Association between periodontitis and blood pressure highlighted in systemically healthy individuals. Results from a nested case-control study.

Short title: Periodontitis and hypertension in systemically healthy individuals

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Abstract

Recent evidence suggests hypertension and periodontitis are closely linked but limited data is available on the nature of the association. We aimed to investigate the relationship between periodontitis and mean arterial blood pressure in a sample of otherwise systemically healthy individuals. A case-control study including 250 cases (participants with periodontitis) and 250 controls (without periodontitis) was designed from a register of clinical trials conducted between 2000-2018 in a university setting. Cases were age, gender and body mass index balanced with controls. Linear, logistic regression and mediation models were planned to test the association between various periodontal measures and arterial blood pressure. We further investigated the role of systemic inflammation assessed by hsCRP and white cell counts. Cases presented with 3.36 mm Hg (95%CI, 0.91-5.82, p=0.007) higher mean systolic blood pressure (SBP) and 2.16 mm Hg (95%CI, 0.24-4.08, p=0.027) higher diastolic blood pressure (DBP) than controls. Diagnosis of periodontitis was associated with mean SBP (β=3.46±1.25, p=0.005) and greater odds of SBP≥140 mm Hg (OR=2.3, 95%CI:1.15-4.60, p=0.018) independent of common cardiovascular risk factors. Similar findings were observed when continuous measures of periodontal status were modelled against SBP. Measures of systemic inflammation although elevated in periodontitis were not found to be mediators of the association between periodontitis and arterial blood pressure values. Periodontitis is linked to higher systolic blood pressure in otherwise healthy individuals. Promotion of periodontal and systemic health strategies in the dental and medical setting could help reduce the burden of hypertension and its complications.

Key words: Periodontitis, bleeding on probing, mean blood pressure, systemic inflammation, case-control study, mediation analysis, hypertension.

1. Introduction

Elevated arterial blood pressure increases the risk of complications from cardiovascular diseases (CVD) such as stroke and myocardial infarction, with more than 7.6 million deaths accounted for every year and 143 million disability adjusted life-years.[1] It is estimated that more than 30% of the overall population suffers from hypertension, and this estimate increases with age.[2] A 15%–50% of individuals however are unaware they are affected by hypertension,[3] whilst many of those with a stablished diagnosis fail to achieve an optimal blood pressure control despite their prescribed medications.[2] The burden and cost of hypertension remain high for any given society. Inflammation is considered an important driver of vascular dysfunction and implicated in the development and progression of hypertension.[4,5]

Periodontitis is a common inflammatory disease caused by a dysbiotic biofilm and affecting the soft and hard tissues around teeth.[6] It is a chronic disease, usually spanning over decades of an individual's life and is characterised by gingival inflammation with associated alveolar bone loss which, if not arrested, will ultimately lead to tooth loss. Almost 750 million people (aged 15 to 99) worldwide present with moderate to severe symptoms of periodontitis,[7] plus the disease is linked to social inequality and it negatively affects patients' quality of life.[8]

Recent evidence suggests a possible causal link between periodontitis and hypertension.[9] Patients with periodontitis often present with higher arterial blood pressure values and a 30-70% higher chance to also present with hypertension,[10] especially when there is active gingival inflammation (i.e. with gingival bleeding).[11] Longitudinal and large interventional studies confirming the nature of this association and the exact pathogenetic mechanisms are scarce.

The aim of this study was to investigate the association between diagnosis of severe periodontitis and arterial blood pressure in a sample of otherwise healthy participants (without a confirmed diagnosis of hypertension). The primary objective was to assess office blood pressure values in patients with periodontitis (cases) compared to controls (participants without periodontitis) and whether a linear relationship exists between measures of periodontitis extent/severity with blood pressure values and whether basic measures of systemic inflammation mediate any association. Further, the prevalence of undiagnosed hypertension between cases and controls was explored.

2. Material and methods

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

2.1.Study design and population:

A nested case-control study was designed, including participants recruited at the UCL Eastman Dental Institute between the years 2001 – 2018. All participants provided written informed consent at the time of study participation including use of data for future analyses. Seventeen clinical trials with the same inclusion criteria (severe periodontitis) or control (no periodontitis) and full-mouth periodontal assessment and arterial blood pressure measurements (same recording protocol) were screened for inclusion in this analysis. This study was approved by the local UCL Research Ethic Committee (Project ID: 16989/001).

Sample inclusion criteria:

Cases were individuals ≥18 years old who had been diagnosed with generalised severe periodontitis (defined as ≥50% of the teeth with probing pocket depths (PPD) of ≥5mm and ≥30% marginal alveolar bone loss) and referred to the periodontal unit for management of their condition[12]. All participants were otherwise systemically healthy (no other systemic condition, as per medical history assessment/interview) and had not undertaken periodontal therapy within 6 months of the specific study assessment.

Controls were individuals ≥18 years old attending the same hospital without a diagnosis of periodontitis (recruited from other dental units but with no active dental infections) who were equally systemically healthy (no other systemic condition, as per medical history assessment/interview).

Sample exclusion criteria:

Possible participants were excluded from this study if presenting with any of the following: i) active infectious diseases such as hepatitis, HIV or tuberculosis, ii) any confirmed systemic

diseases including diabetes, kidney, liver, cardiovascular diseases, hypertension, cancer or on any chronic medication and iii) pregnant or breastfeeding, iv) taking non-steroidal anti-inflammatory drugs on a regular basis or taking antibiotics within 3 months of assessment.

2..2. Periodontal examination:

Periodontal assessment used a standardised protocol carried out by calibrated examiners as previously described.[12] Baseline data on full mouth periodontal assessment was retrieved for all participants. Case definition of periodontitis was confirmed against the latest validated classification.[6] The full-mouth dental plaque (FMPS) and gingival bleeding (FMBS) scores were recorded and the following thresholds for localised (FMBS 10%-29%) and generalized gingival bleeding (FMBS >30%) were adopted. Periodontitis case definition was of generalised severe/stage III/IV periodontitis.[6] Continuous measures of severity and extent of periodontitis were created as follows: a) the extent of periodontal pockets with probing pocket depth (PPD) of ≥ 4mm, ≥5mm, ≥6 mm (number and percentage of sites) and b) extent of loss of periodontal attachment levels (CAL) of ≥3mm, ≥ 4 mm, ≥5mm, ≥6mm (number and percentage of sites).

2.3. Blood pressure assessment:

Office blood pressure measurements were obtained following a standardised protocol using an Omron device M5-1 (HEM-757A-E) by a trained person and recorded in triplicate for each participant as previously described.[13] The patients were advised not to exercise, smoke or consume any caffeine during the 30 min prior to their appointment. Upon arrival, the measurements were recorded after the patients were seated for 5 min and relaxed, with the back resting on the chair and the arm on a desk at the level of the right atrium. Average of the systolic and diastolic arterial pressure (SBP and DBP) readings taken to the nearest value were obtained and used as continuous variables. Unconfirmed hypertension diagnosis was

evaluated applying diagnostic thresholds of the US (values of SBP ≥130 mmHg or DBP ≥80 mmHg) and European (values of SBP ≥140 mmHg or DBP ≥90 mmHg) guidelines.[14,15]

2.4. Additional variables:

Socio-demographics information (age, gender, ethnicity), health lifestyle behaviour [smoking, physical activity (frequency of weekly sessions of being active through walking, cycling, sports and recreation)], and family history of cardiovascular diseases (whether or not any family member had heart or vascular disease) was retrieved from the medical history questionnaires. Anthropometric measurements (body max index: BMI) were collected by trained staff using a standard protocol.[12] Fasting venous blood samples were collected and analysed for White Cell Counts (WBC), high sensitivity C-reactive protein (hsCRP), total cholesterol, low- and high-density lipoprotein, glucose and triglycerides using standard biochemistry procedures and as previously described.[12]

2.5. Statistical Analysis:

A sample size calculation confirmed that a minimum of 248 participants per group was required to detect a difference of 3.5 mm Hg in mean Systolic blood pressure (SBP) between cases and controls, with a standard deviation of 12 mm Hg, to achieve a power of at least 90% assuming an alpha of 0.05.

Cases and controls were balanced based on age ± 3years, gender and BMI (Figure 1).[16] All data was checked for errors, entered in a single dataset and analysed using SPSS (version 25), STATA (version 15) and R Software (version 3.5.2). Descriptive statistics (comparisons between continuous variables used independent t-test/Mann Witney and between categorical variables used Chi squared test) was used to compare general variables and arterial blood pressure levels between cases and controls. Multivariate linear regression analysis was performed to investigate potential associations between: i) periodontitis (categorical and continuous definitions) or systemic markers of inflammation (i.e. hsCRP and WBC) as

exposures and mean SBP and DBP as outcomes, ii) periodontitis (exposure) and systemic markers of inflammation (outcome). Age, BMI, gender, ethnicity, smoking, physical activity and family history of CVD were used as confounders in each model. Similarly, multivariate logistic regression analysis was carried out to test the odds of the following categorical outcome variables: i) SBP≥140, ii) SBP≥130, iii) DBP≥90, iv) DBP≥80 mm Hg and 'undiagnosed' hypertension defined as v) per European guidelines[14] (SBP/DBP≥140/90mmHg) and vi) as per US guidelines[15] (SBP/DBP≥138/80mmHg) in relation to the different exposure variables, i.e. periodontitis diagnosis (categorical yes/no), hsCRP and WBC. β coefficients and ORs with 95% confidence intervals were calculated in unadjusted (Model 1) and adjusted models including age, BMI, gender, ethnicity, smoking, physical activity and family history of CVD (Model 2). A p-value of ≤0.05 were considered statistically significant. Mediation analyses of systemic inflammatory markers were performed if a positive association between CRP or WBC variables with periodontitis and SBP/DBP were found in linear/logistic regression models. Two different and prespecified routes were used, direct (route 1) and indirect (route 2) mediation effects with their 95% CI were estimated: Route 1: Periodontitis (exposure)→ Hypertension (outcome). Route 2: Periodontitis (exposure)→ WBC or hsCRP (mediators)→ Hypertension (outcome).

2. Results

Sample median age [IQR] was 35 [12] years with no substantial differences observed between cases (35 [9] years) and controls (34 [14] years) (p=0.345). Similar gender (p=0.929) and BMI (p=0.209) distributions were confirmed between cases and controls. Higher number of current smokers, with predominant Afro-American/Afro-Caribbean participants, reporting less physical activity and a higher percentage of family history of CVD were observed in cases when compared to controls. Participants with periodontitis exhibited increased glucose, LDL, CRP and WBC levels but lower HDL values when compared with controls (all, p<0.01) (Table 1).

Higher mean differences in SBP (3.36mmHg, 95%CI, 0.91-5.82, p=0.007) and DBP (2.02 mmHg, 95%CI, 0.24-4.08, p<0.027) were observed in cases when compared to controls.

A 14% of cases presented with SBP≥140 mmHg vs a 7% of the controls (p=0.021). Similarly, more than 43% of the cases presented with DBP≥80 mmHg vs 34% of the controls (p=0.035). The percentages of cases with SBP≥130 mmHg or DBP≥90 mmHg was greater than for the controls but not statistically significant (Table 1). Overall a 15.6% of the whole study participants presented with values of SBP/DBP in the range of hypertension (European definition): 17.2% of the cases and 14% of the controls (p=0.324), and a 45.6% (American definition): almost 50% of cases and a 41.6% of controls (p=0.073) (Table 1).

Linear regression analysis confirmed an association between case definition of periodontitis (categorical) and higher mean SBP after adjusting for common risk factors (*ß*±SE=3.46±1.25, *p=0.005*, Model 2) and to DBP (*ß*±SE=2.16±0.98, p=0.027, Model 1) (Table 2). Greater severity of periodontitis as assessed by measure of CAL, PPD and FMBS were associated with higher mean SBP (Models 1 and 2) and with higher mean DBP (Model 1) (Table 2). A similar linear association between different thresholds of periodontal lesions

(PPD thresholds) and SBP (Models 1 and 2) were noted but for different threshold of CAL, this was only seen in Model 1 (Table 2). Periodontitis (categorical and continuous variables) was associated with higher CRP (Model 1) and WBC (Model 1 and 2) levels (Table 2). Similarly, periodontitis (categorical) was associated with higher odds of SBP≥140 mm Hg (OR=1.98, 95%CI, 1.10-3.65, *p*=0.023, Model 1; OR=2.31, 95%CI, 1.17-4.67, *p*=0.018 (Model 2) and DBP≥ 80 mm Hg (OR=1.47, 95% CI, 1.03-2.12, *p*=0.035, Model 1) (Table 3). No significant association was found with SBP≥130 mm Hg, DBP≥90-mm Hg or Hypertension definition according to European or American guidelines.

When CRP and WBC were modelled as exposure variables, no associations were observed with SBP or DBP (Tables 2-3). Lastly, an association between SBP and increasing FMBS irrespective of periodontal diagnosis was observed in the multivariate fitted model for SBP according to bleeding status (Table 4). Participants with generalised bleeding presented with a 5 mmHg greater SBP than those with healthy gums (95%CI, 8.22-1.91, *p*=0.002) (Table 4).

Mediation analyses confirmed that WBC did not act as a mediator of the association between periodontitis (categorical) and SBP (continuous) in either in the unadjusted (β±SE= -0.00±1.21; *p*=0.994) or the fully adjusted (β±SE= -0.03±0.21; *p*=0.900) models (Route 2) (Tables S1 and S2). Similar results were observed when the model was replicated for continuous periodontal (FMBS, CAL, PPD) and categorical BP (SBP≥140 mm Hg) variables.

5. Discussion

The results of this study showed that systemically healthy individuals with periodontitis (cases) presented with higher mean SBP and DBP than participants without periodontitis (controls). Cases presented with more than twice higher likelihood of SBP≥140 mm Hg and almost 50% higher odds of DBP≥80 mm Hg than the controls.

A recent systematic review confirmed a 4.5 mm Hg SBP- and 2.5 mm Hg DBP greater arterial blood pressure values in the general population (including participants with and without other co-morbidities).[10] In a previously reported age- and sex- matched case-control study looking at the association of periodontitis, systemic inflammation and endothelial function, greater differences in SBP (7 mm Hg) and CRP (1.3mg/L) levels between the cases and controls were observed.[17]

Elevated blood pressure remains the main risk factor for heart failure, atrial fibrillation, chronic kidney disease, heart valve diseases, aortic syndromes, and dementia, in addition to coronary heart disease and stroke.[18] It is now understood that biologically normal blood pressure levels are lower than what previously fell within a normal rangel.[15] The observed differences of SBP/DBP between cases and control in this study could be clinically relevant and might represented an overlooked mechanism linking periodontitis with increased future CVD risk.[19]

In agreement with previous studies,[20,21] this study showed that irrespective of periodontal status, bleeding gums was associated with SBP. Similarly, a more recent secondary analysis of NHANES III also reported a 2.6 mm Hg higher mean SBP for gingivitis, also independently associated with 40% greater odds of high/uncontrolled BP.[11] Bleeding gums, the earliest sign of periodontal diseases, has also been linked to increased systemic biomarkers and vascular changes.[22,23] Due to its easy detection by patients and clinicians could represent a valuable parameter in routine BP screening protocols.

Several mechanisms underlying the links between gingival diseases and hypertension have been proposed with dysbiotic subgingival microbiome triggering low-grade systemic inflammation and oxidative stress representing the main pathways.[24] Periodontitis patients express increased local and systemic inflammatory markers such as CRP, TNF- α, neutrophilic enzymes, WBC counts and disparity in T cell subtypes, but also neutrophil dysfunction, which are all mechanisms resulting in vascular changes and endothelial dysfunction.[19,25,26] The presence of periodontal pathogens have been linked to hypertension in epidemiological studies.[27] Pre-clinical evidence originated by experimental animal models, including immunisations with P. gingivalis lysate and Lipopolysaccharide (LPS) - endotoxin from other gram-negative bacteria caused prolonged T-cell activation and elicited increased levels of CRP, TNF-α, and IL-1β, resulting in increased blood pressure.[28] Interaction between oral-gut microbiome can also contribute to amplification of inflammation and metabolic changes.[29] Recent evidence implicates oral bacteria in the nitrate-nitrite-nitric oxide (NO) pathway and pathogenesis of hypertension,[30] with high concentrations of nitrite-reductase bacteria increasing systemic NO and having an effect of lowering SBP.[31]

In the current study, hsCRP/WBC as a proxy of systemic inflammation was associated with periodontitis but not with SBP/DBP. Additionally, WBC did not show a mediation effect between periodontitis and BP. These results are in partial disagreement with a recent analysis of cross-sectional data, based on national health surveys in US and Korea, where a 2-7% mediating effect of WBC and CRP was observed when examining the association between periodontitis and hypertension.[32] A possible explanation for these differences relates to an overall younger population of this study sample (35-years-old) vs 51- and 46- in the American and Korean populations, and possibly due to the systemically healthy status of this sample, when compared to representative samples of those populations, including systemic conditions. Nevertheless, an association of arterial BP with both continuous and categorical

measures of periodontitis in younger and systemically healthy individuals strengthens the evidence in favour of a causal association between the two diseases.[24]

A recent Mendelian Randomisation analysis and results from a short-term pilot RCT on periodontal treatment of resistant hypertensive patients corroborate these findings.[9] Single nucleotide polymorphisms (SNPs) in SIGLEC5, DEFA1A3, MTND1P5, and LOC107984137 loci GWAS-linked to periodontitis and BP phenotype were unravelled and a noticeable reduction in SBP/DBP, endothelial function as well as cytokines and activated T-cells subsets was observed 2-months following the treatment. Similarly, another RCT with 6-months follow-up on a pre-hypertensive population also observed a significant reduction in SBP/DBP following non-surgical periodontal treatment.[33] Oral health promotion strategies such as tooth brushing twice daily has demonstrated very effective, not only in managing and preventing most common oral conditions,[34] but in providing a powerful and affordable tool for hypertension control.[35] Notably, a 14% reduction in CV events have been observed with a 4.4 mm Hg reduction in SBP.[36] Preliminary evidence suggests that periodontal treatment in patients with type 2 diabetes, a common co-morbidity, could result in substantial long-term reduction of medical-related costs for healthcare systems.[37] Thus, given the importance of non-pharmacological and pharmacological blood pressure lowering strategies in decreasing CVD risk and mortality,[38] larger multicentre RCTs and health-economic analyses are warranted to further investigate the benefits of periodontal treatments on blood pressure prevention and control.

Elevated blood pressure is usually asymptomatic and best detected in screening programs or opportunistic measurements of BP, which confirm that a worryingly high number of individuals (> 50%) is unaware of a possible diagnosis of hypertension.[39] The presented study confirmed that a 15-45% of the sample could exhibit undetected hypertension (depending on whether a European or US guideline definition was used), with 54-55% of

these having periodontitis. In a recent cross-sectional study on the association of periodontitis and hypertension, a 15.9% of the study sample presented with undiagnosed high BP (based on a single office measurement), of which a 62.5% had periodontitis.[40] These data confirm that programmes of hypertension screening in the dental settings should not be underestimated.

Whilst this study improves the understanding around the association between periodontitis and arterial blood pressure, it is recognised some limitations exist. The study design and analysis may have introduced some bias namely through selection and assessment biases.[41] Further, in this study we did not account for other factors which might have impacted on blood pressure such as abdominal obesity, salt intake, use of anti-inflammatory drugs, hormone treatments or stress as well as additional oral diseases (i.e. caries). Future analyses should focus on existing or new epidemiological evidence (longitudinal studies) where all possible confounders are appropriately considered. Nevertheless, this study benefits from a robust research methodology in assessing the exposure (periodontitis) and outcome (blood pressure) and sufficient statistical power could have counteracted some of the limitations.[42] Further, using a balanced study design through matching for common confounders of arterial blood pressure facilitated analysis of comparable groups.[43]

6. Conclusions

This study expands current knowledge on the association between periodontitis and elevated blood pressure, pointing at the importance of this link in the generally healthy population. Oral health professionals could play a pivotal role in helping the medical community detecting and tackling the burden and consequences of hypertension.

7. Perspectives:

Periodontal treatments could be well-tolerated novelty non-pharmacological interventions for the management of hypertension. Particularly so when patients are informed that periodontal treatment could be beneficial not only for their oral health but also for their general health and wellbeing in return. Thus, future directions and broad implications of this work will involve liaison of dental and medical health professionals with the following objectives:

1. Raising awareness of the increased risk for high blood pressure among individuals with periodontal diseases.
2. Implementing hypertension screening systems by dental professionals and prompt referral to general practitioners.
3. Implementing periodontal diseases screening systems by medical professionals and referral to dental practitioners.
4. Providing advice for common risk factors: Healthy diet, smoking cessation, promoting physical activity, alcohol reduction, and diabetes management.
5. Early diagnosis and management of gingivitis and periodontitis. Effective prevention and treatment of these conditions is very cost-effective and has shown an effect in reduction of systemic markers of inflammation and improvement in endothelial function.
6. Future research will involve larger multicentre RCTs to test the effects on periodontal treatment on blood pressure levels.

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10. Conflict of interest/disclosures

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12. Novelty and significance

What is New?-

The link between periodontitis and high blood pressure was confirmed in systemically healthy individuals

What is Relevant?-

- Individuals with periodontitis and oherwise healthy presented with higher mean SBP/DBP and odds of SBP>140 mm Hg
- Bleeding gums was associated with higher mean SBP
- Undetected hypertension was a common finding among the population of study

Summary –

The risk of elevated blood pressure was highlighted in systmically healthy periodontal patients. Oral health professionals could play a crucial role in assisting in the screening and management of hypertension.

13. Figure legends:

Figure 1: Study flow chart diagram

Table 1: Main characteristics of participants.

Variables	Overall (500)	Controls (Non-Periodontitis) (250)	Cases (Periodontitis) (250)	*p value*
Categorical N (%)				
Gender (%female)*	263 (52.6)	132 (52.8)	131 (52.4)	0.929
Smoking				
Non-smoker	290 (58.0)	175 (70.0)	115 (46.0)	**<0.001**
Current smoker	96 (19.2)	34 (13.6)	62 (24.8)	
Ex-smoker	114 (22.8)	41 (16.4)	73 (29.2)	
Ethnicity				
Caucasian	299 (59.8)	177 (70.8)	122 (40.8)	**<0.001**
Asian	94 (18.8)	44 (17.6)	50 (20.0)	
Black-African	47 (9.4)	16 (6.4)	31 (12.4)	
Black-Caribbean	41 8.2)	8 (3.2)	33 (13.2)	
Other	19 (3.8)	5 (2.0)	14 (5.6)	
Physical activity				
Daily	62 (12.4)	39 (15.6)	23 (9.2)	**<0.001**
> twice a week	178 (35.6)	118 (47.2)	60 (24.0)	
Once a week	63 (12.6)	30 (12.0)	33 (13.2)	
< once a week	27 (5.4)	4 (1.6)	23 (9.2)	
Never/rarely	170 (34.0)	59 (23.6)	111 (44.4)	
Family History of CVD	156 (31.2)	67 (26.8)	89 (35.6)	**0.034**
SBP≥140 mmHg	54 (10.8)	19 (7.6)	35 (14.0)	**0.021**
DBP≥90 mmHg	57 (11.4)	28 (11.2)	29 (11.6)	0.888
SBP≥130 mmHg	138 (27.6)	65 (47.1)	73 (52.9)	0.424
DBP≥80 mmHg	195 (39.0)	86 (34.4)	109 (43.6)	**0.035**
Hypertension definition (European guidelines)	78 (15.6)	35 (14.0)	43 (17.2)	0.324
Hypertension definition	228 (45.6)	104 (41.6)	124 (49.6)	0.073

(American guidelines)				
Localized gingivitis (BOP:10-29%)	176 (35.2)	143 (57.2)	33 (13.2)	**<0.001**
Generalized gingivitis (BOP≥30)	252 (50.4)	40 (16.0)	212 (84.8)	
Continuous: Mean (SD) or Median [IQR]				
Age (years)*	35 [12]	34 [14]	35 [9]	0.345
BMI (kg/m^2)*	24.01 [5.11]	23.75 [4.92]	24.42 [5.18]	0.209
SBP (mmHg)	122.39 (14.05)	120.70 (12.59)	124.07 (14.33)	**0.007**
DBP (mmHg)	77.02 (10.9)	75.94 (10.76)	78.10 (11.10)	**0.027**
Glucose (mmol/L)	1.4 [2.09]	1.2 [1.1]	1.6 [3.30]	**<0.001**
Total cholesterol (mmol/L)	5.05 [1.30]	5.01 [1.30]	5.10 [1.21]	0.091
HDL (mmol/L)	1.56 (0.43)	1.64 (0.42)	1.49 (0.44)	**<0.001**
LDL (mmol/L)	2.96 (0.83)	2.83 (0.84)	3.09 (0.79)	**<0.001**
Triglycerides (mmol/L)	1.3 [1.49]	1.2 [1.10]	1.3 [2.10]	0.133
Hs-CRP (mg/L)	1.7 [2.39]	1.38 [2.47]	1.86 [2.37]	**0.008**
WBC (thous/µL)	5.93 [2.20]	5.58 [1.99]	6.36 (2.26)	**<0.001**
Probing pocket depth (PPD)	2.56 [1.95]	1.99 [0.43]	3.94 [1.24]	**<0.001**
Clinical attachment level (CAL)	2.74 [2.24]	2.02 [0.47]	4.26 [1.52]	**<0.001**
Full mouth gingival bleeding score (FMBS)	30.65 [26.74]	15.45 [15.27]	53.66 (33.37]	**<0.001**
Full mouth dental plaque score (FMPS)	50.28 [39.38]	49.15 [40.78]	51.11 [39.43]	0.166

BMI indicates body mass index; Hs-CRP, High-sensitive C-reactive protein; CVD, Cardiovascular Diseases; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IQR, Interquartile range; LDL, low-density lipoprotein; SBP, systolic blood pressure; SD, Standard deviation and WBC, White blood cells.*Cases and controls groups balanced according to age, gender and BMI.

Table 2. Linear regression models of SBP, DBP and hs-CRP, WBC according to various indices of Periodontitis or systemic inflammation.

Exposure: Periodontitis	**SBP**		**DBP**	
	Model 1 ß±	**Model 2 ß±**	**Model 1 ß± SE**	**Model 2 ß±**
Categorical variable				
Cases vs controls (perio vs non perio)	**3.36±1.25****	**3.46±1.25****	**2.16±0.98***	1.48±1.04
Continuous variables				
CAL (mean)	**1.51±0.42*****	**0.89±0.42***	**0.69±0.33***	0.14±0.35
N sites with CAL≥	**0.03±0.01****	0.02±0.01	0.02±0.01	0.0002±0.01
N sites with	**0.04±0.01****	0.02±0.01	0.02±0.01	0.004±0.01
N sites with CAL≥	**0.04±0.01****	0.02±0.01	0.02±0.01	0.006±0.01
N sites with CAL≥	**0.06±0.02****	0.03±0.02	0.03±0.02	0.004±0.01
% of sites with	**0.06±0.02****	0.03±0.02	0.02±0.02	-0.006±0.01
% of sites with	**0.06±0.02****	0.03±0.02	0.03±0.02	0.003±0.02
% of sites with	**0.07±0.02****	0.04±0.02	0.03±0.02	0.01±0.02
% of sites with	**0.10±0.03****	0.05±0.03	0.04±0.03	0.01±0.02
PPD (mean)	**1.86±0.52*****	**1.51±0.5****	**0.81±0.41***	0.32±0.44
N sites with PPD≥	**0.04±0.01****	**0.03±0.01***	0.02±0.01	0.008±0.01
N sites with PPD≥	**0.05±0.02****	**0.04±0.02***	0.02±0.01	0.01±0.01
N sites with PPD≥	**0.08±0.02****	**0.06±0.02****	0.03±0.02	0.01±0.02
% of sites with PPD≥	**0.07±0.02****	**0.05±0.02***	0.03±0.02	0.01±0.02
% of sites with PPD≥	**0.07±0.03****	**0.06±0.03***	0.03±0.02	0.01±0.02
% of sites with PPD≥	**0.12±0.04****	**0.10±0.04****	0.05±0.03	0.02±0.03
FMBS (mean)	**0.09±0.02*****	**0.07±0.02****	**0.04±0.02***	0.02±0.02
Exposure: Systemic inflammation	**SBP**		**DBP**	
	Model 1 ß±	**Model 2 ß±**	**Model 1 ß± SE**	**Model 2 ß±**
Hs-CRP	0.22±0.19	0.18±0.17	0.24±0.15	0.21±0.14
WBC	0.17±0.38	0.61±0.34	0.25±0.30	0.44±0.28
Exposure: Periodontitis	**Hs-CRP** (log hs-CRP)		**WBC** (log WBC)	
	Model 1 ß±	**Model 2 ß±**	**Model 1 ß± SE**	**Model 2 ß±**
Categorical variable				
Cases vs controls (Perio vs non perio)	**0.24±0.08****	0.01±0.09	**0.08±0.23*****	**0.08±0.02****
Continuous variables				
CAL (mean)	**0.083±0.029****	0.040±0.032	**0.022±0.008****	**0.023±0.008****
PPD (mean)	**0.112±0.036****	0.05±0.04	**0.031±0.01****	**0.029±0.01****
FMBS (mean)	**0.004±0.002***	0.002±0.002	**0.002±0.000*****	**0.001±0.000****

FMBS, Full-mouth gingival bleeding score; ß, betta coefficient; CAL, Clinical attachment level, Hs-CRP, High-sensitive C-reactive protein; DBP, Diastolic blood pressure; PPD, Probing pocket depth; SBP, Systolic blood pressure; SE, Standard error, and WBC, White blood cells. Model 1: Unadjusted. **Model 2**: Age, BMI, gender, ethnicity, smoking, physical activity, family history of CVD. ***p<0.001; **p<0.01; *p<0.05

Table 3. Multiple logistic regression models of SBP≥140 mm Hg, DBP≥90 mm Hg, SBP≥130 mm Hg, DBP≥80 mm Hg and hypertension definitions according to periodontitis diagnosis, hs-CRP and WBC.

Exposure: Periodontitis	**SBP≥140 mm Hg**	**DBP≥90 mm Hg**	**Hypertension definition (European)**
Model 1 OR (95% CI)	**1.98 (1.10-3.65)***	1.05 (0.59-1.81)	1.28 (0.78-2.08)
Model 2 OR (95% CI)	**2.31 (1.17-4.67)***	1.05 (0.55-1.98)	1.24 (0.71-2.18)
Exposure: Hs-CRP			
Model 1 OR (95% CI)	0.98 (0.89-1.08)	0.97 (0.88-1.08)	0.97 (0.88-1.05)
Model 2 OR (95% CI)	0.95 (0.84-1.06)	0.97 (0.86-1.09)	0.95 (0.85-1.05)
Exposure: WBC			
Model 1 OR (95% CI)	1.00 (0.84-1.18)	0.92 (0.78-1.10)	0.97 (0.84-1.13)
Model 2 OR (95% CI)	1.05 (0.87-1.27)	0.97 (0.80-1.17)	1.02 (0.87-1.20)
Exposure: Periodontitis	**SBP≥130 mm Hg**	**DBP≥80 mm Hg**	**Hypertension definition (American)**
Model 1 OR (95% CI)	1.17 (1.17-1.73)	**1.47 (1.03-2.12)***	1.38 (0.97-1.96)
Model 2 OR (95% CI)	1.26 (0.78-2.00)	1.20 (0.78-1.84)	1.23 (0.81-1.88)
Exposure: Hs-CRP			
Model 1 OR (95% CI)	1.02 (0.96-1.08)	1.06 (0.99-1.12)	1.06 (0.99-1.13)
Model 2 OR (95% CI)	1.02 (0.96-1.08)	1.06 (0.99-1.12)	1.06 (0.99-1.27)
Exposure: WBC			
Model 1 OR (95% CI)	1.00 (0.89-1.13)	1.04 (0.93-1.16)	1.03 (0.93-1.15)
Model 2 OR (95% CI)	1.06 (0.93-1.21)	1.08 (0.96-1.22)	1.09 (0.97-1.23)

Hs-CRP, High-sensitive C-reactive protein; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; and WBC, White blood cells. Model 1: Unadjusted. **Model 2**: Age, BMI, gender, ethnicity, smoking, physical activity, family history of CVD.
***p<0.001; **p<0.01; *p<0.05

Table 4. Multivariate fitted SBP according to bleeding status.

Bleeding status (irrespective of periodontitis)	N (%)	SBP Mean (SD)	Multiple comparisons ΔSBP (95% CI) *p*-value		
			Gingival Health	*Localised Bleeding*	*Generalised Bleeding*
Gingival Health (FMBS<10%)	72 (14.4)	118.71 (11.23)	-		
Localised Bleeding (10%>FMBS≤30%)	176 (35.2)	121.92 (14.68)	3.21 (6.61-0.20) *p*=0.065	-	
Generalised bleeding (FMBS≥30%)	252 (50.4)	123.77 (14.16)	5.06 (8.22-1.91) *p*=0.002)	1,85 (4.65-0.94) *p*=0,192	-

ΔSBP, Mean difference in systolic blood pressure; FMBS, Full-mouth gingival bleeding score.