

# The effect of nonsurgical periodontal therapy on hepcidin and on inflammatory and iron marker levels

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**Abstract:** Serum hepcidin levels may increase in response to infection and inflammation. The present study investigated the effect of nonsurgical periodontal therapy (NSPT) on levels of serum hepcidin, inflammatory markers, and iron markers. An interventional study was conducted on 67 patients (age 30–65 years) without other diseases, except for chronic periodontitis (CP). Patients were allocated to either CP or control groups. The CP group received supragingival and subgingival scaling and root planing procedures, whereas the control group received supragingival scaling. Probing depth (PD), bleeding on probing, clinical attachment level (CAL), visible plaque index (VPI), serum hepcidin and interleukin-6 (IL-6) levels, high-sensitivity C-reactive protein (hs-CRP), hematological markers, and iron markers were measured at baseline and at 90 days after NSPT. The CP group had statistically significant lower mean values for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) ( $p \leq 0.05$ ). The control group had statistically significant reductions in hemoglobin, hematocrit, MCV, and MCH ( $p \leq 0.05$ ). Serum hepcidin, IL-6, and erythrocyte sedimentation rate (ESR) levels were significantly decreased in both groups after NSPT. Periodontal markers were more markedly reduced in the CP group compared with the control group ( $p \leq 0.05$ ). These findings suggest that NSPT may reduce the serum levels of IL-6, hepcidin, and periodontal parameters.

**Keywords:** Chronic Periodontitis; Heparins; Iron; Inflammation.

## Introduction

Periodontal disease is an immunoinflammatory disease of infectious etiology, including gingivitis and periodontitis, which affect the protective and supporting tissues of teeth.<sup>1</sup> Dental biofilm of microorganisms, primarily gram-negative and anaerobic bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans*, is reported to be the primary etiologic factor of most periodontal diseases.<sup>2</sup> Persistent bacterial infection causes the host immune response to disrupt homeostatic mechanisms, resulting in the release of inflammatory mediators, including proinflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor-alpha [TNF- $\alpha$ ]), proteases (metalloproteases), and prostanoids (prostaglandin E2) by macrophages



and monocytes. These inflammatory mediators cause bone loss and destruction of the extracellular matrix in the gingiva and also stimulate the production of acute phase proteins, with IL-6 being recognized as the main inductor.<sup>3,4,5</sup>

The low intensity of the chronic inflammatory process in periodontitis has been suggested as a mechanism that determines the biological plausibility in periodontitis and other systemic diseases.<sup>6,7</sup> Furthermore, evidence indicates that nonsurgical periodontal therapy (NSPT) might influence the development of cardiovascular disease,<sup>8</sup> rheumatoid arthritis,<sup>9</sup> type 1 diabetes mellitus,<sup>10</sup> respiratory disease,<sup>11</sup> and chronic kidney disease.<sup>12</sup>

Thus, hypothetically, some acute phase proteins whose production is increased during infection and inflammation, such as hepcidin, could be reduced after periodontal therapy.<sup>13</sup> Hepcidin is a liver-derived hormone that regulates iron metabolism in the body.<sup>14,15</sup> Increased hepcidin synthesis induces iron sequestration in macrophage stores and decreases tissue iron availability by stimulating ferroportin degradation in lysosomes.<sup>16,17</sup> Body iron load and inflammatory cytokines regulate the production of hepcidin, reflecting the association of this hormone with inflammatory disorders.<sup>18,19</sup>

Prohepcidin, the prohormone of hepcidin, as well as serum IL-6 and high-sensitivity C-reactive protein (hs-CRP) levels, was reported to be significantly decreased after periodontal therapy for chronic periodontitis (CP).<sup>13</sup> However, the effect of periodontal therapy on serum hepcidin levels remains to be elucidated. Therefore, we aimed to evaluate the effect of NSPT on hepcidin, iron markers, hematological parameters, and inflammatory serum levels in individuals with or without CP.

## Methodology

### Study design and groups

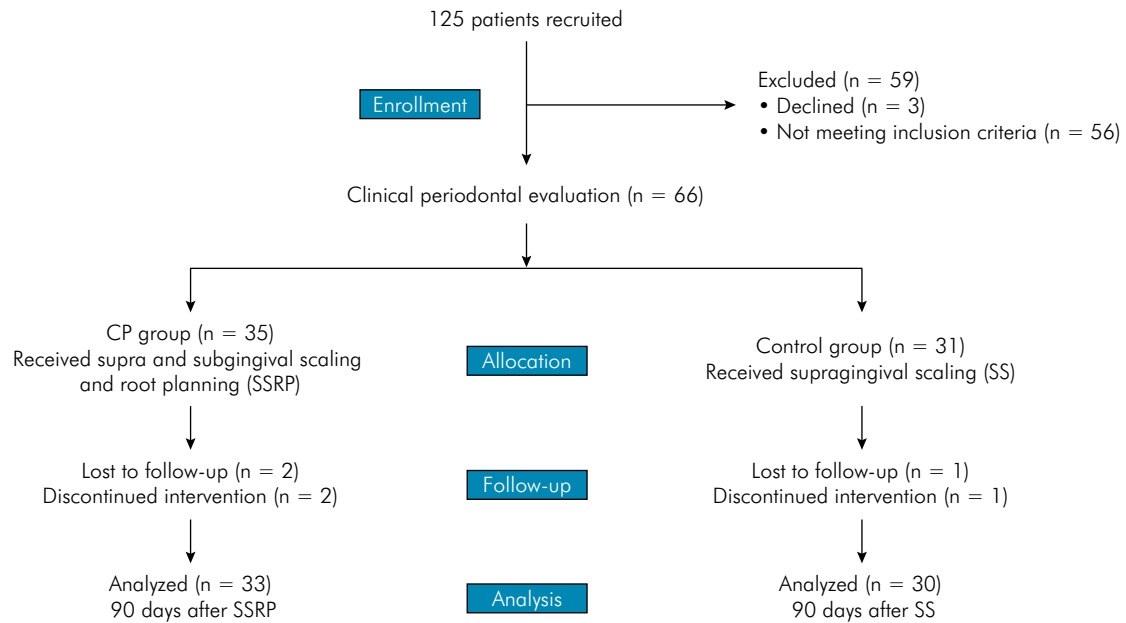
This was an interventional study performed on individuals awaiting care in the outpatient clinics of the Dental School at the Federal University of Maranhão (UFMA), São Luís, Maranhão, Brazil. The study protocol was approved by the Human Research Ethics Committee of the Federal University

of Maranhão. The study is registered at ClinicalTrials.gov (NCT02641210).

Inclusion criteria were as follows: individuals of both sexes aged 30–65 years with no other diseases, except for periodontitis, and with at least 20 teeth. Exclusion criteria were as follows: pregnancy, lactation, immunosuppression, smoking or previous smoking habit with less than 10 years since last smoking, orthodontic appliance use, history of steroid and nonsteroidal anti-inflammatory drug use, and antibiotic therapy 3 months before the study and during the research. In addition, those receiving iron replacement therapy for anemia and who received subgingival scaling and periodontal surgery 6 months before the investigation were also excluded.

Initially, 125 volunteers were recruited. Anamnesis was made to obtain personal data, current and previous medical and dental histories, medications in use, and possible alterations in menstrual flow. Data on blood pressure, weight, and height were collected. All study participants received verminosis therapy (single dose of albendazole 400 mg; Pratti-Dunaduzzi & Cia LTDA, Parana, Brazil) to avoid the confounding bias of anemia caused by verminosis in the study sample. After 15 days, blood and urine samples were obtained from individuals who met the initial selection criteria to evaluate the following biochemical markers: total cholesterol, fasting glycemia, triglycerides, albumin, oxalate transaminase, creatinine, iron, ferritin, transferrin saturation, uric acid, hemoglobin, hematimetric indices, hs-CRP, and urinary sediment examination. Patients with alterations in glycemic parameters, liver function, renal function, and lipid profile, confirmed by laboratory tests, were excluded. A total of 62 patients were excluded from the study: 59 for not meeting the inclusion criteria, three for refusing to participate, and three for discontinued intervention. The recruitment phase synthesis is shown in Figure.

Patients were allocated to two groups: CP group with 33 individuals CP and control group with 30 individuals without systemic diseases or CP. The groups were selected within a period of six months. Individuals with a diagnosis of CP had at least two teeth with a probing depth (PD) of  $\geq 5$  mm and a clinical attachment level (CAL) of  $\geq 6$  mm associated with the presence



**Figure.** Flowchart of study recruitment.

of bleeding on probing at the same site, during 30 s after removal of the periodontal probe.<sup>20</sup> Individuals without CP presented no bleeding on probing, PD up to 3 mm, and visible plaque index (VPI)  $\leq 25\%$ .

### Periodontal status evaluation

Periodontal evaluation was performed by a single trained periodontist (SAML) using a Williams periodontal probe (Hu-Friedy®, Mgf. Co., Inc., Chicago, USA). The following variables were evaluated: PD, gingival recession (GR), CAL, VPI,<sup>21</sup> and gingival bleeding index.<sup>21</sup> PD, GR, and CAL were recorded at six sites around each tooth (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, and mesiolingual),<sup>22</sup> except for the third molars. In both indices, four sites around each tooth (mesiobuccal, midbuccal, distobuccal, and midlingual) were examined. The training process was conducted at the Dental School of UFMA, involving 10 randomly selected patients; the volunteers were examined twice at an interval of 1 week. The intraexaminer agreement coefficient was 0.81 for PD and 0.77 for CAL.

### Laboratory analysis

Twelve-hour fasting venous blood samples (20 mL) were collected in vacuum tubes and were divided

into two portions: a portion of the whole blood was collected in EDTA and a separate aliquot was prepared for blood count evaluation using laser impedance technique (ADVIA 2120 apparatus). The remainder of anticoagulated blood with EDTA was centrifuged for 10 min at 3000 rpm for plasma separation, aliquoted, and stored at  $-70^{\circ}\text{C}$  until further use. Serum samples were collected at baseline and 90 days after periodontal therapy. The following standard laboratory tests were performed: total cholesterol, fasting glycemia, triglycerides, albumin, oxalate transaminase, creatinine, uric acid, hs-CRP, hemoglobin, serum iron, ferritin, transferrin saturation index, hematimetric indices, and urinary sediment examination.

Serum IL-6 and hepcidin levels were assessed by the immunoassay technique using a specific ELISA kit (IBL International, Hamburg, Germany), according to the manufacturer's instructions. The readings were performed using an automatic microplate reader, and the absorbance was read at 450 nm using a MULTISKAN EX spectrophotometer (Multiskan EX, Labsystems, Bucharest, Romania).

### Periodontal therapy

All participants received an oral hygiene kit and attended a motivational lecture regarding

instructions on toothbrushing and flossing. The CP group subjects received NSPT, performed by a single professional, using supragingival and subgingival scaling, and root planing procedures (SSRP), under local anesthesia employing ultrasound device and Gracey and mini-Gracey curettes (5/6, 7/8, 11/12, and 13/14). The therapy was performed in two sessions at an interval of 7 days, with no time limit, according to the need of each periodontal status. In each session, if necessary, individuals received new oral hygiene instructions (OHI).

The control group subjects received supragingival scaling (SS) using an ultrasonic device and periodontal curettes; dental surface polishing; and topical fluoride application. Periodontal maintenance therapy, comprising biofilm control; dental calculus removal, when necessary, through professional prophylaxis; and OHI reinforcement, were performed at 30, 60, and 90 days after the end of NSPT. Ninety days after NSPT, both groups were reassessed using the same baseline clinical periodontal parameters and serum biomarkers.

### Statistical analysis

Data were analyzed using software SAS® system, version 6.11 (SAS Institute, Inc., Cary, USA). Initially, descriptive analysis was performed using measures of frequency, mean, and standard deviation. Categorical variables were compared between the groups using the Chi-square test. The normality of the distribution of continuous variables was verified

using the Lilliefors test. The inferential analysis for evaluating the effect of SSRP or SS on variables of the hemogram, inflammatory markers, iron reserves, and periodontal parameters used the paired Student's *t*-test or Wilcoxon's test. For comparison of the corresponding absolute delta (final–initial) between the groups, the Mann-Whitney test was used. The significance level was set at 5%.

## Results

General characteristics of the study groups are presented in Table 1. The CP group, which received SSRP, consisted of 20 women (60.6%) and 13 men (39.4%), with a mean age of  $41.1 \pm 7.8$  years. The control group, which received SS, consisted of 18 women (60%) and 12 men (40%), with a mean age of  $39.5 \pm 8.9$  years. In general, both groups were homogeneous, comparable, and had no statistically significant differences among the following variables: sex, age, systolic blood pressure, diastolic BP (DBP), body mass index, glycemia, and glomerular filtration rate ( $p > 0.05$ ).

Measures of central tendency and dispersion of serum biomarkers and periodontal variables at baseline and at 90 days after the end of the NSPT (SSRP or SS) are provided in Table 2. The CP group showed a statistically significant decrease in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), IL-6, hepcidin, and erythrocyte sedimentation rate (ESR) levels 90 days after SSRP ( $p \leq 0.05$ ). In the control

**Table 1.** Distribution of general and medical characteristics of the sample.

Variable	CP group (n = 33)		Control group (n = 30)		p-value
Age n (%)					0.65
30-39 years	13	(39.4)	15	(50.0)	
40-49 years	15	(45.4)	12	(40.0)	
50 years or older	5	(15.2)	3	(10.0)	
Sex					0.84
Male	13	(39.4)	12	(40.0)	
Female	20	(60.6)	18	(60.0)	
Systolic blood pressure (mean ± SD)	110.6	± 12.2	109.7	± 14.0	0.78
Diastolic blood pressure (mean ± SD)	68.79	± 9.3	67.33	± 7.8	0.50
Body mass index (mean ± SD)	26.1	± 3.7	25.9	± 4.0	0.78
Glycemia (mean ± SD)	84.9	± 8.2	83.4	± 7.3	0.41
Glomerular filtration rate (mean ± SD)	105.4	± 24.4	104.1	± 26.4	0.86

CP: Chronic periodontitis; ± SD: standard deviation.

group, hemoglobin, hematocrit, MCV, MCH, IL-6, hepcidin, and ESR variables presented a statistically significant difference ( $p \leq 0.05$ ) in their initial mean values when compared with those at the end of the 90-day follow-up. However, when alteration variables ( $\Delta = \text{final} - \text{initial}$ ) were compared between CP and control groups, there was no statistical difference, demonstrating intergroup similarity.

The comparison of periodontal clinical parameters at baseline and at the end of the 90-day follow-up is shown in Table 2. In the CP group, a statistically significant difference ( $p < 0.01$ ) was observed between the mean values of all the parameters obtained in the two examinations. In the control group, this difference was only noticed for PD and CAL ( $p < 0.01$ ). CP group showed a greater reduction in all mean values of periodontal parameters, from the first to the last examination (90 days after SSRP), when compared with those of the control group ( $p < 0.01$ ). The relative variation mean of PD and CAL in the CP group was

-37.5% ( $\pm 9.8$ ) and -37.6% ( $\pm 9.9$ ), respectively, whereas, in the control group, this variation was -27.8% ( $\pm 12$ ) and -29.9% ( $\pm 11.7$ ), respectively.

## Discussion

This study evaluated the effect of NSPT (SSRP or SS) on systemic inflammatory response and levels of iron-related serum markers. Based on the biological evidence that host defense cells release immunoinflammatory mediators such as IL-6, TNF- $\alpha$ , MMPs, and PGE2, and stimulate the production of acute phase proteins such as PCR and hepcidin, in response to periodontal disease, the results of our study are similar to the findings of previous studies.<sup>23,24,25,26</sup> There was a significant reduction in IL-6 and CRP in both groups (with and without periodontal disease), demonstrating that immunoinflammatory mediators and acute phase proteins decrease after inflammation and infection.

**Table 2.** Distribution of serum biomarkers and periodontal variables at baseline and 90 days after nonsurgical periodontal therapy and comparative analysis between groups.

Variable	CP Group (n=33)			Control Group (n=30)			p-value <sup>2</sup>
	Baseline	90 days after SSRP	p-value <sup>1</sup>	Baseline	90 days after SS	p-value <sup>1</sup>	
	mean $\pm$ SD	mean $\pm$ SD		mean $\pm$ SD	mean $\pm$ SD		
<b>Serum biomarkers</b>							
Hemoglobin (g/dl)	13.5 $\pm$ 2.18	13.3 $\pm$ 1.95	0.26	14.2 $\pm$ 1.44	13.9 $\pm$ 1.51	< 0.01	0.46
Hematocrit (%)	41.0 $\pm$ 5.75	40.6 $\pm$ 4.92	0.69	43.4 $\pm$ 4.12	42.1 $\pm$ 4.13	< 0.01	0.08
MCV (fl)	87.1 $\pm$ 12.1	86.9 $\pm$ 7.76	< 0.01	90.8 $\pm$ 4.28	88.8 $\pm$ 4.08	< 0.01	0.35
MCH (pg)	29.0 $\pm$ 3.44	28.3 $\pm$ 3.33	< 0.01	29.7 $\pm$ 3.44	29.3 $\pm$ 1.65	< 0.01	0.21
Serum iron ( $\mu$ g/dl)	88.8 $\pm$ 38.0	88.5 $\pm$ 34.3	0.96	104.6 $\pm$ 31.8	111.9 $\pm$ 34.7	0.26	0.50
Transferrin Saturation (%)	34.5 $\pm$ 16.1	33.7 $\pm$ 13.8	0.78	39.4 $\pm$ 13.5	42.3 $\pm$ 15.0	0.27	0.40
Ferritin (ng/ml)	125.2 $\pm$ 130.6	117.2 $\pm$ 110.4	0.52	127.1 $\pm$ 127.6	138.0 $\pm$ 155.2	0.40	0.29
hs-CRP (mg/dl)	0.27 $\pm$ 0.336	0.24 $\pm$ 0.346	0.17	0.25 $\pm$ 0.389	0.23 $\pm$ 0.343	0.84	0.38
IL-6 (pg/ml)	16.0 $\pm$ 8.6	3.9 $\pm$ 3.3	< 0.01	14.6 $\pm$ 5.2	2.9 $\pm$ 1.1	< 0.01	0.75
Hepcidin (ng/ml)	65.6 $\pm$ 14.3	36.9 $\pm$ 15.6	< 0.01	60.9 $\pm$ 13.0	27.1 $\pm$ 13.0	< 0.01	0.09
ESR (mm/h)	6.64 $\pm$ 5.61	10.5 $\pm$ 11.4	0.04	5.47 $\pm$ 8.17	8.23 $\pm$ 9.50	0.04	0.92
<b>Periodontal variables</b>							
PD (mm)	2.44 $\pm$ 0.48	1.50 $\pm$ 0.22	< 0.01	1.82 $\pm$ 0.24	1.30 $\pm$ 0.17	< 0.01	< 0.01
CAL (mm)	2.96 $\pm$ 0.54	1.82 $\pm$ 0.31	< 0.01	2.05 $\pm$ 0.33	1.44 $\pm$ 0.25	< 0.01	< 0.01
VPI (%)	8.61 $\pm$ 8.33	1.81 $\pm$ 1.75	< 0.01	2.13 $\pm$ 2.62	1.61 $\pm$ 2.01	0.50	< 0.01
BoP (%)	11.6 $\pm$ 9.90	1.61 $\pm$ 1.92	< 0.01	1.18 $\pm$ 1.22	0.89 $\pm$ 0.98	0.38	< 0.01

CP: Chronic periodontitis; SSRP: Supragingival and subgingival scaling and root planing Nonsurgical periodontal therapy; SS: supragingival scaling;  $\pm$  SD: standard deviation; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; hs-CRP: High-sensitivity C-reactive protein; IL: Interleukin; ESR: Erythrocyte sedimentation rate; PD: Probing Depth; CAL: Clinical attachment level; VPI: Visible plaque index; BoP: Bleeding on probing; <sup>1</sup>p-value for comparison between baseline and 90 days in both groups; <sup>2</sup>p-value for comparison of alterations ( $\Delta = \text{final} - \text{initial}$ ) between groups;  $\pm$  SD: standard deviation; \*Values in bold type are significant at the 0.05 level.



The hepatic peptide hormone hepcidin regulates the absorption of iron from the diet; plasma iron concentrations and the distribution of iron in the tissues and its increase are linked to the inflammatory and infectious response, leading to a reduction in iron availability.<sup>27</sup> Hepcidin acts by causing the degradation of its receptor, the ferroportin exporter of cellular iron. Loss of ferroportin decreases plasma iron flux to absorption enterocytes, to macrophages that recycle iron from senescent erythrocytes, and to hepatocytes that store iron, thereby decreasing iron plasma concentration.<sup>15,18</sup> The malfunctioning of the ferroportin-hepcidin axis contributes to the pathogenesis of different anemias.<sup>28</sup> Anemia of chronic disease or anemia of inflammation occurs in chronic infections and inflammatory or neoplastic diseases not caused by marrow deficiencies or other diseases.<sup>16</sup> It occurs even in the presence of adequate intracellular iron and vitamin stores<sup>29</sup> and is characterized by low levels of serum iron (hypoferremia).<sup>30</sup> In this context, the findings suggest that the beneficial effect of periodontal intervention seems to be related to its ability to decrease the levels of inflammatory cytokines, and consequently, of serum hepcidin levels.

The results indicate that NSPT led to a reduction in serum IL-6 levels after 3 months of intervention. The reduction of CRP was not so significant as in IL-6. Comparing the groups with CP and those without CP to some previous studies, IL-6 and CRP increases were observed even when associated with other systemic diseases.<sup>24,31</sup> These findings reinforce the effect of periodontal therapy on the reduction of inflammatory markers such as IL-6 and hepcidin.

In a previous short communication with the same baseline data as those of the present study, no statistically significant differences were observed in the level of hepcidin between the groups with or without CP,<sup>32</sup> whereas another study found differences in serum hepcidin levels between CP and control groups;<sup>33</sup> however, this study also included patients with type 2 diabetes mellitus, and it presented less strict eligibility criteria, which may have contributed to the inclusion of patients with other systemic diseases, leading to differences between the study findings.

Regarding ESR, a measure that demonstrates inflammatory activity, is a significant parameter

related to infection or inflammation. ESR occurred with a greater plasma alteration in several proteins in the CP group compared with the control group. After periodontal therapy in both groups, a significant increase in ESR was observed after 90 days. These findings are at odds with those obtained by Agarwal et al.,<sup>34</sup> who performed serum biomarker analysis 1 year after periodontal intervention. This may have contributed to the difference in results. In addition, in the present study, IL-6 levels were reduced, indicating a decrease in inflammatory activity. Therefore, the isolated increase in ESR may be the result of a late treatment response.

Decreased MCV values suggest the presence of microcytosis, which is more commonly caused by iron deficiency anemia. In our study, MCV values were within the reference range for both groups at baseline and at 3 months after NSPT. Despite that, a statistically significant reduction was detected in both groups, even with normal iron levels in all study participants. This finding suggests that other factors may have led to the reduction of MCV after the intervention, which is in accordance with previous study findings.<sup>35,36</sup>

The decrease in MCH value observed in microcytic anemia is caused by iron deficiency, whereas an increase in MCH value observed in macrocytic anemia is caused by vitamin deficiency. MCH values in our study were within the reference range for both groups at baseline and 3 months after NSPT, indicating that anemia is normocytic. The results are consistent with the findings of Pradeep et al.<sup>35</sup>

The hematocrit value was lower in the CP group and, after periodontal therapy, it was reduced in both groups. In addition, in the group without CP, there was a significant reduction, probably because of a lower number of erythrocytes in individuals with periodontal infection.<sup>37,38,39</sup>

Another important parameter is the iron reserve marker. The results of the present study revealed the influence of periodontal treatment on iron store in individuals without CP, but the increase was not statistically significant. However, in individuals with CP, there was a slight reduction in these reserves, which seems to be compatible with the characteristics of inflammation-related anemia, confirmed by

normal values of MCV in the group of individuals with periodontal infection.

There was no significant difference between hemoglobin values for the CP group, whereas a significant reduction was observed, but within the reference values, in the control group after 90 days of periodontal intervention. These results corroborate those reported by Havemose-Poulsen et al.<sup>38</sup> However, some studies<sup>34,35,36,39</sup> demonstrated a significant increase in hemoglobin and erythrocytes at 1 year of evaluation.

All the clinical parameters analyzed (PD, CAL, BOP, and VPI) in the two groups were affected similarly, with a significant reduction, showing once again the importance of periodontal therapy. These study findings suggest that supragingival biofilm control is an important factor in modifying inflammatory clinical parameters.<sup>40,41</sup>

The limitation of the present study is the small sample size due to eligibility criteria, which might have reduced the statistical power. In addition, the mean PD and CAL levels in the CP group were low, indicating that these patients did not present an extensive periodontal impairment, thereby limiting the effect of SSRP on changes in biomarker levels. As this study is the first to evaluate the effect of periodontal

therapy on serum hepcidin and hemoglobin levels, it may be suggested that periodontal therapy, both SSRP and supragingival biofilm control, influences the reduction of serum IL-6 and hepcidin levels, as well as of all clinical parameters, by significantly decreasing inflammation and infection in CP and control groups. Future studies are necessary to better elucidate the processes that occur during the inflammation phase of periodontal tissues and the possible beneficial effects of periodontal therapy on anemia.

## Conclusion

Our findings suggest that periodontal therapy, both supragingival and subgingival scaling and root planing and only supragingival biofilm control, may reduce serum IL-6 and hepcidin levels, thereby reinforcing the positive effects of periodontal care on systemic health.

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