The impact of non-surgical therapy of periodontal disease on surrogate markers for cardiovascular disease: A literature review

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ABSTRACT: Purpose: To review the literature on the effects of non-surgical periodontal treatment on surrogate markers of cardiovascular diseases (CVDs) and to clarify the impact of periodontal disease on systemic inflammation. **Methods:** PRISMA guidelines for systematic reviews and meta-analyses have been adopted. An electronic search in PubMed up to December 2018 was performed using the following search terms and keywords alone or in combination: non surgical periodontal therapy, atherosclerotic vascular disease (AVD), operative surgical procedures, CVD, IL-6, CRP, cholesterol, LDL, oxidized low density lipoprotein, HDL, endothelial dysfunction, dependent dilatation, carotid intima media thickness, periodontitis, tunica intima. **Results:** The electronic search resulted in the inclusion of 28 articles that were grouped and discussed based on the investigated surrogate markers. Meta-analysis was not carried out due to the heterogeneity of the results. The included studies demonstrated that periodontal treatments contribute to the resolution of oral inflammation and in turn might positively modulate the levels of systemic inflammatory markers. The initial phase of periodontal therapy has a positive impact on the short-term reduction of a series of systemic markers that are considered as surrogate markers of AVD. (*Am J Dent* 2019;32:191-200).

CLINICAL SIGNIFICANCE: The non-surgical therapy of periodontal disease would positively reduce the levels of systemic inflammation markers, decreasing the vascular risk and the possibility of developing CVD or the subclinical progression of the disease.

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Introduction

During the past two decades, a new field of periodontal research has emerged as "periodontal medicine". This term refers to the correlation between chronic periodontitis (CHP), aggressive periodontitis (AgP) and several non-inflammatory pathological conditions, such as cardiovascular diseases (CVDs), diabetes mellitus, preterm pregnancy, obesity and others.[1] The concept behind this statement is that the inflammation that characterizes periodontitis adversely affects the control of systemic diseases. Atherosclerosis or atherosclerosclerotic vascular disease (AVD) is one of the most common CVDs.[2] Only two thirds of the CVDs can be related to proven risk factors (age, sex, hypertension, hypercholesterolemia, diabetes, obesity, smoking, lifestyle). The scientific community is now focusing on the role that inflammation plays.[3,4] Low level chronic inflammation plays a significant role in AVD.[5,6]

Periodontal disease can be defined by means of traditional clinical (probing depth, bleeding on probing, attachment level) and radiographic parameters or markers of poor oral health status (i.e. tooth loss or edentulism). Moreover, some epidemiological studies describe periodontitis by using microbiological profiles or levels of serum antibodies to periodontal bacteria.[7] It should also be taken into account that in some studies, patients are affected by concomitant systemic conditions (i.e. hyperlipidemia, hypertension, poor physical activity) that could act as confounding factors.

CVD surrogate markers are established biomarkers of vascular risk and may contribute to the relative possibility of developing CVD or the subclinical progression of the disease.[4,5] Among other surrogate markers, Interleukin 6 (IL-6) and C-reactive protein (CRP) are strictly linked to inflammatory response, whereas homocysteine, endothelial progenitor cell (or EPC), HDL/LDL cholesterol, endothelial dysfunction and carotid intima-media thickness (CIMT) are circulating markers strongly associated with AVD and CVD in general.[8]

Several authors,[9-12] indicated that levels of white blood cell count and CRP are higher in patients affected by periodontitis than in healthy controls. Slade et al[13] reported an increased level of CRP in 1/3 of patients affected by periodontitis after examining 10,146 subjects with available CRP and cholesterol levels and 4,461 patients with available fibrinogen levels. Beck et al[14] were the first to link periodontitis with subclinical atherosclerosis, which in turn is commonly investigated by the means of CIMT assessment. The author analyzed a group of 6,017 patients, who participated in the ARIC study (Atherosclerosis risk in communities), and showed an augmented odds ratio for CIMT (OD 2.09) related to severe periodontitis. The relationship between cardiovascular measures (i.e. presence of carotid artery plaque and CIMT) and periodontal parameters, including tooth loss, was further assessed by the Oral Infection and Vascular Disease Epidemiology Study (INVEST). Two out of the three INVEST prospective studies[15-17] demonstrated a link between tooth loss[15] and severe bone loss[16] with an increased prevalence of atherosclerotic plaques in a randomly selected tri-ethnic population of 1,056 subjects ≥ 55 years with a negative anamnesis for stroke, MI and chronic inflammatory conditions. The third INVEST report[17] evidenced a tight association between CIMT and bacteria related to periodontal disease, stressing a possible role of periodontal bacteria in atherosclerosis. These data were further supported by recent studies.[18,19]

Finally, several systematic reviews and meta-analyses[20-23] showed an association between periodontal disease and clinical outcomes of cardiovascular disease, consistently concluding that the available evidence suggests a moderate positive association between periodontal disease and CVD. It should be

further investigated whether the relation between periodontal disease and CVD effects can be influenced by comorbidities, such as smoking,[24] or can be completely spurious.[25] A recent case-control study[26] reported an OR of 3.3 for non-fatal stroke among never smokers, with other studies[27,28] supporting these results.

The present study reviewed the literature on the effects of non-surgical periodontal treatment on surrogate markers of CVD and aims to assess the impact of periodontal disease on systemic inflammation.

Materials and Methods

PRISMA guidelines for systematic reviews and meta-analyses were adopted.[29] The current review clearly addresses a focused question by using the participant, intervention, comparison, and outcomes (PICO) criteria.[30] Studies, up to December 2018, evaluating the correlations of periodontal disease with the risk of atherosclerosis were identified by an electronic search on PubMed, Google Scholar, and Scopus, using the following search terms and keywords alone or in combination: non surgical periodontal therapy, AVD, operative surgical procedures, CVD, IL-6, cholesterol, LDL, oxidized low density lipoprotein, HDL, endothelial dysfunction, dependent dilatation, carotid intima media thickness, periodontitis, tunica intima. Moreover, reference lists of the selected studies were manually screened.

Pico criteria

Participants: Participants of any age, healthy or affected by CVD;

Intervention: The intervention evaluated was causal periodontal treatment and its impact on surrogate markers of CVD;

Comparison: No comparison among the analysis methods was made;

Outcome: Variation of plasma levels of surrogate markers, improvement of periodontal parameters, reduction in mm of carotid intima media thickness (CIMT), possible role of periodontal pathogens.

Selection of studies

Only controlled clinical trials were included. Other inclusion criteria were: studies on human beings and English language. Exclusion criteria were: studies on specific ethnic groups and studies assessing diabetic patients in order to avoid a confounding effect.

Data extraction

To ensure homogeneity of data collection and to rule out the effect of subjectivity in data gathering, data extraction was performed independently by two investigators (O.D., A.Q.), according to the following protocol:

1. Electronic search on the digital database (PubMed);
2. Identification of the studies that resulted from the research and reading of the abstract;
3. Studies' authors and year of publication;
4. Number of subjects and mean age (when specified);
5. Population characteristics;
6. Outcome and results;

Discussion and consensus were established to resolve disagreements as the initial approach. In cases where mutual agreement between the two reviewers could not be reached, a third Author (W.L.H.) assessed the study and a final decision was taken.

Quality assessment

Assessment of methodological study quality was performed using the criteria proposed by The Cochrane Handbook for Systematic Reviews of Interventions.[31] Within the random selection of the population, defined inclusion/exclusion criteria, report of loss to follow-up, validated measurements, and statistical analysis were reported, the study was classed with a low risk of bias. When one of these five criteria is missing, the study was classified as having a moderate risk of bias. A high risk of bias is classified if the study lacks two or more of these criteria.

Results

The electronic search, carried out according to the aforementioned strategy, resulted in the inclusion of 28 articles[32-59] (Table).

The included studies are discussed and grouped to the surrogate markers that were investigated in each specific manuscript.

Homocysteine

Bhardwaj et al[32] investigated the plasma levels of homocisteine after non surgical periodontal therapy in a controlled clinical trial. Forty patients were included in this clinical study: 20 affected by CHP (test group) and 20 healthy (control group). The homocisteine plasma levels were significantly higher in the test subjects at the baseline and decreased after non-surgical periodontal treatment (12 weeks post-treatment).

Endothelial progenitor cells

In 2014, Jonsson et al[33] performed a clinical trial to investigate the levels of circulating endothelial progenitor cells in patients affected by CHP. One hundred and twelve patients were enrolled: 56 were periodontally healthy and 56 were affected by CHP. All subjects underwent a full-mouth periodontal examination [measurements on the presence of dental plaque and bleeding on probing (BoP), probing depth (PD) and clinical attachment level (CAL) were collected] and provided a blood sample. Plasma levels of circulating endothelial progenitor cells were approximately 2.3-fold higher in test patients than periodontally healthy controls, after adjustments for age, sex, physical activity, systolic blood pressure, and BMI. Moreover, the levels of circulating endothelial progenitor cells were positively associated with the degree of BOP, PD, and CAL.

In 2009, Li et al[34] evaluated the association between CHP and circulating endothelial progenitor cells on 86 non-smoking subjects (36 males and 50 females, aged 35-80 years), including 23 subjects with no or mild CHP and 63 subjects with moderate to severe CHP. The subjects underwent a full-mouth, comprehensive periodontal examination (i.e. presence or absence of plaque, BoP, PD, gingival recession (GR), CAL). The levels

Table. Evidence table of studies included in the present systematic review.

Authors	Type of study	Surrogate marker	Subjects and treatments	Follow-up	Results
Bhardwaj et al[32] (2015)	Controlled clinical trial	Omocisteine .	40 subjects: 20 healthy; 20 with CHP. Non-surgical therapy (SRP) in the test group (CHP)	12 weeks	The omocisteine plasma levels decreased in the test group after non surgical periodontal treatment
Jonsson et al[33] (2014)	Controlled clinical trial	Endothelial progenitor cells	112 subjects: 56 periodontally healthy; 56 affected by CHP All subjects underwent a full-mouth periodontal examination and provided a blood sample.	None	Plasma levels of circulating endothelial progenitor cells were approximately 2.3-fold higher in test patients than controls. The levels positively correlate with the degree of periodontal parameters.
Li et al[34] (2009)	Controlled clinical trial	Endothelial progenitor cells, CRP, CIMT	86 subjects: 23 with no or mild CHP; 63 with moderate to severe CHP. Full- mouth, comprehensive periodontal examination.	None	Subjects with moderate to severe CHP (tests) exhibited an increased risk of high endothelial progenitor cells count, CPR and thick CIMT, compared with those with no or mild CP (controls).
Zhou et al[35] (2013)	Controlled clinical trial	IL-6, TNF-a, CRP	75 subjects with CHP and CHD: 40 (test) received SRP and oral hygiene instruction, 35(control) received oral hygiene instruction only. All subjects underwent a full-mouth periodontal examination.	3 months	All the analyzed markers decreased after non surgical periodontal therapy in test group
Eickholz et al[36] (2013)	Controlled clinical trial	IL-6, CRP, IL-8, LBV, leukocyte	60 subjects: 31 affected by CHP; 29 affected by AgP. Both groups underwent non surgical periodontal therapy (SRP).	12 weeks	After SRP, markers levels were reduced in AgP but not in CHP subjects
Mariottti et al[37] (2013)	Controlled clinical trial	IL, CRP	64 subjects: 26 CVD and CHP (test); 38 CVD, no CHP (control). All patients underwent periodontal, physical and serum evaluation. CVD and CHP subjects subdivided in treated and untreated group (until 6-week re-evaluation)	6 weeks	Treatment of CHP with Full Mouth Disinfection approach resulted in short term reduction of IL-6 and CRP
Vidal et al[38] (2009)	Controlled clinical trial	IL-6, CRP and fibrinogen	26 patients with CHP and refractory hypertension. At baseline all subjects underwent clinical examination and after 3 months received non-surgical periodontal treatment.	3 & 6 months	CRP, IL-6 and fibrinogen were significantly reduced 6 months after periodontal treatment, as well as other cardiovascular risk markers such as left ventricular mass (LVM), arterial stiffness, systolic and diastolic blood pressure.
Marcaccini et al[39] (2009)	Controlled clinical trial	IL-6, CRP	45 subjects with CHP: 20 (test group) underwent non-surgical periodontal treatment; 25 (control group) were not treated for CHP.	3 months	Decrease in circulating IL-6 and CRP concentrations in the test group after treatment.
D'Aiuto et al[40] (2004)	Pilot intervention study	IL-6, CRP	94 healthy subjects with severe generalized periodontitis. All patients received standard non-surgical periodontal therapy.	2 & 6 months	The periodontal therapy resulted in a significant improvement of all clinical periodontal parameters and a significant reduction of the serum markers, decreasing the CRP-associated CVD risk.
D'Aiuto et al[41] (2004)	Pilot intervention study	IL-6, CRP	94 healthy subjects with severe generalized periodontitis. All patients received standard non-surgical periodontal therapy and evaluation of serological and clinical periodontal parameters.	2 & 6 months	Non-surgical periodontal therapy, significantly decreased serum mediators and markers of acute phase response.
D'Aiuto et al[42] (2004)	Pilot intervention study	IL-6, CRP	94 systemically healthy subjects with severe generalized periodontitis. All patients received standard non-surgical periodontal therapy and evaluation of serological and clinical periodontal parameters.	2 & 6 months	After periodontal therapy, significant reductions in serum IL-6 and CRP and improvements in clinical parameters parameters were observed.
D'Aiuto & Tonetti[43] (2005)	Cohort study	CRP, HDL/LDL cholesterol	55 healthy subjects in need of periodontal therapy. All subjects underwent periodontal therapy.	1 day, 1 week, 1 month	Decrease of the systemic markers following the initial phase of periodontal treatment.

Table (continued).

Authors	Type of study	Surrogate marker	Subjects and treatments	Follow-up	Results
Tawfig[44] (2015)	Controlled clinical trial	CRP, HDL/LDL cholesterol, triglycerides	30 hyperlipidemic subjects with CHP: 15 (test) received SRP and oral hygiene instructions; 15 (control) received only oral hygiene instructions. All patients were evaluated for serum markers and periodontal parameters.	3 months	Non-surgical periodontal therapy improved periodontal health and decreased LDL and CRP levels in hyperlipidemic patients with chronic periodontitis.
Duzagac et al[45] (2016)	Controlled clinical trial	CRP, HDL/LDL cholesterol in serum. IL-6, IL-10, TNF-a and Adiponectin in GCF/serum.	45 subjects: 15 with periodontitis and obesity; 15 with periodontitis and without obesity; 15 healthy controls. All subjects underwent a full periodontal examination; non-surgical periodontal therapy (SRP) was performed in periodontal patients (n=30).	3 months	Periodontal patients (with or without obesity) positively responded to periodontal treatment. However, the systemic condition of obesity seemed to be a major factor suppressing the systemic response to therapy.
Bozoglan et al[46] (2017)	Controlled clinical trial	LDL, HDL, PLT, WBC, fibrinogen, creatinine and hs-CRP	40 patients: 20 with atherosclerosis and CHP (test group); 20 systemically healthy and CHP (control group); All patients had nonsurgical periodontal treatment. The microbiological counts and WBC LDL, HDL, PLT, fibrinogen, creatinine and hs-CRP levels were determined at baseline and 6 months.	Baseline & 6 months	Following periodontal treatment, WBC, LDL, PLT, fibrinogen, creatinine and hs-CRP levels significantly decreased and HDL levels significantly increased in both test and control groups.
De Souza et al[47] (2016)	Controlled clinical trial	CRP	22 patients with CHP (test group) and 22 periodontally healthy individuals (control group). In the test group, oral hygiene instruction and scaling and root planing were carried out.	2 months	After periodontal treatment in the test group, the CRP level decreased significantly in those patients with higher baseline levels of CRP.
Subha et al[48] (2017)	Prospective study	CRP, HDL, LDL, cholesterol	45 subjects with generalized severe periodontitis: Group A (n=15): 0.25% lemongrass oil mouthwash; Group B (n=15): 0.12% chlorhexidine mouthwash; Group C (n=15): Oral prophylaxis only.	3 months	Significant reduction in CRP, PD, CAL, Total cholesterol and LDL was found in Group A
Mercanoglu et al[49] (2014)	Controlled clinical trial	Endothelial dysfunction	54 subjects: 28 with CHP and without any atherosclerotic vascular disease; 26 healthy controls. Brachial artery responses to reactive hyperemia and sublingual nitroglycerin were assessed.	6 weeks	Endothelial functions were impaired in patients with CHP and improved following initial periodontal therapy.
Toregeani et al[50] (2016)	Controlled clinical trial	CIMT	44 subjects: 23 healthy (Group 1); 21 with moderate to severe periodontitis (Group 2). Clinical periodontal parameters and CMIT were recorded. Group 2 also underwent SRP.	6 & 12 months	Periodontal treatments (both SRP and oral hygiene instructions) were effective in improving clinical periodontal parameters promoting reduction in CIMT in the initial phase of therapy.
Tapashetti et al[51] (2014)	Controlled clinical trial	CIMT, CRP	30 healthy subjects: 15 with CHP (test); 15 no CHP (control). All patients were subjected to measurement of CRP levels, CIMT and detailed periodontal evaluation.	None	The CRP levels and CMIT values were significantly higher in CHP group. CIMT had also a large and significant correlation with CRP and with all periodontal indices.
Yu et al[52] (2014)	Cross- sectional study	CIMT, HDL/LDL cholesterol, triglycerides, glucose	847 subjects were evaluated for routine biochemical tests (HDL/LDL cholesterol, glucose, triglycerides), periodontal parameters (PI, PD, CAL, BOP), and maximal CIMT.	None	In hyperglycemic participants, a linear and dose-dependent association between mean CAL and maximal CIMT was recorded. Poor oral hygiene was correlated with maximal CIMT and atherosclerotic plaque in all participants.
Wożakowska-Kapłon et al[53] (2013)	Controlled clinical trial	CIMT, fibrinogen, IL6, TNF-a	179 subjects: 112 with myocardial infarction (MI) (test group); 67 with stable angina (control group). Dental, cardiovascular and biochemical examination were done.	2 days	Test group showed higher level of cardiovascular disease risk factors and poor oral health status in comparison to the control one.
Pinho et al[54] (2012)	Controlled clinical trial	CIMT	50 subjects: 35 (test) with CIMT ≥ 1 mm or presence of any carotid atherosclerotic plaque; 15 (control) with CIMT < 1 mm and absence of atherosclerotic plaques. Full-mouth periodontal evaluation was performed and characterized according to the CAL.	None	This study showed an association between periodontitis severity and carotid atherosclerosis, suggesting that periodontal disease might be a risk indicator for atherosclerotic disease.

Table (continued).

Authors	Type of study	Surrogate marker	Subjects and treatments	Follow-up	Results
Lopez-Jornet et al[55] (2012)	Controlled clinical trial	CIMT, and serum levels of glucose, triglycerides, HDL/ LDL cholesterol, CRP, and HbA1c	60 healthy subjects: 30 with CHP; 30 no CHP. All patients underwent periodontal examination, CMIT measurements and hemostatic parameters assesement.	None	Presence of atheroma plaques was statistically higher in test than control group; the severity of periodontitis influenced the presence of carotid atheroma plaques.
Yakob et al[56] (2011)	Cross- sectional study	CIMT, cIMA	128 subjects: 88 with periodontitis (test);40 without periodontitis (control). Patients underwent periodontal examination, subgingival plaque analysis, common carotid artery evaluation.	18 years	Increased cIMA values were significantly correlated with periodontitis, hypertension, BMI, male gender and poor socioeconomic status, as well as *P. nigrescens* and *P. gingivalis* presence.
Cairo et al[57] (2008)	Controlled clinical trial	CIMT, CRP, glucose, triglycerides, HDL/LDL, cholesterol, HbA1C%	90 subjects: 45 with severe periodontitis (test); 45 healthy (control). Patients underwent periodontal examination, CMIT measurements and blood analysis.	None	Severe periodontitis was associated with sub-clinical atherosclerosis not only in the elderly population but also in young systemically healthy individuals, supporting the hypothesis that periodontal infection may be related to atheroma development.
Cairo et al[58] (2009)	Controlled clinical trial	CIMT, CRP, leucocytes, triglycerides, HDL/LDL cholesterol, glucose, HbA1c%	90 subjects: 45 with severe periodontitis (test); 45 healthy (control). Subclinical atherosclerosis was assessed measuring CMIT; blood samples were provided to assess inflammatory response.	None	Mean PD was a predictor of CMIT and FMBS of CRP levels, providing the risk of sub-clinical atherosclerosis and systemic inflammation in young adults with severe periodontitis.
Söder et al[59] (2009)	Cross- sectional study	CIMT, total cholesterol	111 subjects: 80 CHP (test); 31 no CHP (control). All patients underwent periodontal, physical and serum evaluation.	16 years	Periodontal disease was identified as the principal independent predictor factor for both atherosclerosis and increasing in MMP-9, TIMP-1 and MMP-9/TIMP-1.

Legend
AgP: Aggressive periodontitis
cIMA: Calculated intima-media area
CAL: Clinical attachment loss
CIMT: Carotid intima-media thickness
CHD: Coronary Heart Disease
CHP: Chronic periodontitis
CRP: C-reactive protein
CVD: Cardiovascular disease
FMBS: Full-mouth bleeding score
GCF: Gingival crevicular fluid
HbA1c: Glycated hemoglobin
hs-CRP: High-sensitivity C-reactive protein
IL-: Interleukin-
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
LBV: Lipopolysaccharide-binding protein
PD: Probing depth
PLT: Platelet count
SRP: Scaling and root planning
TNF-α: Tumor necrosis factor alpha
WBC: White blood cells

of circulating endothelial progenitor cells were quantitatively determined, as well as CRP and CIMT. The study showed that moderate to severe CHP is associated with an increased level of circulating endothelial progenitor cells and thicker CIMT than controls. CRP was significantly associated with high levels of circulating endothelial progenitor cells.

Interleukin-6 (IL-6)

Nine studies[35-42] that assessed IL-6 as a systemic marker were included in the present review.

Zhou et al[35] analyzed the effects of non surgical periodontal treatment on the serum levels of TNF-α, IL-6 and CRP. In this trial, 75 patients affected by CHP and CHD were treated. Forty subjects (test) received periodontal non-surgical treatment (scaling and root planing-SRP) and oral hygiene instruction, whereas 35 subjects (control) received oral hygiene instruction only. Clinical periodontal parameters were recorded at baseline and after 3 months; serum levels of TNF-α, IL-6 and CRP were assessed at the same time points. At baseline, there were no statistical differences between the two groups, whereas after non-surgical periodontal therapy a decrease in all the analyzed markers was observed in the test group.

Eickholz et al[36] focused on serum elastase level (CRP, elastase, IL-6, IL-8, lipopolysaccharide-binding protein (LBV) concentrations and leukocyte) after periodontal causal therapy on 31 subjects affected by CHP and 29 subjects affected by aggressive periodontitis (AgP). At baseline serum elastase, CRP and LBV were significantly higher in AgP than CHP patients, and these markers were also significantly elevated 1 day after non-surgical periodontal therapy in both groups. Twelve weeks after treatment, serum elastase levels were reduced in AgP but not in CHP subjects. Mariotti et al[37] carried out a controlled clinical trial on a total of 64 patients to assess the short-term effect of full mouth disinfection protocol on plasma level of systemic inflammation including IL-6 and CRP. Twenty-six patients were affected by both cardiovascular disease and chronic periodontitis (MCV-Perio test group), whereas 38 patients were only affected by cardiovascular disease without periodontitis, (MCV control group). The MCV-Perio group was sub-divided into two sub-groups: Treated MCV-Perio group, which was treated with a Full Mouth Disinfection protocol, and Non-Treated MCV-Perio group, composed of non-treated patients (until the 6-week re-evaluation). Blood samples were collected at baseline and 6

weeks to assess the changes of systemic inflammation markers. Non-surgical periodontal treatment through a Full Mouth Disinfection protocol resulted in a significant improvement in the levels of the IL-6 and CRP.

Vidal et al,[38] in 2009, examined the plasma levels of IL-6, CRP and fibrinogen in 26 patients with CHP and refractory hypertension. Plasma levels of systemic inflammation were assessed at three time points (baseline, 3 and 6 months after baseline); moreover, at the second and third stage (3 and 6 months) all patients received non-surgical periodontal treatment. The median values of CRP, IL-6 and fibrinogen were significantly reduced 6 months after periodontal treatment, as well as other cardiovascular risk markers, such as left ventricular mass (LVM), arterial stiffness, systolic and diastolic blood pressure. The plasma levels of the same markers (IL-6 and CRP) were previously investigated.[39] Forty-five patients with CHP were divided into two groups. The test group (20 patients) underwent non-surgical periodontal treatment, while the control group (25 patients) was not treated for CHP. Periodontal treatment was highly effective and a decrease in circulating IL-6 and CRP concentrations was observed 3 months after therapy in the test group.

D'Aiuto et al[40] analyzed the impact of periodontal therapy on serum inflammatory responses in associated CVD risk. Ninety-four systemically healthy subjects suffering from severe generalized periodontitis received standard non-surgical periodontal therapy. Periodontal parameters, IL-6 and CRP serum levels were monitored 2 and 6 months after the therapy. At the baseline, serum concentrations of IL-6 and CRP were significantly correlated with the extent and severity of periodontitis and associated CVD risk. After 6 months, the periodontal therapy resulted in a significant improvement of all clinical periodontal parameters and a significant reduction of the serum markers. Periodontitis might contribute to the inflammatory burden of the individual, resulting in increased levels of cardiovascular risk based on their serum CRP concentrations.

Another study,[41] conducted by the same authors, assessed the effect of the treatment of severe generalized periodontitis in a population of otherwise healthy individuals, on serological markers of systemic inflammation implicated in cardiovascular atherosclerotic diseases. Ninety-four subjects with severe generalized periodontitis were enrolled in the trial and serological (CRP, IL-6) and clinical periodontal parameters were evaluated at baseline, 2 and 6 months after completion of the non-surgical periodontal therapy. Improvements in all clinical periodontal parameters and significant reductions in serum IL-6 and CRP concentrations were achieved after a 6-month follow-up. Non-surgical periodontal therapy significantly decreased the serum mediators and the markers of acute phase response.

Moreover, D'Aiuto et al[42] assessed 94 systemically healthy subjects with severe generalized periodontitis in a prospective 6-month blind intervention trial. Periodontal parameters and inflammatory markers, CRP and IL-6, were evaluated prior to and 2 and 6 months after delivery of standard non-surgical periodontal therapy and oral hygiene instructions. No significant differences in concentrations were found among different groups according to age, gender, and smoking status. Differences were statistically significant between baseline and 2 and 6 months for IL-6 and between baseline and 6 months for CRP. Six months after treatment, significant reductions in serum IL-6 and CRP and improvements in clinical parameters were observed.

CRP and HDL/LDL cholesterol

D'Aiuto & Tonetti[43] analyzed the CRP and LDL/HDL cholesterol level in the blood samples of subjects with chronic periodontitis. Fifty-five healthy subjects in need of periodontal therapy were enrolled in the study. All subjects underwent periodontal treatment, and were examined after 1 day, 1 week and 1 month to provide a blood sample. An increase of CRP and total cholesterol was observed after 1 day of treatment and was sustained for 1 week; both serum concentrations decreased at 1 month.

Tawfig[44] evaluated the effect of non-surgical periodontal therapy on plasma lipid levels in hyperlipidemic patients with CHP. Thirty hyperlipidemic patients with CHP (age 30-70 years) were included in this study. The test group received non-surgical periodontal therapy (SRP) and oral hygiene instructions. The control group received only oral hygiene instructions. Lipid profile (HDL/LDL and triglycerides), CRP, and periodontal parameters (plaque index (PI), gingival index (GI), PD, CAL) were measured at baseline and after 3 months. Lipid profile and periodontal parameters were comparable between groups at baseline. The test group showed significant decrease in LDL and CRP levels, and a significant decrease in PD, ATL, PI, and GI scores, compared to the baseline; the control group showed significant decrease only in the PI and GI scores. Non-surgical periodontal therapy improved periodontal health and might be considered as an additional control of hyperlipidemia.

Douzagac et al[45] investigated, in a controlled clinical trial, the healing of 30 subjects suffering from periodontitis (15 with and 15 without obesity) after non surgical periodontal therapy. Periodontal patients received non-surgical periodontal therapy (SRP) and the healing process was compared to the control group. Clinical periodontal measurements of all patients were recorded at baseline and after 3 months; moreover, levels of IL-6, IL-10, TNF-a and adiponectin in the gingival crevicular fluid (GCF) and CRP, HDL, LDL in serum were analyzed. Periodontal parameters improved significantly in both periodontitis groups, although the improvement of the same parameters in healthy patients was higher than in the obese ones. HDL significantly increased in both periodontal groups; indeed, HDL levels in the obese group were elevated to levels similar to those of healthy controls. CRP decreased significantly solely in the normal weight group; IL-6, IL-10 and TNF-a levels in GCF improved significantly based on SRP therapy in both periodontal groups. Only serum level of TNF-a decreased significantly in the obese group, while adiponectin, IL-10 and TNF-a improved significantly in the group of periodontal subjects without obesity.

Bozoglan et al[46] determined the relationship between atherosclerosis and periodontopathogenic microorganisms in chronic periodontitis patients following periodontal treatment. Forty subjects were divided into two groups: a test group including patients with atherosclerosis and CHP, and control one with systemically healthy patients with CHP. All patients

underwent non-surgical periodontal treatment and assessment of microbiological counts as well as white blood cells (WBC), LDL, HDL, platelet count (PLT), fibrinogen, creatinine and hs-CRP levels, at baseline and after 6 months. Following periodontal treatment, WBC, LDL, PLT, fibrinogen, creatinine and hs-CRP levels significantly decreased and HDL levels significantly increased in both groups. The association between blood markers levels and the amount of periodontopathogenic microorganisms seemed to support the thesis that periodontal treatment could decrease the risk of atherosclerosis.

De Souza et al[47] evaluated serum CRP levels in 22 chronic periodontitis patients and 22 periodontally healthy individuals and assessed the effect of non-surgical periodontal treatment on them. Sixty days after oral hygiene instruction and scaling and root planing in the test group, CRP level decreased significantly in those patients with higher baseline levels of CRP. Accordingly, Subha et al[48] observed that chronic periodontitis seemed to promote elevated levels of CRP, that could be significantly decreased by non-surgical periodontal treatment and underlined how periodontal disease may be a potential risk factor of CVD.

Endothelial dysfunction

Mercanoglu et al[49] investigated whether there was an endothelial dysfunction in patients with chronic periodontitis and whether recovery was possible with therapy. Twenty-eight patients with CHP and without any atherosclerotic vascular disease (test), and 26 healthy controls were included in the study. Brachial artery responses to reactive hyperemia [endothelium-dependent dilatation (EDD)] and sublingual nitroglycerin [endothelium-independent dilatation (EID)] were assessed using high-resolution vascular ultrasound in both groups. Measurements were taken before and after the initial periodontal therapy in the test group. No significant difference between the groups in terms of cardiovascular risk factors was observed. At baseline, EDD and EID were significantly impaired in the CHP group; however, 6 weeks after periodontal therapy, EDD and EID improved significantly in the tests. This study showed that endothelial functions were impaired in patients with CHP before any evidence of atherosclerosis, and that they improved following the initial periodontal therapy.

Carotid intima media thickness (CIMT)

A recent study by Toregeani et al[50] evaluated the treatment of periodontal disease and its effects on CIMT and expression of laboratory markers related to atherosclerosis. Twenty-three healthy patients (Group 1) and 21 patients with moderate to severe periodontitis (Group 2) were evaluated for clinical periodontal parameters. CMIT of both groups was measured at baseline, and after 6 and 12 months, respectively. All patients received oral hygiene instruction; moreover, Group 2 underwent supragingival and subgingival SRP. A significant improvement in clinical periodontal parameters was observed in both groups, more evident between 6 and 12 months and greater in Group 2. A statistically significant decrease in CIMT in the first 6 months was also detected for all patients.

Another study[51] analyzed the correlation between CIMT and CRP in CHP in 30 systemically healthy subjects aged over 40 years (of which 15 subjects with CHP as tests and 15 subjects with no periodontitis as controls). In all patients, CRP levels and CIMT in addition to clinical periodontal parameters were recorded. The CRP levels and CMIT values were significantly higher in tests than controls. CIMT has also a large and significant correlation with CRP and with all periodontal indices. CRP might be considered as a possible underlying pathway in the association between periodontal disease and CIMT, and as a risk predictor for atherosclerosis in patients with CHP.

Yu et al[52] conducted a cross-sectional study of 847 participants (age 70.64 ± 9.03 years) with ≥ 10 teeth. A questionnaire survey, routine biochemical tests (HDL/LDL cholesterol, glucose, triglycerides), periodontal examination (PI, PD, CAL, BOP), and maximal CIMT measurement were performed for each subject. The authors aimed to verify the association between atherosclerosis and periodontal status. Of the total sample, 245 patients showing maximal CIMT ≥ 1.2 mm presented all the periodontal indexes, except for the bleeding index, significantly higher than the 602 patients showing maximal CIMT < 1.2 mm. In hyperglycemic participants, a statistically significant association was observed between mean CAL and CIMT (each 1 mm increase in mean CAL corresponded to a 0.018 mm increase in maximal CIMT), with a risk of atherosclerotic plaque that increased by 18.3% with each 1 mm increase in mean CAL. Moreover, even poor oral hygiene was correlated with maximal CIMT and atherosclerotic plaque in all participants, regardless of blood glycemic values.

Wożakowska-Kapłon et al[53] evaluated the state of the oral cavity and tested the association between selective parameters of acute, hospital phase MI in patients aged 60 and younger. One hundred and twelve patients with MI aged 60 were involved. The control group consisted of a matched group of patients with stable angina (n= 67). Patients enrolled in the study underwent dental, cardiovascular and biochemical examination. Left ventricular ejection fraction (LVEF) was measured during echocardiographic examination; IMT was assessed by ultrasonographic examination at the same time. The test group showed a higher prevalence of edentulism and advanced periodontal disease, and was characterized by the highest levels of fibrinogen, IL6, TNF-a, increased IMT and numerous atherosclerotic plaque. Moreover, the study revealed a significant association between biomarkers of myocardial injury, LVEF, periodontal parameter and edentulism. Patients of the test group showed a higher level of cardiovascular disease risk factors and poor oral health status in comparison to the control group.

Pinho et al[54] investigated the relationship between the degree of carotid atherosclerosis and severity of periodontitis. Fifty adults underwent assessment of CIMT, were divided into two groups: test (n=35) - CIMT ≥ 1 mm or presence of any atherosclerotic plaque; control (n= 15) - CIMT < 1 mm and absence of atherosclerotic plaques. Periodontitis, classified according to CAL values, as slight (CAL= 1-2 mm), moderate (CAL= 3-4 mm) and severe (CAL ≥ 5 mm), was detected in 22.2%, 44.4% and 33.3% of tests, respectively; whereas, control patients mainly presented slight periodontitis (53.3%), rather than moderate (33.3%) or severe (13.3%). The study reported that severe periodontitis was observed in 70.6% of

patients presenting an atheroma plaque (test group) and that severe forms of periodontal disease were principally associated to atherosclerosis.

Lopez-Jornet et al[55] studied 60 systemically healthy patients > 45 years of age (30 with CHP and 30 periodontally healthy) to explore the effect of periodontitis in relation to markers of carotid atherosclerosis. CIMT as well as periodontal clinical parameters (BOP, PD, CAL, gingival recession) were assessed in all patients. Moreover, glucose, triglycerides, HDL/LDL cholesterol, CRP, and glycated hemoglobin (HbA1c) were quantified after blood tests. A significantly higher total cholesterol and HbA1c levels were found in the CHP group than the controls. Although by comparing CHP and control group the statistical analysis showed no significant differences in CMIT, the presence of atheroma plaques were significantly higher in the CHP group than in the control. The authors concluded that the severity of periodontitis, as well as the patient age, might influence the occurrence of carotid atheroma plaques.

A cross sectional study[56] analyzed subgingival microorganisms and early carotid lesions in subjects with and without periodontitis. Eighty-eight subjects with periodontitis and 40 subjects without periodontitis were periodontally evacuated (the number of remaining teeth, PI, GI, PD, CAL). The presence of the periodontal microorganisms, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Tannerella forsythia*, was analyzed from subgingival plaque using PCR amplification. The calculated intima-media area (cIMA) of common carotid artery was measured by means of ultrasound. All clinical periodontal parameters showed significant differences between tests and controls. The more frequent microorganisms were *P. gingivalis*, *P. intermedia* and *T. forsythia*; moreover, a significant difference in the presence and types of periodontal microorganisms between test and control group was found. Increased cIMA values were significantly correlated with periodontitis, hypertension, BMI, male gender and poor socioeconomic status, as well as *P. nigrescens* and *P. gingivalis* presence.

Cairo et al,[57,58] in two different studies on the same sample, evaluated the effects of periodontal status on sub-clinical atherosclerosis. Ninety systemically healthy young subjects, 45 affected by severe periodontitis (test group) and 45 without a history of periodontal disease (control group) were enrolled. CIMT, traditional cardiovascular risk factors[57] as well as inflammatory markers (i.e. leucocytes, triglycerides, HDL cholesterol, LDL cholesterol, total serum cholesterol, high sensitivity CRP, glucose and HbA1c%)[58] were evaluated in both groups. In the first trial,[57] the authors reported that CIMT was significantly greater in the test group and that periodontitis as well as regular physical activity were predicting variables of overall mean CIMT. The subsequent cross-sectional study,[58] demonstrated that mean PD was a predictive factor for CMIT, and full-mouth bleeding score (FMBS) of CRP levels, supplying the risk of sub-clinical atherosclerosis and systemic inflammation in subjects with severe periodontal disease. In a cross-sectional study, Söder et al[59] analyzed the relationship between periodontitis and expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in blood samples as systemic inflammatory markers. Subjects presenting CHP (n=80) were allocated to the test group, while subjects with no periodontal disease (n=31) to the control group. After 16 years, the test group showed early signs of carotid atherosclerosis and significantly higher values of CIMT than the controls. Moreover, the plasma of test patients with atherosclerosis presented greater levels of MMP-9 and TIMP-1. Periodontal disease might be identified as a predictable risk factor for both atherosclerosis and the increased blood levels of inflammatory systemic markers.

Discussion

Periodontal disease is mostly responsible for tooth loss in adults. The emerging interest for the correlation between periodontal disease and systemic conditions has led to a new way to interpret this pathology, both from the diagnostic and therapeutic point of view. The systemic field of periodontal research has been explored during the last decade and, within the limits of these studies, there is clear evidence on the involvement of periodontal disease in the onset of CVD. Although there is still much to clarify about the impact of periodontal disease on systemic inflammation and the effect of non-surgical periodontal therapy on surrogate markers of systemic conditions, it can cautiously be stated that the evaluation of specific systemic markers in periodontal patients could be considered as a useful tool to assess the risk of sub-clinical atherosclerosis. Consequently, in the near future, it would be indicated to focus on the systemic phase of periodontal therapy which should include regular counseling with health practitioners and management of behavioral aspects. Successful management of such patients could be therefore based on the understanding of the periodontal-systemic correlation, the improvement of our knowledge about the disease process and the motivational interviewing and counseling with the patient about the importance of adopting a correct lifestyle.

In the present review, we designed the search strategy in order to avoid confounding factors; for this reason, studies having assessed diabetic patients were not included. The present review clearly shows that periodontal treatment can positively modulate the levels of systemic markers that play an important role in the atherogenesis and contribute to the resolution of inflammatory oral foci thus decreasing the level of systemic inflammatory markers in the blood.

D'Aiuto et al[60] showed that intensive periodontal treatment produced an acute systemic inflammatory response of 1 week duration and might represent an alternative to classic endotoxin-challenge or drug-induced models to study acute inflammation in humans. Moreover, the same authors[61] showed that the levels of circulating IL-6 and CRP were reduced, although IL-6 had more significant reduction when patients were treated with local antibiotics, whereas this further improvement was not observed in CRP. The Periodontitis and Vascular Events (PAVE) study[62,63] is the only multicenter pilot study assessing the results of periodontal therapy on cardiac events during a 25-month follow-up period; the study randomized subjects with CHP and severe CVD to either community care or oral hygiene instruction and mechanical periodontal therapy treatment protocol and concluded that cardiovascular adverse occurrences happened with similar fre-

quency in both groups.

The plasma levels of the surrogate markers analyzed in this review have shown to decrease levels within 6 months following non-surgical periodontal therapy. IL-6 was the most extensively investigated marker and its plasma level variations were explored in association to TNF-α, CRP, serum elastase and fibrinogen. The study by Duzagac et al[45] showed how the surrogate marker alteration in obese patients was reflected in reduced clinical healing.

The influence of the lipidic profile on the potential atherogenetic role of CRP in periodontal patients was studied by Tawfig[44] and revealed that local non-surgical periodontal therapy resulted in improvement of periodontal health, with significant decrease in the LDL cholesterol and CRP levels in hyperlipidemic patients with chronic periodontitis. The sample included patients with a wide age range (35 to 70 years). In all subjects, blood level markers decreased after periodontal therapy, strengthening the hypothesis that the resolution of inflammation helps in maintaining a healthy lipidic profile and reduces the risk of atherogenesis.

As carotid intima-media thickness (CIMT) has proven to be a valuable predictor of myocardial infarction and ischemic stroke that is independent from traditional risk factors, it is considered to be a marker of subclinical atherosclerosis.[64] It is worth noting that this approach may be controversial, as a CIMT increase, especially in the initial stages, results mainly from the thickening of the carotid media and may occur with aging without concomitant formation of atherosclerotic plaques.[65] The role of age and shear stress caused by hemodynamic factors, such as blood pressure components and tachycardia, is also emphasized. Recent large meta-analyses gave rise to controversies regarding the impact of traditional cardiovascular (CV) risk factors on CIMT and the predictive value of CIMT.[66] According to our review, CIMT was reliably used as a predictor factor and the trials included revealed how pathological periodontal variables or an overt AgP may predict subclinical atherosclerosis. In particular, Cairo et al[58] paired the test and control group for age, gender, body mass index (BMI) and smoking habits giving a clear patient profile. In the context of this argument, the work by Yakob et al[56] about the influence of periodontal pathogens, traces an interesting research field.

In conclusion, within the limitations of the present review, it is possible to conclude that the initial phase of periodontal therapy has a positive impact on the short-term reduction of a series of systemic surrogate markers of CVD. This effect may be positive in controlling the levels of systemic inflammation. It is, however, in the opinion of the authors that the current scientific evidence is not conclusive with regard to the long-term effects of non-surgical periodontal therapy on the surrogate markers of AVD. Further studies with a longer follow-up are definitely needed to obtain additional information on this topic of wide and inter-disciplinary interest.

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References

1. Buhlin K, Gustafsson A, Pockley AG, Frostegård J, Klinge B. Risk factors for cardiovascular disease in patients with periodontitis. *Eur Heart J* 2003;24:2099-2107.
2. Kumar V, Abbas A, Aster J. *Robbins basic pathology*. 9th ed., Philadelphia: Saunders, 2013.
3. Hegde R, Awan KH. Effects of periodontal disease on systemic health. *Dis Mon* 2018; S0011-5029(18)30130-30135.
4. Zebrack JS, Anderson JL. The role of inflammation and infection in the pathogenesis and evolution of coronary artery disease. *Curr Cardiol Rep* 2002;4:278-288.
5. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999; 138:S419-S420.
6. van Leuven SI, Franssen R, Kastelein JJ, Levi M, Stroes ES, Tak PP. Systemic inflammation as a risk factor for atherothrombosis. *Rheumatology* 2008;47:3-7.
7. Papapanou PN, Lindhe J. Epidemiology of periodontal disease. In: Lindhe J, Lang NP. *Clinical periodontology and implant dentistry*. Hoboken: Wiley-Blackwell, 2015;125-156.
8. Wierzbicki AS. Surrogate markers, atherosclerosis and cardiovascular disease prevention. *Int J Clin Pract* 2008;62:981-987.
9. Kweider M, Lowe GD, Murray GD, Kinane DF, McGowan DA. Dental disease, fibrinogen and white cell count; links with myocardial infarction? *Scott Med J* 1993;38:73-74.
10. Loos BG, Craandijk J, Hoek FJ, Werterheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000; 7:1528-1534.
11. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res* 2000;79:49-57.
12. Wu T, Trevisan M, Genco RJ, Dorn JP, Falkner KL, Sempos CT. Periodontal disease and risk of cerebrovascular disease: The first national health and nutrition examination survey and its follow-up study. *Arch Intern Med* 2000;169:2749-2755.
13. Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the atherosclerosis risk in communities study. *Arch Intern Med* 2003;163:1172-1179.
14. Beck JD, Offenbacher S. The association between periodontal diseases and cardiovascular diseases: A state-of-the-science review. *Ann Periodontol* 2001;6:9-5.
15. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobson Jr, Papanou PN, Sacco RL. Relationship between periodontal disease, tooth loss, and carotid artery plaque: The Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Stroke* 2003;34:2120-2125.
16. Engebtretson Sp, Lamster IB, Elkind MS, Rundek T, Serman NJ, Demmer RT, Sacco RL, Papanou PN, Desvarieux M. Radiographic measures of chronic periodontitis and carotid artery plaque. *Stroke* 2005; 36:561-566.
17. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR Jr, Sacco RL, Papapanou PN. Periodontal microbiota and carotid intima-media thickness: The Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation* 2005;111:576-582.
18. Beck JD, Offenbacher S. Systemic effects of periodontitis: Epidemiology of periodontal disease and cardiovascular disease. *J Periodontol* 2005; 76(11S):2089-2100.
19. Desvarieux M, Demmer RT, Jacobs DR, Papapanou PN, Sacco RL, Rundek T. Changes in clinical and microbiological periodontal profiles relate to progression of carotid intima-media thickness: The Oral Infections and Vascular Disease Epidemiology Study. *J Am Heart Assoc* 2013; 2:e000254.
20. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease and stroke: A systematic review. *Ann Periodontol* 2003;8:38-53.
21. Janket SJ, Baird AE, Chuang SK, Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;95:559-569.
22. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: A systematic review and meta-analysis. *J Periodontol* 2007;78:2289-2302.
23. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: A systematic review and meta-analysis. *J Gen Intern Med* 2008;23:2079-2086.

24. Spiekerman CF, Hujoel PP, DeRouen TA. Bias induced by self-reported smoking on periodontitis-systemic disease associations. *J Dent Res* 2003;82:345-349.
25. Hujoel PP, Cunha-Cruz J, Kressin NR. Spurious associations in oral epidemiological research: The case of dental flossing and obesity. *J Clin Periodontol* 2006;33:520-523.
26. Sim SJ, Kim HD, Moon JY, Zavras AI, Zdanowicz J, Jang SJ, Jin BH, Bae KH, Paik DI, Douglass CW. Periodontitis and the risk for non-fatal stroke in Korean adults. *J Periodontol* 2008;79:1652-1658.
27. Okoro CA, Balluz LS, Eke PI, Ajani UA, Strine TW, Town M, Mensah GA, Mokdad AH. Tooth loss and heart disease: Findings from the Behavioral Risk Factor Surveillance System. *Am J Prev Med* 2005;29:50-56.
28. Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J. Systemic exposure to Porphyromonas gingivalis predicts incident stroke. *Atherosclerosis* 2007;193:222-228.
29. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Int J Surg* 2010;8:336-341.
30. Miller SA, Forrest JL. Enhancing your practice through evidence-based decision making: PICO, learning how to ask good questions. *J Evid Base Dent Pract* 2001;1:136-141.
31. Higgins JPT, Altman DG. Assessing risk of bias in included studies. In: Higgins JPT, Green S. *Cochrane handbook for systematic reviews of interventions.* Chichester, England: The Cochrane Collaboration and John Wiley & Sons Ltd, 2008;187-241.
32. Bhardwaj S, Prabhuji ML, Karthikeyan BV. Effect of non-surgical periodontal therapy on plasma homocysteine levels in Indian population with chronic periodontitis: A pilot study. *J Clin Periodontol* 2015;42:221-227.
33. Jönsson D, Spinell T, Vrettos A, Stoecklin-Wasmer C, Celenti R, Demmer RT, Kebschull M, Papapanou PN. Circulating endothelial progenitor cells in periodontitis. *J Periodontol* 2014;85:1739-1747.
34. Li X, Tse HF, Yiu KH, Jia N, Chen H, Li LS, Jin L. Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. *J Clin Periodontol* 2009;36:933-939.
35. Zhou SY, Duan XQ, Hu R, Ouyang XY. Effect of non-surgical periodontal therapy on serum levels of TNF-a, IL-6 and C-reactive protein in periodontitis subjects with stable coronary heart disease. *Chin J Dent Res* 2013;16:145-151.
36. Eickholz P, Siegelin Y, Scharf S, Schacher B, Oremek GM, Sauer-Eppel H, Schubert R, Wohlfeil M. Non-surgical periodontal therapy decreases serum elastase levels in aggressive but not in chronic periodontitis. *J Clin Periodontol* 2013;40:327-333.
37. Mariotti G, Quaranta A, Merli M, Paterno Holtzman L, Piemontese M. Chronic periodontitis and cardiovascular disease: A controlled clinical trial. *European J of lnflammation* 2013;11:459- 467.
38. Vidal F, Cordovil I, Figueredo CM, Fischer RG. Non-surgical periodontal treatment reduces cardiovascular risk in refractory hypertensive patients: A pilot study. *J Clin Periodontol* 2013;40:681-687.
39. Marcaccini AM, Meschiari CA, Sorgi CA, Saraiva MC, de Souza AM, Faccioli LH, Tanus-Santos JE, Novaes AB, Gerlach RF. Circulating interleukin-6 and high-sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. *J Periodontol* 2009;80:594-602.
40. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J Periodontal Res* 2004;39:236-241.
41. D'Aiuto F, Parkar M, Andreou G, Brett PM, Ready D, Tonetti MS. Periodontitis and atherogenesis: Causal association or simple coincidence? *J Clin Periodontol* 2004;31:402-411.
42. D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, Tonetti MS. Periodontitis and systemic inflammation: Control of the local infection is associated with a reduction in serum inflammatory markers. *J Dent Res* 2004;83:156-160.
43. D'Aiuto F, Tonetti MS. Contribution of periodontal therapy on individual cardiovascular risk assessment. *Arch Intern Med* 2005;165:1920-1921.
44. Tawfig A. Effects of non-surgical periodontal therapy on serum lipids and C-reactive protein among hyperlipidemic patients with chronic periodontitis. *J Int Soc Prev Community Dent* 2015;5:S49-S56.
45. Duzagac E, Cifcibasi E, Erdem MG, Karabey V, Kasali K, Badur S, Cintan S. Is obesity associated with healing after non-surgical periodontal therapy? A local vs. systemic evaluation. *J Periodontal Res* 2016;51:604-612.
46. Bozoglan A, Ertugrul AS, Taspınar M, Yuzbasioglu B. Determining the relationship between atherosclerosis and periodontopathogenic micro-organisms in chronic periodontitis patients. *Acta Odontol Scand* 2017; 75:233-242.
47. de Souza AB, Okawa RT, Silva CO, Araújo MG. Short-term changes on C-reactive protein (CRP) levels after non-surgical periodontal treatment in systemically healthy individuals. *Clin Oral Investig* 2017;21:477-484.
48. Subha DS, Pradeep T. Periodontal therapy with 0.25% lemongrass oil mouth-wash in reducing risk of cardiovascular diseases: A 3-arm prospective parallel experimental study. *Ethiop J Health Sci* 2017;27:531-540.
49. Mercanoglu F, Oflaz H, Oz O, Gökbuget AY, Genchellac H, Sezer M, Nişanci Y, Umman S. Endothelial dysfunction in patients with chronic periodontitis and its improvement after initial periodontaltherapy. *J Periodontol* 2004;75:1694-1700.
50. Toregeani JF, Nassar CA, Nassar PO, Toregeani KM, Gonzatto GK, Vendrame R, Castilhos JS, Rotta LS, Reinheimer AC, Longoni A, Barcella MW. Evaluation of periodontitis treatment effects on carotid intima-media thickness and expression of laboratory markers related to atherosclerosis. *Gen Dent* 2016;64:55-62.
51. Tapashetti RP, Guvva S, Patil SR, Sharma S, Pushpalatha HM. C-reactive protein as predict of increased carotid intima media thickness in patients with chronic periodontitis. *J Int Oral Health* 2014;6:47-52.
52. Yu H, Qi LT, Liu LS, Wang XY, Zhang Y, Huo Y, Luan QX. Association of carotid intima-media thickness and atherosclerotic plaque with periodontal status. *J Dent Res* 2014;93:744-751.
53. Wożakowska-Kapłon B, Włosowicz M, Gorczyca-Michta I, Górska R. Oral health status and the occurrence and clinical course of myocardial infarction in hospital phase: A case-control study. *Cardiol J* 2013; 20:370-377.
54. Pinho MM, Faria-Almeida R, Azevedo E, Manso MC, Martins L. Periodontitis and atherosclerosis: An observational study. *J Periodontal Res* 2013;48:452-457.
55. López-Jornet P, Berná-Mestre JD, Berná-Serna JD, Camacho-Alonso F, Fernandez-Millan S, Reus-Pintado M. Measurement of atherosclerosis markers in patients with periodontitis: A case-control study. *J Periodontol* 2012;83:690-698.
56. Yakob M, Söder B, Meurman JH, Jogestrand T, Nowak J, Söder PÖ. Prevotella nigrescens and Porphyromonas gingivalis are associated with signs of carotidatherosclerosis in subjects with and without periodontitis. *J Periodontal Res* 2011;46:749-755.
57. Cairo F, Castellani S, Gori AM, Nieri M, Baldelli G, Abbate R, Pini-Prato GP. Severe periodontitis in young adults is associated with sub-clinical atherosclerosis. *J Clin Periodontol* 2008;35:465-472.
58. Cairo F, Nieri M, Gori AM, Rotundo R, Castellani S, Abbate R, Pini-Prato GP. Periodontal variables may predict sub-clinical atherosclerosis and systemic inflammation in young adults. A cross-sectional study. *J Clin Periodontol* 2009;36:933-939.
59. Söder PO, Meurman JH, Jogestrand T, Nowak J, Söder B. Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in blood as markers for early atherosclerosis in subjects with chronic periodontitis. *J Periodontal Res* 2009;44:452-458.
60. D'Aiuto F, Nibali L, Mohamed-Ali V, Vallance P, Tonetti MS. Periodontal therapy: A novel non-drug-induced experimental model to study human inflammation. *J Periodontal Res* 2004;39:294-299.
61. D'Aiuto F, Graziani F, Tetè S, Gabriele M, Tonetti MS. Periodontitis: From local infection to systemic diseases. *Int J Immunopathol Pharmacol* 2005;18(S3):1-11.
62. Beck JD, Couper DJ, Falkner KL, Graham SP, Grossi SG, Gunsolley JC, Madden T, Maupome G, Offenbacher S, Stewart DD, Trevisan M, Van Dyke TE, Genco RJ. The Periodontitis and Vascular Events (PAVE) pilot study: Adverse events. *J Periodontol* 2008;79:90-96.
63. Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, Couper DJ, Stewart DD, Falkner KL, Graham SP, Grossi S, Gunsolley JC, Madden T, Maupome G, Trevisan M, Van Dyke TE, Genco RJ. Results from the Periodontitis and Vascular Events (PAVE) Study: A pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. *J Periodontol* 2009;80:190-201.
64. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam study. *Circulation* 1997;96:1432-1437.
65. Finn AV, Kolodgie FD, Virmani R. Correlation between carotid intimal/ medial thickness and atherosclerosis: A point of view from pathology. *Arterioscler Thromb Vasc Biol* 2010;30:177-181.
66. Fraser AG, Kyaw Y, Kozakova M, Palombo C. Ultrasonic imaging of the carotid arteries, from intima-media thickness to histological markers for plaque vulnerability: What do we know? *Dialog Cardiovasc Med* 2013;18:87-98.