

# Association between markers of cardiovascular risk and clinical parameters of periodontitis

*Associação entre marcadores do risco cardiovascular e parâmetros clínicos da periodontite*

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## Resumo

**Introdução:** A doença periodontal é uma resposta inflamatória a bactérias que residem no tecido gengival podendo apresentar repercussão sistêmica. **Objetivo:** O objetivo deste estudo foi conhecer a relação entre a periodontite e marcadores do risco cardiovascular. **Material e método:** Noventa pacientes selecionados foram divididos em dois grupos de acordo com os níveis de profundidade de sondagem (PS) e nível clínico de inserção (NCI). Grupo controle, n = 45 (< 4 sítios com PS ≥ 4.0 mm e NCI ≥ 3.0 mm) e grupo casos, n = 45 (≥ 30% dos sítios com PS ≥ 4.0 mm e NCI ≥ 3.0 mm). Todos os indivíduos foram submetidos a dosagens plasmáticas de Proteína C reativa de alta sensibilidade, HDL-c e Lipoproteína de baixa densidade eletronegativa (LDL<sup>-</sup>). Dados do histórico médico e nível socioeconômico foram também coletados. **Resultado:** Os níveis plasmáticos de HDL-c foram maiores nos indivíduos com doença periodontal (p = 0,016) e inversamente associados com o número de sítios periodontais com OS ≥ 3 mm (rho = -0.325) e números de sítio com OS ≥ 3 mm e NCI ≥ 3 mm (rho = -0.216). NCI ≥ 3 mm. Estas associações permaneceram após ajuste para biofilme e uso de cigarro usando análise de covariância univariada (p < 0.05). O *odds ratio* ajustado entre a doença periodontal e os níveis de HDL-c foi 0.94 (CI95% 0.88–0.99) após ajuste para idade, fumo e biofilme. Os demais marcadores do risco vascular não foram relacionados com a doença periodontal. **Conclusão:** Os parâmetros clínicos da periodontite foram inversamente associados com as concentrações de HDL-c.

**Descritores:** Doenças periodontais; HDL-c; doença cardiovascular coronariana; risco cardiovascular.

## Abstract

**Introduction:** Periodontal disease is an inflammatory response to bacteria that reside in the gum tissue and can have systemic repercussion. **Objective:** The aim of this study was to assess the relationship between periodontitis and markers of cardiovascular risk. **Material and method:** Ninety selected patients were assigned into two groups in accordance with their levels of probing pocket depth (PPD) and Clinical Attachment Level (CAL): control group, n = 45 (< 4 sites with PPD ≥ 4.0 mm and CAL ≥ 3.0 mm) and case group, n = 45 (≥ 30% of sites with PPD ≥ 4.0 mm and CAL ≥ 3.0 mm). Plasma concentrations of C-reactive protein, high sensitive CRP, high-density lipoproteins (HDL-c) and electronegative low density lipoproteins (LDL) were assessed in all participants. Data from medical history and socioeconomic level were also collected from patients. **Result:** Plasma levels of HDL-c were lower in subjects with periodontal disease (p = 0.016) and were inversely associated with the number of sites with PPD ≥ 3 mm (rho = -0.325) and number of sites with PPD ≥ 3 mm and CAL ≥ 3 mm (rho = -0.216). These associations remained significant after adjustments for dental plaque and smoking using Univariate Analysis of Covariance (p < 0.05). Adjusted odds ratio between periodontal disease and levels of HDL-c was 0.94 (CI95% 0.88–0.99) after adjusting for age, smoking and dental plaque. Other investigated markers of cardiovascular risk were not related to periodontal disease. **Conclusion:** Clinical parameters of periodontitis were inversely associated with plasma concentrations of HDL-c.

**Descriptors:** Periodontal diseases; HDL-c; coronary heart disease; cardiovascular risk.

## INTRODUCTION

Periodontal pathogens can produce and transmit toxins to the blood stream, which can invade blood vessels, triggering host immune defense processes, directed by inflammatory cytokines and acute phase proteins<sup>1</sup>. Such processes can produce cellular defense changes, and these pathogens migrating through the blood stream may be associated with the increase in lipid metabolism and atherogenesis processes in blood vessels<sup>2</sup>.

Several studies have investigated whether periodontal disease was associated with risk predictors for cardiovascular disease<sup>3-8</sup>. Among the markers of cardiovascular disease, C-Reactive Protein (CRP), homocysteine, fibrinogen, High-density lipoproteins (HDL-c) and Low-density lipoproteins (LDL-c) have been most frequently investigated. CRP is an acute phase protein, which plays a crucial role in the reestablishment of homeostasis after infections and inflammatory processes because of its microbicidal and phagocytic functions<sup>9</sup>. It is also considered an independent biochemical predictor of several diseases, such as cardiovascular disease<sup>10</sup>.

HDL-c represents a class of lipoproteins composed of a wide range of different subpopulations with particle sizes varying from less than 7 nm to 14 nm, which carry fatty acids and cholesterol from the body tissue to the liver. HDL-c can remove cholesterol from atheroma within arteries and transport cholesterol back to the liver for excretion or re-utilization. A high level of HDL-c seems to protect against cardiovascular diseases, and low HDL-c levels increase the risk for heart disease<sup>11</sup>. There is a study that suggests that periodontal treatment can change the anti-atherogenic metabolism of HDL-c<sup>12</sup>. A minimum oxidized fraction of the LDL in plasma, electronegative LDL or LDL<sup>-</sup> (minus), is a possible predictor of cardiovascular risk<sup>13</sup>. LDL<sup>-</sup> is considered a more accurate measure for cardiovascular disease risk than LDL<sup>+</sup>. This is because LDL<sup>-</sup> contains higher amounts of cholesterol oxides, conjugated dienes, TBARS and decreased levels of  $\alpha$ -tocopherol in comparison to native LDL (n-LDL)<sup>14</sup>. Despite some studies evaluated the association between LDL and periodontal disease<sup>15</sup> no previous study investigated LDL<sup>-</sup> as a putative risk factor for periodontitis.

The use of the ratio between independent risk predictors for cardiovascular disease is also a strategy used to increase the power of a test in the identification of subjects at risk for cardiovascular disease<sup>16</sup>.

The findings on the association between periodontal disease and markers of cardiovascular disease are not consistent, suggesting the need for further studies using appropriate methodology. The hypothesis of this study was that subjects with periodontal disease are more likely to have higher levels of CRP and LDL<sup>-</sup>, and lower levels of HDL-c compared with those without periodontal disease. The aim of this study was to analyze the association between clinical parameters of periodontal disease and markers of cardiovascular risk factors, namely serum C-Reactive Protein, HDL-c and LDL<sup>-</sup>.

## MATERIAL AND METHOD

### 1. Subjects

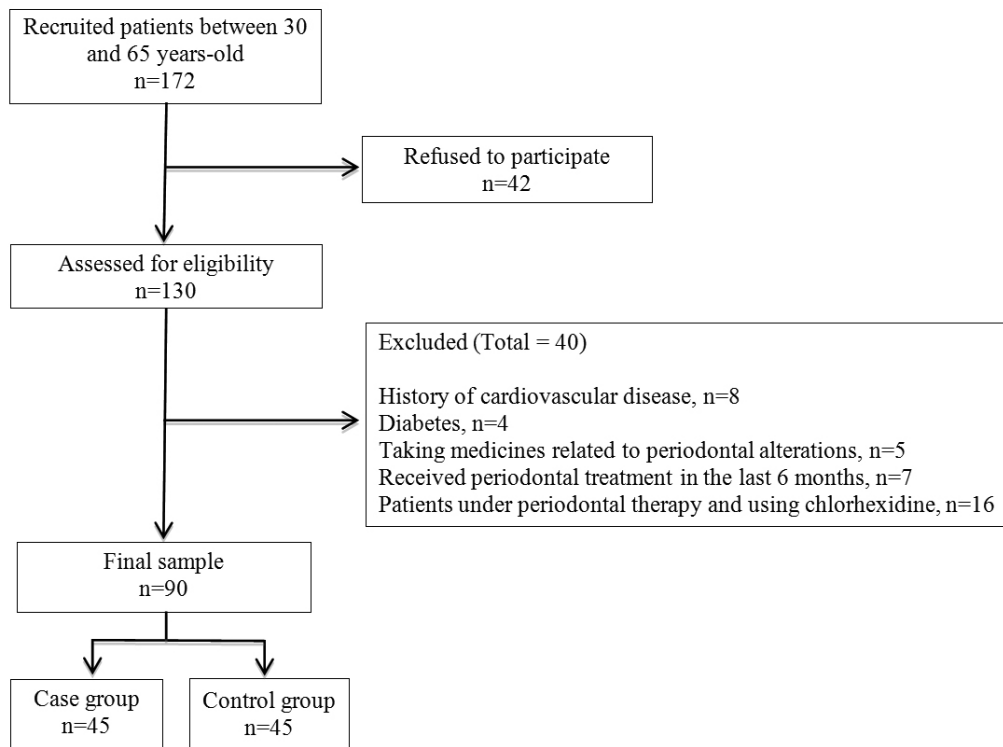
A cross-sectional study was conducted on a convenience sample of patients referred to the Dental School at Federal University of Amazonas (UFAM), Manaus (AM), Brazil. This study was approved by the Committee of Ethics and Research of the Federal University of Amazonas (UFAM).

The nature of the investigation was explained to selected individuals attending the Clinical Dentistry Department of the Dental School of the UFAM. Initially, a pilot study including 45 patients being over 30 years of age and presenting at least four sites with periodontal pocket depth (PPD) > 4.0 mm was conducted. Intraclass correlation coefficient of agreement findings for PPD and Clinical Attachment Level (CAL) were 0.86 and 0.77, respectively. No changes were needed in either questionnaires.

The inclusion criteria for the main study consisted of participants between 30 to 65 years-old, with at least 15 natural teeth and did not participate in the pilot study. Subjects were excluded if they: had a history of cardiovascular disease, systemic conditions associated with periodontal disease or if they were taking medicines related to periodontal alterations (antibiotics, cyclosporine A, steroidal and non-steroidal anti-inflammatory); pregnancy; received periodontal therapy in the last six months; and used chlorhexidine during the last month. Subjects with history of cardiovascular disease were excluded because their concentrations of cardiovascular risk markers are probably altered. The exclusion of such individuals was in order to avoid the potential confounder effect of previous cardiovascular disease on the relationship between periodontal disease and markers of cardiovascular risk.

Initially, 172 patients between 30 to 65 years-old and with at least 15 natural teeth from the Clinical Dentistry Department were invited based on information from dental records. The acceptance was 75.6%. Of the 130 patients who agreed to participate, 40 were excluded based on the selection criteria. Therefore, 90 individuals were considered suitable and were assigned to one of two groups in accordance with their levels of PPD and CAL. The flowchart of the sample is presented in Figure 1. The Case group included 45 patients were with at least 30% of sites with PPD  $\geq$  4.0 mm and CAL  $\geq$  3.0 mm. The Control group was composed by 45 patients had less than four sites with PPD  $\geq$  4.0 mm and CAL  $\geq$  3.0 mm in non-adjacent teeth. Bleeding on probing was not considered in the definition of cases and controls because this parameter usually represents a current inflammatory periodontal condition instead of the periodontal status in the long term<sup>17</sup>.

All suitable patients received and signed a written informed consent form regarding the study aims, procedure and the voluntary character of their participation. Patients were selected between February and November 2007. All subjects considered suitable were interviewed to obtain socio-demographic data including age, sex, ethnicity, marital status, schooling and familial income. In addition, tobacco-use and information concerning previous and current systemic diseases was gathered.



**Figure 1.** Flow chart of the sample.

A pre-tested questionnaire was used to obtain these data. Immediately after the interview, one examiner previously calibrated for periodontal clinical parameters examined the patients, and finally blood samples were collected to quantify the risk predictors for cardiovascular risk. The examiner conducting the periodontal clinical examinations was masked concerning the laboratory results of risk predictors for cardiovascular disease.

## 2. Periodontal Clinical Examination

Periodontal clinical measurements registered by one calibrated examiner included Visible Plaque Index (VPI), Bleeding on Probing Index (BOP), PPD and CAL measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) for all teeth, excluding third molars. PPD and CAL were recorded to the nearest higher millimeter by means using the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in diameter. Plain oral mirrors (Hu-Friedy®) were used for periodontal examinations. VPI and BOP were dichotomic measures assessed by visually examining the presence or absence of dental biofilm and gingival bleeding on probing, respectively<sup>18</sup>.

## 3. Collection of Blood Sample and Assays

After periodontal clinical examination, 5 mL of venous blood sample was collected individually and placed in separate tubes containing anticoagulant solution of sodium citrate 0.15%. The tubes were stored at 4 °C and transported to the laboratory of Biochemistry (São Paulo University, São Paulo, Brazil). First, the samples were centrifuged as 800 g for 15 minutes to obtain the blood plasma.

The investigated risk predictors for cardiovascular disease were C-reactive protein (CRP), high sensitive C-reactive protein (hs-CRP), high-density lipoproteins (HDL-c), electronegative low-density lipoproteins (LDL-) and the proportion of hs-CRP/HDL-c.

CRP, hs-CRP and HDL-c were measured by immunoturbidimetry automated methodology using the equipment Cobas Mira plus® (Roche, Mannheim, German) and commercial Kits (Labtest diagnostic, Minas Gerais, Brazil). LDL- antibodies levels were assessed through immunoenzymatic assay with specific monoclonal antibody against LDL<sup>-13</sup>. All methods were validated using internal controls. The interview, periodontal clinical exam, collection of blood samples and laboratorial analysis were conducted between March and November 2007.

## 4. Statistical Analysis

The continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. The comparison concerning socio-demographic variables as well as tobacco-use habits and systemic diseases between groups were performed by two sample t-test, Chi-square and Fisher's exact tests. Periodontal clinical parameters including the average of VIP, BOP Index, PPD and CAL level were computed for each subject and then averaged across subjects in the two groups.

Differences among periodontal clinical parameters were examined in the subset of sites according to their PPD ( $\geq 4$  mm and  $\geq 5$  mm), CAL ( $\geq 3$  mm,  $\geq 4$  mm and  $\geq 5$  mm) and the use of a combination of both PPD  $\geq 4$  mm and CAL  $\geq 3$  mm. The values for each clinical parameter were averaged separately within each PPD and CAL level category for each subject and then

averaged across participants in the two groups. The significance of differences between the two groups was checked by Mann-Whitney tests. The mean levels of markers of cardiovascular risk CRP, hs-CRP, HDL-c and LDL<sup>c</sup> presented normal distribution and were compared between groups by two sample t-test. The comparisons of proportions of subjects with different levels of risk for CRP, hs-CRP and HDL-c between groups were performed by Chi-square test.

Possible associations between periodontal clinical parameters and risk predictors for cardiovascular disease were sought after using the Spearman coefficient correlation (adjusted for multiple testing analysis). Multivariate logistic regression was performed to examine the association between periodontal disease and risk predictors for cardiovascular disease, adjusting for covariates. The covariates that presented  $p \leq 0.20$  in the bivariate analysis as well as dental plaque and smoking were used in the logistic regression. Dental plaque and smoking were forced in the analyses because of their potential confounding effect on the relationship between periodontal disease and risk markers for cardiovascular disease. The procedure used was stepwise backward selection of risk factors. In addition, univariate analysis of covariance was

carried out for all periodontal parameters and risk predictors for cardiovascular disease adjusting for dental plaque and smoking (covariates). Effect modification analysis was performed among periodontal disease measures and covariates in both analyses. All statistical analyses were carried out with SPSS 10.0 (Statistical Package for the Social Sciences for Windows®, SPSS Inc., Chicago, IL, USA) with a significance level of 5% ( $p \leq 0.05$ ).

## RESULT

The study sample comprised 90 participants (37.5% males) from the Dental School of the Federal University of Amazonas. In this study the prevalence of subjects with altered levels of HDL-c was 82%. Assuming that the sample size was equal to 90 to detect 15% of the differences between groups, with 5% Type I error probability, the power of this study was 84.4%. Sample population age ranged from 30 to 65 years, with a mean age of  $40 \pm 8.1$  years. There was no statistically significant difference between cases and controls for socio-demographic variables, smoking and diabetes (Table 1).

**Table 1.** Demographic and socioeconomic characteristics, smoking and diabetes data of participants in control and test groups

	Total	Control group (N = 45)	Case group (N = 45)	<i>p</i>
Age (mean $\pm$ SD) <sup>a</sup>	40 $\pm$ 8.1	41.7 $\pm$ 8.9	39.0 $\pm$ 7.1	0.111
Sex (%) <sup>b</sup>				0.515
Male	37.8	33.3	42.2	
Female	62.2	66.7	57.8	
Ethnicity (%) <sup>c</sup>				0.984
White	17.8	17.8	17.8	
Brown	72.2	73.3	71.1	
Black	7.8	6.7	8.9	
Indigenous	2.2	2.2	2.2	
Marital status (%) <sup>c</sup>				0.327
Married/Partner	66.7	60.0	73.3	
Unmarried	24.4	31.1	17.8	
Divorced/widow	8.9	8.9	8.9	
Schooling (years) (%) <sup>b</sup>				0.963
< 8	15.7	15.6	15.9	
$\geq$ 8	84.3	84.4	84.1	
Monthly familial income (US\$) (mean $\pm$ SD) <sup>a</sup>	641 $\pm$ 453	660 $\pm$ 349	623 $\pm$ 537	0.709
Smoking (%) <sup>c</sup>				0.772
Never smoked	63.6	60.0	66.7	
Former smoker	27.8	31.1	24.4	
Smoker	8.9	8.9	8.9	
Diabetes (%) <sup>c</sup>	3.3	2.2	4.4	1.000

<sup>a</sup>Two-sample t test, <sup>b</sup>Chi-square test, <sup>c</sup>Fisher's exact test.

Table 2 presents data for clinical parameters for the case and control groups. No statistically significant difference was found for BOP means between the two groups ( $p > 0.05$ ). Statistically significant differences were found for the two groups with respect to mean values for VPI, PPD and CAL ( $p < 0.01$ ). Sites were grouped according to PPD categories of  $\geq 3$  mm,  $\geq 4$  mm and  $\geq 5$  mm, CAL categories of  $\geq 3$  mm,  $\geq 4$  mm and  $\geq 5$  mm, the combination of PPD  $\geq 3$  mm and CAL  $\geq 3$  mm, and the combination of PPD  $\geq 4$  mm and CAL  $\geq 3$  mm (Table 2). There was a significant difference in the number of sites with different categories of PPD and CAL for the two groups ( $p < 0.05$ ).

The risk predictors for cardiovascular disease investigated in patients with and without periodontal disease were analyzed by comparing the mean levels of each component between groups (Table 3). The levels of HDL-c were statistically lower in the case group than in control group ( $p < 0.05$ ).

Nonparametric Spearman linear correlation was used to identify possible associations between periodontal clinical

measures and risk predictors for cardiovascular disease (Table 4). Number of sites with dental plaque, number of sites with PPD  $\geq 3$  mm and number of sites with PPD and CAL  $\geq 3$  mm were inversely associated with low levels of HDL-c ( $p < 0.05$ ).

Unadjusted odds ratio between periodontal disease and levels of HDL-c was 0.94 (95% confidence interval 0.88–0.99). Periodontal disease maintained an inverse association on the odds of HDL-c after adjusting for age, smoking and dental plaque with the same odds ratio magnitude (Table 5).

Univariate analysis of covariance was performed on the number of sites with PPD  $\geq 3$  mm and number of sites with PPD and CAL  $\geq 3$  mm using HDL-c levels as the between-subjects factor. Adjustments were made for dental plaque and smoking. Number of sites with PPD  $\geq 3$  mm ( $F = 4.535$ ,  $p = 0.036$ ) and number of sites with PPD and CAL  $\geq 3$  mm ( $F = 4.350$ ,  $p = 0.040$ ) were inversely associated with lower levels of HDL-c. No effect modification was observed among periodontal measures, dental plaque, smoking and socioeconomic characteristics.

**Table 2.** Clinical periodontal parameters (mean  $\pm$  SD) of subjects in case and control groups

	Total	Control group (N = 45)	Case group (N = 45)	$p^a$
Visible Plaque Index	0.30 $\pm$ 0.18	0.20 $\pm$ 0.12	0.39 $\pm$ 0.18	< 0.001
Bleeding on Probing	0.93 $\pm$ 0.05	0.92 $\pm$ 0.04	0.93 $\pm$ 0.06	0.198
Periodontal Pocket Depth				
Mean $\pm$ SD	2.4 $\pm$ 0.4	2.1 $\pm$ 0.2	2.8 $\pm$ 0.4	< 0.001
n sites $\geq 3$ mm	49.3 $\pm$ 25.1	28.4 $\pm$ 10.5	70.1 $\pm$ 16.5	< 0.001
n sites $\geq 4$ mm	11.6 $\pm$ 13.6	3.3 $\pm$ 3.7	19.8 $\pm$ 14.8	< 0.001
n sites $\geq 5$ mm	4.4 $\pm$ 7.3	1.2 $\pm$ 2.4	7.7 $\pm$ 9.0	< 0.001
Clinical Attachment Level				
Mean $\pm$ SD	3.0 $\pm$ 0.7	2.8 $\pm$ 0.8	3.1 $\pm$ 0.6	< 0.001
n sites $\geq 3$ mm	70.3 $\pm$ 23.7	57.2 $\pm$ 20.8	83.4 $\pm$ 18.7	< 0.001
n sites $\geq 4$ mm	28.1 $\pm$ 21.8	23.0 $\pm$ 21.2	33.4 $\pm$ 21.3	< 0.005
n sites $\geq 5$ mm	12.5 $\pm$ 15.7	10.2 $\pm$ 15.3	14.8 $\pm$ 15.9	< 0.05
n sites PPD $\geq 3$ mm and CAL $\geq 3$ mm	48.1 $\pm$ 24.7	27.8 $\pm$ 10.4	68.4 $\pm$ 16.8	< 0.001
n sites PPD $\geq 4$ mm and CAL $\geq 3$ mm	11.4 $\pm$ 13.4	3.2 $\pm$ 3.7	19.5 $\pm$ 14.7	< 0.001

<sup>a</sup>Mann-Whitney test. PPD, Periodontal Pocket Depth; CAL, Clinical Attachment Level.

**Table 3.** Plasma levels (mean  $\pm$  SD) of some markers of cardiovascular risk of subjects in case and control groups

	Total	Control group (N = 45)	Case group (N = 45)	$p^a$
HDL-c (mg/dL)	32.4 $\pm$ 8.3	34.5 $\pm$ 8.3	30.3 $\pm$ 7.9	0.016
CRP (mg/dL)	8.2 $\pm$ 2.2	8.4 $\pm$ 2.2	8.1 $\pm$ 2.1	0.566
hs-CRP (mg/L)	2.4 $\pm$ 2.7	2.5 $\pm$ 2.6	2.3 $\pm$ 3.0	0.802
LDL <sup>-</sup> (mg/L)	1.7 $\pm$ 1.2	1.6 $\pm$ 1.3	1.8 $\pm$ 1.0	0.414
hs-CRP/HDL-c	0.07 $\pm$ 0.09	0.09 $\pm$ 0.10	0.07 $\pm$ 0.08	0.207

<sup>a</sup>two-sample t test. CRP, C Reactive Protein; hs-CRP, ultra sensitivity C Reactive Protein; HDL-c, High density lipoprotein; LDL<sup>-</sup>, Low density lipoprotein.



**Table 4.** Correlation matrix (Spearman coefficient, adjusted for multiple testing) between periodontal clinical parameters and markers of cardiovascular disease

	CRP	us-CRP	HDL-c	hsCRP/HDL-c	LDL-c
Number of sites with dental plaque	0.113	0.191	-0.325 <sup>a</sup>	0.248	0.052
Number of sites BOP	0.097	0.086	-0.121	0.146	0.029
Periodontal pocket depth					
Mean	-0.041	0.060	-0.152	0.122	-0.143
N sites ≥ 3 mm	0.009	0.009	-0.223 <sup>a</sup>	0.146	-0.143
N sites ≥ 4 mm	-0.065	0.018	-0.134	0.058	-0.104
N sites ≥ 5 mm	-0.171	0.061	-0.135	0.087	-0.102
Clinical attachment level					
Mean	-0.052	0.069	-0.078	0.097	-0.037
N sites ≥ 3 mm	0.004	0.096	-0.169	0.123	-0.056
N sites ≥ 4mm	-0.115	-0.031	0.089	-0.038	-0.008
N sites ≥ 5mm	-0.045	0.146	-0.081	0.149	0.078
N sites with PPD ≥ 3 mm and CAL ≥ 3 mm	-0.080	0.073	-0.216 <sup>a</sup>	0.140	-0.138
N sites with PPD ≥ 4 mm and CAL ≥ 3 mm	-0.080	0.004	-0.132	0.044	-0.094

<sup>a</sup> $p \leq 0.05$ . CRP, C Reactive Protein; HDL-c, High density lipoprotein; LDL-c, Low density lipoprotein; BOP, bleeding on probing; PPD, periodontal pocket depth; CAL, clinical attachment level.

**Table 5.** Results of analysis of periodontal disease with HDLD, adjusted for age, smoking and dental plaque

Independent variables	Unadjusted OR		Adjusted OR	
	OR	95% CI	OR	95% CI
HDLD	0.94 <sup>*</sup>	0.88 – 0.99	0.94 <sup>*</sup>	0.88 – 0.99
Age <sup>a</sup>	0.96	0.91 – 1.01	-	-
Dental Plaque <sup>b</sup>	1.03	0.94 – 1.12	1.00	0.91 – 1.10
Smoking <sup>c</sup>				
Never smoker	1	-	1	-
Previous smoker	0.90	0.21 – 3.96	0.73	0.16 – 3.36
Current smoker	0.71	0.28 – 1.82	0.84	0.31 – 2.28

OR, odds ratio; CI, confidence interval; <sup>\*</sup> $p < 0.05$ ; <sup>a</sup>variable removed on step 1; <sup>b</sup>variable removed on step 2; <sup>c</sup>variable removed on step 3.

## DISCUSSION

During recent decades the epidemiology of periodontal disease has changed focus from determinants of periodontal disease to periodontal medicine; the potential harmful effects of periodontal disease on systemic health<sup>19</sup>. Systematic reviews on this subject have been conducted considering adverse pregnancy outcomes, poor glycemic control, pulmonary disease, cardiovascular disease and risk predictors for cardiovascular disease as the main outcomes<sup>12,20,21</sup>. Despite the increasing number of studies on periodontal medicine, most review papers emphasize the need for more studies. Among the systemic conditions related to periodontal disease, from a public health perspective, cardiovascular disease is the most important as it accounts for high mortality rates in most countries.

One of the main results observed in this study was the inverse relationship between periodontal clinical measures and plasma concentrations of HDL-c. HDL-c is a lipoprotein fraction responsible for the reverse transport of cholesterol and has an antioxidant effect on LDL, potentially inhibiting the formation of atheromatous plaque on endothelial vessels. The oxidation of LDL molecules is considered one of the main initiating events in the atherosclerosis process because of its capacity to provoke endothelial dysfunction<sup>10</sup>.

Consistent with our findings, previous studies have also detected a negative association between clinical parameters of periodontal disease and HDL-c<sup>5,15,22</sup>. The agreement between ours, and previous findings, can be based on the methodological similarities including mean age of the participants, the methodology applied measuring HDL-c levels and the full

mouth periodontal examination. Other studies found no association between HDL-c and periodontal disease<sup>23-27</sup>. A possible explanation for these conflicting findings include: the low number of subjects enrolled in some studies<sup>23,24</sup>; a wide age range of the participants<sup>3,24-26</sup>; mean age of participants<sup>25</sup> and the lack of adjustment for confounders, such as dental plaque<sup>4,23-26</sup> and smoking<sup>24-26</sup>. The methodology applied to assess periodontal disease in some studies may be another important source of differences in findings among studies. Some studies that did not find an association between periodontal disease and HDL-c used CPITN<sup>25,26</sup>. The CPITN index is considered an inadequate method for measuring periodontal disease in analytic studies. The CPITN index overestimates the severity of periodontal disease and its use produces non differential misclassification bias, which, in turn decreases the possibility of detecting statistical differences when they in fact exist<sup>28</sup>. In this study, bleeding on probing did not differ between case and control groups, which may be considered an unexpected finding. However, the clinical parameters used to define periodontal disease were selected to reflect the past history of periodontal disease since the aim of the study was to investigate the relationship between periodontal disease and markers of cardiovascular risk.

The findings of the present study showing no association between LDL<sup>-</sup> and periodontal disease may be relevant because this was the first study that tested such relationship. Further studies are needed to confirm this hypothesis once LDL<sup>-</sup> has been considered a more accurate measure for cardiovascular disease<sup>13</sup>.

In the present study, there was no association between periodontitis and CRP using two different measurement methods. Similar results were reported by previous studies<sup>5,27</sup>. This finding is not consistent with previous studies where an ultra sensitive methodology was employed<sup>4-6,23</sup> or when other methods were used<sup>3,29,30</sup>. Some studies that found an association between

periodontal disease and CRP included younger individuals<sup>6,29</sup>, and subjects from specific populations, such as students and health professionals<sup>29</sup>, and other ethnic groups<sup>23</sup>. That may explain in part the observed differences between findings.

Some limitations of the present study included the cross-sectional design and the relatively small sample size, which was selected from a dental clinic. On the other hand, one noteworthy positive methodological aspect was the full mouth periodontal examination method used to measure periodontal disease conducted by a calibrated examiner. Another relevant aspect of the present study was the use of plasmatic analysis by immunoturbidimetry and ELISA method to assess risk predictors for cardiovascular disease. These methods possibly prevented measurement bias. In addition, selection criteria and adjusted analysis prevented the effect of potential confounders on the results.

## CONCLUSION

Within the limits of this study, clinical measures of periodontitis in adult subjects were found to be inversely associated with plasma concentrations of HDL-c.

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## CONFLICTS OF INTERESTS

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The authors declare no conflicts of interest.

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