0.4±1.22	0.58**	0.94±1.49	0.95**
	FM-SRP	-0.86±0.92		0.86±1.08		0±2.52	
ΔFMD best (%)	*Q-SRP*	0.07±1.75	0.49**	2.32±1.99	0.86**	2.39±2.26	***0.04****
	FM-SRP	-0.75±2.07		1.44±2.75		0.69±1.97	
ΔGTN-FMD	*Q-SRP*	-0.58±1.86	0.42***	1.58±2.68	0.51***	1±2.24	0.97***
	FM-SRP	-1.44±2.78		2.34±3.10		0.9±1.35	

Abbreviations: SD, Standard Deviation; HbA1c, glycated hemoglobin; CRP, C-reactive protein; FMD, flow-mediated dilation; GTN-FMD, glyceryl-trinitrate Flow Mediated Dilation.

* p-values adjusted for baseline characteristics (age, BMI, smoking, gender, baseline body temperature)

** p-values adjusted for baseline diameter.

*** p-values adjusted for baseline GTN diameter.

Table 5: best model of the linear multivariate regression analysis evaluating the impact of the relative CRP increase at Day1 on the change in HbA1c levels at Day90 (ΔHbA1c baseline-Day90).

Best Model (Mallow's Cp= 4.31)						
					95% CI	
Predictors	**Coefficients**	**SE**	**t**	***p*-value**	**Lower**	**Upper**
(Constant)	-1.73	7.44	-0.23	0.82	-17.52	14.06
Relative CRP increase (Day1)	0.02	0.004	2.069	*0.02*	0.002	0.03
Age	0.03	0.11	0.32	0.75	0.19	0.26
Smoking	1.90	1.46	1.30	*0.01*	1.20	5.00
Group	0.95	1.31	0.49	*0.03*	0.43	2.13
BMI	1.04	1.27	0.82	0.43	-1.66	3.73
				Analysis of Variance		
			Source	**Sum of squares**	**df**	**Mean Square**
R^2	0.4984		Model	75.50	5	15.10
Adjusted R^2	0.4104		Residual	113.99	16	7.12
Root MSE	2.6692		Total	189.5	21	9.02
			*F=2.12; p=0.04**			

Abbreviations: CI, Confidence Interval; SE, Standard Error; Root MSE, Root Mean Square Error; df, degrees of freedom.