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ORIGINAL ARTICLE

Periostin in the relation between periodontal disease and atherosclerotic coronary artery disease: A pilot randomized clinical study

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Abstract

Objective: The aim of this study was to analyze the effects of periodontal treatment on markers of atherosclerotic coronary artery disease and circulating levels of periostin.

Background: Periostin is necessary for periodontal stability, but it is highly present in atherosclerotic plaques. Treatment of periodontal disease, with low levels of local periostin, is thought to reduce systemic levels of periostin. Thus, this may contribute to cardiovascular health.

Methods: A pilot randomized controlled clinical trial was designed to include patients with severe periodontal disease and history of atherosclerotic coronary artery disease. Samples of gingival crevicular fluid (GCF) and serum were collected before and after periodontal treatment by periodontal surgery or non-surgical therapy. The levels of several markers of inflammation and cardiovascular damage were evaluated including CRP, IFN-γ, IL-1ß, IL-10, MIP-1α, periostin, and TNF-α in GCF and CRP, Fibrinogen, IFN-γ, IL-1ß, IL-6, IL-10, L-Selectin, MIP-1α, Periostin, TNF-α, and vWF in serum.

Results: A total of 22 patients with an average of 56 years old were recruited for participating in this study. Twenty of them were male. Most of them (82%) had suffered an acute myocardial event and underwent surgery for placing 1, 2, or 3 stents in the coronary arteries more than 6 months ago but less than 1 year. The treatment of periodontal disease resulted in an overall improvement of all periodontal parameters. Regarding the evaluation of GCF and serum, a significant increase of periostin in the GCF was observed after periodontal surgery. In contrast, although other markers in GCF and serum improved, no significant correlations were found.

Conclusion: Treatment of periodontal disease through periodontal surgery induces a local and transient increase in the levels of periostin in the gingival crevicular fluid. The effects on systemic markers of inflammation and cardiovascular function have not been confirmed.

KEYWORDS
atherosclerosis, coronary heart disease, extracellular matrix, periodontal disease, periodontal therapy, periostin

1 | INTRODUCTION

Periodontal diseases are chronic localized infectious and inflammatory diseases that affect the periodontium. Severe or moderate forms affect 45%–84% of adults in the European Union (EU) and are the main cause of tooth and bone loss.[1] The worldwide prevalence has remained stable in the last 30 years.[2,3] On the other hand, cardiovascular diseases (CVD) are the largest cause of death in the EU, accounting for 40% of deaths or 2 million per year. CVD are estimated to cost the EU economy almost EUR 110 billion a year, and they are one of the leading causes of long-term sickness and loss to the labor market (CVD Statistics). Atherosclerotic cardiovascular diseases (ACVD), specifically, are due to the generation of an atheroma plaque in the wall of any artery with fatal consequences in the coronary arteries (CAD). ACVD and periodontal disease have been associated in numerous epidemiological and interventional studies.[4–9] However, their specific biological interactions are yet to be fully understood.

Inflammation plays a determinant role in both diseases.[10,11] Periodontal disease is initiated by the ecologic succession of the dental plaque that triggers a host immune and inflammatory response. Additional to genetic and environmental common risk factors, periodontal diseases result in bacteremia and endotoxemia that produce direct effects on platelet aggregation, endothelial damage by crossed autoimmune responses, macrophage and endothelial cell invasion, and endocrine effects on vascular tissues by pro-inflammatory mediators.[12] Thus, periodontal diseases are known to increase the risk for ACVD and, subsequently, also correlate with infarct size.[13]

The maintenance of periodontal tissues is the result of a sensitive balance. Matricellular molecules, such as periostin, play a determinant role in this. Periostin (gene POSTN) is a 90 kDa, 835 aminoacids protein highly expressed in the periosteum.[14] In the oral cavity, periostin is highly specific for the periodontal ligament (PDL) and essential for its homeostasis.[15] Its expression is regulated by mechanical stimulation through TGF-ß1. Periostin interacts with collagen to increase its cross-linking, fiber diameter and, therefore, tissue mechanical strength. It has been used as a marker of periodontal functionality after regeneration processes[16,17] and it is known to increase cell migration and proliferation.[18,19] However, the expression of periostin is reduced in vitro by periodontal bacteria and pro-inflammatory cytokines.[20] In vivo, in the presence of periodontal disease, its expression is reduced and contributes to the progression of the inflammatory infiltrate and alveolar bone destruction.[21] Interestingly, it is fibrillary distributed in healthy human tissues while it is diffused in diseased tissues.[15] These effects suggest a protective role of periostin in periodontal disease pathogenesis while its reduction constitutes a concomitant cumulative effect for disease progression. Interestingly, after periodontal surgery, levels of periostin increase in gingival crevicular fluid (GCF) and decrease in serum.[15] This inverse effect is explained by the fact that periostin increases locally by PDL cells to promote tissue healing and restore the structure and function. At a systemic level, however, periostin is associated with fibrotic and inflammatory processes, such as asthma, allergy, and kidney inflammatory diseases.[22,23] Therefore, the reduction of circulating periostin after periodontal surgery could help to reduce the negative effects on distant tissues.

In the cardiovascular system, periostin is required during cardiac development.[14] Adult heart, however, does not show high levels of periostin. These levels of periostin only increase after acute events, fibrotic remodeling, and cardiomyocyte senescence.[24] This is, periostin has been referred to as a "heterofunctional regulator of cardiac development and disease"[25] as it is necessary for tissue development but it becomes pathologic if maintained. As a consequence, high serum levels of circulating periostin have been detected after acute myocardial infarction[26] and are associated with reduced cardiac function and increased risk of new cardiac events.[27] Furthermore, periostin is also increased in atheroma plaques[28] and it has been associated to their maturation by calcification.[29] It additionally contributes to atherogenesis by increasing smooth muscle cell migration, angiogenesis, and MMPs production and increases the risk of CAD.[30]

The relevance of periostin in periodontal disease and CAD and the plausibility of the proposed biological interaction should, therefore, be investigated. Thus, we hypothesized that periodontal treatment improves systemic biomarkers of atherosclerotic coronary artery disease in association with a reduction of circulating levels of periostin and an increase of local periostin in the gingival crevicular fluid. So, our overall goal was to evaluate the effects of periodontal treatment on atherosclerotic coronary artery disease based on the reduction of circulating periostin. In addition, to analyze which could be a sufficient therapy, a randomized controlled clinical trial was designed to perform either a localized periodontal surgery combined with full-mouth non-surgical therapy, or only full-mouth non-surgical periodontal therapy.

2 | MATERIALS AND METHODS

2.1 | Study design and settings

We used the CONSORT checklist (Appendix S2) when writing our report.[31]

To achieve the goals of the study, a randomized controlled clinical trial was conducted in patients with periodontal disease and atherosclerotic coronary artery disease. They received either localized

periodontal surgery (PS) on the worst quadrant and non-surgical therapy (NST; scaling and root planning) on the rest of the mouth or only a session of full-mouth NST. Both groups were followed up at 2 weeks, 1 month, 3 months, and 6 months post-intervention. Before any procedure was initiated, all patients were informed of the aims, procedures, risks, benefits, rights, and responsibilities associated with the study and provided their voluntary signed informed consent. Recruitment of patients was performed at the Cardiovascular Clinic of the Hospital Virgen de las Nieves (Granada, Spain) and the School of Dentistry – University of Granada (Spain), while periodontal treatment and sample evaluation was conducted at the School of Dentistry – University of Granada and the Centre for Biomedical Research – University of Granada, respectively. All procedures were conducted under the supervision of the Ethics Committee on Human Research of Granada (Junta de Andalucia, Spain) (registration number 0296-M1-16) and the Ethics Committee on Human Research of the University of Granada (registration number 937). The study was registered at the International Standard Randomized Controlled Trial Number site (registration number ISRCTN17884414).

2.2 | Study population

As a pilot study, and based on our previous studies,[15] a total of 10 patients per group were considered necessary to detect significant changes in periostin over time after periodontal treatment. To be included in the study, patients had to be under 75 years old with atherosclerotic coronary artery disease defined as more than 50% blockage of a coronary artery or recent coronary event (6–36 months before), including myocardial infarction, coronary bypass, or stent. They also have to present what is currently classified as localized or generalized Stage III, Grade B periodontal disease.[32] In any case, at least one quadrant had to be susceptible to periodontal surgery due to more than 30% of teeth with more than 6 mm of probing depths. Exclusion criteria included additional coronary event less than 6 months before enrolment, renal failure (creatinine higher than 1.5 mg/dL), uncontrolled liver or pulmonary disease, malignant tumor, autoimmune disease, neurologic or psychiatric disorder, uncontrolled diabetes mellitus (HbA1c higher than 8), presence of infectious disease (aside from periodontal), antibiotic or periodontal therapy 3 months before, alcoholism or drug abuse, other on-going oral condition (orthodontic treatment, caries, etc.), any other contraindication for treatment or unwilling or unable to provide informed consent and participate in the study. After inclusion, patients would be secondarily excluded if periodontal disease progression of more than 2 mm at the 3 months re-evaluation, (re) infarction, or cardiovascular (re)intervention was observed.

2.3 | Randomization and study procedures

After enrolment, patients were randomized to a control group (NST) or intervention group (PS). Randomization was performed by using the software Minimpy[33] to balance age, gender, and smoking status. Patients assigned to the NST group received a full mouth supragingival tooth cleaning and scaling and root planning. Patients in the PS group received surgical treatment of periodontal disease by access flap or modified Widman flap and bone surgery (to smooth shallow boney craters), if required.[34] If more than one quadrant was susceptible to receive periodontal surgery, it was conducted only in the more severe one. In both cases, the provider was the main author of the study (MP-M). Sutures were removed 7 days later. Post-surgical and oral hygiene instructions were provided in all cases, including recommendations for analgesics if required and chlorhexidine mouth rinse for 15 days.

2.4 | Data collection

A complete oral and medical history was recorded, including age, gender, maximum level of study, smoking status, diabetes, type and time since the cardiovascular event, weight, height, body mass index (BMI), resting blood pressure, and glycemia. An oral and periodontal exam was performed by an experienced provider (GG-P) at baseline, 3 and 6 months, including probing depth (PD), gingival recession (GR), clinical attachment level (CAL) (all in mm), and bleeding on probing (BOP, dichotomous) in 6 sites per tooth. Additionally, Löe's gingival (GI) and plaque indexes (PI)[35] were also registered in four sites per tooth. After treatment, wound healing (WHI) was registered as: 0 = Mature wound healing; 1 = Erythema; 2 = Spontaneous bleeding; 3 = Flap mobility; 4 = Suppuration; and 5 = Necrosis.[36] In addition, patients registered overall pain in a 100 mm scale from 0 (no pain at all) to 100 (maximum pain imaginable). WHI and pain were registered only for the 1-, 2- and 4-week post-baseline visits.

2.5 | Sample collection and analysis

At baseline, 2 weeks, and 3 and 6 months, gingival crevicular fluid (GCF) and serum were collected. Levels of periostin in GCF and serum after periodontal treatment are considered the primary variables of the study while levels of other markers are secondary variables.

2.5.1 | Gingival crevicular fluid

For GCF collection, we followed the same methodology used previously by our group.[15] Briefly, to avoid contamination, before any periodontal measurement was conducted, the area with the three sites with the deepest PD, as determined in the screening visit, were isolated with cotton rolls, air-dried, tooth plaque removed, and a GCF paper strip carefully inserted in the sulcus for 30 s. Then, the strip was retrieved and immediately stored at −80°C until assayed. Fluid extraction from the strip consisted of 5 sequential washes with 20 μL of a PBS solution containing PMSF (1:200), aprotinin (1:100),

and human albumin (30%) that were spun at 760*g*, 4°C, 5 min each. The final volume (100 μL) was stored at −80°C until analysis.

2.5.2 | Serum

Peripheral venous blood was extracted and collected into BD Vacutainer® blood collection tubes, and immediately separated in plasma and serum by spinning for 10 min at 1000*g*. Serum was collected and stored at −80°C until assayed.

2.5.3 | Biomarker determination

Sample processing and biomarker determination were conducted by a blinded study member (NM-M). Once the samples from both GCF and serum were prepared and ready to be analyzed, the concentration of each marker of interest was quantified by using an ELISA-type multiplex assay based on Luminex® xMAP® magnetic beads technology (EMD Millipore's MILLIPLEX® MAP, Merck). We used three different personalized kits for the different groups of markers to be analyzed: cytokines (HCYTOMAG-60 K: IFN-γ, IL-1ß, IL-6, IL-10, MIP-1α, TNF-α), markers related to cardiovascular health (HCVD3MAG-67 K: CRP, Fibrinogen, L-Selectin, vWF), and periostin (HCMBMAG-22 K). CRP, IFN-γ, IL-1ß, IL-10, MIP-1α, periostin, and TNF-α were evaluated in GCF while CRP, Fibrinogen, IFN-γ, IL-1ß, IL-6, IL-10, L-Selectin, MIP-1α, Periostin, TNF-α, and vWF were evaluated in serum. The assay for periostin has a sensitivity of 0.12 ng/mL and no cross-reactivity, according to the manufacturer.

At the manufacturer recommendations, after the preparation of the reactants necessary in the analysis (microspheres with immobilized antibodies, washing buffer, standards) the assay procedure was initiated. The 96 wells of the plate provided by the kit were previously washed with the wash buffer, and then the standards were added to the appropriate wells. Subsequently, the test buffer and the matrix solution (if required depending on the type of kit) were added to the appropriate wells. Then, the GCF or serum samples were added (with the appropriate dilutions following the manufacturer's recommendations) and then the magnetic microspheres. The plates were incubated at 4°C throughout the night and after that, the detection antibodies were added. After 1 h of incubation at room temperature, streptavidin-phycoerythrin was added, the plate was incubated for 30 min at room temperature and several plate washes were performed. Finally, the reading buffer was added, and the plate was read in a Luminex 200 device that measures the fluorescence of each well. The concentration of each marker was calculated against the standard curve. Each sample was analyzed in duplicate.

2.6 | Statistical analysis

IBM SPSS-MacOS 28.0 (SPSS Inc.) was used for the analyses. Results are presented as mean (standard deviation) for continuous variables and as percentage (frequency) for categorical data. Given the limited sample size, all comparisons were evaluated by non-parametric tests. Differences between groups were investigated by the Independent Samples Mann–Whitney U Test; differences over time within each group were analyzed by the Related-Samples Friedman's Two-Way Analysis of Variance by Ranks and pairwise comparisons further evaluated by applying the Bonferroni correction for multiple tests. Correlations between variables were evaluated by calculating the *p*-value for the Spearman's rank correlation coefficient. Differences were considered statistically significant when *p*-values were below .05. Graphics were constructed on Microsoft® Excel for Mac v16.76 and Prism 7 for Mac.

3 | RESULTS

3.1 | Study population

A total of 878 patients were pre-screened from either direct interview during follow-up at the Cardiovascular Clinic of the Hospital Virgen de las Nieves or clinical records review at the School of Dentistry – University of Granada. Of those, 835 did not meet the inclusion criteria, 3 declined to participate, and 4 were being treated for other pathologies. Thus, 36 patients were fully screened for potential inclusion. Only 22 were randomized, 11 to each group, because 13 did not meet the inclusion criteria and another one did not show to the baseline visit. All patients included were followed for the whole duration of the study. A flow diagram of patients in the study is presented in Figure 1.

Demographics and clinical basic data of randomized patients are presented in Table 1. As noted, no statistically significant differences were detected between groups for any variable except for diabetes. All six patients with diabetes were assigned to the PS group. Most patients were men in their mid-fifties, overweighted, with high school or lower education level, former smokers, who had suffered an acute myocardial infarction that made them go under surgery for placement of a stent in any of the coronary arteries less than a year before.

3.2 | Status and evolution of periodontal and atherosclerotic disease after treatment

Full-mouth average clinical attachment level was not statistically different between groups at any time point. More than 50% of sites had more than 5 mm of calculated CAL at baseline, while only 30.88 (26.75) % and 38.06 (32.49) % at 6 months presented that measure or above in the NST and PS groups, respectively ($p = .898$ and $p = .905$, baseline and 6 months, respectively; Independent Samples Mann–Whitney U Test). The change over time was significant in both groups ($p < .001$, Related-Samples Friedman's Two-Way Analysis of Variance by Ranks) when comparing the baseline data against the 3 or 6 months but not between 3 and

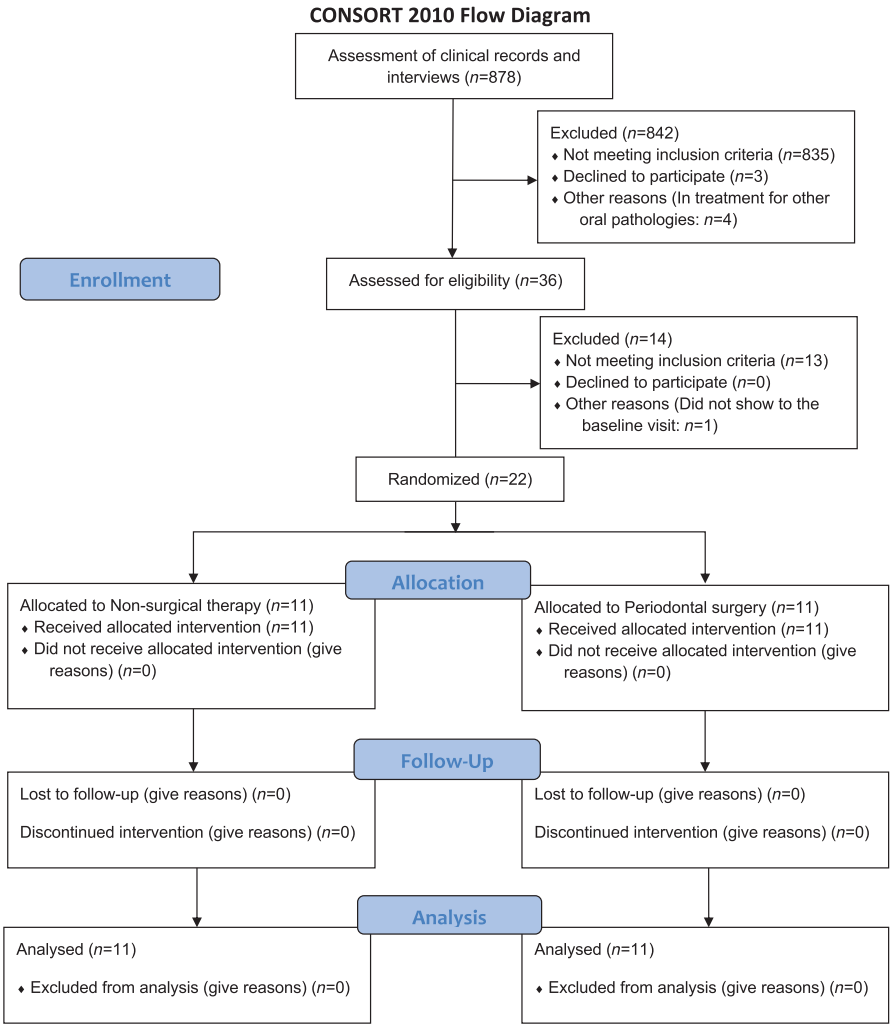

FIGURE 1 Flow diagram of patients in the study.

6 months (Bonferroni correction for multiple comparisons). In all cases, probing depth decreased from 21.32 (15.11) % of sites with more than 6 mm in the NST group and 17.89 (11.30) % in the PS group at baseline ($p = .606$, Independent Samples Mann–Whitney U Test) to 1.97 (3.44) % and 1.71 (1.73) % at 6 months ($p = .604$, Independent Samples Mann–Whitney U Test). The change over time was statistically significant ($p < .001$, Related-Samples Friedman's Two-Way Analysis of Variance by Ranks) and, again, specifically when comparing the baseline data against the 3 or 6 months but not between 3 and 6 months (Bonferroni correction for multiple comparisons). FGM and PD data are represented in Figure 2. Although overall CAL change from baseline to either 3 or 6 months was not statistically different between groups, the difference in PD reduction from baseline to 3 months was statistically significant (1.18 (0.67) mm vs. 1.41 (0.59) mm; NST vs. PS, respectively; $p < .001$, Independent Samples Mann–Whitney U Test). Accordingly, sites with positive BOP decreased from 39.60 (29.65) % and 42.90 (23.88) % at baseline to 1.48 (3.12) % and 0.00 (0.00) % at 6 months (NST and PS, respectively). Differences in BOP were not statistically significant between groups but were significant over time. Similarly, differences in both PI and GI were not statistically significant between groups but were significant over time (Figures S1 and S2). No differences were detected between groups in any other periodontal variable, except WHI at 7 days.

All periodontal data, WHI, and pain over time are available in Tables S1–S3.

Regarding other cardiovascular parameters such as blood pressure and glycemia, no differences were observed neither between groups nor over time (Table S4; Figures S3 and S4).

3.3 | Biomarkers in gingival crevicular fluid and serum

The levels of all biomarkers in the GCF and the statistical comparisons, both between groups and over time, are summarized in Table S5 and graphically represented in Figure 3. Similarly, the analyses in serum are presented in Table S6 and Figure 4.

The levels of periostin in the GCF of patients with periodontitis who underwent surgery increased significantly over time ($p = .019$, Related-Samples Friedman's Two-Way Analysis of Variance by Ranks) and with respect to the group who received non-surgical therapy. Specifically, the concentrations of periostin at 1, 2, and 4 weeks after

TABLE 1 Characteristics of patients included in the study.

	Total–*n* = 22	NST–*n* = 11	PS–*n* = 11	*p* value*
Age (years)				
Mean	56.32	57.73	54.91	.300
Range	45–68	45–68	48–65	
Gender (*n* (%))				
Male	20 (90.91)	10 (90.91)	10 (90.91)	1.000
Female	2 (9.09)	1 (9.09)	1 (9.09)	
Education level (*n* (%))				
Less than high school	15 (68.18)	10 (90.91)	5 (45.45)	.069
High school	6 (27.27)	1 (9.09)	5 (45.45)	
More than high school	1 (4.55)	0 (0.00)	1 (9.09)	
Smoking status (*n* (%))				
Never smoked	4 (18.18)	2 (18.18)	2 (18.18)	.054
Former (<10 cigarettes)	4 (18.18)	4 (36.36)	0 (0.00)	
Former (>10 cigarettes)	11 (50.00)	3 (27.27)	8 (72.73)	
Current (<10 cigarettes)	3 (13.64)	2 (18.18)	1 (9.09)	
Diabetes (*n* (%))				
No	16 (72.73)	11 (100.00)	5 (45.45)	.004
Yes	6 (27.27)	0 (0.00)	6 (54.55)	
Cardiovascular event (*n* (%))				
AMI	3 (13.64)	1 (9.09)	2 (18.18)	.459
AMI + Stent	18 (81.82)	10 (90.91)	8 (72.73)	
Stent	1 (4.55)	0 (0.00)	1 (9.09)	
Time since cardiovascular event (*n* (%))				
>6 months to ≤1 year	18 (81.82)	10 (90.91)	8 (72.73)	.121
>1 year to ≤2 years	3 (13.64)	0 (0.00)	3 (27.27)	
>2 years to ≤3 years	1 (4.55)	1 (9.09)	0 (0.00)	
Weight (kg) (mean (SD))	79.55 (14.50)	75.27 (12.63)	83.82 (15.55)	.270
Height (cm) (mean (SD))	167.32 (8.46)	168.09 (6.91)	166.55 (10.07)	.898
BMI (mean (SD))	28.34 (4.25)	26.57 (3.99)	30.10 (3.89)	.056

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index.

*: *p* value (NST vs. PS): Independent Samples Mann–Whitney U Test for continuous variables; Chi-square for categorical variables.

surgery were significantly higher in the PS group compared to the NST group (0.5 (0.38) vs. 3.16 (3.36) ng/mL, 1.4 (2.12) vs. 6.99 (6.36) ng/mL, and 0.6 (0.52) vs. 4.72 (4.44) ng/mL; $p = .010$, $p = .007$, and $p < .001$, respectively). Serum levels of periostin, on the other hand, did not vary significantly over time, nor between groups. Analysis of this primary outcome confirmed that the statistical power reached was low.

The concentrations of cytokines and chemokines characteristic of the inflammatory process in periodontal disease and atherosclerosis showed no significant differences either over time or between the two groups. This was observed both in GCF and in serum.

Regarding specific markers of atherosclerotic disease and cardiovascular health, including CRP, fibrinogen, L-selectin, and vWF, we found no significant differences neither over time nor between groups.

Finally, the analysis of possible correlations between the different biomarkers analyzed and clinical data showed no clear trend; isolated significant correlations were found though.

4 | DISCUSSION

This study was designed to evaluate a possible reduction of periostin levels in serum associated to increased periostin in the GCF because of periodontal treatment. A temporary significant increase in local periostin levels was observed after surgical treatment of periodontal disease. A reduction of some markers associated with periodontal and atherosclerotic disease (e.g., IL-6, MIP-1α, and TNF-α), particularly in the group who received periodontal surgery, was observed, although the differences were not statistically significant. The importance of this study with respect to previous studies that analyzed

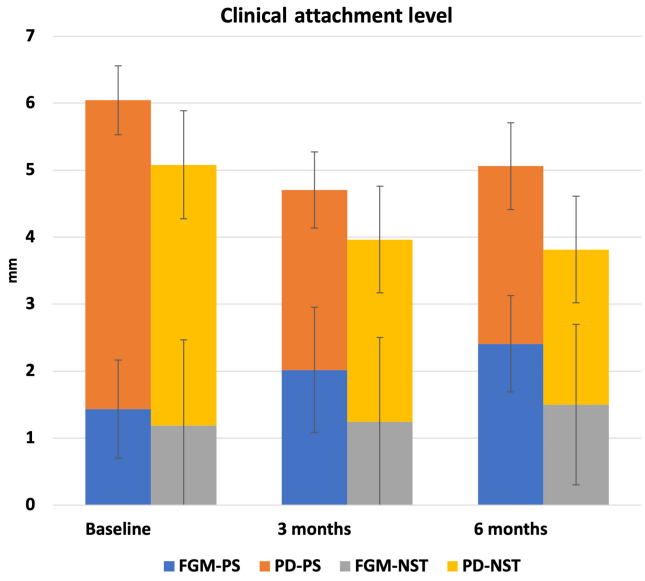

FIGURE 2 Graphical representation of clinical attachment level (CAL) as a piled composition of FGM and PD over time.

how periostin levels vary in periodontal disease lies in the inclusion of patients who present, in addition to periodontitis, atherosclerotic disease of the coronary arteries. Only a previous cross-sectional study has studied patients with both diseases and has found a potential role of periostin in the association.[37]

Levels of periostin in the GCF of sites with periodontitis are significantly lower than in sites with gingivitis and periodontal health.[38] Periostin has been particularly proposed as a marker of susceptibility and activity of periodontal disease because it negatively correlates with the clinical parameters indicative of periodontal health.[39–41] In the presence of systemic diseases such as diabetes, levels of periostin in the GCF in cases of periodontal disease are even lower.[42] Our group made important contributions to the understanding of this association in a number of in vitro, animal, and clinical studies.[15,18–21] In summary, factors associated to periodontal disease, such as inflammation and bacterial byproducts, can reduce the expression of periostin by periodontal ligament fibroblasts. Consequently, cell migration and extracellular matrix organization are impaired. Aggressive forms of periodontal disease demonstrate even lower levels of periostin, which confirms the double role in the maintenance of periodontal structure.[40,43] These are: (1) periostin is essential for maintaining the strength of the periodontal structure; (2) if periostin is reduced either by the action of inflammation or bacterial byproducts, the tissue destruction is initiated. Fortunately, periodontal therapy has been shown to promote an increase in those levels of periostin in the GCF.[15,44,45] This transient increase would serve as a booster for cell migration and extracellular matrix organization. Once the healing is finalized, periostin levels should go back to the levels of a healed site; this is higher than in disease. In the current study, although other markers of inflammation and tissue destruction have followed a non-regular pattern and statistical differences have not been clearly demonstrated, the behavior of periostin in the GCF has followed the same pattern as described in the aforementioned studies. This confirms, once more, its role in periodontal disease, therapy, and healing.

In contrast, the levels of periostin that are released to the surroundings and reach systemic areas follow the opposite scheme: periostin detection is higher in the saliva of patients with periodontal disease, even higher if the periodontal disease is an aggressive form.[40] More distantly, besides the finding of periostin in atheroma plaques,[28] an animal study has found that periostin knock-out mice did not develop aortic atherosclerotic plaque lesions as big as wild-type mice.[46] Furthermore, macrophage recruitment, extracellular matrix organization, and fibrotic caps were reduced in the absence of periostin while MMP-2 and MMP-13 were increased. Overall, the authors proposed therapeutic reduction of periostin as a potential target for the treatment of atherosclerotic plaques.

Thus, although the levels of periostin in serum are not different between different periodontal conditions,[41] according to a previous study, after periodontal surgery, levels of periostin decrease in serum following a similar transient pattern as the increase in the GCF.[15] This was hypothesized as an indication of a reduction of the release of periostin from the local periodontal surgical site to the systemic blood circulation. As a consequence, due to the hetero-functional aspect of periostin in health and disease,[22,25,47,48] those sites where periostin would induce a negative effect, such as an atheroma plaque, would benefit from this reduction of periostin after the periodontal therapy. However, in the current study, no significant changes between groups at the different time points nor over time were detected. These results indicate that there might be no systemic influence of periodontal surgery on the expression of periostin in patients with periodontitis and atherosclerosis, since its levels do not change in serum, in contrast with those observed in a previous study.[15] Even more, unfortunately, in the current study, we have not been able to demonstrate changes in other markers nor any correlation with the levels of periostin, neither in the GCF nor in serum.

Together with periostin, we have evaluated CRP, IFN-γ, IL-1ß, IL-10, MIP-1α, and TNF-α in GCF, and CRP, Fibrinogen, IFN-γ, IL-1ß, IL-6, IL-10, L-Selectin, MIP-1α, TNF-α, and vWF in serum. Markers of inflammation such as CRP, IFN-γ, IL-1ß, IL-10, MIP-1α, and TNF-α have been shown to be associated with the adaptive and innate response. In turn, systemic levels of CRP, Fibrinogen, IL-6, L-selectin, TNF-α, and vWF have been associated with atherogenesis, endothelial dysfunction, and, thus, predictors of coronary artery disease.[49] In the current study, although not significantly, some such as IL-6, MIP-1α, and TNF-α showed a reduction in serum over time after periodontal treatment. Particularly, IL-6 is directly related to myocardial damage in the chronic and not acute phase after infarction such as troponin.[50] The observed reduction may indicate long-term benefit rather than immediate benefit. TNF-α is a widely known inflammatory mediator that plays a determining role in the regulation of cardiac contractility and peripheral resistance, the main determinants of myocardial function. Elevated levels have been associated with ischemia–reperfusion damage, myocarditis, and progression of congestive heart failure.[51] Finally, high levels of MIP-1α are associated

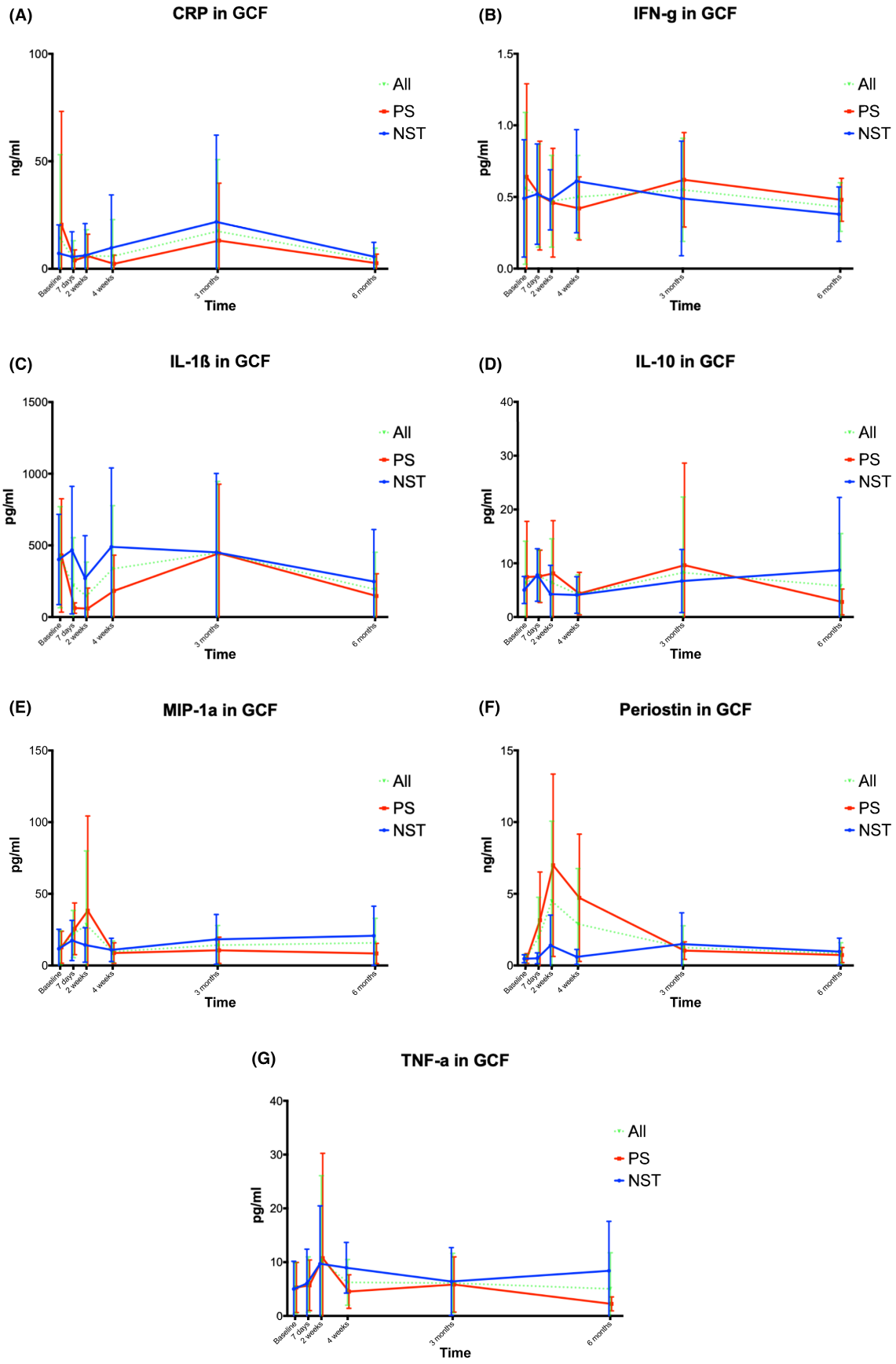

FIGURE 3 Graphical representation of the different markers analyzed in the gingival crevicular fluid (GCF) over time, including (A) CRP, (B) IFN-γ, (C) IL-1ß, (D) IL-10, (E) MIP-1α, (F) periostin, and (G) TNF-α.

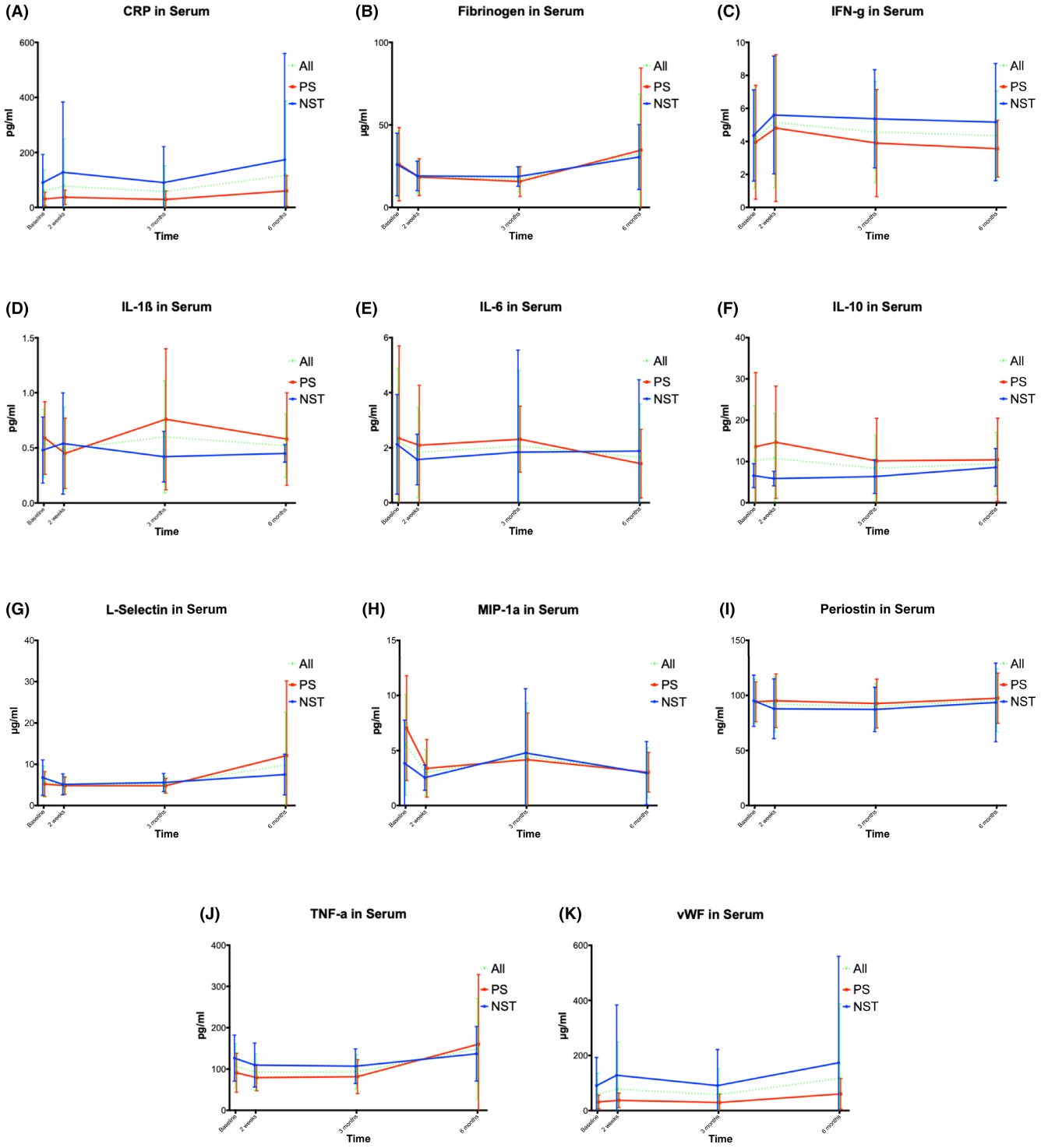

FIGURE 4 Graphical representation of the different markers analyzed in serum over time, including (A) CRP, (B) fibrinogen, (C) IFN-γ, (D) IL-1ß, (E) IL-6, (F) IL-10, (G) L-selectin, (H) MIP-1α, (I) periostin, (J) TNF-α, and (K) vWF.

with increased risk of new cardiovascular events independently with other risk factors as it increases the recruitment of monocytes to the zone of inflammation and may contribute to increased atheromatous plaque.[52] Therefore, the observed reduction of these markers may indicate that periodontal treatment induces a systemic reduction of inflammatory markers and an improvement in the maintenance of overall cardiovascular health. For some of those markers, we observed differences at baseline, which could be related to other conditions not factored in the current study. Regardless, periodontal disease might not influence systemic levels of cytokines in patients with acute coronary syndrome, as commonly thought.[53]

In any case, although the direct effects of periodontal treatment on cardiovascular health and reduction of pro-atherogenesis markers through periostin, according to the current study, might not be strong

enough, we must keep in mind the actions of periostin in the cardiovascular system as a whole. Particularly, periostin is necessary and highly expressed during cardiac development. In the adult heart it is not expressed at high levels but increases after acute events,[54,55] reflecting fibrotic remodeling processes[56] and subsequent short-term loss of function.[57] Thus, its use as a marker of the severity of cardiac damage has been suggested.[27,58] It may contribute to generate vulnerability to acute myocardial infarction in cardiomyocytes[59] but it also induces the recruitment of cardiomyocytes to the infarcted area,[47] showing that the balance between beneficial effects and low levels that shall prevent the fibrosis process is under study with conflicting results. It all seems to be dependent on the time after infarction.[60] In atherosclerotic plaques periostin is increased[28] and contributes to atherogenesis[61] by increasing smooth muscle cell migration[62] and calcification,[29] adventitial remodeling,[63] and angiogenesis and MMP production,[30] which increases the risk of atherosclerotic coronary heart disease.[64] Thus, the goal would be to keep periostin high in the GCF associated to periodontal health that would be associated to a limited release to the system; in case of an acute atherosclerotic event, the low levels of periostin may help reduce the consequences of such event.

Certain limitations of this study could affect the results presented. Among them, the main one is the low number of individuals who participated in the study, which ultimately affected the statistical power to detect differences in the primary variable (differences in serum levels of periostin over time). However, the study was designed as a pilot study because of the limited information in the specific hypothesis presented. In fact, the only study we know of in which the serum levels of periostin were evaluated after periodontal treatment had a similar sample size,[15] and was, in fact, the base for the current study. Moreover, we must keep in mind that periodontal disease may induce severe alteration of the systemic inflammatory profile because of the continuous low-level inflammatory burden and bacteremia with higher defensive cell counts and an increased risk of systemic diseases, in comparison with healthy patients.[65] However, cardiovascular diseases are highly multifactorial, and patients often suffer from other concomitant systemic pathologies and are undergoing chronic pharmacological treatments. This could be masking the reduction of the biomarkers we have evaluated in these patients. So much so that, although the influence of diabetes, for example, on serum periostin levels has not been specifically analyzed in the current study, there is biological plausibility for the existence of this relationship, similar to that shown by Morsy and Ali.[42] In turn, diabetes influences cardiovascular disease by altering, among others, the normal healing capacity because of changes in the inflammatory capacities and control mechanisms. In this way, periostin could have a role in regulating both. Several studies show that diseases such as diabetes or obesity and habits such as smoking are related to periodontal disease affecting its progression and evolution while at the same time affecting the cardiovascular system; the interaction between all of them ultimately modifies the potential benefits on the cardiovascular health of treating periodontal disease.[4–6] In addition, if we consider the type of treatment conducted in the current study, we must also mention a recent systematic review of reviews that concludes that the evidence regarding the impact of periodontal therapy on improvement of systemic diseases is not strong. In fact, it is concluded that the evidence and reviews frequently lack proper quality.[66]

Regardless of these limitations, the current study, for the first time, makes an approximation to the biological plausibility of the interaction of periodontal and coronary artery diseases through the actions of periostin. Although the results might be inconclusive, they offer opportunities for future studies with higher sample size and wider biomarkers under study.

5 | CONCLUSION

Treatment of periodontal disease through periodontal surgery induces a local and transient increase in the levels of periostin in the gingival crevicular fluid. The effects on systemic markers of inflammation and cardiovascular function have not been confirmed.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest, either directly or indirectly, in any of the products listed in the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

INFORMED CONSENT

Written informed consent for this randomized clinical study was obtained from each patient before any study procedures were initiated. The protocol was previously approved by the Ethics Committee on Human Research from the University of Granada on January 27, 2015, and was registered with number 937. Additionally, the protocol was registered in a WHO and ICMJE approved registry with the code ISRCTN17884414 (https://doi.org/10.1186/ISRCTN17884414).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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