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Association of vitamin D in patients with periodontitis: A cross-sectional study

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Abstract

Background and Objective: Vitamin D has been considered to possess anti-inflammatory and antimicrobial activity, which may be a link for the known interaction of periodontitis (CP) and coronary heart disease (CHD). This study investigated the association between serum vitamin D levels and periodontitis in patients with CP and with CHD. Furthermore, the objective was to determine whether periodontitis and CHD had an impact on serum vitamin D levels.

Material and Methods: Using a cross-sectional design, a total of 46 patients with CP, 45 patients with CHD, 45 patients with both CP and CHD, and 43 healthy patients were enrolled in the present study.

Results: Patients in the CP (17.4 ± 5.2 ng/mL) and in the CP + CHD (16.5 ± 5.6 ng/mL) group presented a significantly lower mean serum level of 25(OH)vitamin D compared to patients in the CHD (24.6 ± 3.7 ng/mL) and healthy control groups (29.9 ± 5.4 ng/mL) ($P < .001$). 25(OH)vitamin D levels were positively correlated with the number of teeth and negatively with C-reactive protein (CRP) and all periodontal parameters ($P < .001$). In all patients, there was a proportional increase of 25(OH)vitamin D levels with a progressive increase in number of teeth (P-trend <.001) while there were a proportional decrease in 25(OH)vitamin D levels with a progressive increase in clinical attachment level (CAL, P-trend = .001), probing depth (PD, P-trend = .006), and bleeding sites (BOP, P-trend <.001) levels.

Conclusion: Patients with CP and CP + CHD presented significantly lower serum levels of vitamin D compared to CHD and healthy controls. Moreover, the presence of CP negatively influenced serum vitamin D levels.

KEYWORDS

cardiovascular disease, coronary disease, cross-sectional studies, periodontal disease, vitamin D

1 | INTRODUCTION

Periodontitis (CP) is an inflammatory periodontal disease caused by specific micro-organisms which result, through the elicited inflammatory host response, in attachment loss and alveolar bone re-absorption.[1]

Periodontitis has been associated with different systemic disorders such as coronary heart disease (CHD),[2] diabetes,[3] and metabolic syndrome.[4] However, other factors have also been investigated as influencers of periodontal disease progression.

Vitamin D (25(OH)vitamin D) is a fat-soluble vitamin obtained from food intake or an endogenous production by the skin following exposure to sunlight.[5] It has been reported a protective effect of vitamin D as a modulator of the inflammatory response in various systemic conditions such as CHD, endothelial dysfunction, cancer, and diabetes.[5,6]

Mounting evidence supports the hypothesis that high vitamin D levels are favorable for good conditions of oral health. Recent studies have shown that both higher or lower serum vitamin D levels can lead to increase caries,[7] gingival inflammation,[8] periodontal diseases and, finally, tooth loss.[9]

Furthermore, it has been hypothesized that vitamin D can influence and prevent the progression of periodontal disease through an antimicrobial and anti-inflammatory response following the bacterial insult on the periodontium.[10,11] More specifically, it has been shown that, during CP, the vitamin D receptor presents in the cells of the immune system protects the endothelium of periodontal tissues and by reducing the production of B and T lymphocytes which are released following the insult of periodontopathogenic bacteria and during CVD.[12,13]

Given the critical role played by vitamin D during CP, there is an ongoing interest in the role exerted from serum vitamin D during CP, which could improve our knowledge on the process of endothelial damage during CP.

During the last decades, the different studies that have analyzed the impact of vitamin D on periodontal tissues have, however, reported conflicting results.[14-16] A large National Health and Nutrition Examination Survey (NHANES III) study which analyzed serum concentrations of vitamin D in CP patients found an independent association between low serum vitamin D levels and CP.[14] Similarly, a Buffalo OsteoPerio study on 920 postmenopausal women demonstrated that a cutoff serum 25(OH)vitamin D level ≥50 nmol/L is associated with a lower odds ratio of gingival bleeding and a lower risk of developing severe CP.[15]

Conversely, another cohort NHANES III study in a matched population younger than 50 years old did not find any significant association between vitamin D levels and CP, even if reported a decrease in the 10% odds of gingival bleeding for each 30 nmol/L increase in serum vitamin D levels.[16]

In light of these results, the aim of the present study was to evaluate the association between serum vitamin D levels, CP, and CHD and which were the greatest determining factors that influenced periodontal health status in patients with CP and with CHD. Furthermore, the objectives were (a) to determine whether vitamin D levels have an impact on CP and tooth loss and (b) if periodontitis significantly influenced serum vitamin D levels.

2 | MATERIALS AND METHODS

2.1 | Study design

A total of 389 patients with CP, CHD, and healthy controls were enrolled from February 2016 to January 2019 at the Department of Odontostomatology of the University of Messina, Messina, Italy. The local ethical committee (IRB University of Messina, Italy) approved the study protocol (#17-09). All patients were informed about the characteristics of the study and provided their informed written consent. The study was registered at clinicaltrials.gov (NCT03873935). The study was performed following the guidelines of the Declaration of the World Medical Association 1975 in Helsinki, revised in 2000. The study was reviewed and checked following the STROBE (STrengthening the Reporting of OBservational Studies in Epidemiology) guidelines (Appendix 1).

Patients with a diagnosis of CP[1,17] were enrolled for the CP group. Inclusion criteria for the CP group were as follows: (a) presence of at least twenty teeth; (b) CP with a minimum of 40% of sites with a clinical attachment level (CAL) ≥2 mm and probing depth (PD) ≥4 mm[18]; (c) presence of at least ≥2 mm of crestal alveolar bone loss verified on digital periapical radiographs; and (d) presence of ≥40% sites with bleeding on probing (BOP).[19]

Patients with a diagnosis of CHD[20] were enrolled for the CHD group. Inclusion criteria for the CHD group were as follows: at least ≥18 years old with a diagnosis of CVD, as defined by previous or current diagnosis of at least ≥50% of stenosis of at least one coronary artery diagnosed by coronary angiography or previous or current coronary artery bypass surgery or percutaneous coronary intervention. CHD diagnosis was performed with a coronary angiogram carried out at the Department of Cardiology of the University of Messina, Italy. During this stage, the presence of hypertension, diabetes mellitus, dyslipidemia, or other systemic diseases were recorded.

Healthy individuals, matched for age and gender, who presented no systemic disease, no drugs are taken, no sites with PD ≥4 mm or CAL ≥4 mm, or radiographic signs of bone loss were enrolled, such as control group.

The exclusion criteria for all patients were as follows: (a) intake of contraceptives; (b) intake of immunosuppressive, antibiotics or anti-inflammatory drugs throughout the last three months prior to the study; (c) status of pregnancy or lactation; (d) previous history of excessive drinking; (e) allergy to local anesthetic; (f) intake of drugs that may potentially determine gingival hyperplasia such as hydantoin, nifedipine, cyclosporin A, or similar drugs.

Clinical and medical characteristics (sex, age, and a complete medical anamnesis) and medications were obtained from all enrolled patients and recorded. Participants reported their smoking status as (a) never smokers; (b) previous smokers (≥5 years); or (c) current smokers. In patients who were current smokers, the number of cigarettes consumed (per day) was recorded.

2.2 | Study population

Using a cross-sectional design, after the first screening, 210 patients were excluded from the final sample because they did not meet the inclusion criteria (n = 153), declined to participate (n = 35), or missed the first appointment (n = 22). Thus, for this study, a total of 46 patients with CP, 45 patients with CHD, 45

patients with both CP and CHD, and 43 healthy patients were finally enrolled (Figure 1).

2.3 | Clinical data collection

The intake of vitamin D measured as an international unit (IU) per day was explored by a self-administered food frequency questionnaire (FFQ), which was previously validated for the evaluation of the vitamin D intake in adults.[21-23] The FFQ was adopted in the present study and comprised several domains aimed at measuring the average common food intake and included a domain that specifically investigated the regular use of vitamin and multivitamin supplements.

The inter-examiner reliability test resulted in an agreement of 86.1% (k = 0.64) for the primary outcome chosen, CAL. The intra-examiner agreement was evaluated by the measurement of Cohen's k coefficient, which was 0.856, and which equaled a high degree of reliability. The kappa coefficient was also calculated for the measurements performed by two examiners (GI, GM), and an acceptable degree of intra-class correlation coefficient reliability (ICC = 0.812) was obtained.

2.4 | Clinical and medical characteristics

The clinical and medical characteristics (sex, age, body mass index, and a complete medical history) and medications were assessed from all enrolled patients. The presence of diabetes mellitus was diagnosed from the history of the patient or if the patient presented a fasting blood glucose ≥7 mmol/L. Body mass index (BMI) was estimated based on the patient's height and weight. The periodontal evaluation comprised the recording of PD, CAL, plaque score (PI),[24] and BOP that was evaluated, during PD assessment, by the presence of bleeding up to 30 seconds after probing. CAL was recorded as PD plus recession with the cementoenamel junction as a reference for CAL measurements. All clinical periodontal parameters were recorded at six sites per tooth on all teeth present excluding third molars.

In each group, duplicate full-mouth periodontal evaluations were randomly performed in five patients, by two calibrated examiners not involved in the subsequent data analysis with a manual periodontal probe (GM, ER; UNC-15, Hu-Friedy). In the case of discordant measurements ≥2 mm PD, a new clinical assessment was carried out. Intra- and inter-examiner reproducibility of CAL was assessed from randomly selected patients.

2.5 | Laboratory analyses

During the first clinical examination, all patients underwent venous blood sampling at 8.30 AM Extensive chemical analyses were performed at the medical center after overnight fasting in all patients. Glucose, plasma lipids, and fibrinogen were determined by routine laboratory methods analysis. CRP levels expressed as milligrams per deciliters (mg/dL) were obtained by a commercially available enzyme-linked immunoassay (ELISA) kit.

The levels of 25-hydroxyvitamin D3 – 25(OH)vitamin D (vitamin D) were measured by using a commercial ELISA kit according to the manufacturer's instructions (Immunodiagnostics System). The data of 25(OH)vitamin D are expressed as nanograms per milliliters (ng/mL) with a wide measuring range of 9.7-66.2 ng/mL.

A masked duplicate plasma control sample was included in each batch and presented a coefficient of variation within-one pair of 4.9% for vitamin D. The inter-assay variation for 25(OH)vitamin D was 5.2%. The study was conducted each year (2016, 2017, and 2018), from March to July, in order to reduce the bias regarded to the seasonal variation in the intensity of sunlight.

2.6 | Power and sample size

The sample size was established considering four groups, an effect size of 0.26 for CAL (that represents the primary outcome variable), an expected standard deviation of 0.5,[19] a 2-sided significance level of .05, and a power of 80%. It was determined that approximately

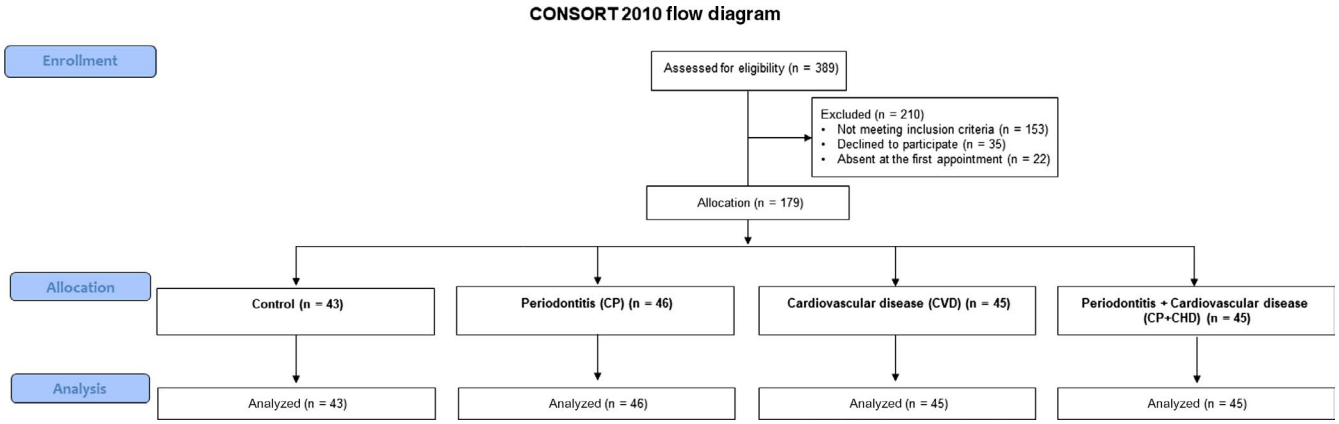

FIGURE 1 Flowchart of the study

23 patients per group would be needed. The enrolled patients were about 43 per group so that the achieved power value was 81%.

2.7 | Statistical analysis

For each of the four groups, numerical data are expressed as mean ± SD and categorical variables as number and percentage. Examined variables did not present normal distribution, as verified by the Kolmogorov–Smirnov test. Consequently, the non-parametric approach was used.

The Kruskal–Wallis test was applied in order to compare the four groups with regard to all numerical variables, so two-by-two comparisons between groups were performed using the Mann–Whitney test. The chi-squared test (χ^2) was used to compare groups for categorical variables. Bonferroni's correction was applied for the multiple comparisons, for which the significant alpha level .050 was divided by the number of possible comparisons. The "adjusted" significance level for this analysis was equal to 0.050/6 = 0.008. For each periodontal parameter, we report the regression coefficient, the 95% confidence interval (CI), and the relative *P*-value are reported.

For all groups and then for every single group of patients, the non-parametric Spearman correlation test was applied in order to assess the existence of significant interdependence between 25(OH)vitamin D and all periodontal parameters. For each periodontal parameter (number of teeth, CAL, PD, BOP, and PI), a stepwise multivariable linear regression model was estimated in order to assess the dependence of each periodontal parameter by potentially explicable variables such as age, sex, BMI, current smoking status, 25(OH)vitamin D, dietary vitamin D intake, supplementary vitamin D intake, total cholesterol, HDL-cholesterol, LDL-cholesterol, and CRP. Moreover, the possible existence of multicollinearity between 25(OH)vitamin D, dietary vitamin D intake, and supplementary vitamin D intake was explored. In particular, variance inflation factors (VIF) was used to explain how much amount of multicollinearity exists among variables.

Quartiles of vitamin D levels were evaluated, and for each 25(OH) vitamin D quartile, we calculated mean and standard deviation (±SD) of all periodontal parameters. In order to estimate a p-trend for ordered 25(OH)vitamin D quartiles, theJonckheere-Terpstra test was applied for all variables. More specifically, we wanted to assess if the periodontal parameters significantly increase or decrease with a 25(OH)vitamin D increase. Statistical analyses were performed using a statistic software program (SPSS 17.0 for the Windows package, SPS srl). $P < .05$, two sides were considered statistically significant.

3 | RESULTS

3.1 | Study participants

Sociodemographic variables of the study participants are represented in Table 1. All enrolled patients were matched for age ($P = .065$), gender ($P = .122$), percentage of smokers ($P = .331$) and for the BMI ($P = .061$) (Table 1). All enrolled patients were Caucasians.

Patients in the CP (0.44 ± 0.5 mg/dL), CHD (0.66 ± 0.6 mg/dL), and CP plus CHD (0.83 ± 0.9 mg/dL) presented higher mean CRP levels compared to healthy controls (0.29 ± 0.7 mg/dL), with a $P < .001$ for all comparisons (Table 1).

3.2 | Primary outcome vitamin D

Most patients in all groups showed a deficiency in 25(OH)vitamin D levels; only 13% (n = 6) of patients with CP and 11.1% (n = 5) of healthy controls presented 25(OH)vitamin D levels of ≥20 ng/mL. Regarding the differences in mean 25(OH)vitamin D levels, patients in the CP (17.4 ± 5.2 ng/mL) and in the CP + CHD (16.5 ± 5.6 ng/mL) group presented a significantly lower mean serum level of 25(OH) vitamin D compared to patients in the CHD (24.6 ± 3.7 ng/mL) and healthy control groups (29.9 ± 5.4 ng/mL) ($P < .001$) (Tables 1 and 2). There were no differences among groups in mean dietary ($P = .112$) and supplementary ($P = .531$) vitamin D intake.

The mean values of the periodontal parameters are presented in Table 1. Patients in the CP and CP + CHD group presented a significantly lower number of teeth and higher PD, CAL, and BOP compared to CHD and healthy control patients ($P < .001$), while there were no differences in the mean values of PI among four groups ($P = .455$).

The Spearman correlation test performed in the whole sample showed that 25(OH)vitamin D levels were positively correlated with the number of teeth (coeff. = 0.658, $P < .001$) and negatively with CRP levels (coeff. = −0.431, $P < .001$), CAL (coeff. = −0.687, $P < .001$), PD (coeff. = −0.669, $P < .001$), BOP (coeff. = −0.612, $P < .001$), and PI (coeff. = −0.604, $P < .001$) (Figure 2).

Further univariate and multivariate stepwise regression analyses were performed to assess the possible associations between 25(OH) vitamin D levels and the different features of periodontal health status in the whole sample.

The results of the univariate regression models highlighted that, in all enrolled patients, 25(OH)vitamin D levels significantly influenced the number of teeth to CAL, PD, and BOP with $P < .001$ for all outcomes (data not shown). Table 3 shows the results of the multivariate regression.

In the stepwise multivariate regression model, all potential confounding variables (age, gender, BMI, CRP, smoking, HDL-cholesterol, LDL-cholesterol, total cholesterol, 25(OH)vitamin D levels, supplementary vitamin D, and vitamin D intake) were inserted (Figure 3). The results showed that the number of teeth, CAL, PD, and BOP was significantly related to 25(OH)vitamin D levels ($P < .001$) (Table 3). In addition, in the whole sample, the number of teeth was significantly dependent also on age ($P = .019$), male gender ($P = .006$), and CRP ($P < .001$); the CAL was significantly dependent on age ($P = .033$), 25(OH)vitamin D levels ($P < .001$), CRP levels ($P < .001$), and LDL-cholesterol levels ($P = .028$). The PD was significantly dependent on 25(OH)vitamin D levels ($P < .001$) and CRP levels ($P < .001$), while

TABLE 1 Descriptive statistics of numerical variables for four groups and comparison among them. $P < .05$, statistically significant

Clinical features	Controls (n = 43)	CP (n = 46)	CHD (n = 45)	CP + CHD (n = 45)	*P*-value
Male, n. (%)	17 (37.8)	16 (35.9)	18 (39.4)	17 (39.4)	.131
Age, mean ± SD	53.7 ± 4.5	53.1 ± 4.2	52.4 ± 3.9	52.9 ± 3.9	.074
Education level					
Primary school, n (%)	14 (27)	16 (28.2)	15 (26.3)	16 (26.3)	.644
High school, n (%)	17 (27)	16 (30.7)	17 (34.2)	16 (28.9)	.556
College/university, n (%)	15 (18.9)	14 (15.4)	14 (13.1)	13 (18.4)	.671
Ethnicity					
White, n (%)	43 (100)	46 (100)	45 (100)	45 (100)	.999
BMI, kg/m^2, mean ± SD	24.4 ± 4.4	24.1 ± 4.3	24.2 ± 3.9	23.9 ± 3.8	.084
Glucose, mg/dL, mean ± SD	97.1 ± 9.2	104.4 ± 8.1	108.4 ± 10.4	109.5 ± 10.2	.065
Uric acid, mg/dL, mean ± SD	2.3 ± 3.7	3.9 ± 1.5	4.1 ± 1.2	4.7 ± 1.3	<.001
Albumin, g/L ± SD	37.4 ± 6.6	39.2 ± 5.3	40.4 ± 4.6	40.5 ± 5.8	.355
Fibrinogen, mg/dL, mean ± SD	296.1 ± 74.3	301.2 ± 66.4	319.6 ± 61.4	312.1 ± 70.3	.578
Apolipoprotein A, mg/dL, mean ± SD	137.9 ± 22.5	138.6 ± 20.4	132.3 ± 21.2	140.3 ± 23.5	.412
Total cholesterol, mg/dL, mean ± SD	182.9 ± 22.1	185.5 ± 24.5	196.1 ± 20.4	192.3 ± 23.8	.764
HDL-cholesterol, mg/dL, mean ± SD	52.5 ± 8.4	54.5 ± 6.4	57.7 ± 8.7	57.9 ± 7.5	.558
LDL-cholesterol mg/dL, mean ± SD	118.9 ± 18.3	124.4 ± 18.5	128.5 ± 21.8	126.5 ± 18.8	.646
BUN, mg/dL, mean ± SD	29.2 ± 13.4	31.2 ± 11.5	32.7 ± 12.4	33.9 ± 13.8	.451
CRP, mg/dL, mean ± SD	0.32 ± 0.5	0.42 ± 0.5	0.61 ± 0.4	0.83 ± 0.9	<.001
Systolic pressure, mm Hg, mean ± SD	120.7 ± 10.2	122.3 ± 7.5	140.5 ± 8.4	138.5 ± 9.2	<.001
Diastolic pressure, mm Hg, mean ± SD	86.1 ± 8.5	83.8 ± 7.1	93.6 ± 10.8	86.4 ± 10.5	.019
Ferritin, ng/mL, mean ± SD	81.2 ± 20.5	90.1 ± 27.5	95.8 ± 25.7	96.1 ± 27.9	.067
Smoker, n. (%)	11 (29.7)	13 (25.6)	12 (28.9)	13 (31.6)	.325
Current, n. (%)	9 (24.3)	10 (17.9)	9 (21)	9 (21)	.281
Never, n. (%)	32 (43.2)	33 (48.7)	33 (31.6)	32 (42.1)	.209
Past, n. (%)	2 (5.4)	3 (7.7)	2 (7.9)	4 (10.5)	.597
Comorbidities					
Diabetes, n (%)	—	4 (7.7)	8 (18.4)	7 (18.4)	<.001
Atrial fibrillation, n (%)	—	—	8 (21)	10 (26.3)	<.001
Angina pectoris, n (%)	—	—	19 (47.4)	12 (31.6)	<.001
Stroke, n (%)	—	—	10 (26.3)	10 (23.7)	<.001
Heart failure, n (%)	—	—	11 (28.9)	12 (31.6)	<.001
Drug treatment of CVD					
Antihypertensive, n (%)	—	—	15 (36.8)	12 (31.6)	<.001
Statins, n (%)	—	—	13 (34.2)	11 (28.9)	<.001
Low-dose aspirin, n (%)	—	—	12 (31.6)	15 (36.8)	<.001
Beta blockers, n (%)	—	—	13 (34.2)	12 (31.6)	<.001
Vitamin D, ng/mL, mean ± SD	29.9 ± 5.4	17.4 ± 5.2	24.6 ± 3.7	16.5 ± 5.6	<.001
Dietary vitamin D intake (IU/Day), mean ± SD	207.1 ± 8.1	204.5 ± 5.6	202.3 ± 4.7	199.4 ± 4.8	.134
Supplementary vitamin D intake (IU/Day), mean ± SD	247.2 ± 9.5	232.5 ± 10.7	237.5 ± 9.3	252.4 ± 41.5	.766
Total vitamin D intake (IU/Day), mean ± SD	447.5 ± 10.4	442.5 ± 10.3	444.5 ± 41.2	452.3 ± 41.6	.538
Periodontal parameters					
Number of teeth, n°, mean ± SD	26.4 ± 0.9	17.2 ± 1.4	22.3 ± 2.2	16.5 ± 3.2	<.001
CAL, mm, mean ± SD	1.5 ± 0.6	3.89 ± 0.6	2.31 ± 0.3	4.11 ± 0.5	<.001

(Continues)

TABLE 1 (Continued)

Clinical features	Controls (n = 43)	CP (n = 46)	CHD (n = 45)	CP + CHD (n = 45)	*P*-value
% of sites with CAL 4-5 mm, mean ± SD	—	36.5 ± 3.1	2.8 ± 1.3	34.3 ± 4.1	<.001
% of sites with CAL ≥6 mm, mean ± SD	—	20.8 ± 2.3	4.4 ± 2.1	20.7 ± 5.5	<.001
PD, mm, mean ± SD	1.51 ± 1.4	4.51 ± 0.7	2.59 ± 2.2	4.39 ± 0.5	<.001
% of sites with PD 4-5 mm, mean ± SD	—	44.6 ± 4.7	8.5 ± 2.6	48.7 ± 6.4	<.001
% of sites with PD ≥6 mm, mean ± SD	—	21.5 ± 4.2	2.2 ± 1.4	24.3 ± 4.7	<.001
BOP, mean % ± SD	9.5 ± 8.4	47.8 ± 3.5	15.5 ± 4.3	50.4 ± 6.9	<.001
Plaque index, mean ± SD	0.84 ± 0.4	0.85 ± 0.5	0.81 ± 0.7	0.84 ± 0.7	.459

BOP was significantly dependent on age (*P* = .008), 25(OH)vitamin D levels (*P* < .001), and CRP levels (*P* < .001) (Table 3). Supplementary vitamin D was also inserted into the models but did not result in statistically significant for any analyzed periodontal parameter (Table 3). There was no multicollinearity between 25(OH)vitamin D, dietary vitamin D intake, and supplementary vitamin D intake because the results of VIF for these variables resulted lower than 2 (25(OH)vitamin D and dietary vitamin D, VIF = 1.03, 25(OH)vitamin D, and supplementary vitamin D, VIF = 1.57; supplementary vitamin D and dietary vitamin D, VIF = 1.07).

Moreover, for all outcomes, the other confounders such as BMI, HDL-cholesterol, total cholesterol did not result statistically significant. Regarding the smoking variable, it was found that *P*-values were borderline, but not significant. More specifically, the number of teeth (*P* = .172), PD (*P* = .079), CAL (*P* = .092), and BOP (*P* = .103) resulted not statistically significant.

Moreover, in order to assess if the periodontal parameters significantly influenced 25(OH)vitamin D levels (quartiles), a *P*-trend for the ordered alternative hypothesis was estimated using the Jonckheere–Terpstra test. In all patients, there was a proportional increase of 25(OH)vitamin D levels with a progressive increase in number of teeth (*P*-trend <.001) while there were a proportional decrease in 25(OH)vitamin D levels with a progressive increase in CAL (*P*-trend = .001), PD (*P*-trend = .006), and BOP levels (*P*-trend < .001) (Figure 4).

4 | DISCUSSION

This cross-sectional study analyzed the association between serum 25(OH) vitamin D levels, CP, and CHD and which were the greatest determining factors that influenced the periodontal health status in patients with CP and CHD. The objectives were also to evaluate whether vitamin D levels have an impact on CP and tooth loss and if periodontitis significantly influenced serum vitamin D levels.

The present study showed that patients in the CP and the CP + CHD groups presented a significantly lower serum level of vitamin D compared to patients in the CHD and healthy control group.

During the last decades, several evidences showed the "perio-protective" effects of vitamin D for periodontal tissues, highlighting a low serum vitamin D levels such as a critical step for periodontal health, especially during CP.[12,25]

It has been previously reported in a large prospective cohort study with a 20-year follow-up on a population of 42.730 adults aged between 40-75 years, that patients who presented high vitamin D values had a lower incidence (20%) of CP and tooth loss.[12] Moreover, another cohort study on pregnant women without CP found a double chance of risking (OR 2.1) developing moderate to severe CP on patients who presented serum vitamin D levels <30 ng/mL.[26] Thus, a greater effort has been made to better understand the role of vitamin D during CP.

The primary function of vitamin D has long been recognized as a key role in the regulation of serum calcium levels and to regulate the alveolar bone growth and periodontal ligament homeostasis.[27] Vitamin D was reported to possess specific anti-inflammatory, antimicrobial activity. It has been shown that vitamin D modulates the adaptive immune response by selective stimulation of the production of cytokines, T-helper lymphocytes, monocytes, and macrophages to release peptides, such as defensins and cathelicidin, that possess good anti-inflammatory effects on periodontal tissues.[25,28,29] The anti-inflammatory action has been demonstrated to be one of the most protective effects of vitamin D, in a dose-dependent manner, against periodontal pathogenic bacteria.[29] More specifically, Grenier et al[30] demonstrated a selective inhibition by vitamin D on Porphyromonas gingivalis growth and on virulence factor gene expression that would thus significantly reduce the inflammatory response and periodontal damage during CP.

In the present study, the number of teeth, CAL, PD, and BOP was significantly related to vitamin D levels; in particular, the main periodontal parameters (PD, CAL, and BOP) were significantly dependent on vitamin D levels (*P* < .001).

Our findings are consistent with those investigated the relationship between CP and vitamin D levels. Studies on a large cohort have shown that the presence of mild or severe attachment loss or gingival bleeding is associated with low serum vitamin D levels.[13,15,30,31]

TABLE 2 *P*-value of two-by-two comparison between groups performed by the Mann–Whitney test. Adjusted α level = .008. $P < .05$, statistically significant

Variables	CNT vs CP	CNT vs CHD	CNT vs CP + CHD	CP vs CHD	CP vs CP + CHD	CHD vs CP + CHD
Age, mean ± SD	0.966	0.065	0.729	0.132	0.2123	0.328
BMI, kg/m^2, mean ± SD	0.152	0.223	0.191	0.319	0.284	0.185
Glucose, mg/dL, mean ± SD	0.959	0.654	0.115	0.628	0.548	0.179
Uric acid, mg/dL, mean ± SD	0.003	0.001	0.003	0.084	0.089	0.436
Albumin, g/L ± SD	0.384	0.322	0.078	0.981	0.747	0.328
Fibrinogen, mg/dL, mean ± SD	0.474	0.298	0.429	0.358	0.223	0.529
Apolipoprotein A, mg/dL, mean ± SD	0.569	0.922	0.349	0.379	0.587	0.165
Total Cholesterol, mg/dL, mean ± SD	0.474	0.555	0.358	0.254	0.328	0.478
Triglycerides, mg/dL, mean ± SD	0.322	0.289	0.431	0.282	0.347	0.226
BUN, mg/dL, mean ± SD	0.536	0.436	0.428	0.759	0.491	0.257
CRP, mg/dL, mean ± SD	<0.001	<0.001	<0.001	0.658	0.547	0.003
Systolic pressure, mm Hg, mean ± SD	0.598	<0.001	<0.001	<0.001	<0.001	0.399
Diastolic pressure, mm Hg, mean ± SD	0.614	<0.001	0.005	<0.001	<0.001	0.317
Ferritin, µ/L, mean ± SD	0.747	0.554	0.445	0.465	0.512	0.587
Vitamin D, ng/mL, mean	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Dietary vitamin D intake (IU/d), mean ± SD	0.823	0.978	0.846	0.978	0.888	0.524
Supplementary vitamin D intake (IU/d), mean ± SD	0.764	0.662	0.588	0.441	0.532	0.647
Total vitamin D intake (IU/d), mean ± SD	0.554	0.729	0.629	0.523	0.447	0.681
Periodontal parameters						
Number of teeth, n°, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.554	<0.001
CAL, mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.587	<0.001
% of sites with CAL 4-5 mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.453	<0.001
% of sites with CAL ≥6 mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.335	<0.001
PD, mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.536	<0.001
% of sites with PD 4-5 mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.624	<0.001
% of sites with PD ≥6 mm, mean ± SD	<0.001	<0.001	<0.001	<0.001	0.331	<0.001
BOP, mean % ± SD	<0.001	<0.001	<0.001	<0.001	0.328	<0.001
Plaque index, mean ± SD	0.125	0.223	0.151	0.213	0.166	0.329

Based on these pivotal observations, we designed the present study to assess whether periodontal parameters significantly influenced serum vitamin D levels.

Our study showed that there was a proportional increase of vitamin D levels with a progressive increase in number of teeth (*P*-trend < .001) while there was a proportional decrease in vitamin D levels when patients presented a progressive increase in CAL (*P*-trend = .001), PD (*P*-trend = .007), and BOP levels (*P*-trend < .001).

In accordance with our results, Millen et al[15] found a 33% lower ratio of periodontitis and 42% lower ratio of presenting ≥50% of gingival bleeding sites among patients with good (≥50 nmol/L) levels compared with deficient (<50 nmol/L) serum vitamin D levels. Teles et al[32] who analyzed the relationship between vitamin D and CP, showed that patients who had higher serum vitamin D levels presented lower BOP and CAL levels and fewer missing dental elements, as well as low proportions of periodontopathogenic bacteria, compared with those who presented low levels of vitamin D. Zhan et al,[33] in a longitudinal study, showed that the increase of 25-µg/L (25 nmol/L) of vitamin D serum was associated with a proportional reduction of 13% of tooth loss highlighting, in accordance with our results, that high vitamin D levels may represent a protective factor against periodontal tissue damage during CP.

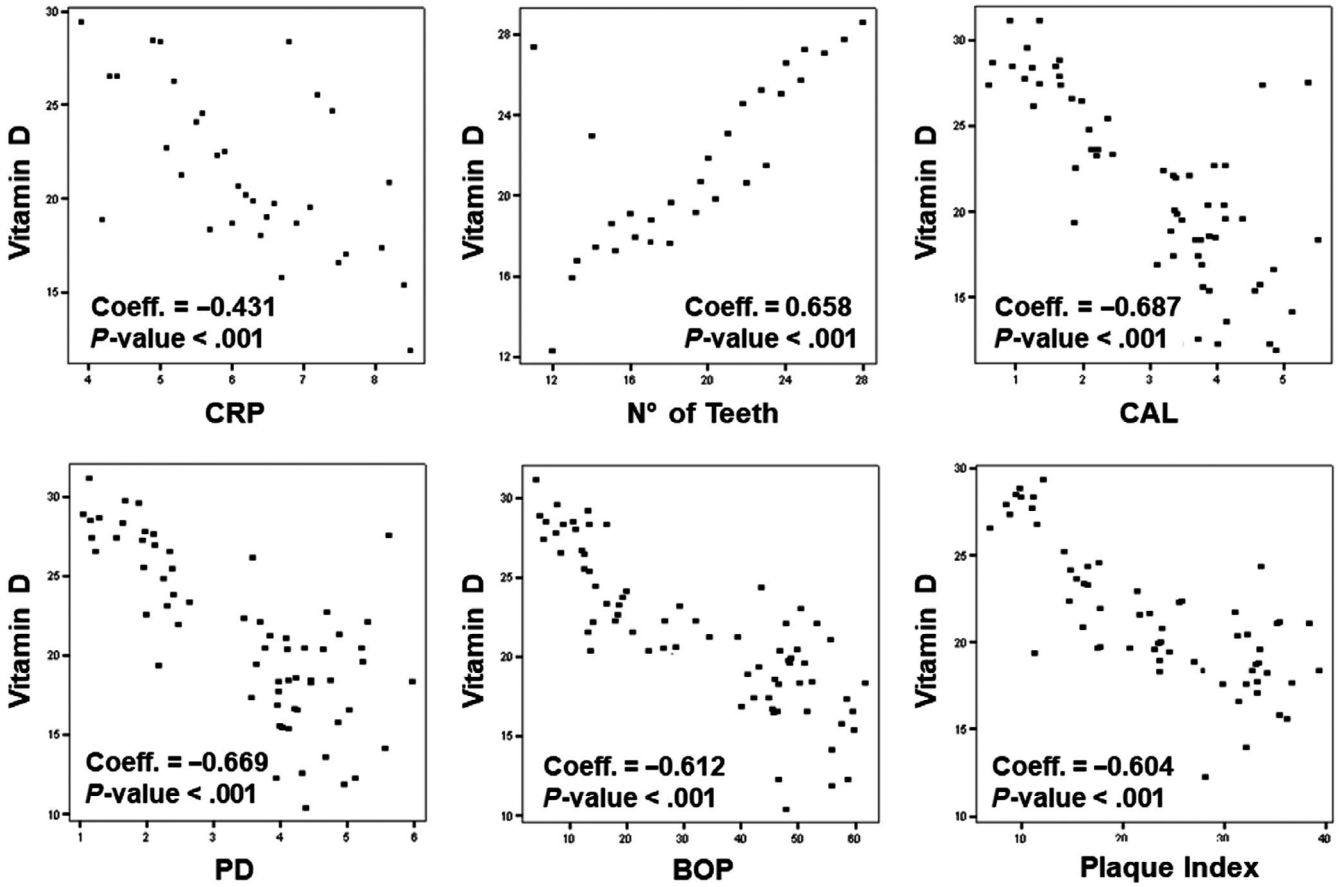

FIGURE 2 Spearman correlation between vitamin D levels, CRP, and periodontal parameters

TABLE 3 Stepwise (backward elimination) linear regression models for periodontal parameters

	Number of teeth			CAL		
Variables	**Coeff.**	**95% CI**	***P*-value**	**Coeff.**	**95% CI**	***P*-value**
Age	−0.15	−0.28; −0.05	.019	0.04	0.04; 0.80	.033
Gender	1.64	0.45; 2.74	.006	—	—	n.s.
Vitamin D	0.33	0.17; 0.47	<.001	−0.12	−0.16; −0.62	<.001
Dietary vitamin D	−0.12	−0.23; 0.16	.86	—	—	n.s.
CRP	−2.47	−3.25; −1.85	<.001	0.48	0.28; 0.65	<.001
LDL-cholesterol	—	—	n.s.	0.01	0.01; 0.36	.028
	PD			**BOP**		
Variables	**Coeff.**	**95%CI**	***P*-value**	**Coeff.**	**95%CI**	***P*-value**
Age	0.06	−0.03; 0.95	.054	0.79	0.19; 1.35	.008
Vitamin D	−0.13	−0.14; −0.55	<.001	−1.75	−2.28; −1.20	<.001
CRP	0.45	0.23; 0.66	<.001	8.44	5.42; 11.37	<.001
LDL-cholesterol	−0.13	−0.01; 0.23	.077	—	—	n.s.

Note: Age, BMI, vitamin D, dietary vitamin D, CRP, LDL-cholesterol, HDL-cholesterol, total cholesterol, and smoking were included as independent variables. Only significant covariates in the models were shown. For gender, female served as reference. n.s., not significant. $P < .05$, statistically significant.

In this regard, it has been demonstrated that additional calcium and vitamin D supplementation therapy had a positive effect on patients with CP during periodontal maintenance therapy.[34,35] However, in our study, all patients presented the same values in mean dietary and supplementary vitamin D intake.

Interestingly, our study also indicated that the number of teeth, PD, CAL, and BOP, which were dependent on vitamin D levels, was also positively correlated with CRP levels, even after multiple adjustments.

The ability of vitamin D to strongly reduce the risk of developing CVD has been previously demonstrated by different reports.[36,38,39-41] A possible explanation of the association between vitamin D and CVD could be described by the strong impact of CP and some other oral diseases on systemic inflammation.[37,42-45] We hypothesized that stimulating oxidative stress conditions, such as CP and CVD, may have led to the high production of high levels of CRP. In accordance with our results, Tsioufis et al[46] in a sample of 108 untreated hypertensive patients with CP found that serum CRP levels were associated in a dose-dependent manner in a patient with CP. In the study of Tsioufis et al,[46] after adjustment for potential confounders, CRP levels were significantly associated with mean CAL, PD, and BOP.

The results of the present study have, however, some limitations. One limitation is represented by the study design. The cross-sectional design does not permit to analyze the temporal association between vitamin D levels and periodontitis that should be assessed with a longitudinal observation. Moreover, only some confounders were included, in the present study, for the stepwise multivariate regression model, such as smoking, which, however, resulted borderline not significant. In this regard, some further analyses with a larger number of confounders on consecutively enrolled patients should be performed in order to better understand the association between vitamin D, CP, and CHD.

In recent years, several studies have focused their interest in the analysis of the relationship between vitamin D and CP. There are ongoing researches to examine and develop new methods that can help the clinicians and researchers in understanding

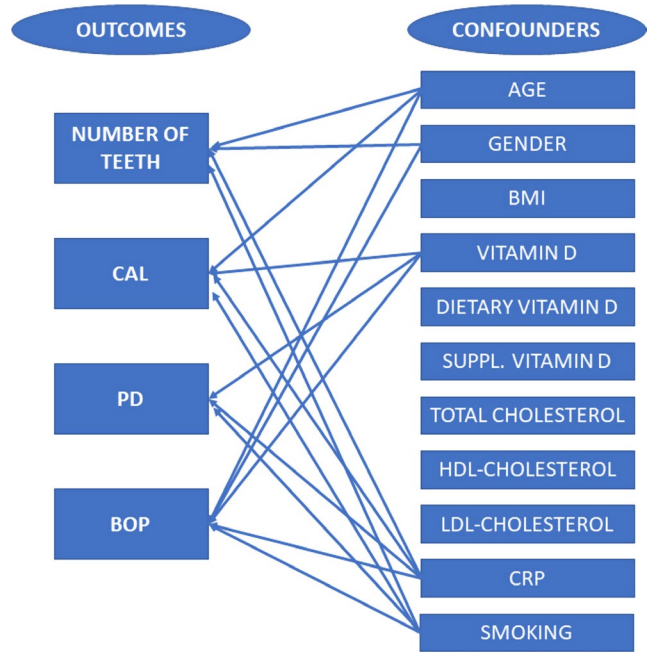

FIGURE 3 Statistical model developed

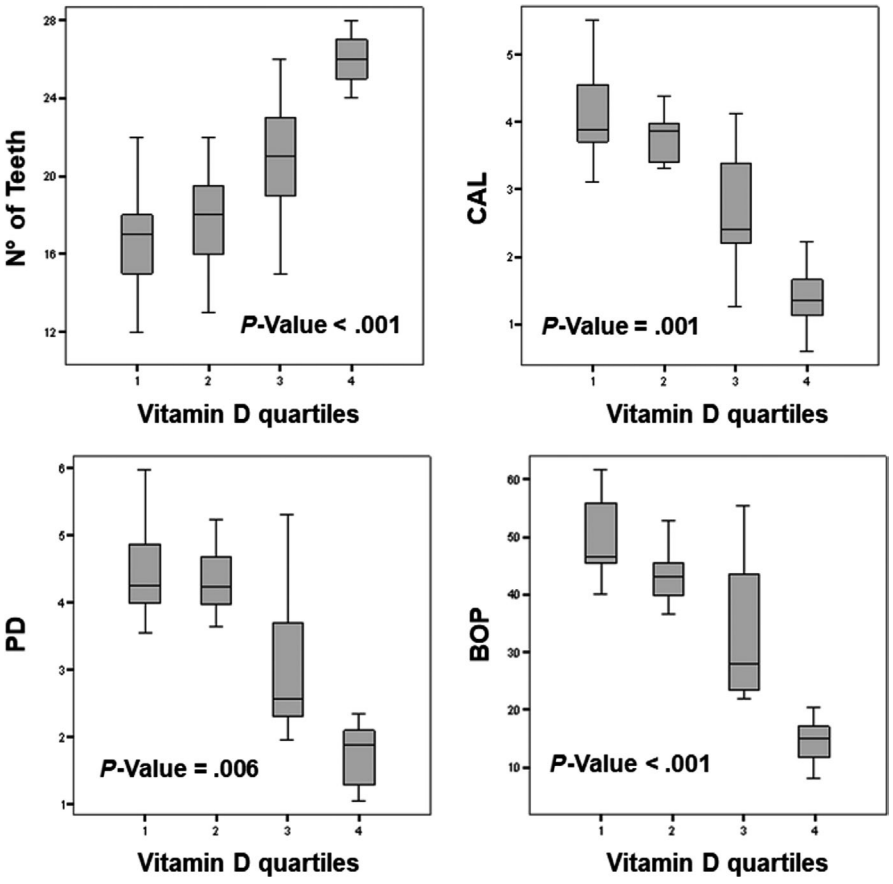

FIGURE 4 Boxplot of periodontal parameters according to vitamin D quartiles and *P*-trend. Each *P*-trend value referred to an increase/decrease of periodontal parameters according to vitamin D quartiles and was obtained using the Jonckheere–Terpstra test for the ordered alternative hypothesis

the causes which determine CP and tooth loss. Identifying new agents that influence the CP pathway should be encouraged in order to find alternative treatment strategies to prevent CP progression.

5 | CONCLUSION

This study indicated that patients who have CP and CP plus CHD presented lower serum levels of vitamin D compared to CHD and healthy patients. Moreover, vitamin D was significantly correlated with CP and was negatively influenced by CP.

Since this study supports the evidence that low serum vitamin D levels are associated with periodontitis and tooth loss, an assessment of vitamin D levels, especially in patients with CP, should be recommended at the beginning of periodontal therapy as it could predict and reduce the possible progression and risk of developing CP.

However, future studies with a larger sample and a prospective design should attempt to determine, with greater accuracy, the role potential benefits of vitamin D on periodontal health in patients affected by periodontitis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in the present study.

AUTHOR CONTRIBUTIONS

Gaetano Isola conceived the idea of the work, planned and performed the experimental procedures. Angela Alibrandi performed the statistical analysis concealment and critical revision of the manuscript. Ernesto Rapisarda and Giovanni Matarese analyzed and summarized the experimental results. Gaetano Isola and Ray C. Williams wrote the paper. Rosalia Leonardi performed the acquisition and interpretation of revised data and critically revised the manuscript. The final approval of the version to be published was obtained by all co-authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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