THE EFFECT OF TWICE DAILY KIWIFRUIT CONSUMPTION ON PERIODONTAL AND SYSTEMIC CONDITIONS BEFORE AND AFTER TREATMENT: A RANDOMIZED CLINICAL TRIAL

L'EFFETTO DEL CONSUMO DI DUE KIWI AL GIORNO SULLE CONDIZIONI PARODONTALI E SISTEMICHE PRIMA E DOPO IL TRATTAMENTO: STUDIO CLINICO RANDOMIZZATO

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Running title: Kiwifruit effect on periodontal inflammation

Key words: Periodontal disease, inflammation, kiwifruit, nutraceutical, periodontal treatment

Abstract

Aim: To assess the nutraceutical effects of twice/daily intake of kiwifruit on periodontal parameters and systemic health before and after initial periodontal treatment (IPT).

Matherials and Methods: Included subjects were randomized at baseline (BL) to consumption of 2 kiwifruits or not for 5 months. In the first two months, no periodontal treatment was delivered (2M). Subsequently, a session of full-mouth IPT within 24 hours was performed. Subjects were then re-assessed after 3 months (5M). Blood collection, evaluating lipids profile and C-reactive protein, and vital signs were also collected at BL, 2M and 5M.

Results: Groups were balanced at baseline. At 2M no differences could be detected for any parameter but bleeding score which decreased significantly by 6.67±11.90% ($p<0.01$) in the kiwifruit group. Kiwifruit consumption was also related to lower values of plaque and attachment loss. After treatment both groups experienced significant clinical benefits and no differences were noted but in terms of plaque and gingival inflammation. In the kiwifruit group, a reduction of diastolic blood pressure ($p\leq0.01$) and HDL ($p=0.026$) was noted.

Conclusions: Kiwifruit consumption reduces gingival inflammation despite the lack of any periodontal instrumentation or patient's behavioural changes. No adjunctive effect to periodontal treatment of dietary intake of kiwifruit was noted.

Abstract

Obiettivo: Valutare gli effetti nutraceutici dell'assunzione di 2 kiwi/dì sui parametri parodontali e sistemici prima e dopo trattamento parodontale non-chirurgico (TPNC).

Materiali e Metodi: I soggetti inclusi sono stati randomizzati a baseline (BL) in base al consumo o no di 2 kiwi/dì. Nei primi due mesi, nessun trattamento parodontale è stato effettuato (2M). Successivamente, è stata eseguita una sessione di full-mouth TPNC. I soggetti sono stati rivalutati dopo 3 mesi (5M). I prelievi ematici per la valutazione del profilo lipidico e della proteina C-reattiva e i segni vitali sono stati raccolti a BL, 2M e 5M.

Risultati: I due gruppi erano comparabili a BL. A 2M solo il sanguinamento al sondaggio si è ridotto in modo significativo (6.67 ± 11.90% [$p<0.01$]) nel gruppo kiwi. Il consumo di kiwi è stato associato a valori più bassi di placca e perdita di attacco. Dopo il TPNC entrambi i gruppi hanno tratto significativi benefici clinici e sono emerse differenze solo in termini di placca e infiammazione gengivale. Nel gruppo kiwi, è stata osservata una riduzione della pressione diastolica ($p\leq0.01$) e dell'HDL ($p = 0,026$).

Conclusioni: In assenza di TPNC il consumo di kiwi riduce l'infiammazione gengivale ma non apporta alcun effetto aggiuntivo al TPNC.

Introduction

Periodontitis, a destructive inflammatory disease of the supporting tissues of the teeth, is caused by an imbalance between the host defence and environmental factors like bacteria, smoking and poor nutrition. Nowadays the etiological role of bacteria has been changed from putative periodontal pathogens to periodontal pathobionts (Cugini et al. 2013). A pathobiont is a symbiont that is able to promote pathology only when specific genetic or environmental conditions are altered in the host (Chow & Mazmanian 2010). This insight may revolutionize prevention and treatment of periodontitis. Focus should not only be on plaque control and removal of bacteria but also on improving host resistance through smoking abstention, stress reduction and a healthy diet. The importance of micronutrients has been extensively reviewed and it was concluded that for prevention and treatment of periodontitis daily nutrition should include sufficient antioxidants, vitamin D and calcium (Van der Velden et al. 2011).

Regarding antioxidants, vitamin C has attracted the attention of periodontal researchers. The relationship between necrotizing ulcerative gingivitis and vitamin C deficiency is well-known (Melnick et al. 1988). Furthermore, it has been shown that plasma vitamin C levels are inversely related to the severity of periodontitis (Amarasena et al. 2005, Panjamurthy et al. 2005, Staudte et al. 2005, Amaliya et al. 2007, Chapple et al. 2007). The results of a recent case control study also showed that periodontitis patients have lower plasma vitamin C levels than subjects without periodontal breakdown (Kuzmanova et al. 2012). Moreover, 19% of the periodontitis patients appeared to be depleted of vitamin C (plasma values 2.0-3.9 mg/l), although the estimated dietary intake of vitamin C was comparable. In contrast, Staudte et al. (2012) found that periodontitis patients had lower plasma vitamin C level compared to healthy controls but that they had a reduced intake of vitamin C.

Up to now there is limited available research investigating the effect of vitamin C supplementation on the periodontal condition. Experimental vitamin C depletion/repletion studies in young subjects showed that gingival inflammation was directly related to the vitamin C status (Leggot et al. 1986, Jacob et al. 1987). No effect of vitamin C supplementation was found in relation to the development of gingivitis during experimental gingivitis (Vogel et al. 1986). However, in subjects with gingivitis, vitamin C supplementation resulted in a decrease of gingivitis (Gokhale et al. 2013) while in untreated periodontitis patients vitamin C supplementation had no effect on gingivitis and pocket depth.

Recently, a significant increase of medical literature on the effect of nutraceutical (a term indicating both nutrition and pharmaceutical) dietary aliments on general health has been noted. A nutraceutical "*identifies a food or part of a food, which can be of vegetal or animal origin, that has a beneficial pharmaceutical activity beyond its nutritional value"* (Santini et al. 2016) and, to our knowledge, only one study evaluated in untreated periodontitis patients the nutraceutical effect of vitamin C by means of increasing intake through natural dietary sources i.e. two grapefruits daily for two weeks (Staudte et al. 2005). Results showed that the intake of

grapefruit leads to an increase in plasma vitamin C levels and improved sulcus bleeding scores but no change in pocket depth.

Good sources of vitamin C include peppers, strawberries, broccoli, Brussels sprouts, oranges, grapefruits and kiwifruit. Kiwifruit (Van der Velden et al. 2011) seems most attractive since it contains 93 mg vitamin C/100 g fruit whereas oranges and grapefruits contain 45 and 33 mg/100 g fruit respectively (USDA 2010). Furthermore, it has been reported that consuming two or three kiwifruits per day may reduce blood pressure (Karlsen et al. 2013), platelet aggregation and triglycerides levels (Duttaroy & Jørgensen 2004) and may increase HDL-cholesterol levels (Chang & Liu 2009, Gammon et al. 2013).

Therefore, the aim of the present study was twofold. The first objective was to investigate the effect of twice-daily kiwifruit consumption as sole treatment modality in untreated periodontitis, followed after two months by initial periodontal therapy (IPT) supported by continued kiwifruit consumption. The second objective was to investigate the effect of kiwifruit consumption on periodontal and systemic parameters of these periodontitis patients 3 months after treatment.

Materials e Methods

Experimental patient population

This study was a single-centre randomized, parallel design, clinical trial with a 5-month follow up involving subjects affected by periodontitis. The protocol of the study was approved by the local ethical committee (#3729/2012) and it was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects.

Eligible patients were identified from those referring to the Sub-Unit of Periodontology, Halitosis and Periodontal Medicine of the University Hospital of Pisa, Italy. All subjects gave written informed consent, full medical history was recorded and oral examination was completed. Patients presenting with proximal attachment loss of ≥3 mm in ≥2 non-adjacent teeth (Tonetti & Claffey 2005), PPD≥4 mm and BoP on at least 25 % of their total sites, and documented radiographic bone loss were considered eligible to participate in this study. Subjects were excluded from the study if they were (i) younger than 18 years or older than 70 years, (ii) pregnant or lactating females, (iii) females using contraceptive pharmacological medications, (iv) reported diagnosis of any systemic illnesses including cardiovascular, renal, and liver diseases, (v) in need of antibiotic treatment during initial periodontal treatment (IPT), (vi) IPT in the previous 6 months, (vii) allergic to latex, kiwifruit and fruits in general, (viii) suffering of eating/digestive disease or food intolerances and (ix) smoking more than 20 cigarettes per day.

Study design

Subjects who accepted to participate were invited to another clinical session in which a clinical examination was performed and blood collection was taken (Figure 1). Allocation envelopes were then opened and subjects in the test group were prescribed twice daily kiwifruit. They were instructed to eat two kiwifruits per day during the whole study period. The kiwifruits had to be provided by the subjects themselves. No recommendation on the type of kiwifruit was delivered. Kiwifruit was suggested to be taken as a whole and not mixed with sugar or other. No further dietary changes were requested. Subjects were given diaries to annotate the kiwifruit intake and eventual side-effects. In the control group no dietary recommendations were given.

No treatment was delivered in the following 8 weeks which is the approximate waiting list period to receive treatment in our hospital in Pisa. During this period no clinical sessions were performed unless urgency appointment if requested. After 2 months (2M) blood was again withdrawn and another clinical examination was performed. Subsequently, subjects received full mouth IPT within 24h. Subjects were seen once a month to re-enforce oral hygiene. Three months after IPT (5M) another clinical examination was performed and blood samples taken.

Randomization procedures, allocation concealment, masking and sample size calculation

Study participant numbers were assigned in ascending order at the enrolment visit. Subjects were randomly assigned in a 1:1 ratio to either test or control group using a computer-generated table (www.random.org). No stratification on cigarettes/day and years of smoking was made. The randomization table was saved by a research fellow not directly involved in the experimentation. Allocation to treatment was concealed to the clinical examiner and statistician with sealed opaque envelopes which were opened by a clinical staff member on the day of the allocation.

The clinical examiner and the therapists were masked to the allocation. Subjects were asked not to indicate their group allocation. The sample size calculation was based on data of Staudte et al. (2005) showing a drop in bleeding scores from 1.68±0.6 to 1.05±0.6 mg/l after grapefruit consumption. Thus, 24 subjects per treatment arm would be needed to provide 95% power to detect a difference of 0.6 between test and control using the bleeding score after two months of kiwifruit consumption as the primary outcome variable assuming that the standard deviation is 0.6. Thus, a sample of 50 subjects, 25 per arm were recruited to compensate for possible drop-out.

Clinical parameters

Both systemic and periodontal parameters were collected at BL, 2M and 5M.

Periodontal clinical parameters were assessed using a UNC 15-mm periodontal probe by the clinical examiner at six sites/tooth excluding third molars. Calibration of the examiner was performed on a total of 10 non-study subjects affected by periodontitis. The examiner was judged to be reproducible after meeting a percentage of agreement of CAL recording within ≤2 mm between two repeated measurements in separate occasions of at least 98% (Graziani et al. 2010b). During the trial, full-mouth pocket probing depth (PPD) and recession of the gingival margin (REC), positive and negative, were recorded with measurements rounded off to the nearest millimetre. Clinical attachment level (CAL) was calculated as the sum of PPD and REC. The full-mouth plaque score (FMPS) was measured as the percentage of the total surfaces showing plaque assessed dichotomously on six surfaces per tooth (O'Leary et al. 1972). Similarly, a full-mouth percentage bleeding score (FMBS) was calculated after assessing dichotomously the presence of bleeding on probing (Torfason et al. 1979).

During the study, blood samples were also collected and vital signs including systolic (SBP) and diastolic blood pressure (DBP) were measured in triplicates by using an automatic oscillometric device (OMRON- 705IT, Omron, Kyoto, Japan). Average BP was then calculated from the last two measurements. Weight and height were measured and body mass index (BMI) was calculated. Body temperature was measured with tympanic reading by using an ear canal thermometer (Genius TM 2, Covidien LLC, USA). Smoking history was registered dichotomously as current or never/former.

Blood collection and analysis of the serum markers

Serum samples were collected from a venepuncture in the antecubital fossa before 9.00 AM and after an overnight fast for all patients. Blood samples were immediately processed and serum aliquots were stored at -80°C. All laboratory analyses were performed at the laboratory of the University Hospital of Pisa. Glycated haemoglobin, lipid fractions including total cholesterol, HDL, LDL, and triglycerides were measured using standard laboratory procedures. Serum C-Reactive Protein (CRP) was measured by immunoturbidometry (Cobas, Roche Diagnostic, Mannheim, Germany). To assess Vitamin C, within 10 min after collection, sampling tubes were centrifuged with a low-speed centrifuge 3500 rpm for 15 min (Heraeus Megafuge 40R, Thermo Fisher Scientific, Waltham, USA) to separate plasma from blood cells. Immediately thereafter, plasma was stabilized by means of a precipitation reagent in order to minimize the oxidation and subsequently prepared for vitamin C assessment by high-pressure liquid chromatography according to the manufacturer's instructions (Eureka, Chiaravalle. Italy). All samples were analysed at the end of the study by non-staff members masked to the group allocation.

Initial periodontal treatment

Supra- and sub-gingival mechanical instrumentation of the root surface was performed by a single periodontist. Treatment was provided using both hand and ultrasonic instrumentation with periodontal tips (EMS, Nyon, Switzerland). Local anaesthesia was used when needed and no time constraints were enforced. Subjects received treatment within 24 h in two sessions, one side of the mouth for each session.

Oral hygiene instructions (OHI) consisting of electric tooth brushing and interdental brushes were explained and shown to the participants the first day of the IPT. OHI were further re-enforced after one week and once a month during follow-up.

Statistical analysis

Descriptive statistics and data analyses were performed with statistical software from SPSS (version 21.0, SPSS Inc., Chicago, IL, USA). All data are presented as mean and standard deviation unless otherwise specified. Changes of oral and systemic parameters over time within test and control group were analysed with ANOVA for repeated measures at different time points unadjusted for confounding factors. Least significant difference post hoc corrections were adopted. Differences in changes between test and control group over time were analysed with a linear mixed model analysis including regimen variable, session variable and the interaction between regimen and session, adjusting for the particular outcome variable at baseline and the potential confounding factors age, gender, smoking status, BMI, Vitamin C and HbA1c. This model was used because it takes into account the correlation between the repeated measures within the subject.

Results

Subjects accountability, baseline characteristics and dietary compliance

A total of 96 subjects were screened from May 2013 to May 2015 and 50 were finally included. All subjects recruited were Caucasians and they all completed the study providing data for the final database analyses.

Both groups were comparable for age, gender and smoking habits. Included subjects were mainly at their fifties, female subjects and smokers accounted to 60% and 64% and 48% and 40% of the test and control group respectively. No differences in terms of systemic parameters were observed between the two groups. No subjects were undergoing hormone replacement, anti-coagulant or anti-aggregation treatment. Overall subjects self-reported systemically healthy with a tendency for high-end levels of cholesterol, HbA1c and CRP. Low levels of

baseline Vitamin C were noted. Differences were noted for baseline level of HbA1c indicating a lower glycemic control in the test group being at the threshold of prediabetes. Included subjects appeared to be affected by CAL higher than 4 mm on average, FMPS above the 70% and inflammation observed in more than 50% of the sites analysed (Table 1). No differences between the two groups were noted at baseline.

Daily kiwifruit intake was positively perceived by the study population as self-reported at 2M and 5M and adherence to dietary changes was high. The overall percentage of self-reported kiwifruit intake per day on the total of expected assumption (2 kiwis/day over 5 months) was from baseline to month 2 87.62 ± 14.63 % and from month 2 to month 5 78.37 ± 17.35 % (difference BL-2M versus 2M-5M, p= 0.055). No major side-effects were reported. One patient experienced 3 days of diarrhoea and one patient described two days of itching lips 9 days after the beginning of the consumption but kiwifruit intake was not discontinued.

Periodontal parameters

In the first 2M period, in which no treatment was performed, the control group did not show statistically significant changes of the periodontal parameters. In this group FMPS remained above 80%, FMBS above 60% and no changes of PPD and CAL could be assessed (Table 2). Conversely, in the test group, FMBS decreased significantly by 6.67 ± 11.90% ($p \leq 0.01$). The number of pockets also showed a minor reduction in the test group ($p<0.05$). Comparison of test and control group showed for FMPS, FMBS and CAL significant differences in favour of the test group (Table 2). In the second period of the study, IPT appeared to be successful in both test and control group. The only difference in this period was that treatment resulted in more reduction of FMBS, FMPS and CAL in the control group (Table 2).

Systemic biomarkers

In the first 2M no differences were noted among systemic biomarkers and vital signs in both groups compared to baseline. When comparing changes among test and controls, SBP showed a greater reduction in the first 2M ($p=0.034$). After periodontal treatment an increased level of triglycerides ($p \leq 0.01$) and a reduction of HDL ($p \leq 0.05$) were also noted in the test compared to the control group.

Discussion

This clinical trial compared the clinical effect of the consumption of two kiwifruits per day on both untreated and treated periodontal disease. This is the first nutraceutical trial evaluating this nutritional effect in such a particular model. Our data indicate that kiwifruit consumption

establishes a significant decrease of gingival inflammation in the absence of any treatment. This finding is in agreement with a previous observational study indicating reduction of gingival bleeding after two weeks of two grapefruit intake in periodontitis affected subjects, particularly in non-smokers subjects (Staudte et al. 2005). In the present study, comparison of test and control group with regard to the attachment level measurements indicated a significant difference in favour of the test group. The control group experienced a small loss during the two months without treatment whereas the kiwifruit showed a small gain. Although these changes were not statistically significant for each group, the comparison of the two groups by means of linear mixed model analysis adjusted for confounding factors indicated that kiwifruit consumption had a positive influence. Both the reduction in FMBS and the CAL data suggest that due to this supplementation in the diet probably less inflammation is present in the periodontal tissues.

The reason for less inflammation might be due to the high supplementation of vitamin C, provided by kiwifruit, which has a robust modulatory effect of gingival inflammation (Leggott et al. 1991). Indeed, over 5 months a significant increase of Vitamin C level was observed in the test group. Vitamin C consumption through kiwifruit intake may vary among 60 mg to 160 mg per 100 mg of pure fruit flesh according to the type of kiwi with an approximate daily intake of 100-200 Vitamin C per day in this trial (Ma et al. 2017). Vitamin C may actively diminish inflammation through its role in the oxidative stress as potent antioxidant, down regulating inflammation and improving endothelial function (Ashor et al. 2015, Ellulu et al. 2015). Moreover, it has been shown that neutrophil chemotaxis and oxidant generation increased by 20% after kiwifruit supplementation (Bozonet et al. 2015). Recently, it was found in an untreated periodontally diseased population deprived from regular dental care that supplementation with 200 mg vitamin C daily reduced both the subgingival load of all studied periodontal bacteria as well as the serum CRP levels suggesting less inflammation (Amaliya et al. 2015). Furthermore, vitamin C may act as a robust mediator of collagen synthesis as it has been observed in periodontal tissues (Aurer-Kozelj et al. 1982). In that study, vitamin C supplementation in periodontitis-affected subjects was associated with histological repair of the interdental papilla. Furthermore, kiwifruit is also rich of other numerous antioxidants such as lutein, an oxycarotenoid and alpha-linolenic acid, an omega-3 fatty acid (Drummond 2013). Indeed, carotenoids and fatty acids have both anti-inflammatory properties (Helmersson et al 2009).

The reduction of inflammation in the study period without any treatment may also have had an effect on the FMPS. Several studies have reported an increased plaque accumulation in the presence of gingival inflammation (Lang et al. 1973, Goh et al. 1986, Ramberg et al 1994, 1995, Rowshani et al 2004). Also in experimental gingivitis studies it has been shown that subjects develop plaque more rapidly in the presence of gingivitis (Quirynen et al. 1991, Ramberg et al. 1994, Daly & Highfield 1996). Therefore, it may be suggested that a reduction in inflammation may also result in a reduction of plaque. This assumption is supported by the present study

results since in the study period without treatment the FMPS of the test group decreased by 4% whereas the FMPS in the control group increased by 2.3%, a difference that was statistically significant.

No significant effect as adjunctive to periodontal treatment was noted. It is likely that the magnitude of the treatment effect would hide a possible benefit of the fruit consumption. The only difference was that treatment resulted in more reduction of FMBS and FMPS in the control group (Table 2). However, this should be seen in the light of the lower FMBS and FMPS already obtained by the test group after two months of kiwifruit consumption without treatment.

Interestingly, a significant reduction on SBP was also noted in the kiwifruit group when compared to control group. This is in agreement with previous reports indicating significant reduction of both SBP and DBP modulated by kiwifruit consumption (Karlsen et al. 2012, Svendsen et al. 2015). The reason for no variation of the DBP might be due to the different dosage; our trial used two kiwifruits per day versus three in the hypertension studies. Moreover, our population was not constituted by subjects affected by hypertension in which the effects of kiwifruit consumption on blood pressure levels are milder (Gammon et al. 2014). The mechanisms for such effects are hypothesized to rest in the increase of both potassium (Aburto et al. 2013) and the vitamin C (Juraschek et al. 2012) intake provided by the kiwifruit.

Unexpectedly, no beneficial effects on lipid metabolism were seen but a deterioration of the triglycerides in the kiwifruit group. This finding cannot be clearly explained. Kiwifruit consumption usually lessens the plasma values of triglycerides (Rocio-Rodriguez et al. 2015) by approximately 15% (Duttaroy & Jørgensen 2004). However, although significant higher triglyceride values were found compared to month 2 at the start of treatment, they were not different compared to the baseline triglycerides values.

The authors are aware of the strength and the intrinsic limitations of the study. Especially, the sample size was calculated on the effect on the bleeding score. Therefore, the number of subjects in the present study may not be adequate for the other studied parameters. Nevertheless, our findings are of uttermost importance as no other nutraceutical trials are present within the periodontal literature. In particular, the present research model allows measuring the effect of the dietary consumption on both untreated and treated periodontal disease.

In conclusion, daily kiwifruit consumption determines a significant reduction of gingival inflammation in untreated periodontal disease. This may provide support for improved nutritional approaches in the prevention of periodontal diseases.

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Table 1. Periodontal and systemic characteristics of the study sample at baseline, 2 and 5 months (unadjusted p-values).

Variable	*Test Group (N=25)*			*Control Group (N=25)*		
	BL	**2M**	**5M**	**BL**	**2M**	**5M**
FMBS, %	54.3±16.4	47.0±17.9**	12.1±9.3*	62.9±20.4	65.3±19.0	13.3±5.6*
FMPS, %	74.8±23.8	70.1±23.3	19.3±15.4*	83.0±19.1	84.6±15.6	18.0±11.1*
Recession, mm	0.4±0.5	0.4±0.6	0.8±0.6*	0.4±1.5	0.4±0.5	0.9±0.5*
Probing depth, mm	3.8±0.5	3.7±0.5	2.6±0.3*	3.8±0.5	3.8±0.5	2.5±0.3*
Probing depth ≥ 5mm	5.8±0.4	5.8±0.5	4.9±1.2*	5.7±0.5	5.7±0.4	5.3±0.3*
Number of pockets ≥ 5 mm	48.9±17.3	45.8±21.6†	6.4±10.1*	54.3±23.5	53.6±20.1	6.1±6.3*
Clinical attachment level, mm	4.0±0.6	4.0±0.8	3.3±0.7*	4.2±0.7	4.3±0.8	3.4±0.6*
BMI	23.9±4.4		23.8±4.4	24.4±3.6		24.6±3.3
Vitamin C, mg/l	2.2±3.2	4.3±5.2	6.8±6.4**	3.2±5.2	3.0±5.1	5.6±6.0
CRP, mg/l	2.3±2.8	2.4±2.4	2.0±2.6	1.5±1.6	1.6±2.3	1.1±1.2
Triglycerides, mmol/l	94.6±42.4	82.4±24.4	101.1±46.4	87.4±34.2	92.7±41.0	89.2±30.2
Total Cholesterol, mmol/L	205.2±35.3	205,0±41.1	209.0±40.0	216.6±36.9	207.6±33.7	213.0±30.0
HDL, mmol/L	59.5±15.7	60.2±15.7	58.8±15.2	61.1±13.6	60.0±12.6	63.4±13.3
HbA1c, mmol/mol	38.9±4.7	39.0±5.0	39.0±4.6	35.8±3.6	36.4±3.6	36.0±3.6
Systolic BP, mmHg	125.0±20.5	125.2±25.3	122.0±18.7	120.8±18.7	119.0±19.3	122.7±18.6
Diastolic BP, mmHg	80.8±10.5	77.2±10.8	77.0±10.1	76.0±11.3	77.4±13.1	78.3±12.0

Body temperature °C	36.5±0.5	36.6±0.4	36.7±0.4	36.7±0.6	36.5±0.5	36.5±0.5
Heartrate, beat/minute	72.6±8,8	73.8±10.9	73.0±12.7	72.4±7.5	71.3±7.1	70.8±8.7

* p<0.001 from baseline & 2M

** p<0.01 from baseline

† p<0.05 from baseline

FMPS, Full Mouth Plaque Score; FMBS, Full Mouth Bleeding Score; Rec, PD, Probing depth; BP, blood pressure; BMI, body mass index; CRP, C reactive protein; HbA1C= glycated hemoglobin

Table 2. Clinical periodontal parameters of groups at various time points and differences between groups analysed by linear mixed model analysis (adjusted for baseline values of age, gender, smoking status, vitamin C, BMI and HbA1c)

Variable (mean ± SD)	Time	**Within Test Group Difference**	**Within Control group Difference**	**Differences Test vs Control p-value**
FMBS, %	BL – 2M	6.67 ± 11.90	-2.68 ± 8.23	<0.001
	2M – 5M	36.16 ± 20.39	51.49 ± 15.99	0.009
FMPS, %	BL-2M	3.95 ± 12.95	-2.25 ± 11.1	0.001
	2M-5M	51.40 ± 20.92	65.10 ± 13.97	0.014
Recession, mm	BL-2M	-0.03 ± 0.52	-0.02 ± 14.9	0.09
	2M-5M	-0.40 ± 0.62	-0.46 ± 0.36	0.19
Probing depth, mm	BL-2M	0.04 ± 12.95	-0.01 ± 0.03	0.38
	2M- 5M	1.14 ± 0.44	1.29 ± 0.48	0.15
Probing depth ≥5mm	BL – 2M	-0.03 ± 0.18	0.03 ± 0.15	0.42
	2M – 5M	0.96 ± 1.18	0.46 ± 0.54	0.10
Number of pockets ≥5mm	BL – 2M	3.04 ± 7.25	0.68 ± 9,25	0.059
	2M – 5M	39.48 ± 21.39	47.56 ± 19.88	0.16
Clinical attachment level, mm	BL- 2M	0.03 ± 0.41	-0.06 ± 0.39	0.039
	2M – 5M	0.66 ± 0.46	0.89 ± 0.83	0.031
Vitamin C, mg/l	BL – 2M	-1.98±5.67	0.16±6.52	0.87

	2M – 5M	-2.62±7.24	-2.58±6.67	0.78
CRP, mg/l	BL – 2M	-0.3±2.4	0.4±1.8	0.86
	2M – 5M	-0.2±1.9	0.5±1.9	0.20
Tryglicerids, mmol/l	BL – 5M	8.67 ± 30.68	-0.68 ± 31.64	0.15
	2M – 5M	-15.88 ± 34.54	1.40 ± 39.10	0.008
Total Cholesterol, mmol/l	BL – 2M	-2.96 ± 15.35	9.54 ± 18.42	0.81
	2M – 5M	-2.39 ± 25.71	-7.67 ± 18.15	0.88
HDL, mmol/l	BL – 2M	-3.25 ± 15.10	2.30 ± 6.10	0.48
	2M – 5M	3.33 ± 14.51	-4.37 ± 7.41	0.024
HbA1c, mmol/l	BL – 2M	0.09 ± 1.74	-0.83 ± 2.31	0.14
	2M – 5M	-0.09 ± 1.68	0.72 ± 1.90	0.059
Systolic BP, mmHg	BL – 2M	-0.20 ± 13.81	0.00 ± 12.99	0.034
	2M – 5M	3.20 ± 20.09	-1.46 ± 14.93	0.30
Diastolic BP, mmHg	BL – 2M	3.60 ± 10.05	0.40 ± 9.35	0.36
	2M – 5M	0.20 ± 11,32	-2.25 ± 11.49	0.41
Body temperature °C	BL – 2M	-0.11 ± 0.52	0.24 ± 0.10	0.38
	2M – 5M	-0.12 ± 0.47	0.03 ± 0.71	0.42
Heartrate, beat/minute	BL – 2M	-1.20 ± 9.63	1.08 ± 7.84	0.27
	2M – 5M	0.78 ± 12.49	0.58 ± 9.33	0.70

CRP, C-reactive Protein; BP, blood pressure; HDL, high-density lipoprotein BL, baseline, 2M; 2 months; 5M, 5 months.

Figure 1. Flowchart of the study