Periodontitis as inflammatory burden to systemic diseases

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Cover:

- The cover background is the photo of evening at the island of Schiermonnikoog (by Hendri Susanto, 2009)

Cover design : Fajar Ibnu Tufeil

ISBN : 978-90-367-5715-7

rijksuniversiteit groningen

Periodontitis as inflammatory burden to systemic diseases

Proefschrift

ter verkrijging van het doctoraat in de
Medische Westenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken
in het openbaar te verdedigen op
17 oktober 2012
om 11.00 uur

door

Hendri Susanto

geboren op 02 september 1976

te Sumenep, Indonesië

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Chapter 1

General Introduction

Although the link between oral and overall health had already been reported thousands of years ago by the ancient Egyptians and Greek, for many years this link didn't get too much attention. The last decades, however, the number of studies on the association between periodontal disease and systemic disease is rapidly increasing. Many studies demonstrate an association between periodontal disease and several systemic conditions and diseases. Well known examples are associations between periodontal disease and diabetes mellitus, cardiovascular diseases, adverse pregnancy outcome, osteoporosis, respiratory disease and rheumatoid arthritis[1-3].

The nature of the association between periodontal disease and systemic diseases is considered bidirectional. Periodontal disease can be initiated or deteriorated by certain systemic diseases, but can also initiate or deteriorate certain systemic diseases[4]. Although there may not always be a cause and effect association[5], many reports have shown that an increased severity of systemic disease is associated with increased periodontal disease severity and vice versa[6, 7].

Increased prevalence and severity of periodontitis in some systemic diseases, e.g. in diabetes mellitus, may reflect increased vulnerability to infection through systemic dysregulation[8, 9, 10]. Additionally, the link between periodontitis and systemic disease may reflect epiphenomena or confounding by factors such as age, sex, genetics, socioeconomic status and smoking. [11]. Finally, the increased prevalence and severity of periodontitis in some systemic diseases may reflect periodontitis as a risk factor for these diseases. Periodontitis poses an inflammatory and infectious burden as evidenced by increased serum levels of c-reactive protein (CRP). As such, periodontitis may initiate or deteriorate systematic diseases by causing a proinflammatory and procoagulatory state.

A major drawback of the various clinical studies assessing the association between periodontal disease and systemic diseases is that a variety of methods has been used to define periodontitis and these studies have been conducted in different populations. Although most studies demonstrated an association between periodontitis and systemic diseases, the results also revealed that the existence of such associations are not unequivocal. As such, the method used for clinical measurement of periodontal disease might have

affected the observed associations[12-15]. Clinical measures of periodontitis, such as probing pocket depth (PPD), bleeding on probing (BOP), clinical attachment loss (CAL) are commonly used for defining periodontal diseases[16]. These tools reflect the extent of the periodontal disease process but do not reflect the amount of inflammatory periodontal tissue[17]. Recently a new clinical measure for periodontitis, the periodontal inflammatory surface area (PISA), reflecting the surface area of bleeding and inflamed pocket epithelium in square millimeters, has been introduced[18]. In addition to the different definitions of periodontal disease that were used in these studies, the difference in associations between periodontitis and systemic diseases may be due to the variety in the subject populations such as ethnicity, genetics, nutrition and cultural background. Therefore, the result of one study often can not be generalized to other populations[19].

Valuable contributions to the discussion on the nature of association between periodontal disease and systemic diseases stem from studies assessing the effect of periodontal treatment on systemic diseases. Treatment of periodontal disease may be beneficial not only for the health of periodontal tissue it self, but may also reduce the severity of certain systemic diseases. For example, periodontal treatment has been shown to improve glycaemic control (HbA1c) in diabetes mellitus[19] and to reduce disease activity score (DAS28) in rheumatoid arthritis (RA) patients (0.5-1.0 point).[20] Periodontal treatment has also been shown to reduce systemic inflammation markers such as c-reactive protein[21]. Therefore, decreased severity of systemic disease after periodontitis treatment may be mediated by the effect of periodontal treatment on serum CRP level[22, 23]. Indeed some intervention studies have shown effects of periodontal treatment on serum level of CRP, not only in periodontitis patients[24-27] but also in patients suffering from systemic diseases such as cardiovascular disease[28-30], RA[20] and diabetes mellitus[31, 32]. However, a systematic review summarising the effect of treatment of periodontitis on disease activity in patients suffering from cardiovascular diseases is not yet available.

In summary, studies on the association between periodontitis and systemic diseases show conflicting results, possibly influenced by the differences in background of the study population and the definition of

periodontitis used. Therefore, the results of one study may not be generalized to other populations. In this respect it is striking that only limited data are available for Asia, while Asians compose 60% of the world's population and the prevalence of both systemic diseases and periodontitis has been reported to be high in Asian countries[33],[34].

The overall aim of the PhD research described in this thesis was to investigate associations between periodontitis and systemic diseases as diabetes mellitus type 2 (DM2) and rheumatoid arthritis (RA) in Indonesia as well as to systematically review whether patients suffering from systemic diseases, in particular cardiovascular diseases and metabolic disorders, eg DM2, might benefit from treatment of periodontitis. Therefore, a number of studies was performed:

1. To assess the prevalence and severity of periodontitis among DM2 patients in Indonesia, using multiple, commonly used methods to operationalize both periodontitis prevalence and severity (**chapter 2**).

2. To assess whether periodontitis severity and CRP predict HbA1c levels in healthy Indonesians and Indonesian DM2 patients (**chapter 3**).

3. To compare periodontitis prevalence and severity in Indonesian RA patients with Indonesian healthy controls using a variety of definitions for periodontitis severity as well as a variety of serum parameters (**chapter 4**).

4. To systematically assess the effect of treatment of periodontitis on disease activity in patients suffering from atherosclerotic and metabolic diseases like DM2 (**chapter 5**).

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Chapter 2

Periodontitis prevalence and severity in Indonesians with type 2 diabetes

Abstract

Background: The prevalence of diabetes mellitus type 2 (DM2) in Indonesia is high and still rising. Periodontitis is associated with DM2. No study has investigated this association in Indonesia, nor has any study investigated this association using a variety of methods to operationalize periodontitis. The present study compares prevalence and severity of periodontitis in DM2 patients to healthy controls, using different methods to operationalize periodontitis.

Methods: A total of 78 subjects with DM2 and 65 healthy control subjects underwent a full mouth periodontal screening assessing probing pocket depth, gingival recession, plaque index and bleeding on probing. Using these measurements, periodontitis prevalence and severity was operationalized in various ways. Differences in periodontitis prevalence and severity between DM2 and healthy subjects were analyzed using univariate analyses. In regression analyses, prevalence and severity of periodontitis were predicted on the basis of DM2 presence, controlling for confounders and effect modification.

Results: Prevalence of periodontitis was significantly higher in subjects with DM2 compared to healthy subjects, showing odds ratios of 5.0 and 6.1. Likewise, periodontitis severity was significantly higher in DM2 subjects.

Conclusion: Indonesian DM2 subjects had more prevalent and more severe periodontitis than Indonesian healthy subjects, independent of confounding factors or the methods used to operationalize periodontitis.

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by dysregulation of carbohydrate, protein and lipid metabolism. An elevation of blood glucose level (hyperglycemia) is the primary feature of DM and results from either a defect in insulin secretion by pancreatic beta cells, a decrease in insulin sensitivity, or a combination of both. The most common form of DM is DM type 2 (DM2), which accounts for 85% of all diabetes patients. [1] The estimated world wide prevalence of DM is 220.5 million, or 2.8 % of the world's population. DM currently is the twelfth leading cause of death in the world. The prevalence is estimated to rise up to 4.4%, putting DM in the top ten leading causes of death by 2030. [2, 3] With the increasing prevalence of DM, this already vast and world wide epidemic will increasingly pose serious problems to public health. These problems mostly arise from the complications associated with DM like myocardial infarction, cerebrovascular disease, retinopathy, nephropathy and neuropathy. [4]

Periodontitis is more prevalent and severe among patients with DM2 than among healthy controls. [5-7] Thus, DM2 may initiate or deteriorate periodontitis. However, the reverse could also be true, i.e. periodontitis may initiate or deteriorate DM2. The strongest support for this comes from studies showing that treatment of periodontitis improves glycaemic control in DM2 patients. [8-13] Thus, there is an association between DM2 and periodontitis that appears to be bilateral causal in nature (i.e. one causes or deteriorates the other and vice-versa).

The strength of associations between periodontitis and DM2 appears to differ geographically. Studies performed in different locations, i.e. performed among different ethnic groups, show different associations between periodontitis and DM2. [5, 14-17] These differences in the strength of associations may, apart from differences in study design and data analysis, be based on genetic, dietary, cultural and other differences between ethnic groups. [18] Therefore, findings among one ethnic population cannot automatically be generalized to another ethnic population.

South East Asia hosts approximately 10% of the world's current population. With 240 million inhabitants, Indonesia is the 4th most populous

country in the world. The prevalence of DM in South East Asia is 5.3%. [19] In Indonesia, the prevalence of DM in 2008 was 5.7%, [20] putting Indonesia in the top ten of countries with the highest number of DM patients in the world. By the year 2030, the estimated number of patients with DM in Indonesia will be over 20 million (approximately 10% of the population). [2] With this high and rising prevalence of DM2 in Indonesia, periodontitis prevalence and severity may also rise. Only three studies report on the association between periodontitis and DM2 in South East Asia: Thailand, [21] Singapore, [22] Indonesia. [23] Unfortunately, in the latter study, the way in which periodontitis was measured and defined remains unclear. Neither did this study make a distinction between DM type 1 and DM type 2 patients, leaving many questions unanswered.

Also remaining unanswered is the influence of using a particular method to operationalize periodontitis on the strength of an association between periodontitis and DM2. The use of different methods to operationalize periodontitis prevalence and severity may influence not only the strength of the associations between a given disease and periodontitis, but may even influence whether an association is observed at all. For example, in research linking periodontitis to preterm low birth weight, 13 different methods have been used to operationalize periodontitis. Depending on the method used, either an association [24, 25] or no association [26] between periodontitis and preterm low birth weight was observed. The same may be true for periodontitis and DM2.

This study assesses prevalence and severity of periodontitis among DM2 patients in Indonesia, using multiple, commonly used methods to operationalize both periodontitis prevalence and severity.

Subjects and methods

DM2 patients were recruited at three different sites: 1) Internal Medicine Dept. of the Dr. Sardjito Hospital, 2) Prof. Soedomo Dental Hospital, Faculty of Dentistry, Gadjah Mada University and 3) Diabetes Center of Jogjakarta International Hospital, Indonesia. DM2 patients were diagnosed according to World Health Organization criteria: fasting blood glucose level $\geq$126 mg/dl and/or a postprandial blood glucose level $\geq$200 mg/dl. [27] Healthy controls

were recruited in Prof. Soedomo Oral and Dental Hospital, Gadjah Mada University. Inclusion criteria for all subjects in this study were an age 18 years or over, and having ≥8 remaining teeth. This study was approved by the Ethical Committee for Research of the Medical Faculty of Gadjah Mada University and was conducted from July 2008 until February 2009.

To assess whether periodontitis prevalence and severity differed between DM2 patients and healthy controls, 78 DM2 patients (35 males and 43 females) and 65 healthy controls (22 males and 43 females), who gave informed consent, underwent a full mouth periodontal examination. Full mouth periodontal probing pocket depth (PPD), gingival recession, plaque score and bleeding on probing (BOP) measurements were performed on all teeth, on six sites per tooth. All permanent fully erupted teeth were examined with a manual periodontal colour coded standard probe (Dentsply, London, UK). Measurements were made in millimetres and were rounded to the nearest whole millimetre. CAL was defined as the distance from the cemento enamel junction (CEJ) to the bottom of the pocket/sulcus, and calculated as the mathematical sum of the PPD and gingival recession measurements [28]. BOP was recorded as either present or absent within 30 seconds after probing at 6 sites per tooth. The number of missing teeth was also recorded. Plaque score was defined as being present or absent at 6 points on each tooth. [29]

Periodontitis prevalence, extent and severity were operationalized using a variety of methods, all of which are currently used in literature studying the association between periodontitis and other diseases. All methods used to operationalize periodontitis prevalence and severity were calculated using conventional clinical measurements obtained during the full mouth periodontal examination. Periodontitis prevalence was operationalized by using two diagnostic threshold values; 1) having one site with PPD ≥4 mm and CAL ≥3 mm, [26] and 2) having one site with PPD ≥5 mm and CAL ≥2 mm. [30] The following methods to operationalize periodontitis extent and severity were calculated using an online spreadsheet (www.parsprototo.info, Microsoft Excel 2003 spreadsheet for Windows); the number of sites with PPD ≥4, ≥5 and ≥6 mm, the numbers of sites with CAL ≥3, ≥4, ≥5 and ≥6 mm, mean PPD, mean CAL and the percentage of sites with BOP. [26]

Table 1. Characteristics of healthy subjects and DM2 and potential determinants of periodontitis severity

Patient Characteristics	Controls (n=65)	DM2 (n=78)	Difference (95%CI)	*p* value
Age:				
mean (SD) yr	50.5(10.6)	56.7(9.4)	6.2 (2.8 to 9.5)	<0.001‡
Sex: n (%)				0.180*
Male	22(34)	35(45)		
Female	43(66)	43(55)		
Smoking: n (%)	10(15)	14(18)		0.683*
Java origin: n (%)	60(92)	73(94)		0.765*
Education: n (%)				0.762*
Low	17(26)	18(23)		
Middle	26(40)	29(37)		
High	22(34)	31(40)		
BMI (SD) (kg/m^2)	24.6(3.9)	25.1(3.8)		0.460
Number of tooth (SD) (n)	24.5(5.5)	22.9(6.1)		0.112
Plaque score (SD) (%)	92.5(9.2)	90.8(7.6)		0.211
Medical conditions: n (%)†	12 (18)	26 (33)	15% (0.3 to 29)	<0.05*‡
1. Hypertension				
2. Gastritis	4 (6)	10 (13)		0.182*
3. Anemia	2 (3)	0 (0)		0.119*
4. Angina Pectoris	0 (0)	3 (4)		0.110*

*Results of Chi-square test, other results are the results of the independent samples t test,

†Only diseases with a prevalence of at least 1% (i.e. 2 patients) were analyzed

‡statistically significant difference ($p \leq 0.05$) between DM2 and controls

95%CI: 95% Confidence Interval

BMI: Body Mass Index

DM2: Diabetes Mellitus type 2

Education: (low: elementary & junior school, middle: high school, high: university) HbA1c: Glycosilated/Glycated Hemoglobin

n: number of participants

SD: Standard Deviation

yr: year

Additionally, two recently introduced measures of periodontitis severity, the Periodontal Epithelial Surface Area (PESA) and the Periodontal Inflamed Surface Area (PISA) [31] were both calculated using another freely downloadable spreadsheet (www.parsprototo.info). PESA reflects the surface area of *all* pocket epithelium in square millimetres, whereas PISA reflects the surface area of *bleeding* pocket epithelium in square millimetres. PESA and PISA are calculated using conventional CAL, gingival recession and BOP measurements. PISA quantifies the amount of inflamed periodontal tissue and it is suggested that PISA thereby quantifies the inflammatory burden posed by periodontitis.

Furthermore, all participants completed a validated general health assessment questionnaire, [32-34] to identify other medical conditions that might be a risk factor for periodontitis. The original questionnaire was translated from English into Indonesian, a reverse translation to English was made to check for potential differences. No substantial differences were found. Additionally, ethnicity[35], Body Mass Index (BMI)[36], dental plaque, age, sex, smoking (pack years), and Socio Economic Status (SES, operationalized using level of education) were recorded for each participant, since these are potential determinants of periodontitis. [37]

To ensure that healthy controls were not undiagnosed patients with diabetes, all participants underwent venipuncture to obtain a blood sample. Blood glucose and glycosilated glycated haemoglobin (HbA1c) were determined for both DM2 patient and healthy controls. Controls with a blood HbA1c level of ≥6.5% were excluded from the analysis, to exclude latent DM2 status.

Statistical analysis

Differences in periodontitis prevalence, extent and severity between DM2 and healthy subjects were analyzed first using univariate analyses, the independent sample t-test or Chi-square test as appropriate. Likewise, differences in potential predictors of periodontitis (namely: age, sex, BMI, SES, smoking, plaque score, number of teeth, ethnicity and other medical conditions) between DM2 patients and healthy controls were tested for

significance using univariate analyses. In case both periodontitis prevalence, extent and severity and any of the potential predictors of periodontitis differed significantly between DM2 patients and healthy controls, the predictors of periodontitis other than DM2 might act as confounders or effect modifiers.

Table 2. Differences in periodontitis prevalence between healthy subjects and DM2.

Periodontitis prevalence	Controls n=65	DM2 n=78	Difference (95%CI)	OR (95%CI)	*p* value†
PPD4&CAL3 (yes)	46(71%)	72(92%)	21% (9% to 32%)	5.0 (1.8 to 13.3)	0.001*
PPD5&CAL2 (yes)	20(31%)	57(73%)	42% (26% to 55%)	6.1 (2.9 to 12.6)	<0.001*

* Result of Chi-square test

†statistically significant difference ($p \leq 0.05$) between DM2 and controls

OR: Odds Ratio's were calculated using binary logistic regression analyses controlling for confounders

95%CI: 95% Confidence Interval

DM2: Diabetes Mellitus type 2

PPD: Probing Pocket Depth

CAL: Clinical Attachment Loss

PPD4&CAL3: participants with one site exhibiting both PPD 4 mm & CAL 3 mm,

PPD5&CAL2: participants with one site exhibiting both PPD 5 mm & CAL 2 mm

Table 3. Differences in periodontitis severity between Healthy Controls and subjects with DM2.

Periodontitis severity	Controls (n=65) Mean (SD)	DM2 (n=78) Mean (SD)	Difference (95%CI)	*p* value*
PESA (mm^2)	863.0(259.4) mm^2	1190.5(1161.5) mm^2	327.5(58.3 to 596.6) mm^2	0.018
PISA (mm^2)	154.1(192.1) mm^2	429.4(964.9) mm^2	275.3(52.9 to 497.6) mm^2	0.016
Number of sites with:				
CAL > 3 mm	35.8(23.1) sites	63.5(29.9) sites	27.6(18.8 to 36.4) sites	<0.001
CAL > 4 mm	12.3(15.1) sites	37.1(27.2) sites	24.8(17.7 to 31.9) sites	<0.001
CAL > 5 mm	5.9(10.2) sites	22.9(21.4) sites	16.9(11.5 to 22.4) sites	<0.001
CAL > 6 mm	3.1(7.3) sites	13.7(16.2) sites	10.6(6.6 to 14.7) sites	<0.001
PPD > 4 mm	4.5(7.9) sites	16.6(21.2) sites	12.1(6.9 to 17.3) sites	<0.001
PPD > 5 mm	1.4(3.7) sites	7.7(12.5) sites	6.3 (3.4 to 9.2) sites	<0.001
PPD > 6 mm	0.8(2.3) sites	4.5(9.3) sites	3.7(1.5 to 5.9) sites	0.001
BOP (%)	14.2(13.3) %	24.9(16.1) %	10.7(5.8 to 15.5) %	<0.001
CAL mean (mm)	2.2(0.9) mm	3.1(1.3) mm	0.9(0.5 to 1.3) mm	<0.001
PPD mean (mm)	1.8(0.4) mm	2.2(0.6) mm	0.4(0.2 to 0.5) mm	<0.001

*statistically significant difference ($p \leq 0.05$) between DM2 and controls

95%CI: 95% Confidence Interval

BOP: Bleeding On Probing

CAL: Clinical Attachment Loss

DM2: Diabetes Mellitus type 2

PESA: Periodontal Epithelial Surface Area

PISA: Periodontal Inflamed Surface Area

PPD: Probing Pocket Depth

SD: Standard Deviation

Because periodontitis severity was operationalized as several interval variables (i.e. PISA, or number of sites with PPD $\geq$4mm), linear regression analyses (backward stepwise) were performed to predict periodontitis extent and severity on the basis of DM2 presence and the other potential predictors (age, sex, BMI, SES, smoking, plaque score, number of teeth, ethnicity and

other medical conditions). To facilitate clinical interpretation of presented analyses, age was centered to it's mean (53.86). Because periodontitis prevalence was operationalized as two dichotomous variables, logistic regression analyses were performed in a similar way. Odds ratios and 95% confidence intervals were calculated using these logistic regression analyses. Interaction between different predictors of periodontitis was explored. Statistics were calculated using SPSS 16.0.

Results

Of the original 76 healthy controls, 11 subjects were excluded because of HbA1c levels >6.5%, leaving 65 healthy controls and 78 DM2 patients (table 1). The prevalence of periodontitis in DM2 subjects was significantly higher than healthy controls, regardless of the definition used (table 2). The extend and severity of periodontitis was also significantly higher in participants with DM2 when compared to controls, again independent of the method used to operationalize periodontitis severity (table 3).

Table 4. DM2 and age as statistical predictors of periodontitis extent and severity:

Results of multiple linear regression analyses with periodontitis operationalized according to commonly used definitions

Dependent variable Model predictors	β Unstandardized	β Standardized	p-value of β (†)	R^2	95% CI of β
PESA				0.03	
DM2	327.49	0.18	<0.05		36.65 to 618.33
Constant	863.04		<0.001		648.25 to 1077.84
PISA				0.04	
DM2	275.29	0.19	<0.05		34.69 to 515.89
Constant	154.06		0.089		-23.63 to 331.76
BOP%				0.11	
DM2	10.65	0.34	<0.001		5.71 to 15.60
Constant	14.23		<0.001		10.58 to 17.89
*PPD > 4 mm				0.12	
DM2	12.10	0.34	<0.001		6.60 to 17.60
Constant	4.92		<0.05		0.43 to 8.56

*PPD > 5 mm				0.10	
DM2	6.30	0.31	<0.001		3.13 to 9.47
Constant	1.35		0.254		-0.98 to 3.69
*PPD > 6 mm				0.07	
DM2	3.70	0.26	0.002		1.36 to 6.03
Constant	0.75		0.388		-0.97 to 2.47
PPD mean				0.11	
DM2	0.37	0.33	<0.001		0.19 to 0.54
Constant	1.83		<0.001		1.70 to 1.96
*CAL > 3 mm				0.24	
DM2	24.25	0.40	<0.001		14.99 to 33.49
Age centered	0.54	0.19	0.017		0.10 to 0.98
Constant	37.7		<0.001		31.0 to 44.4
*CAL > 4mm				0.29	
DM2	20.85	0.41	<0.001		13.31 to 28.40
Age centered	0.64	0.26	0.001		0.28 to 1.00
Constant	14.5		<0.001		9.0 to 19.9
*CAL > 5 mm				0.25	
DM2	14.12	0.37	<0.001		8.31 to 19.94
Age centered	0.46	0.25	0.002		0.18 to 0.74
Constant	7.5		0.001		3.26 to 11.68
*CAL > 6 mm				0.19	
DM2	8.82	0.32	<0.001		4.42 to 13.22
Age centered	0.29	0.22	0.008		0.08 to 0.50
Constant	4.03		0.013		0.85 to 7.22
*CAL mean				0.21	
DM2	0.71	0.29	<0.001		0.33 to 1.09
Age centered	0.03	0.27	0.001		0.01 to 0.05
Constant	2.27		<0.001		2.00 to 2.54

* = Number of sites with

† p-value of ≤0.05 was considered statistically significant

p = probability, β: unstandardized coefficient

95%CI: 95% Confidence Interval

Independent variables:

Age centered = Age minus Mean age

Constant = sites with PPD≥ that are not dependent on DM2 presence or increasing age

DM2: Diabetes Mellitus type 2

Dependent variables (periodontitis severity measures) are underlined:

BOP: Bleeding On Probing

Number of sites with CAL: Clinical Attachment Loss ≥3, ≥4, ≥5 & ≥6 mm

Number of sites with PPD: Probing Pocket Depth ≥4, ≥5 & ≥6 mm
PESA: Periodontal Epithelial Surface Area
PISA: Periodontal Inflamed Surface Area

Age and hypertension were the only potential predictors of periodontitis prevalence and severity that differed significantly between DM2 and healthy subjects in the univariate analysis (table 1). In the multiple linear regression analyses, controlling for age and hypertension as potential confounders, DM2 remained a significant predictor of all measures of periodontitis severity (table 4). Age was an additional predictor of periodontitis severity, together with DM2, whenever periodontitis severity was operationalized using CAL. Age did not modify the effect of DM2 on periodontitis severity. Hypertension was not a predictor of periodontitis prevalence or severity.

Discussion

This study revealed that Indonesian DM2 subjects had significantly increased prevalence, extent and severity of periodontitis compared to healthy Indonesian subjects. Moreover, these increases, were independent of the methods used to operationalize periodontitis. Furthermore, these increases seemed to be independent of confounding factors i.e.: age, sex, smoking, BMI, ethnicity, SES and other medical conditions.

Age was an additional predictor of all methods that used CAL to operationalize periodontitis severity. This could have been expected since CAL reflects the accumulation of damage sustained by the periodontium over time. In other words, with increasing age, CAL increases. Nevertheless, DM2 remained a significant predictor of every method used to operationalize periodontitis prevalence and severity.

Before the study we did not perform a formal sample size calculation, although this study is the second on periodontitis and DM2 in Indonesia. It is not clear how periodontitis was measured and defined in the first study and no distinction was made among patients with DM1 and DM2. [23] In a post-hoc power analysis it seemed that we had a power of .92 to find a difference in prevalence of periodontitis of 21%, between controls (n=65, prevalence=71%)

and DM2 patients (n=78, prevalence=91%). A sample of 50 DM2 patients and 50 controls would have been enough to detect this difference (power, 0.80).

A limitation of this study is that we did not use a population based sampling scheme to select DM2 patients. However, Indonesian DM2 patients regularly visit hospitals to make use of the laboratory facilities. Thus, selection of DM2 patients at two hospitals does not mean a subset of patients with more severe DM2 patients was selected. Rather, these subjects may be thought to represent a sample of diagnosed and treated Indonesian patients with DM2 . However, true DM2-associated increased periodontitis risk may be underestimated, because a substantial portion of DM2 often goes undiagnosed. Likely, these patients have worse blood sugar control and consequently worse periodontal status. On the other hand, the DM2 patients that were recruited from a dental hospital may have visited this hospital for periodontitis, overestimating DM2 associated periodontitis prevalence in this subgroup. Likewise, because controls were all selected from the same dental hospital, the prevalence of periodontitis in controls may also have been overestimated. Because the prevalence in both groups might be overestimated, the overall effect on calculated DM2 associated periodontitis risk may have been small. Finally, selection of healthy controls at a dental hospital may not be representative of the general Indonesian population without DM2. Thus, although some threats to the generalizability of our results remain, the increased DM2 associated periodontitis risk does appear to have sufficient generalizability and might sooner have been underestimated rather than overestimated.

The finding that DM2 subjects have an increased prevalence and severity of periodontitis is in accordance with other studies. [30, 38, 39] A major achievement of present study is that a large variety of methods to operationalize periodontitis prevalence and severity has been applied, and that the conclusions that could be drawn from the results were irrespective of the measures used. This indicates that this association is robust. Because the prevalence of DM2 in Indonesia, and South East Asia, is already high and is predicted to rise further, the prevalence and severity of periodontitis may also rise. Due the vast number of people living in Indonesia, and South East Asia,

and the proposed bilateral association between DM2 and periodontitis, this will increasingly pose serious problems to public health.

Two main mechanisms are thought to underlie the proposed bilateral association between DM2 and periodontitis. One underlying mechanism is that DM2 may alter local immune responses within periodontal tissue. DM2 may result in small vessel damage within the periodontium, resulting in poor nutrient delivery, decreased oxygen diffusion and decreased elimination of metabolic waste products. [40] Furthermore, hyperglycaemia alters collagen metabolism which predisposes to impaired wound healing. In general, hyperglycemia results in the formation of proteins known as advanced glycation end products (AGEs). AGEs may be associated with a state of enhanced oxidative stress, thereby accelerating tissue injury. AGEs also function as a chemotactic for monocytes, thereby magnifying the inflammatory response, delaying wound healing and tissue repair and inducing connective-tissue damage and bone resorption. Finally, hyperglycaemia and the imbalance in lipid metabolism impair neutrophil and monocyte functioning. [41, 42-44] All of these factors may contribute to DM2 predisposing to periodontitis.

The second underlying mechanism of the association between DM2 and periodontitis is that periodontitis may play a role in initiating, or exacerbating DM2. [7, 43, 45] Periodontitis poses an inflammatory burden consisting of increased serum levels of inflammatory mediators, like C-reactive protein and Interleukin-6. [46] This inflammatory burden in turn leads to deteriorating blood glucose control in DM2 patients. [43] The higher the amount of inflamed periodontal tissue, the higher the inflammatory burden, and the poorer blood glucose control in DM2 patients may be thought to be.
PISA quantifies the amount of inflamed periodontal tissue (representing it as the surface area of inflamed periodontal epithelium in square millimeters), and it is suggested that PISA thereby quantifies the inflammatory burden posed by periodontitis. [31] It was shown that there is indeed a dose-relationship between PISA and HbA1c in DM2 patients in the Dutch Caribbean. [47] Likewise, the finding of a significantly higher PISA among DM2 subjects in the present study may mean that periodontitis is a risk factor for poor glucose control. Treating periodontitis might improve blood glucose control, [8-13] and

prevention and treatment of periodontitis in patients with DM2 might contribute to better general health in patients with DM2 . [48, 49]

Conclusions

This study shows that periodontitis prevalence is significantly higher in a group of Indonesian patients with DM2 patients compared to a group of healthy Indonesians. Furthermore, Indonesian subjects with DM2 have more extended and more severe periodontitis than healthy Indonesian subjects. Given the already high and increasing prevalence of DM2 in Indonesia, patients with DM2 should be screened for periodontitis and preventive oral health care should become part of the regular care provided to Indonesian patients with DM2. Given the proposed bilateral association between DM2 and periodontitis, such care may contribute to better oral and overall health.

Acknowledgement

We would like to thank the following institutions for their cooperation: the Internal Medicine Dept. and the Dental Clinic of the Dr. Sardjito Hospital, the Prof. Soedomo Dental Hospital of the Faculty of Dentistry of Gadjah Mada University, the Diabetes Center of Jogjakarta International Hospital, the Prodia Laboratory in Yogyakarta, Indonesia. Additionally, we would like to thank Yvonne Huijser van Reenen and Evelien Hoedemaker for their contributions and support.

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Chapter 3

Periodontal inflamed surface area and c-reactive protein as predictors of HbA1c: a study in Indonesia

Abstract

Objectives: Periodontitis may exert an infectious and inflammatory burden, evidenced by increased c-reactive protein (CRP). This burden may impair blood glucose control (HbA1c). The aim of our study was to analyze whether periodontitis severity as measured with the periodontal inflamed surface area (PISA) and CRP predict HbA1c levels in a group of healthy Indonesians and a group of Indonesians treated for type 2 diabetes mellitus (DM2).

Materials and methods: A full mouth periodontal examination, including probing pocket depth, gingival recession, clinical attachment loss, plaque index and bleeding on probing, was performed in 132 healthy Indonesians and 101 Indonesians treated for DM2. Using these data, PISA was calculated. In addition, HbA1c and CRP were analyzed. A validated questionnaire was used to assess smoking, body mass index (BMI), education and medical conditions. In regression analyses, it was assessed whether periodontitis severity and CRP predict HbA1c, controlling for confounding and effect modification (i.e. age, sex, BMI, pack years, education).

Results: In healthy Indonesians, PISA and CRP predicted HbA1c as did age, sex, and smoking. In Indonesians treated for DM2, PISA did not predict HbA1c.

Conclusions: Periodontitis may impair blood glucose regulation in healthy Indonesians in conjunction with elevated CRP levels. The potential effect of periodontitis on glucose control in DM2 patients may be masked by DM2 treatment.

Clinical relevance: periodontitis may impair blood glucose control through exerting an inflammatory and infectious burden evidenced by increased levels of CRP.

Introduction

Periodontal inflamed surface area (PISA) quantifies the amount of inflamed periodontal tissue and is supposed to quantify the inflammatory and infectious burden resulting from periodontitis [1]. Periodontitis may cause an inflammatory burden through the production of local inflammatory mediators entering the systemic circulation. This inflammatory burden is evidenced by increased serum c-reactive protein (CRP) levels found in patients suffering from periodontitis [2-7].

Periodontitis may also cause an infectious burden through bacteria and their products entering the systemic circulation. This burden may endanger overall health by for example causing atherosclerosis [8] and cardiovascular diseases [9]. Circulating oral bacteria and lipopolysacharides are also able to stimulate hepatocytes to secrete CRP [10-12]. Thus, increased levels of CRP associated with periodontitis may be considered as a common pathway for both the inflammatory and infectious burden as a resulting from periodontitis. The high levels of CRP accompanying periodontitis, may lead to insulin resistance and thereby to poor control of blood glucose in type 2 diabetes mellitus (DM2) and healthy subjects [13-16] resulting in increased levels of glycated haemoglobin (HbA1c) [17, 18]. Accordingly, high levels of CRP are correlated with increased HbA1c levels in DM2 patients [19, 20]. Increased CRP levels have also been associated with an increased risk of cardiovascular disease (CVD) in DM2 patients, the major cause of the increased mortality in DM2 patients [21, 22].

Given the importance of blood glucose control in preventing CVD in DM2 patients, relatively few studies assessed whether the specific combination of periodontitis and CRP as a reflection of inflammatory burden, predict HbA1c levels [23, 24]. Furthermore, no study has investigated this issue in Indonesia, while this country is in the top ten of countries with the highest prevalence of DM in the world with a prevalence of DM of 5,7 % in 2005. It has been estimated that by the year 2030, the prevalence of DM in Indonesia will be about 10%, corresponding with 20 million DM patients [26]. In addition to a high DM prevalence, in Indonesia a high prevalence of periodontitis has been reported [27]. Finally, depending on the definition used, our previous study showed that Indonesian DM2 patients have a

prevalence of periodontitis of 72 to 93% and an increased severity of periodontitis [28]. Therefore, the aim of this study was to assess whether periodontitis severity, as measured with the periodontal inflamed surface area (PISA) method and CRP predict HbA1c levels in healthy Indonesians and Indonesian treated for DM2 patients.

Materials and methods

The participants in this study were recruited from three different sites, namely 1) The Internal Medicine Department Dr. Sardjito Hospital, Yogyakarta 2) Prof. Soedomo Dental Hospital, Faculty of Dentistry, Gadjah Mada University, Yogyakarta and 3) Diabetes Center of Jogjakarta International Hospital, Yogyakarta Indonesia. All participants had to be aged $\geq$ 18 years and had to have $\geq$ 8 remaining teeth. The latter inclusion criteria was proposed prior to commencing the study, since current inflammatory burden posed by periodontitis requires the presence of at least a minimum number of teeth affected by periodontitis. This study was approved by the Ethical Committee for Research of the Medical Faculty of Gadjah Mada University, Yogyakarta, Indonesia.

All participants completed a validated general health assessment questionnaire [29, 30] to check for other medical conditions that might be a risk factor for periodontitis. Information on age, gender, height and weight (for body mass index (BMI) calculation), education and smoking were obtained from each participant.

All participants underwent a periodontal examination including periodontal probing pocket depth (PD), gingival recession, plaque score and bleeding on probing (BOP) measurements by trained and calibrated examiners (HS, YHR, EH). All measurements were performed on all teeth, on six sites per tooth using a manual periodontal colour coded standard probe (Dentsply ™, London, UK).

Clinical attachment loss (AL) was defined as the distance from the cemento enamel junction (CEJ) to the bottom of the pocket/sulcus, and calculated as the mathematical sum of the PD and gingival recession measurements [31]. Measurements were made in millimeters and were rounded of to the nearest millimeter. BOP was recorded as either present or

absent within 30 seconds of probing at 6 sites per tooth. Plaque score was defined as being present or absent at 6 points on each tooth [32]. The number of missing teeth was also recorded.

Periodontitis extent and severity were operationalized using a variety of methods, all of which are currently used in literature studying the association between periodontitis and other diseases. All measurements were calculated using conventional clinical measurements obtained during the full mouth periodontal examination (HS and WN). The number of sites with probing PD $\geq$4, $\geq$5 and $\geq$6 mm, the numbers of sites with clinical AL $\geq$3, $\geq$4, $\geq$5 and $\geq$6 mm, mean PD, mean AL [33] and the percentage of sites with BOP were calculated. Additionally, a recently introduced measure of periodontitis severity, the periodontal inflamed surface area (PISA) [1] was calculated, PISA quantifies the amount of inflamed periodontal tissue, and it is suggested that PISA thereby quantifies the inflammatory burden exerted by periodontitis [1]. Finally, all participants underwent a venapuncture to obtain a blood sample. Both blood glucose (fasting blood glucose), determined by glucose oxidase enzymatic method, and glycosilated/glycated hemoglobin (HbA1c) values, determined using low pressure cation ion exchange chromatography (DIASTAT™, Bio-Rad, USA), were determined for all participants. Additionally, CRP, determined by a high-sensitivity chemiluminescent immunometris assay (Immulite 2000™, Diagnostic Products Corp., Los Angeles, CA, USA), were determined for all participants.

Statistical analysis

Differences in periodontitis severity between DM2 and healthy subjects were analyzed first using univariate analyses (independent sample t-test, Mann-Whitney U-test or Chi-square test as appropriate). Likewise, differences in potential predictors of periodontitis (age, gender, body mass index (BMI), education, smoking, plaque score, number of teeth, ethnicity and other medical conditions) between DM2 patients and healthy subjects were tested for significance using univariate analyses. Since periodontitis severity was operationalized in 12 different ways, significance level α of 0.05 was corrected for multiple comparisons according to the Bonferroni-Holm method.

To assess periodontitis and CRP as potential predictors of HbA1c in subjects with and without DM2, a multiple linear regression analysis was performed, using a backward stepwise method. HbA1c was the dependent. As independent variables, i.e. predictors of HbA1c: age, sex, BMI, pack years, education, CRP and all measures of periodontitis severity. The latter were introduced in the model one by one. Statistics were calculated using SPSS 16.0.

Results

In total 101 participants (40 men and 61 women) with a mean age of 54 years treated for DM2, diagnosed according to WHO criteria [34] were included (Table 1). Most of the participants were of Javanese origin (93.0 %). The mean BMI in this group was 25.5, labeling them as being overweight according to the World Health Organization classification (overweight: BMI 23-27.5) [35]. The DM2 patients had an average of 24 teeth with a mean plaque score of 91% (Table 2). The mean HbA1c in the DM2 group was 8.9%. In addition, a 132 healthy controls was enrolled, 34 men and 98 women, with a mean age of 48 years. Again, the healthy subjects were mainly of Javanese origin (90%) and were on average ranked as being overweight according to the BMI index for Asian populations (BMI: 24.4) [35]. The healthy subjects had an average of 26 teeth and a mean plaque score of 93 %. The average HbA1c level of this group was 5.5% (below 6.5% classified as healthy/non diabetic).

The severity of periodontitis was significantly higher in participants with DM2 when compared to controls, again independent of the method used to operationalize periodontitis severity (table 2). In the DM2 patients, none of the measures of periodontitis severity predicted HbA1c. Only SES (β = -0.752, 95% CI = -1.345 to -0.158) was a predictor of HbA1c, (r^2 of 6.0%) (data not shown). By contrast, in healthy Indonesians, PISA (ß = 0.0004, 95% CI = 0.00004-0.00076) was a predictor of HbA1c together with age, sex, smoking and CRP, (r^2 = 21%) in the model. (Table 3).

Table 1. Characteristics of the participants.

Variables	DM2 (n=101)	Healthy controls (n=132)	p value
Demography			
Age (yrs) mean (SD)	54.4 (10.7)	47.9 (10.1)	<0.001
Smoking (pack year) median (IQR)†	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.109
BMI (kg/m^2) mean (SD)	25.5(4.2)	24.4 (3.8)	0.054
Java origin n (%)*	94 (93)	119 (90)	0.431
Gender n (%)*			<0.05
Male	40 (40)	34 (26)	
Female	60 (60)	98 (74)	
Education n (%)*	2.18 (0.79)	2.13 (0.77)	0.662
Low (6-9yrs)	32 (24)	24 (23)	
Middle (9-12 yrs)	51 (39)	34 (34)	
High (12-17 yrs)	49 (37)	43 (43)	
Hypertension (yes) n (%)*	26 (26)	24 (18)	0.164
Medication n (%)			
Sulfonylurea	55 (54)	NA	NA
Insulin	41 (41)	NA	
Metformin	37 (37)	NA	
Acarbose	33 (33)	NA	
Laboratory serum markers			
HbA1c (%) Mean (SD)	8.9 (2.4)	5.5 (0.4)	<0.001
CRP (mg/l) Median (IQR)†	2.8(1.6 to 7.0)	1.1 (0.6 to 2.3)	<0.001

*Result of Chi-square test; † result of Mann Whitney U-test, other results are the results of the independent samples t-test; p: probability, p values were Bonferroni-Holm corrected, a *p*-value of < 0.05 was considered statistically significant; BMI: body mass index; CRP: c-reactive protein; DM2: diabetes mellitus type 2; HbA1c: glycated hemoglobin; IQR: inter quartile range; n: number of participants; NA: not applicable; SD: standard deviation; Education: low: elementary & junior school, middle: high school, high: university), yrs: years

Table 2. Periodontal status of healthy controls and DM2.

Variables	Healthy controls (n=132)	DM2 (n=101)	p value
Periodontal prevalence: n (%)*			
PD ≥4mm AL ≥3mm	89 (67)	89 (88)	<0.001
PD ≥5mm AL ≥2mm	43 (33)	67 (66)	<0.001
Periodontal severity: median (IQR)†			
PISA (mm^2)	83.9 (35.2 to 206.4)	170.4 (91.5 to 392.6)	<0.017
AL (mm)	1.8 (1.7 to 2.3)	2.4 (2.0 to 3.4)	<0.001
PD (mm)	1.7 (1.6 to 1.9)	1.9 (1.7 to 2.4)	<0.001
Number of sites: median (IQR)†			
AL ≥ 3 mm	30.0 (17.0 to 47.0)	54.0 (32.50 to 74.50)	<0.001
AL ≥ 4 mm	5.0 (1.0 to 15.8)	25.0 (9.0 to 48.0)	<0.001
AL ≥ 5 mm	1.0 (0.0 to 4.8)	13.0 (2.0 to 31.50)	<0.001
AL ≥ 6 mm	0.0 (0.0 to 1.0)	4.0 (0.0 to 19.5)	<0.001
PD ≥ 4 mm	2.0 (0.0 to 4.0)	6.0 (2.0 to 18.0)	<0.001
PD ≥ 5 mm	0.0 (0.0 to 1.0)	2.0 (0.0 to 8.0)	<0.001
PD ≥ 6 mm	0.0 (0.0 to 0.0)	1.0 (0.0 to 3.0)	0.001
BOP (n %)	9.0 (4.0 to 20.0)	18.0 (10.0 to 31.5)	<0.001
Mean (SD)			
Plaque Score (%)	93 % (8.5)	91 % (7.9)	

* Result of Chi-square test; † result of Mann Whitney U-test; other results are the results of the independent samples t-test; p: probability, p values were Bonferroni-Holm corrected, a *p*-value of < 0.05 was considered statistically significant; BOP: bleeding on probing; AL: clinical attachment loss; DM2: diabetes mellitus type 2; IQR: inter quartile range; n: number of participants; PISA: periodontal inflamed surface area; PD: probing pocket depth; SD: standard deviation

Table 3. Results of the multiple linear regression analysis healthy control group.

Model predictors	β	*p*-value of β	R^2	95% confidence interval of β
Model			0.21	
PISA (mm^2)*	0.0004	<0.05		0.00004 to 0.00076
Age (year)	0.0105	0.001		0.00431 to 0.01674
Male/female (1/0)	-0.1378	0.08		-0.29170 to 0.01595
Smoking(pack year)	0.0086	0.07		-0.00074 to 0.01805
CRP (mg/l)	0.0212	0.06		-0.00093 to 0.04343
Constant	4.9591	<0.001		4.62706 to 5.29121

* Other measures of periodontitis severity were not predictors of HbA1c; *p* = probability, a *p*-value of < 0.05 was considered statistically significant; β: unstandardized coefficient; Dependent variable: HbA1c :glycated hemoglobin; Independent variables: PISA, age, sex, smoking/pack years, CRP : C-reactive protein; Constant .

Discussion

This study showed that PISA is a predictor of HbA1c, in conjunction with CRP, age, sex and smoking, in healthy Indonesians. Thus, periodontitis may play a role in inducing impaired blood glucose control. Indeed there is evidence that periodontitis may induce insulin resistance [18]. It is thought that periodontitis exerts an inflammatory and infectious burden as evidenced by increased levels of CRP [3, 5, 6] which may increase HbA1c levels [19, 20, 36]. Accordingly, PISA predicted HbA1c together with CRP.

It is striking that out of the various methods to operationalize periodontitis severity, only the PISA emerged as a predictor of HbA1c. The other methods for determining the severity and extent of periodontitis did not contribute significantly to a model that predicts HbA1c. It may be that the PISA is a predictor of HbA1c because it reflects the amount of inflamed

periodontal tissue, thereby predicting both infectious and inflammatory burden more accurately than other methods used to operationalize periodontitis [1, 18].

In Indonesian DM2 patients,no measure for periodontitis severity predicted HbA1c. The observed differences between DM2 patients and healthy Indonesians may be explained as follows. First, almost all DM2 patients used a combination of medication to control blood sugar, drugs that healthy Indonesians obviously did not take [37]. Use such medication may mask an effect of periodontitis on the HbA1c level in DM2 patients. Second, DM2 patients may have dietary restrictions, e.g. avoiding sugar rich foods. Such a diet may impact blood sugar and may mask an affect of periodontitis on HbA1c level.

Previously, a dose-response relationship was observed between PISA and HbA1c in DM2 patients from the Caribbean island, Curacao [18]. Additionaly, an increased HbA1c was observed to be associated with increased severity of periodontitis. [38]. Moreover, it has been reported that periodontal treatment leads to improvement of glycemic control in DM2 patients [39]. These findings appear to be in sharp contrast with the findings in this study, i.e. PISA is not a predictor of HbA1c in a group of Indonesian DM2 patients. The difference between the current study and the study in Curacao [18] could be explained by differences in populations. First, substantial differences were observed in the frequency of antidiabetics used between DM2 from Indonesia and Curacao. Acarbose (33 vs. 5%), insulin (41 vs. 21%) and sulfonylurea (54 vs. 35%) were used more often in DM2 patients from Indonesia, while metformin 37 vs. 67% was used more often in DM2 patients from Curacao. Additionally 16% of DM2 patients from Curacao used tolbutamide, while none of Indonesian DM2 patients used it. Differences in the frequency of antidiabetics used, may also explain the differences in results. As reported by Teeuw et al [38] and Simpson et al [39], HbA1c levels might be affected by the treatment DM2 patients receive. i.e. both the drugs they use and the periodontal treatment the patients have received. Thus a drug-related reduction in HbA1c levels in patients not yet adequately periodontally treated might be responsible for the failure of PISA to predict HbA1c levels in our

Indonesian DM2. Second, the average BMI of DM2 patients from Curacao was 31, while the average BMI of Indonesian DM2 patients was substantially lower, namely 25. BMI may be an effect modifier, i.e. a higher BMI may increase the potential insulin resistance inducing effect of periodontitis in DM2 patients [40, 41]. Third, there is a clear difference in ethnicity between the two groups, i.e. one from Indonesia of Javanese origin and the other from Curacao of African-American origin. Possible differences in (health promoting) behavior, diet and genes, related to the differences in ethnicity, may be another explanation for the differences in results [42, 43].

Conclusions

PISA was shown to be a predictor of HbA1c in healthy Indonesians in conjunction with CRP, age, sex, and smoking. This implies that periodontitis might contribute to insulin resistance through the inflammatory and infectious burden leading to DM2. In Indonesian patients, treated for DM2, PISA was not able to predict HbA1c.

Acknowledgement

We would like to thank the following institutions for their cooperation in Yogyakarta, Indonesia: the Internal Medicine Department and the Dental Clinic of the Dr. Sardjito Hospital, the Prof. Soedomo Dental Hospital of the Faculty of Dentistry of Gadjah Mada University, the Diabetes Center of Jogjakarta International Hospital and the Prodia Laboratory. This study was supported by Bernouilli Foundation Grant from the University of Groningen.

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Chapter 4

Prevalence and severity of periodontitis in Indonesian rheumatoid arthritis patients

Abstract

Background: Patients with rheumatoid arthritis (RA) may have more prevalent and severe periodontitis than healthy controls. Periodontitis may increase the systemic inflammation in RA.

Aim: To assess periodontitis prevalence and severity and its potential association with systemic inflammation in Indonesian RA patients.

Methods: A full mouth periodontal examination including probing pocket depth, gingival recession, plaque index and bleeding on probing was performed in 78 Indonesians with RA and 132 Indonesian controls. A validated questionnaire was used to assess smoking, body mass index, education and medical conditions. In addition, in all participants the use of drugs was noted and erythrocyte sedimentation rates and serum levels of high sensitivity test c-reactive protein, rheumatoid factor and anti citrullinated protein antibodies were measured. Differences in periodontitis prevalence and 12 measures of periodontitis severity between RA patients and controls were analyzed using univariate analyses.

Result: There were no significant differences in periodontitis prevalence and 12 measures of periodontitis severity between RA patients and controls, except the number of sites with clinical attachment level ≥3mm, which was significantly larger in controls (p=0.041). On the other hand, there was a tendency towards a lower surface area of healthy pocket epithelium in RA patients versus controls (p=0.052) as well as a trend towards higher high sensitivity c-reactive protein levels in RA patients with severe periodontitis compared to RA patients with no/mild or moderate periodontitis (p=0.087). It has to be noted that all RA subjects used anti-inflammatory drugs, while none of the controls used such drugs.

Conclusion: Prevalence and severity of periodontitis in Indonesian RA patients is comparable to controls, but with a tendency of less healthy pocketepithelium than in controls and a higher inflammatory state in RA subjects with severe periodontitis.

Introduction

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by symmetric inflammation of mainly hand and wrist joints, which leads to permanent deformity and destruction of these joints[1]. RA impairs quality of life and is associated with early mortality. The cause of RA is still unknown[2].
Studies in Australia, America, Europe and Africa have shown that RA patients have more prevalent and severe periodontitis than non-RA controls when controlling for important confounders like dental plaque, age, sex and smoking[3-10]. Studies in Sweden, Brazil, USA, Turkey, and Japan, however, did not find a higher prevalence and severity of periodontitis in RA patients[11-15]. In other words, the prevalence and severity of periodontitis in RA patients may be influenced by genetic, dietary, cultural and other differences associated with differences in nationality and ethnicity[16]. Although Caucasian, African American, Latin American, North-African and Japanese populations have been studied[3-9, 12], no study has yet been performed in a South East Asian population.

In addition to differences in results due to variations in nationality and ethnicity, the association between RA and periodontitis may also differ because a variety of definitions for periodontitis prevalence and severity has been used[17]. Although various studies have assessed the association between periodontitis and RA, no study has yet investigated the effect of using several definitions for periodontitis prevalence and severity on the association between periodontitis and RA.

Recently a few pilot-intervention studies have pointed towards periodontitis as a risk factor for RA[18-21]. After treating periodontitis, a reduction in RA disease activity was shown, possibly related to a reduction in periodontitis associated inflammatory burden. Periodontitis poses an inflammatory burden, as evidenced by increased levels of erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) in RA patients with periodontitis[3-6]. Thus, periodontitis may increase systemic inflammation, which may in turn lead to increase RA severity[17, 22]. Likewise *Helicobacter pylori* infections may contribute to increase RA severity by posing inflammatory burden, while eradication of this bacterium in RA patients improves clinical condition and laboratory markers of RA disease activity[23].

Thus, the aims of this study were to compare periodontitis prevalence according to the case definitions of Page and Eke (2007)[24] and periodontitis severity in Indonesian RA patients with Indonesian controls, using a variety of definitions for periodontitis severity. In addition, it was analyzed whether inflammatory burden differed between participants with or without periodontitis, both for RA patients and controls.

Subjects and methods

Between July 2008 and February 2009, all consecutive RA patients visiting the Internal Medicine Department of the Dr. Sardjito Hospital Yogyakarta, the Clinic for rheumatology of PKU Muhammadiyah Hospital Yogyakarta, or a private rheumatology clinic in Surabaya, Indonesia that matched the inclusion criteria were informed about the nature of the study and were asked to participate. All RA patients had been diagnosed by rheumatologists (NK and JS) according to revised American College of Rheumatology (ACR) 1987 criteria[1] and were on a regular recall schedule for RA. During the same period, controls were recruited from the consecutive patients that visited the Prof. Soedomo Dental Hospital, Faculty of Dentistry Gadjah Mada University, Yogyakarta for their routine dental check-up. Likewise, these controls were informed about the nature of the study and asked to participate. The inclusion criteria for RA patients and controls were an age ≥18 years and having ≥ 8 remaining teeth. The number of 8 remaining teeth was chosen since there should at least be a minimum periodontitis-associated inflammatory burden, given this inflammatory burden decreases with decreasing number of teeth (e.g. edentulous patients don't have any periodontitis-associated inflammatory burden). Exclusion criteria were presence of other systemic diseases or conditions (e.g diabetes) which are known as risk factors for periodontitis[25] and a history of treatment for periodontal disease. With regard to controls, additional exclusion criteria were the use of medication or consuming drugs which are known to be risk factors for periodontitis. This study was approved by the Ethical Committee for Research of the Medical Faculty of Gadjah Mada University, Yogyakarta, Indonesia. Informed consent was obtained from all patients and controls. A ratio of about 1:2 between RA

patients and controls was aimed, but as recruiting of control subjects was limited to the same period as recruiting RA patients, we ended with a 1:1.7 ratio. We considered this ratio as sufficient for the purpose of our study.

All participants had to complete a validated general health assessment questionnaire assessing the presence of other diseases and the use of medication. This questionnaire was composed of questions assessing symptoms related to systemic diseases such as heart disease, pulmonary disease, endocrine disorders, hematologic disease, gastro-intestinal disorders, genitourinary disorders and neurological disease, and the use of medications[25]. Additionally, information about age, sex, smoking (current and pack years), education level and body mass index (BMI) was obtained by means of a questionnaire.

All participants underwent a full mouth periodontal examination on six sites per tooth assessing probing pocket depth (PPD), gingival recession, plaque score, bleeding on probing (BOP) and clinical attachment loss (CAL)[26]. All permanent fully erupted teeth were examined with a manual periodontal colour coded standard probe[*]. Measurements were made in millimetres and were rounded to the nearest whole millimetre. BOP was recorded as either present or absent within 30 seconds of probing. Plaque score was defined as being present or absent at 6 points on each tooth[3] The number of missing teeth was also recorded.

Periodontitis prevalence was established according to Page and Eke (2007)[24] case definitions. Periodontitis severity was operationalized using a variety of methods (number of sites with PPD $\geq$4, $\geq$5 and $\geq$6 mm, number of sites with CAL $\geq$3, $\geq$4, $\geq$5 and $\geq$6 mm, mean PPD, mean CAL and percentage of sites with BOP anywhere in the dentition) all currently used to study the association between periodontitis and other diseases[27-29]. To facilitate calculation a freely accessible spreadsheet[♣] was used online. Furthermore, two recently introduced measures of periodontitis severity, the periodontal epithelial surface area (PESA) and the periodontal inflamed surface area (PISA) [30] were calculated, again using the same spreadsheet[†♣]. PESA reflects

[*] Dentsply, London, United Kindom

[†] www.parsprototo.info; Microsoft Excel 2003 spreadsheet for Windows

the surface area of all pocket epithelium in square millimetres, whereas PISA reflects the surface area of bleeding pocket epithelium in square millimetres. PESA and PISA were calculated using conventional CAL, gingival recession and BOP measurements. PISA quantifies the surface area of inflamed periodontal tissue in square mm. PISA is a measure of inflammatory burden posed by periodontitis[28, 30]

Finally, a blood sample via vena puncture was taken from all participants (RA patients and controls) to determine CRP, ESR, RF, and ACPA. High sensitivity c-reactive protein (hsCRP) was determined by chemiluminescent enzyme immunoassay[‡÷] (Immulite 2000™, Diagnostic Products Corp., Los Angeles, CA, USA), ESR was determined by Westergren, rheumatoid factor (RF) was determined by latex agglutination methods and anti citrullinated protein antibody (ACPA) was determined by an enzyme link immunosorbent assay[§°].

Statistical analysis

Data are presented as mean and standard deviation (SD) in case of normal distribution, medians and interquartile range (IQR) in case of non-normal distribution and percentages for categorical data. Differences between RA patients and controls were analyzed using independent sample t-test or Mann Whitney U, in case of a non-normal distribution, and Chi-square test. Differences between periodontal status groups within RA patients and controls were analyzed using non-parametric analyses, i.e. Kruskall-Wallis and Chi-square tests as appropriate. Statistics were calculated using SPSS 16.0.

Results

Prevalence and severity of periodontitis in RA patients and controls

In total, 78 RA patients (median disease duration 4 years (IQR 2.0; 6.5, range; 1- 15 years) and 132 controls were included. All consecutive RA patients and controls that fulfilled the inclusion criteria during the recruiting period agreed

‡ Immulite 2000™, Diagnostic Products Corp., Los Angeles, CA, USA

§ ELISA; Euroimmun™, Medizinische Labordiagnostika AG, Germany

to participate in this study. The characteristics of the participants, including the use of medication, are summarized in table 1. Mean BMI of RA patients was significantly lower than controls (p<0.001) and there was a tendency towards a significantly higher plaque score in RA patients (p=0.062). None of controls used medication, other than birth control pills or antihypertensives. No significant differences were observed regarding periodontitis prevalence. Periodontal disease severity was significantly lower in RA group compared to control group when defined as number of sites with CAL ≥3 mm. There was a tendency towards a significantly (p=0.052) lower PESA, the surface area of healthy pocket epithelium, for RA patients compared to controls. (table 2).

Serum laboratory markers in RA patients and controls according to periodontal status

RA patients with severe periodontitis had a lower plaque score (p=0.014) and were older (p=0.075) than RA patients with no, mild or moderate periodontitis. There was a trend towards higher hsCRP levels in RA patients with severe periodontitis (p=0.087).

Low education level and smoking were more common among RA patients with severe periodontitis (p-values resp. 0.044 and 0.092). Smoking was more common among controls with severe periodontitis (p-value 0.081) (table 4).

Table 1. Characteristic of RA patients and controls including factors associated with periodontitis severity.

Patient characteristics	RA patients (n=78)	Controls (n=132)	p value	Difference (95% CI)
Sex (woman) n (%)	62 (80%)	98 (74%)	0.389*	
Education (low) n (%)	46 (59%)	83 (63%)	0.574*	
Java origin (yes) n (%)	66 (85%)	119 (90%)	0.231*	
Smoking (yes) n (%)	5 (6%)	14 (11%)	0.306*	
Age (yr) mean (SD)	45.5 (12.2)	47.9 (10.1)	0.145†	2.4 (-0.84 to 5.6)
BMI (kg/m^2) mean (SD)	22.3 (3.8)	24.4 (3.8)	<0.001†	2.2 (1.1 to 3.3)
Number of teeth median (IQR)	27 (22; 29)	27 (24; 29)	0.379‡	

Plaque score (%) mean (SD)	97 (94; 99)	96 (92; 98)	0.062‡
Medication:	76%	0%	
- Corticosteroid (prednisolone)	67%	0%	
- Methotrexate	50%	0%	
- Cloroquine	37%	0%	
- NSAIDs (diclofenac)	23%	0%	
- Meloxicam	9%	0%	
- Sulfasalazin	8%	0%	
- Leflunomide	5%	0%	
- Celecoxib	6%	8%	
- Antihypertensives	0%	1%	
- Birth control pills			

*Results of Chi-square test; †results are the results of the independent samples t-test; 95% CI: 95% confidence interval; ‡result of Mann-Whitney U test; BMI: body mass index; Education: low: elementary, junior & high school; p: probability; RA: rheumatoid arthritis; SD: standard deviation; yr: year

Table 2. Differences in periodontitis prevalence and severity between RA patients and controls.

Variables	RA patients (n=78)	Controls (n=132)	p value	Difference (95% CI)
Periodontitis prevalence	n (%)	n (%)		
- No/mild periodontitis§	25 (32%)	35 (27%)	0.420*	
- Moderate periodontitis	37 (47%)	75 (57%)		
- Severe periodontitis	16 (21%)	22 (17%)		
Periodontitis severity: mean (SD)				
PESA (mm^2)	816.7 (305.8)	892.2 (247.1)	0.052†	75.5 (-151.6 to 0.6)
PISA (mm^2)	96 (42; 176)	84 (35; 206)	0.832‡	
Number of sites:				
CAL ≥ 3 mm	22 (9; 41)	30 (17; 47)	0.041‡	
CAL ≥ 4 mm	5 (1; 15)	5 (1; 16)	0.872‡	
CAL ≥ 5 mm	1 (0; 5)	1 (0; 5)	0.838‡	
CAL ≥ 6 mm	0 (0; 1)	0 (0; 1)	0.688‡	
PPD ≥ 4 mm	1 (0; 4)	2 (0; 4)	0.296‡	
PPD ≥ 5 mm	0 (0; 1)	0 (0; 1)	0.632‡	
PPD ≥ 6 mm	0 (0; 0)	0 (0; 0)	0.709‡	
BOP	10 (6; 20)	9 (4; 20)	0.255‡	
PPD (mm)	1.7 (0.4))	1.8 (0.3)	0.148†	0.07 (-1.2 to 0.03)
CAL (mm)	2.0 (0.8)	2.0 (0.7)	0.841†	0.02 (-0.2 to 0.2)

*Result of Chi-square test; †results of the independent samples t-test; ‡result of Mann-Whitney U test; §According to the criteria of Page and Eke (2007); 95% CI: 95% confidence interval; BOP: bleeding on probing; CAL: clinical attachment loss; p: probability; PESA: periodontal epithelium surface area; PISA: periodontal inflammation surface area; PPD: probing pocket depth; RA: rheumatoid arthritis; SD: standard deviation

Table 3. Biomarkers and characteristics of RA patients according to periodontal status.

	No or mild Periodontitis (n=25)		Moderate Periodontitis (n=37)		Severe Periodontitis (n=16)		p-value
	Med	IQR	Med	IQR	Med	IQR	
Age in years	42.0	29.0; 51.0	49.0	39.0; 54.0	50.5	43.0; 55.5	0.075
BMI	21.0	19.4; 25.0	22.2	19.8; 25.2	22.6	20.2; 24.6	0.828
Plaque score (%)	98.0	96.0; 99.0	97.0	95.0; 98.0	92.5	91.0; 97.5	0.014
RA Since (years)	5.5	2.0; 10.0	4.0	2.0; 6.0	3.0	2.0; 5.0	0.222
ACPA igG (RU/ml)	1.2	0.0; 46.1	0.0	0.0; 31.6	1.5	0.0; 78.4	0.588
ESR (mm/hour)	30.0	17.0; 40.0	36.0	18.0; 60.0	41.5	24.0; 69.5	0.399
hsCRP (mg/l)	4.4	2.6; 6.6	3.3	0.8; 24.9	15.4	6.3; 21.8	0.087
	%	N	%	N	%	n	
Gender (women)	84%	21	84%	31	63%	10	0.134
Education (low)	40%	10	68%	25	69%	11	0.044
Smoking (yes)	0%	0	8%	3	13%	2	0.092
RF (yes)	36%	9	16%	6	38%	6	0.838

Med: median; IQR: Interquartile range; %: collum percentage. Differences in medians are tested using Kruskal-Wallis test. Difference in percentages are tested using chi- square analyses (Linear by linear association), except differences in percentages of smokers, which is tested exactly because of expected cell count was not sufficient to meet the assumptions of the chi- square test. ACPA: anti citrullinated protein antibody; BMI: body mass index; hsCRP: high sensitivity c-reactive protein; ESR: erythrocyte sedimentation rate; hr: hour; n: number; p: probability; RU: relative unit; RF: rheumatoid factor; n: number

Table 4. Biomarkers and characteristics of controls according to periodontal status.

	No or mild Periodontitis		Moderate Periodontitis		Severe Periodontitis		p-value
	n=35		n=75		n=22		
	Med	IQR	Med	IQR	Med	IQR	
Age (years)	46.0	40.0; 54.0	46.0	41.0; 54.0	49.5	45.0; 56.0	0.346
BMI	23.9	21.1; 26.2	24.6	22.1; 26.7	24.6	20.4; 28.1	0.771
Plaque score (%)	97.0	95.0; 99.0	96.0	91.0; 98.0	95.0	89.0; 98.0	0.132
ACPA igG (RU/ml)	0.0	0.0; 0.6	0.0	0.0; 1.0	0.0	0.0; 0.0	0.967
ESR (mm/hour)	20.0	12.0; 35.0	23.0	13.0; 35.0	23.5	15.0; 39.0	0.572
hsCRP (mg/l)	0.9	0.5; 1.7	1.2	0.6; 2.5	1.4	0.6; 3.4	0.152
	%	N	%	n	%	n	
Sex (women)	77%	27	71%	53	82%	18	0.880
SES (low)	74%	26	59%	44	59%	13	0.183
Smoking (yes)	3%	1	12%	9	18%	4	0.081
RF (yes)	6%	2	0%	0	0%	0	0.096

Med: median; IQR: Interquartile range, %: collum percentage. Differences in medians are tested using Kruskal-Wallis test. Difference in percentages are tested using chi- square analyses (Linear by linear association), except differences in percentages of smokers, which is tested exactly because of expected cell count was not sufficient to meet the assumptions of the chi- square test. ACPA: anti citrullinated protein antibody; BMI: body mass index; hsCRP: high sensitivity c-reactive protein; ESR: erythrocyte sedimentation rate; hr: hour; n: number; p: probability; RU: relative unit; RF: rheumatoid factor; n: number

Discussion

The prevalence of moderate to severe periodontitis in RA patients in the current study is fairly high (68%), but similar to those reported in other studies[3, 9]. However, the prevalence of moderate to severe periodontitis in controls was also high (73%) and not significantly different. Likewise, some other studies reported no significant differences in periodontitis prevalence between RA patients and controls[11-15]. The vast majority of studies did find a higher prevalence of periodontitis in RA patients as compared to controls[3, 7-9], however. Furthermore, an association between periodontitis and RA also has been shown in animal studies[31-33]. Both human and animal studies point towards an association between periodontitis and RA that might

be due to sharing similar inflammatory markers, viz. increased cytokines, matrix metalloproteinase and c-reactive proteins.

The fact that the number of sites with CAL ≥3mm was significantly higher in Indonesian controls than in RA patients is apparently in sharp contrast with the above mentioned studies since it points towards healthy controls having more severe periodontitis than RA patients. However, the cut-off point of CAL ≥3mm is low and could even be considered as borderline pathology. Thus, the number of sites with CAL ≥3mm could simply be higher because RA patients were older than controls, given CAL increases with age. Furthermore, the surface area of healthy pocket epithelium (PESA) trended to be lower in RA patients than controls (p=0.052), pointing towards more severe periodontitis in RA patients. Another possible explanation for our findings may be the remarkably high periodontitis prevalence in Indonesian controls. This is not surprising, as the prevalence of periodontitis in Indonesian general population may, depending on the definition used, has been reported to be as high as 80%[34]. Furthermore, our findings might also in part be explained by the use of medication in RA patients. Since drugs used for RA reduce periodontitis severity[12]. More than half of the RA patients in our study were on corticosteroids (table 1). Corticosteroids have anti inflammatory activities by inhibiting proinflammatory protein as cyclooxygenase 2, interleukins (IL) 1, 2 and 6, tumor necrosis factor alpha and adhesion molecules[35]. Some RA patients used diclofenac (non-steroidal anti-inflammatory drug; NSAID). Diclophenac has anti-inflammatory activity inhibiting cyclooxygenase, an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins and tromboxanes. NSAIDs also have been shown to reduce alveolar bone loss in periodontitis[36, 37]. A high number of RA patients (table 1) also used disease modifying anti-rheumatoid drugs (DMARD) such as methotrexate, sulfasalazine, chloroquine, leflunomide. These drugs are taken to reduce the inflammatory component in RA[36]. Methotrexate in combinations with prednisolone decrease blood levels of Il-1β and IL-6 and inhibits the intensity of free radical-mediated processes in RA[38], which also may decrease periodontal inflammation.

Noteworthy is the fact that there was a tendency towards higher hsCRP levels in RA patients with moderate to severe periodontitis compared to those with no/mild periodontitis. Other studies also found higher levels of hsCRP in RA patients with periodontitis[3, 6]. No significant difference in hsCRP was observed between controls

with periodontitis and controls without periodontitis (table 4). Two main explanations can be given for these findings. Firstly, periodontitis may aggravate RA. Since periodontitis is accompanied by higher CRP levels[39-43]. The elevation of inflammatory cytokines (such as IL-1, IL-6) that are locally induced by periodontitis [44] is thought to induce systemic inflammation by increasing serum CRP levels and thus to contribute to an increased systemic inflammation in RA[45]. Secondly, RA may aggravate periodontitis. Since more severe RA is also accompanied by higher CRP levels[45, 46], higher CRP levels may be a reflection of active RA, which may contribute to an increased inflammatory state in periodontitis. Interestingly, CRP level reduces in RA patients after periodontal therapy[21], lending support to the hypothesis that periodontitis may contribute to an increased systemic inflammation in RA.

Another explanation of higher CRP levels in RA patients with moderate to severe periodontitis compared to RA patients with no/mild periodontitis may be confounding by impaired maintenance of oral hygiene, smoking and low education level. Regarding oral hygiene, RA affects the wrist joint and the small joints of the hand. The joint afflictions may impair motor function of the hand and as a result may impair maintenance of proper oral hygiene resulting in periodontitis[47]. However, RA patients with moderate to severe periodontitis had lower mean plaque score than RA patients with no/mild periodontitis. Therefore, impaired maintenance of oral hygiene in RA patients has probably not confounded the association between periodontitis and higher hsCRP levels.

Smoking and low education level on the other hand may be negatively associated with general health and may thus confound the observed association between moderate to severe periodontitis and increased hsCRP levels in RA patients.
Periodontitis prevalence and severity are not higher in Indonesian RA patients than in controls. However, the presence of moderate to severe periodontitis in RA patients appears to be associated with an increased hsCRP level. Recognition of the fact that periodontitis could promote a whole that is greater than the sum of its parts, i.e. a combination of hyperinflammation, hypercoagulation, hyperglycaemia, hypertriglyceridaemia and hypertension, could lead to new insights into the causes of cardiovascular and auto-immune diseases including RA.

Acknowledgement

We would like to thank the following institutions for their cooperation in Yogyakarta, Indonesia: the Internal Medicine Department and the Dental Clinic of the Dr. Sardjito Hospital, Clinic for rheumatology of PKU Muhammadiyah Hospital Yogyakarta, the Prof. Soedomo Dental Hospital of the Faculty of Dentistry of Gadjah Mada University, Yogyakarta and the Prodia Laboratory, Yogyakarta.

Disclaimers and sources of support

The authors declare that they have no conflict of interest. Funding has been made available from the authors` institutions. This study is supported by a Bernouille Bursary from the University Medical Center Groningen, University of Groningen.

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Chapter 5

Treatment of periodontal disease improves the atherosclerotic profile: A systematic review and meta-analysis

Abstract

Background - We designed a systematic review and meta-analysis to study the robustness of observations that treatment for periodontitis improves the cardiovascular risk profile.

Methods and Results - A literature search (until January 2012) was performed. Selection of publications was based on two criteria: 1) original investigations, and 2) controlled periodontal intervention trials where the non-intervention group received no treatment. An independent screening of 3463 unique titles and abstracts (retrieved from three libraries) resulted in 14 publications, representing 19 intervention trials that met the eligibility criteria. These studies enrolled 1034 periodontitis patients. Seven trials enrolled periodontitis patients that were otherwise healthy, whereas 12 trials recruited periodontal patients also suffering from cardiovascular diseases (CVD) and/or metabolic disorders. In our meta-analysis, periodontitis was the predictor and inflammatory, metabolic and/or vascular markers were defined as outcomes. The meta-analysis, that included all available trials, demonstrated significant weighted mean differences (WMD) for hsCRP (-0.43 mg/L, CI: -0.70;-0.16; p=0.002), total cholesterol (-0.14 mM, CI: -0.24;-0.03; p=0.009) and HbA_{1c} (-0.45%, CI:-0.70; -0.19; p=0.0006) favoring periodontal intervention. Importantly, periodontitis patients suffering from CVD and/or metabolic disorders benefited most from periodontal therapy; significant WMD were observed for levels of hsCRP (-0.62 mg/L, CI:-0.97;-0.27; p=0.0005), IL-6 (-1.15 ng/L, CI:-1.63;-0.67; p<0.00001), total cholesterol (-0.15 mM, CI:-0.29;-0.01; p=0.03) and HbA_{1c} (-0.45%, CI:-0.70;-0.19, p=0.0006).

Conclusions - Our systematic review and meta-analysis demonstrates that periodontal treatment reduces biomarkers of atherosclerotic disease in periodontitis subjects, especially in those already suffering from CVD and/or metabolic disorders. This emphasizes the need for periodontal diagnosis and therapy in atherosclerotic and diabetic individuals to improve their cardiovascular risk profile to prevent the future occurrence of CVD events.

Introduction

A large number of trials have reported on the relationship between periodontal (PD) and cardiovascular disease (CVD).[1] PD is a common chronic multifactorial infectious disease of the supporting structures of the teeth (periodontal ligament and alveolar bone) and a major cause of tooth loss. This condition occurs in about 10-15% of the population. The increase in relative risk estimates for developing CVD in subjects with PD ranges from 1.24 to 1.34.[2] These estimates increase to 1.44 in subjects younger than 65 years of age.[3] In addition, the risk for stroke in PD patients is estimated to be 1.85 compared to subjects without PD.[3-4] Several causal mechanisms have been proposed whereby bacterial pathogens, antigens, endotoxins, and/or inflammatory cytokines from periodontal lesions in the oral cavity contribute to the process of atherogenesis as well as to thromboembolic events and thereby increase the risk for CVD.[1-2, 5-6] Treatment of periodontitis includes mechanical removal of supra- and subgingival bacterial plaque deposits with scalers, curettes and ultrasonic devices (scaling and root planing [SRP]) and intensive oral hygiene instructions. Optimal oral hygiene is the only way to prevent formation of a new dental plaque biofilm on the roots of the teeth and re-infection of the supporting tissue of the teeth. Albeit effective in selected cases, the routine use of systemic or local antibiotics as an adjunctive therapy to SRP is still controversial.[7-9] Regularly, periodontal surgery is needed to reduce or eliminate residual periodontitis lesions.

In the last two decades, clinical studies have investigated the effect of periodontal therapy on primary and/or secondary outcomes of CVD. These trials were often uncontrolled, provided conflicting data and many report only short-term results. However, because of the chronic nature of CVD, may be only long-term results (e.g. 6 month follow-up) of periodontal intervention should be considered. Therefore, we hypothesized that, if periodontitis is somehow causally related to the common atherosclerotic form of CVD, then periodontal treatment should effect primary or surrogate parameters for CVD, including inflammatory and thrombotic markers, markers for lipid and glucose metabolism, and vascular markers. We therefore performed a systematic review of clinical intervention trials (RCT/CCT designs) to assess the question whether periodontal treatment affects surrogate parameters of cardiovascular risk.

Methods

This systematic review was conducted in accordance with the guidelines of Transparent Reporting of Systematic Reviews and Meta-Analyses [PRISMA statement[10]]. The focused question was as follows: What is the effect of periodontal therapy on cardiovascular disease and/or surrogate markers in patients suffering from periodontitis?

Search Strategy

Three literature databases were used to search for appropriate papers that satisfied the study purpose. These included the National Library of Medicine, Washington, D.C. (Medline-PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL) and EMBASE (Excerpta Medical Database by Elsevier). The databases were searched for trials conducted in the period up to and including January 31, 2012. The structured search strategy was designed to include any publication that evaluated the effect of periodontal therapy on cardiovascular disease markers (for detail on the used search terms, see BOX 1). The following eligibility criteria for publications were used:

(a) Original Clinical Trials

(b) Randomized Controlled Trials (RCT's) or Controlled Clinical Trials (CCT's), containing the following aspects:

- Human subjects with periodontitis
- Intervention group receiving periodontal treatment and a non-intervention group receiving no periodontal treatment
- Outcome parameters must be clinical CVD parameters (i.e. clinical event, such as angina pectoris, myocardial infarction, stroke, death) and/or surrogate CVD markers, including systemic inflammation and thrombosis, lipid and glucose metabolism and vascular function.

Box 1. Search terms used for PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE. The search strategy was customized according to the database being searched.

Search terms:
{(periodontal diseases [MesH] OR periodontal disease OR periodontitis OR periodontal infection OR periodont* [textword])

AND

(cardiovascular diseases [MesH] OR cardi OR cardiac disease OR stroke OR cerebro OR cerebrovascular accident OR stroke OR Atherosclerosis OR arthero* OR myocardial* OR Myocardial ischemia OR myocardial disease OR chronic heart disease OR cardiovascular disease OR cardio* OR acute myocardial infarction OR coronary vascular disease OR peripheral arterial disease OR CRP OR c-reactive protein OR inflammatory cytokine OR cytokine* OR interleukin OR IL-* OR Endothelial OR endothelial function OR triglyceride OR cholesterol OR flow mediated dilation OR fmd OR imt OR intima media thickness [textword]).

AND

(therapy OR therapeutics OR intervention trials [Mesh]) OR (treatment OR therapy OR therapeutics OR intervention [textword])}

Limits:
Human
Controlled study

Screening and Selection

Two reviewers (W.J.T. and H.S.) independently screened titles and abstracts for eligible papers. If information relevant to the eligibility criteria was not available in the abstract, or if the title was relevant but the abstract was not available, the paper was selected for full reading of the text. Next, full-text papers that fulfilled the eligibility criteria were identified and included into this study. The two reviewers hand-searched the reference lists of all the selected trials for additional published papers that could possibly meet the eligibility criteria of this study. Papers that fulfilled all of the selection criteria were processed for data extraction.

Heterogeneity of trials.

The heterogeneity across the trials was detailed according to the following factors:

- Population characteristics
- Study design
- Definition of diseases
- Type of periodontal intervention

Quality Assessment

Two reviewers (W.J.T. and D.E.S.) scored the methodological quality of the included trials, as proposed by Van der Weijden et al.[11]

Data Extraction

Data from the papers that met the selection criteria were processed for analysis. Outcomes for CVD and/or surrogate markers were extracted with regard to the effects of periodontal therapy in comparison to no periodontal therapy. For the trials that presented intermediate assessments, the baseline and final evaluations were used for this review. All data were extracted by W.J.T. and D.E.S. If some mean values and standard deviations of outcome data could not be extracted from a paper (e.g. data were presented in a graph, median values were used, etc.) the corresponding author of the publication were asked to provide the requested data.

Data Analysis

A descriptive manner of data presentation was used for all trials included in the analyses. Where appropriate, a meta-analysis was performed for trials in which the 'intervention' group received periodontal treatment and the 'non-intervention' group

received no periodontal therapy. When not reported in the publication, the difference (Δ) in mean values of a given parameter (x) between baseline and end, was calculated for both the 'intervention' and 'non-intervention' groups by with the formula:[12]

$$\Delta x = x_{end} - x_{baseline}$$

Further, if not reported for some trials, the variance (S) of Δx was estimated as follows:

$$S_{\Delta x} = (SD_{x\,end})^2 + (SD_{x\,baseline})^2 - 2r \cdot SD_{x\,end} \cdot SD_{x\,baseline}$$

where SD is the reported standard deviation and r is the correlation between the baseline and end values; we assumed r to be 0.5 as was used by us before.[13] The $SD_{\Delta x}$ was calculated as $\sqrt{S_{\Delta x}}$. Subsequently from the Δx and corresponding $SD_{\Delta x}$ and seize of study population, weighted mean difference (WMD) values between intervention and non-intervention at both baseline and end were calculated using a *random* (≥ 5 trials included) or *fixed effect* model (2-4 trials included) (Review Manager (RevMan) [Computer program] Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011).

Grading the 'Body of Evidence'

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system as proposed by the GRADE working group[14] was used for grading evidence merging from this review. Two reviewers (W.J.T. and D.E.S.) rated the quality of the evidence and strength of recommendations on the following aspects: risk of bias of the individual trials, consistency and precision among the study outcomes, directness of the study results and the detection of publication bias. Any disagreement between the two reviewers was resolved after additional discussion.

Results

Search and Selection Results

The combined MEDLINE (via PubMed), Cochrane CENTRAL and EMBASE searches resulted in 3464 potentially eligible manuscripts (Figure 1). Subsequently, 14 publications were identified as eligible for inclusion in this review according to the defined criteria for the study design, participants, intervention and outcome. Of these 14 studies, 1 article described 3 different treatment groups together with 3 different non-intervention groups using the same study design,[15] 1 article described 2 different trials together with 2 different non-intervention groups using a different study design[16] and 2 articles used 2 different treatment groups with 1 non-intervention group and 1 study design.[17-18] Therefore, all together, 19 trials were identified as eligible for inclusion in this review and were processed for data extraction.

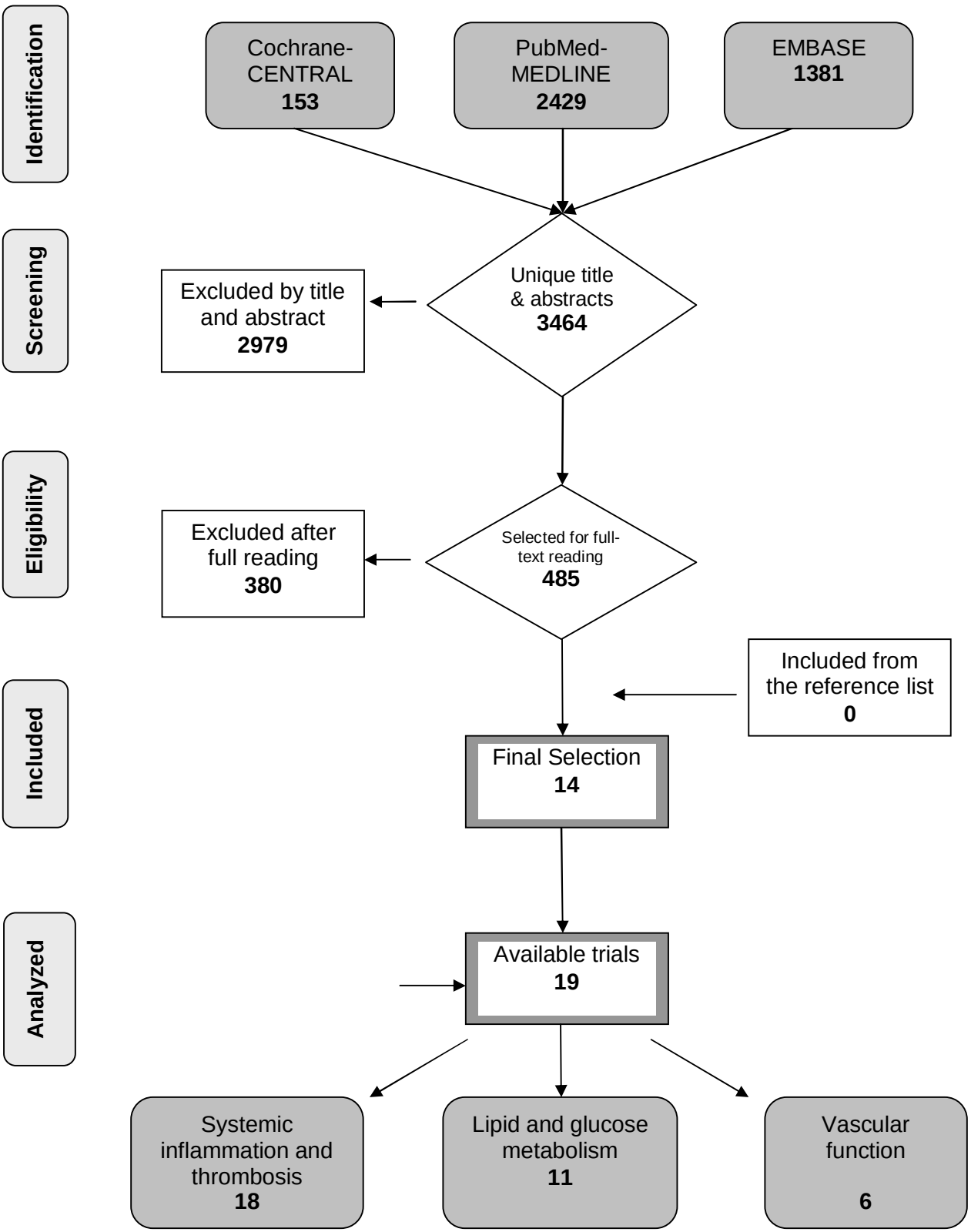


Figure 1. Flowchart of literature search and study selection

General trial characteristics

Detailed information regarding the trial characteristics, including study population, periodontal treatment and the medical and oral status of the subjects, is summarized in Supplemental Table 1. All trials described a study population suffering from periodontitis. Some of the trials used an otherwise healthy population,[16, 18-22] whereas other trials described a diseased population. The systemic diseases can be divided in the following categories: i) CVD (ideopathic edema,[23] coronary artery disease,[24]), ii) a metabolic disorder (diabetes mellitus,[15, 17, 25-26] metabolic syndrome,[27] impaired glucose tolerance,[15] hypertension,[16] hypercholesterolemia[28]) or iii) a combination of CVD and a metabolic disorder.[15]

All subjects in the intervention group received SRP, whereas no subject of the non-intervention group received any form of periodontal therapy. In 14 trials adjunctive local or systemic administration of antibiotics or antiseptics, extraction of hopeless teeth and/or periodontal surgery in addition to SRP were applied.[15-16, 18, 21-25, 27-28] The follow-up time among the 19 included trials ranged from 4 weeks to 12 months.

Further, the authors' estimated risk of bias is formulated and also included in Supplemental Table 1. Details of quality assessment values, including the internal, external, and statistical validity, are presented in Supplemental Table 2.

Study outcomes

None of the trials used a *real* endpoint for CVD as the outcome parameter (i.e. clinical event, such as angina pectoris, myocardial infarction, stroke, death). The outcome parameters of all trials were known as *surrogate* endpoint markers for CVD and can be divided in three groups: systemic inflammation and thrombosis, lipid and glucose metabolism and vascular function.

Periodontal intervention and systemic inflammation and thrombosis.

Eighteen of the 19 trials reported changes in hsCRP levels as risk marker for CVD. Eleven trials showed a significant decrease of hsCRP levels in the treatment group after periodontal treatment compared with baseline levels.[15-17, 20, 23-24, 27] In addition, two of these trials showed also a significant decrease in the non-intervention group compared to baseline levels after the study period.[23, 27] The majority of the trials that showed a decrease in hsCRP levels after periodontal treatment used a systemically diseased population.[15 , 16-17, 23-25, 27] Baseline levels of hsCRP for the experimental groups ranged from 1.0 ± 0.8 mg/L to 12.5 ±

15.9 mg/L, and the baseline values for the non-intervention groups ranged from 0.8 ± 1.0 mg/L to 6.6 ± 1.2 mg/L (Supplemental Table 3A). The significant reduction of hsCRP levels after periodontal treatment compared to baseline ranged from -0.5 ± 0.7 mg/L to -8.8 ± 13.7 mg/L. No treatment group showed an significant increase in hsCRP levels compared to baseline levels after the study period.

Similar results as reported above were obtained by 10 trials using IL-6 as an outcome parameter. Six trials showed a significant decrease in IL-6 levels after periodontal treatment compared to baseline levels.[15-16, 18, 24] This significant reduction ranged from -0.4 ± 0.8 ng/L to -1.4 ± 1.5 ng/L and was more often seen in systemically diseased subjects.[15-16, 24] No treatment group showed a significant increase in IL-6 levels compared to baseline levels after the study period (Supplemental Table 3B).

Five trials used TNF-α as an outcome to study the effect of periodontal treatment on the inflammatory state. Two trials showed a significant decrease in TNF-α levels after periodontal treatment compared to baseline levels (-1.4 ± 1.2 pg/L, -1.5 ± 2.2 pg/L[15]) (Supplemental Table 3C). Both trials used a systemically diseased population.

Several other inflammatory markers have been used as study outcome to determine the effect of periodontal treatment on the systemic inflammatory state. In summary, IL-1β,[19] sialic acid,[19] soluble E-selectin (sE-selectin),[22] number of leukocytes[21-22] and number of lymphocytes.[21] Only the levels of sE-selectin were slightly reduced after periodontal treatment compared to the non-treatment group.[22] Regarding the other markers, no change was observed after periodontal treatment.

Some trials also used markers reflecting the pro-thrombotic state, like fibrinogen,[19, 27] Von Willibrand factor,[22] plasminogen activator inhibitor-1 (PAI)-1[22] and tissue plasminogen activator (tPA).[22] No changes in these surrogate parameters after periodontal treatment were observed in 2 available trials.[19, 22] In contrast, 1 trial showed a slight reduction in fibrinogen levels after periodontal treatment compared to baseline levels, but this was not significantly different from the non-treatment group.[27]

Periodontal intervention and lipid and glucose metabolism

Eleven trials used at least 4 different lipid parameters (triglycerides, total cholesterol [TC], high density and low density lipoproteins [HDL-C and LDL-C, respectively]) to study the effect of periodontal treatment on lipid metabolism. One trial showed a significant decrease

of triglycerides levels after periodontal treatment compared to baseline levels (-0.8 ± 1.5 mmol/L),[17] while one trial showed a significant increase compared to baseline levels for this biochemical parameter in the non-intervention group after the study period (0.2 ± 0.7 mmol/L), whereas the treatment group showed no difference (0.0 ± 1.3 mmol/L)[28] (Supplemental Table 3D). Further, two trials showed a significant reduction in TC levels after periodontal treatment compared to baseline, whereas the non-intervention group showed no significant change after the study period. The reduction in both trials was similar (-0.3 ± 0.7 mmol/L[18]and -0.3 ± 0.3 mmol/L)[28] (Supplemental Table 3E). One article described in 2 intervention groups and 1 non-intervention group, a significant reduction in HDL-C levels compared to baseline levels, regardless the type of periodontal treatment (Range ΔHDL-C: -0.2 ± 0.5 mmol/L to -0.1 ± 0.4 mmol/L)[17] and one trial demonstrated a significant reduction for HDL-C in the non-intervention group after the study period compared to baseline (-0.03 ± 0.1 mmol/L)[28] (Supplemental Table 3F). The latter two publications showed significant reductions of LDL levels after periodontal treatment compared with baseline levels;[17, 28] notably, one trial showed also a reduction of LDL-C in the non-treatment group after the study period (Supplemental Table 3G).[17] No trial showed an increase after periodontal treatment compared to baseline for any of the levels of lipid biomarkers. Some trials reported on other lipid markers, including Very Low Density Lipoprotein (VLDL-C),[28] Apoliproteins, Lipoproteins[21] and Malondialdehyde-LDL-C,[24] however no significant changes were observed after periodontal treatment compared to baseline levels and the non-treatment group.

Several trials used markers reflecting glucose metabolism. The main marker used in diabetes care and related to CVD is the percentage of glycosylated hemoglobin (HbA_{1c}). Five trials, using a diabetes study population, reported absolute changes in HbA_{1c} after periodontal treatment. Four trials showed a significant reduction in HbA_{1c} after periodontal treatment compared to baseline levels and the non-intervention group receiving no periodontal treatment (Range ΔHbA_{1c}: -0.72 ± 0.93 % to -0.42 ± 1.39 %)[15, 17, 26] (Supplemental Table 3H). The majority of the trials showed no differences in glucose levels after periodontal treatment compared to baseline levels and no treatment,[16, 22, 24-25] while only one trial showed a significant decrease in glucose levels after periodontal treatment and intensive maintenance period for 6 months compared to baseline levels (-0.73 mmol/L).[17] Other markers, like albumin[23] and insulin[16, 24] did not change after periodontal treatment. One article, with 3 trials included, described the effect of periodontal treatment

on adiponectin levels.[15] The authors showed that after periodontal treatment the levels of adiponectin were increased in a population diagnosed with impaired glucose tolerance or diabetes mellitus.[15] In a study population with diabetes mellitus together with CVD no changes after periodontal treatment were observed.[15]

Periodontal intervention and vascular function

The primary outcome of several trials was vascular function, assessed through the effects of periodontal treatment on endothelial function. One trial showed that periodontal treatment positively affected the endothelial function as demonstrated by an increase in flow-mediated dilation (FMD) compared to the non-treated group after 6 months (2.0%; CI: 1.2-2.9).[22] Comparable results were described in 3 other trials.[16, 24] Forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, after periodontal treatment were also evaluated. All trials showed that ACh-FBF was significantly increased in the periodontal treatment groups compared to baseline, whereas the non-treatment groups showed no differences.[16, 24] Furthermore, one trial described a reduction of $CD34^+$ cells after periodontal treatment; these are endothelial progenitor cells and closely related to the risk of CVD.[21]

Other markers of vascular function, like nitroglycerine-mediated dilation, brachial-artery diameter, reactive hyperemia ratio,[22] FBF, sodium-nitroprusside-dependent FBF, ACH-FBF in the presence of a NO-synthase-inhibitor[16, 24] and pulse amplitude tonometry,[21] systolic and diastolic blood pressure[16, 22, 24, 27] (Supplemental Table 3I) did not change after periodontal treatment compared to baseline levels of the treatment group and/or end levels of the non-treatment group.

Meta-analyses

The effect of periodontal treatment on primary or secondary markers of inflammation (hsCRP, IL-6, TNF-α), lipid and glucose metabolism (triglycerides, TC, HDL-C, LDL-C and HbA_{1c}) and vascular function (systolic and diastolic blood pressure) could be analyzed in a series of meta-analyses. The weighted mean differences (WMD) and 95% confidence intervals (CI) baseline to end between intervention groups and non-intervention groups for each parameter were calculated (Supplemental Figures 1-12) and summarized in Table 1. A significant WMD including all trials was found for hsCRP (-0.43 mg/L), TC (-0.14 mmol/L) and HbA_{1c} (-0.45%). Notably, sub-analyses showed that periodontitis patients suffering from cardiovascular and/or metabolic disease benefited the most from periodontal therapy;

significant WMD were observed for hsCRP (-0.62 mg/L, CI:-0.97;-0.27; *p*=0.0005), IL-6 (-1.15 ng/L, CI:-1.63;-0.67; *p*<0.00001), TC (-0.15 mM, CI:-0.29;-0.01; *p*=0.03) and HbA_{1c} (-0.45%, CI:-0.70;-0.19; *p*=0.0006). Significant WMD were not observed in systemically healthy periodontitis patients. Figure 2 depicts the WMD for hsCRP of all studies and sub-analyses.

In trials which recruited periodontitis patients with cardiovascular and/or metabolic disease, we observed differences in WMD for studies with different follow-up periods (Supplemental Table 4). For example, 4 trials[15, 23] with <6 months duration, showed for hsCRP a WMD of -0.81 mg/L (CI:-1.06 ; -0.56; *p*<0.00001), while another 7 trials[16-17, 24-27] with ≥6 months follow-up showed for hsCRP a WMD of -0.24 mg/L (CI:-0.47 ; -0.01; *p*=0.04). For Il-6, a significant WMD was found for 3 trials[15] with <6 months follow-up (-1.16 ng/L, CI:-1.66 ; -0.66; p<0.00001).

With the available trials, no significant WMD were observed regarding triglycerides, HDL-C, LDL-C, systolic and diastolic blood pressure.

Grading the 'Body of Evidence'

Since the data regarding the inflammatory markers and glucose metabolism is consistent (Supplemental Table 5) with on average a low to moderate risk of bias, the precision is good and the study results are general applicable. The quality of 'Body of Evidence' that periodontal treatment affects levels of systemic inflammatory markers and glucose metabolism is high (Supplemental Table 6). Because of the inconsistent data regarding lipid metabolism and blood pressure with on average a moderate to high risk of bias, the precision is moderate and the study results cannot be generalized. The quality of 'Body of Evidence' that periodontal treatment does not affect lipid metabolism and blood pressure is moderate.

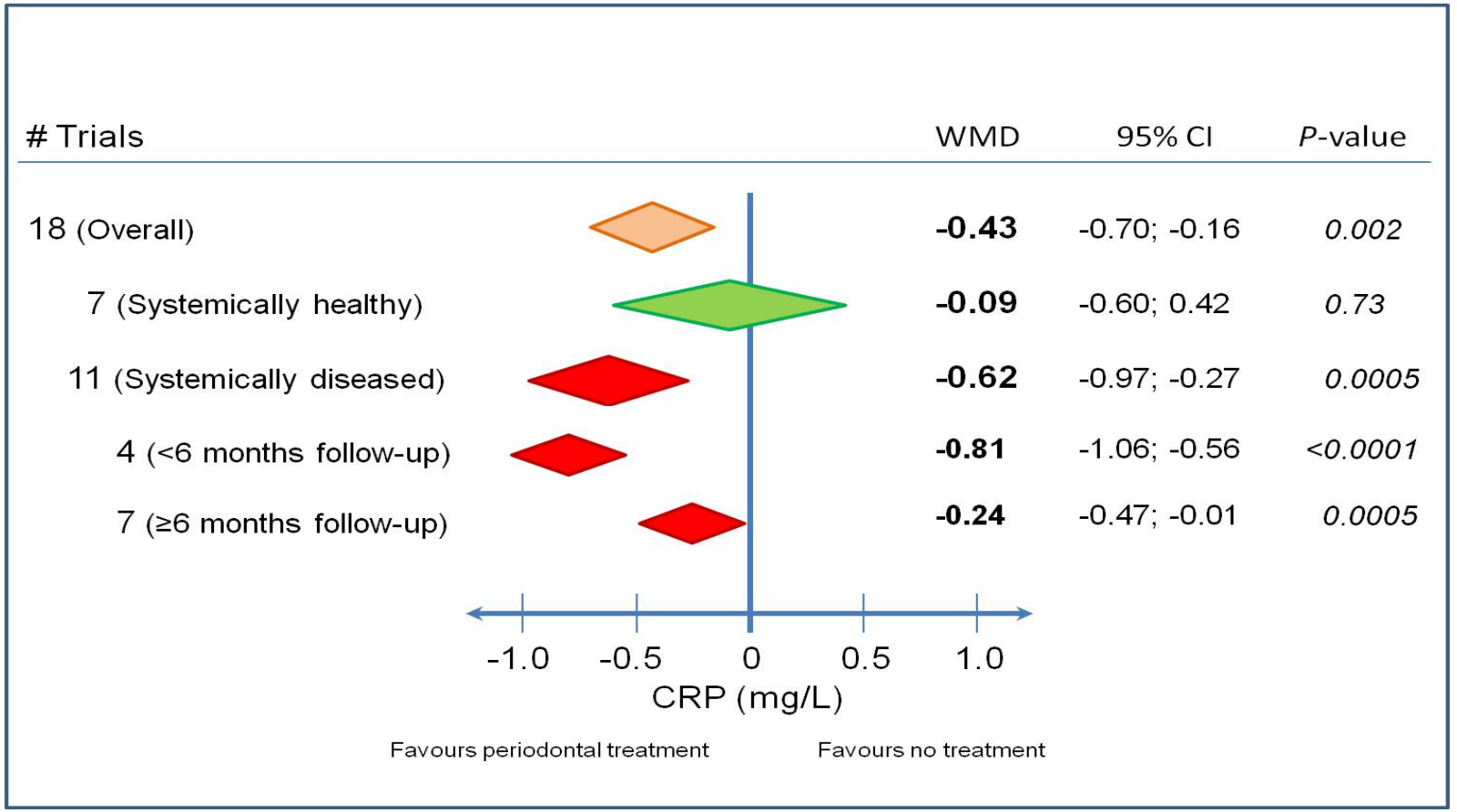
Table 1. Results of meta-analyses of selected biomarkers.

Parameter of interest	Group	References for included trials	Model[1]	# subjects		WMD	95% CI	Test for overall effect (*p*-value)	Test for heterogeneity			
				I	C					*p*-value	I^2 -value %	
CRP (mg/L)	**Overall**		R	545	451	-0.43	[-0.70 ; -0.16]	0.002	0.002	55		
	Non diseased	16, 18-22	R	182	154	-0.09	[-0.60 ; 0.42]	0.73	0.06	51		
	Diseased	15-17, 23-27	R	363	297	-0.62	[-0.97 ; -0.27]	0.0005	0.005	60		
IL-6 (ng/L)	**Overall**		R	258	222	-0.38	[-0.88 ; 0.13]	0.14	0.01	57		
	Non diseased	16, 18-19, 22	R	139	111	0.10	[-0.26 ; 0.47]	0.58	0.45	0		
	Diseased	15-16, 24	R	119	111	-1.15	[-1.63 ; -0.67]	<0.00001	0.97	0		
TNF-α (pg/mL_	**Overall**	15, 17, 19	R	187	134	-0.77	[-1.57 ; 0.04]	0.06	0.002	74		
Triglycerides (mM)	**Overall**		R	366	296	-0.01	[-0.13 ; 0.11]	0.84	1.00	0		
	Non diseased	16, 18, 20, 22	R	133	114	0.00	[-0.14 ; 0.15]	0.95	0.97	0		
	Diseased	16-17, 24, 27-28	R	233	182	-0.05	[-0.27 ; 0.17]	0.64	0.95	0		
TC (mM)	**Overall**		R	366	296	-0.14	[-0.24 ; -0.03]	0.009	0.72	0		
	Non diseased	16, 18, 20, 22	R	133	114	-0.12	[-0.27 ; 0.04]	0.14	0.65	0		
	Diseased	16-17, 24, 27-28	R	233	182	-0.15	[-0.29 ; -0.01]	0.03	0.48	0		
HDL (mM)	**Overall**		R	366	296	0.01	[-0.03 ; 0.05]	0.58	0.97	0		
	Non diseased	16, 18, 20, 22	R	133	114	-0.02	[-0.08 ; 0.05]	0.62	0.71	0		

	Diseased	16-17, 24, 27-28	R	233	182	0.03	[-0.02 ; 0.08]	0.27	1.00	0
LDL (mM)	**Overall**	R	366	296	-0.13	[-0.27 ; 0.00]	0.06	0.14	32	
	Non diseased	16, 18, 20, 22	R	133	114	-0.13	[-0.29 ; 0.04]	0.14	0.34	12
	Diseased	16-17, 24, 27-28	R	233	182	-0.09	[-0.32 ; 0.13]	0.42	0.11	44
Systolic BP (mm Hg)	**Overall**	R	197	188	-0.11	[-1.87 ; 1.65]	0.90	0.99	0	
	Non diseased	16, 22	F	74	72	-0.45	[-3.83 ; 2.92]	0.79	0.76	0
	Diseased	16, 24, 27	F	123	116	0.02	[-2.04 ; 2.09]	0.98	0.96	0
Diastolic BP (mm Hg)	**Overall**	R	197	188	0.59	[-0.47 ; 1.65]	0.27	0.74	0	
	Non diseased	16, 22	F	74	72	-0.62	[-2.91 ; 1.66]	0.59	0.68	0
	Diseased	16, 24, 27	F	123	116	0.92	[-0.27 ; 2.11]	0.13	0.80	0
HbA_{1c} (%)	**Overall (Diseased)**	15, 17, 25-26	R	200	141	-0.45	[-0.70 , -0.19]	0.0006	0.06	56

[1] A random effect model was used if at least 5 trials per analysis could be included. Otherwise a fixed effect model was performed.

Abbreviations: BP = Blood Pressure; C = Non-intervention Group; CI = Confidence Interval; CRP = C-Reactive Protein; F= Fixed; HbA_{1c} = Glycated Hemoglobin; HDL = High Density Lipoprotein; I = Intervention group; IL-6 = Interleukin-6; LDL = Low Density Lipoprotein; R = Random; TC = Total Cholesterol; TNF-α = Tumor Necrosis Factor α; WMD = Weighted Mean Difference



-1 article described 3 different RCTs[15]
-1 article described 2 different RCTs[16]
-2 articles described 2 different treatment groups with 1 non-treatment group[17-18]
+5

Figure 2. Weighted mean differences (WMD) and confidence intervals (CI) of CRP levels between the intervention groups (periodontal therapy) and non-intervention groups (no periodontal therapy).

Supplemental Table 1. Characteristics of included trials

Trial reference	Design Blinding Evaluation period Risk of bias	Population Location Mean age (SD) Gender Mean BMI (SD) # Smokers (%)	Definition disease Periodontal Inclusion criteria Mean mm PPD (SD) Mean mm CAL (SD) Mean % BOP (SD) Mean # Teeth (SD) Mean # or % sites with PPD ≥4mm (SD)	Definition disease Cardiovascular	Intervention # subjects base(end)	Author Conclusion
[20]	RCT NR 3 months Moderate	Irbid, Jordan	≥6 teeth with PPD ≥5mm and ≥3 sites with CAL ≥3mm	CVD: No CVD risk markers: No		Nonsurgical periodontal therapy results in a significant reduction in serum CRP levels.
		I: Age: 47 (3) years Gender: ♀: 8 ♂:10 BMI: 25 (2) kg/m^2 # Smokers: 0 (0)	**I:** PPD: NR CAL: NR BOP: NR # Teeth: 27 (1) % Sites ≥4mm: 39 (NR)		**I:** SRP # 18 (18)	
		C: Age: 45 (3) years Gender: ♀: 8 ♂:10 BMI: 25 (1) kg/m^2 # Smokers: 0 (0)	**C:** PPD: NR CAL: NR BOP: NR # Teeth: 27 (1) % Sites ≥4mm: 41 (NR)		**C:** No periodontal treatment # 18 (18)	

[17]	RCT Single blind 6 months Low	Guangzhou, China	≥16 teeth Mean CAL ≥1mm	CVD: No CVD risk markers: - Type 2 diabetes; Diagnosis ≥1 year		Non-surgical periodontal treatment can effectively improve periodontal and circulating inflammatory status.
		I (IPT): Age : 60 (9) years Gender: ♀:19 ♂:23 BMI: 24 (3) kg/m^2 # Smokers: 7 (17)	**I (IPT):** PPD: 2.7 (0.7) CAL: 3.6 (1.3) BOP: 38 (20) # Teeth: NR % Sites ≥4mm: 17 (NR)		**I (IPT):** SRP + subgingival debridement at 3 months follow-up # 45 (42)	
		I (SPT): Age: 58 (11) years Gender: ♀:17 ♂:26 BMI: 24 (4) kg/m^2 # Smokers: 10 (23)	**I (SPT):** PPD: 2.6 (0.7) CAL: 3.0 (1.2) BOP: 32 (17) # Teeth: NR % Sites ≥4mm: 15 (NR)		**I (SPT):** SRP + supragingival prohylaxis at 3 months follow-up # 45 (43)	
		C: Age: 63 (9)years Gender: ♀:24 ♂:17 BMI: 24 (3) kg/m^2 # Smokers: 7 (17)	**C:** PPD: 2.5 (0.6) CAL: 3.4 (1.2) BOP: 34 (19) # Teeth: NR % Sites ≥4mm: 12 (NR)		**C:** No periodontal treatment # 44 (41)	
[27]	RCT Single blind 12 months	Santiago, Chile	≥14 teeth ≥ 4 teeth with ≥1 pocket with PPD and with CAL ≥3mm	CVD: No CVD risk factors: - Metabolic syndrome (obesity, dyslipidemia, high blood pressure and		Elimination of periodontal inflammation by using SRP and systemic

	Low	**I:** Age: 54 (9) years Gender: ♀:58 ♂:24 BMI: 30 (4) kg/m^2 # Smokers: 24 (29)	**I:** PPD: NR CAL: 3.8 (1.5) BOP: 52 (17) # Teeth: 21 (4) % Sites ≥4mm: 32 (20)	elevated fasting glucose)	**I:** SRP + extraction of hopeless teeth + systemic antibiotics (Metronidazol 250mg and Amoxicillin 500mg 3 times a day for 1 week) # 82 (81)	antibiotics or using plaque control and supragingival scaling significantly decreases CRP in patients with metabolic syndrome. The elimination of periodontal inflammation in patients with metabolic syndrome may contribute to reduce cardiovascular risk
		C: Age: 56 (9) years Gender: ♀:17 ♂:13 BMI: 30 (4) kg/m^2 # Smokers: 22 (27)	**C:** PPD: NR CAL: 3.5 (1.4) BOP: 51 (17) # Teeth: 20 (5) % Sites ≥4mm: 31 (21)		**C:** Supra-gingival scaling and 2 placebo tablets for antibiotics (3 times a day for 1 week) # 83 (79)	
[26]	RCT Single blinded 6 months Low	Athens, Greece	≥16 teeth ≥8 sites with PPD ≥6mm and ≥4 sites with CAL ≥5mm distributed in at least two quadrants	CVD: No CVD risk factors: - Type 2 diabetes (HbA_{1c} 7-10%)		SRP significantly improved HbA_{1c} levels in patients with type 2 diabetes, but did not result in

		I: Age: 60 (8) years Gender: ♀:13 ♂:17 BMI: 28 (4) kg/m^2 # Smokers: 4 (13)	**I:** PPD: NR CAL: NR BOP: 72 (27) # Teeth: 24 (4) % Sites ≥4mm: 67 (NR)		**I:** SRP # 30 (26)	improvement of hsCRP, d-8-iso, MMP-2 and MMP-9 levels
		C: Age: 59 (10) years Gender: ♀:16 ♂:14 BMI: 28 (4) kg/m^2 # Smokers: 7 (23)	**C:** PPD: NR CAL: NR BOP: 69 (26) # Teeth: 24 (4) # Sites ≥4mm: 64 (NR)		**C:** Supra-gingival scaling # 30 (27)	
[23]	CCT NA 4 weeks High	Calicut, India	≥10 teeth ≥1 pocket with PPD ≥4mm and ≥4 teeth with CAL ≥3mm	CVD: - Ideopathic edema		Periodontal disease, could affect the pathogenesis of idiopathic edema. Successful elimination of periodontal inflammation leads to a clinical benefit in patients who are distressed by idiopathic edema
		I: Age: 40 (10) years Gender: ♀:15 ♂:0 BMI: 27 (4) kg/m^2 # Smokers: NR	**I:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4mm: NR		**I:** SRP + extraction of hopeless teeth + systemic antibiotics (doxycycline) + mouthwash twice a day (chlorhexydine) # 15 (15)	
		C: Age: 34 (8) years Gender: ♀:15 ♂:0 BMI: 28 (6) kg/m^2 # Smokers: NR	**C:** PPD: NR CAL: NR BOP: NR # Teeth: NR		**C:** no periodontal treatment # 15 (15)	

			# Sites ≥4mm: NR			
[21]	RCT Single blind 3 months Low	Hong Kong, China	NR	CVD : No CVD risk factors: No		Periodontal treatment has neutral effects on peripheral endothelial function but significantly decreases circulating $CD34^+$ cell count.
		I: Age: 59 (12) years Gender: ♀:15 ♂:10 BMI: 25 (4) kg/m^2 # Smokers: 0 (0)	**I:** PPD: NR CAL: NR BOP: 45 (19) # Teeth: NR % Sites ≥4mm: 13 (14)		**I:** SRP + chlorhexydine gel in all sites with PPD ≥4mm + extraction of hopeless teeth # 25 (24)	
		C: Age: 60 (10) years Gender: ♀:12 ♂:13 BMI: 23 (4) kg/m^2 # Smokers: 0 (0)	**C:** PPD: NR CAL: NR BOP: 46 (21) # Teeth: NR % Sites ≥4mm: 10 (10)		**C:** no periodontal treatment # 25 (23)	
[15]	RCT Not reported 3 months Moderate	Hangzhou, China	- ≥20 teeth - PPD ≥5mm - ≥30% of teeth with CAL >4mm, or ≥60% of teeth with PPD >4mm and CAL >3mm		**I:** SRP + periodontal surgery when indicated + extraction of hopeless teeth + antibiotics (tinidazole 1.0g , bid and ampicillin 0.25g qid 3 days before	Periodontal intervention is helpful for glucose control, which may be associated with increase serum

		Trial 1: **I:** Age: 56 (12) years Gender: ♀:11 ♂:14 BMI: 23 (3) kg/m^2 # Smokers: 0 (0) **C:** Age: 56 (13) years Gender: ♀:11 ♂:14 BMI: 23 (3) kg/m^2 # Smokers: 0 (0)	**Trial 1:** **I+C:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4mm: NR	**Trial 1:** CVD: No CVD risk markers: - Impaired glucose tolerance	and after periodontal intervention) **C:** no periodontal treatment **Trial 1:** **I:** # 25 (25) **C:** # 25 (25)	adiponectin levels and decreased inflammatory cytokine levels.
		Trial 2: **I:** Age: 56 (11) years Gender: ♀:12 ♂:17 BMI: 23 (3) kg/m^2 # Smokers: 0 (0) **C:** Age: 58 (11) years Gender: ♀:12 ♂:17 BMI: 24 (3) kg/m^2 # Smokers: 0 (0)	**Trial 2+3:** **I+C:** PPD: 4.3 (0.7) CAL: 4.5 (1.3) BOP: 94 (11) # Teeth: NR # Sites ≥4mm: NR	**Trial 2:** CVD: No CVD risk markers: - Type 2 diabetes; Diagnosis ≥1 year; Good glucose control (Fasting glucose <7.0 mmol/L and HbA_{1c} between 6.5% and 7.5%)	**Trial 2:** **I:** Similar as group 1 # 29 (29) **C:** Similar as group 1 # 29 (29)	

		Trial 3: **I:** Age: 58 (11) years Gender: ♀:10 ♂:14 BMI: 24 (3) kg/m^2 # Smokers: 0 (0) **C:** Age: 56 (10) years Gender: ♀:10 ♂:14 BMI: 24 (3) kg/m^2 # Smokers: 0 (0)		**Trial 3:** CVD: - Heart disease (#20) - Carotid artery atherosclerosis (#12) - Lower extremity atherosclerosis (#14) - CHD (#2) CVD risk markers: - Type 2 diabetes; Diagnosis ≥1 year; Good glucose control (Fasting glucose <7.0 mmol/L and HbA_{1c} between 6.5% and 7.5%)	**Trial 3:** **I:** Similar as group 1 # 24 (24) **C:** Similar as group 1 # 24 (24)	
[25]	RCT Not reported 6 months Moderate	Tokyo, Japan **I:** Age: 60 (10) years Gender: ♀:11 ♂:21 BMI: 24 (4) kg/m^2 # Smokers: 0 (0)	- ≥ 11 teeth - ≥ 2 sites with PPD ≥ 4mm **I:** PPD: 3.0 (0.9) CAL: NR BOP: 37 (23) # Teeth: 24 (5) # Sites ≥4mm: NR	CVD: No CVD risk markers: - Type 2 diabetes (HbA_{1c}: 6.5%-10.0%)	**I**: SRP + local application of antibiotics (Minocyline 10mg in every periodontal pocket) + extraction op hopeless teeth # 32 (32)	The results suggest that periodontal treatment with topical antibiotics improves HbA_{1c} through the reduction of CRP, which may relate to amelioration of insulin resistance, in

		C: Age: 59 (5) years Gender: ♀:11 ♂:6 BMI: 26 (5) # Smokers: 0 (0)	**C:** PPD: 2.8 (0.9) CAL: NR BOP: 25 (15) # Teeth: 24 (3) # Sites ≥4: NR		**C**: No periodontal treatment # 17 (17)	type 2 diabetic patients with periodontal disease
[24]	RCT Not reported 6 months Moderate	Hiroshima, Japan	Self-reported questionnaire: gingival swelling and bleeding, purulent discharge, and tooth mobility Measurements: ≥2 teeth with PPD ≥4mm and CAL ≥3mm	CVD: - Coronary artery disease (stenosis in ≥70% in at least one proximal epicardial coronary artery and with objective evidence of myocardial infarction or at least one coronary stenosis ≥80% and classic angina without provocative testing		Periodontitis is associated with endothelial dysfunction in patients with coronary artery disease
		I: Age: 63 (12) years Gender: ♀:5 ♂:19 BMI: 24 (3) kg/m^2 # Smokers: 0 (0)	**I:** PPD: 5.1 (0.8) CAL: 6.0 (1.1) BOP: 51 (18) # Teeth: NR # Sites ≥4mm: NR		**I:** SRP + antibiotics for 4 to 7 days + mouthwash every day for 6 months # 24 (24)	
		C: Age: 62 (13) years Gender: ♀: 6 ♂: 18 BMI: 24 (3) kg/m^2 # Smokers: 0 (0)	**C:** PPD: 4.8 (0.7) CAL: 5.7 (1.0) BOP: 54 (17) # Teeth: NR # Sites ≥4mm: NR		**C:** no periodontal treatment # 24 (24)	

[16] (Trial 1)	RCT Not reported 6 months Moderate	Hiroshima, Japan	Self-reported questionnaire gingival swelling and bleeding, purulent discharge, and tooth mobility Periodontitis definition : NR	CVD : No CVD risk factors: No		Periodontitis is associated with endothelial dysfunction in systemically healthy subjects
		I: Age: 25(3) years Gender: ♀:0 ♂:16 BMI: 23 (2) kg/m^2 # Smokers: 0 (0)	**I:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4mm: NR		**I:** SRP + antibiotics for 4 to 7 days + mouthwash every day for 6 months # 16 (16)	
		C: Age: 25(4) years Gender: ♀:0 ♂:16 BMI: 24 (2) kg/m^2 # Smokers: 0 (0)	**C:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4mm: NR		**C:** no periodontal treatment # 16 (16)	

[16] (Trial 2)	CCT Not reported 6 months High	Hiroshima, Japan	Self-reported questionnaire gingival swelling and bleeding, purulent discharge, and tooth mobility Periodontitis definition : NR	CVD: - Hypertension: Medication (≥ 6 months): Calcium antagonist (n=42) Rennin-angiotension inhibitors (n=16) β-blockers (n=9) Diuretics (n=8)		Periodontitis is associated with endothelial dysfunction in hypertensive patients
		I: Age: 53(14) years Gender: ♀:6 ♂:11 BMI: 23 (3) kg/m^2 # Smokers: 0 (0)	**I:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4: NR		**I:** SRP + antibiotics for 4 to 7 days + mouthwash every day for 6 months # 17 (17)	
		C: Age: 55(11) years Gender: ♀:2 ♂:7 BMI: 23 (3) kg/m^2 # Smokers: 0 (0)	**C:** PPD: NR CAL: NR BOP: NR # Teeth: NR # Sites ≥4mm: NR		**C:** no periodontal treatment # 9 (9)	
[28]	RCT Not reported 3 months Moderate	Ankara, Turkey	>3 sites with PPD ≥4mm	CVD: No CVD risk factors: -hypercholesterolemia (Triglyceride <200 mg/dL, HDL <35 mg/dL, LDL<130 mg/dL, VLDL <40 mg/dL blood fasting glucose <126 mg/dL)		Periodontal treatment has potential effects on lipid metabolism

		I: Age: 49 (6) years Gender ♀:15 ♂:10 BMI: NR # Smokers: 3 (12)	**I:** PPD: 3.4 (0.9) CAL: 2.7 (0.9) BOP: 62 (20) # Teeth: NR # Sites ≥4mm: NR	**I:**Hypercholesterolimia duration : 5.5 ± 2.6 years	**I**: SRP + 5 patients received open flap debridement # 25 (25)	
		C: Age: 52 (8) years Gender: ♀:16 ♂:9 BMI: NR # Smokers: 4 (16)	**C:** PPD: 2.8 (0.8) CAL: 2.4 (0.6) BOP: 62 (18) # Teeth: NR # Sites ≥4mm: NR	**C:**Hypercholesterolimia duration : 4.8 ± 2.7 year	**C**: no periodontal treatment # 25 (25)	
[22]	RCT Single blind 6 months Low	London, United Kingdom	≥50% of teeth with PPD ≥6mm and ≥30% ABL	CVD : No CVD risk factor: No		Periodontal treatment results in improvement in endothelial function after 6 months
		I: Age: 48 (8) years Gender: ♀:31 ♂:30 BMI: 27 (5) kg/m^2 # Smokers: 18 (30)	**I:** PPD: NR CAL: NR BOP: 66 (18) # Teeth: 27 (3) # Sites ≥4mm: 82 (27)		**I**: SRP + local application of antibiotics (Arestin®) + extraction op hopeless teeth # 61 (58)	
		C: Age: 48 (6) years Gender: ♀:29 ♂:30 BMI: 27 (5) kg/m^2 # Smokers: 20 (34)	**C:** PPD: NR CAL: NR BOP: 68 (17) # Teeth: 27 (3) # Sites ≥mm4: 84 (26)		**C**: Supragingival scaling and polishing # 59 (56)	

[18]	RCT Single blind 2 months Low	London, United Kingdom	≥50% of teeth with PPD ≥6mm and ≥30% ABL	CVD : No CVD risk factor: No		Periodontitis might cause moderate systemic inflammation in systemically healthy subjects
		I (IPT): Age : 49 (7) years Gender: ♀:8 ♂:12 BMI: 26 (4) kg/m^2 # Smokers: 5 (25)	**I (IPT):** PPD: 4.5 (0.8) CAL: 5.5 (1.5) BOP: NR # Teeth: NR # Sites ≥4mm: 80 (25)		**I (IPT):** SRP + local delivery of minocycline (Arestin®) # 20 (20)	
		I (SPT): Age: 48 (7) years Gender: ♀:10 ♂:11 BMI: 26 (4) kg/m^2 # Smokers: 6 (29)	**I (SPT):** PPD: 4.3 (0.8) CAL: 5.3 (1.5) BOP: NR # Teeth: NR # Sites ≥4mm: 79 (28)		**I (SPT):** SRP # 21 (21)	
		C: Age: 48 (6)years Gender: ♀:9 ♂:15 BMI: 25 (3) kg/m^2 # Smokers: 7 (29)	**C:** PPD: 4.6 (0.8) CAL: 5.5 (1.6) BOP: NR # Teeth: NR # Sites ≥4mm: 80 (26)		**C**: no periodontal treatment # 24 (24)	
[19]	RCT Not reported 3 months	London, United Kingdom	≥20 teeth without periapical lesions ≥5 teeth with PPD ≥6mm and radiographic evidence of ABL	CVD : No CVD risk factor: No		Improvement in periodontal health do not influence the levels of vascular

	Moderate	**I** : Age: 48 (8) years Gender: ♀:11 ♂:13 BMI: NR # Smokers: 0 (0)	**I:** PPD: NR CAL: NR BOP: 42 (17) # Teeth: 27 (3) # Sites ≥4mm: NR		**I:** SRP # 24 (24)	markers
		C: Age: 46 (6) years Gender: ♀:5 ♂:10 BMI: NR # Smokers: 0 (0)	**C:** PPD: NR CAL: NR BOP: 45 (23) # Teeth: 28 (3) # Sites ≥4mm: NR		**C:** no periodontal treatment # 15 (15) :	

Abbreviations: ABL = Alveolar Bone Loss; BMI = Body Mass Index; BOP = Bleeding On Probing; C = Non-intervention group; CAL = Clinical Attachment Level; CCT = Controlled Clinical Trial; CHD = Coronary Heart Disease; CRP = C-Reactive Protein; CVD = Cardiovascular Disease; d-8-iso = d-8-iso prostaglandin F2a; HbA_{1c} = Glycated Hemoglobin; HDL = High Density Lipoprotein; hsCRP = high-sensitive C-Reactive Protein; I = Intervention group; IPT = Intensive Periodontal Treatment; MMP = Matrix Metalloproteinase; LDL = Low Density Lipoprotein; NR = Not Reported; PPD = Probing Pocket Depth; RCT = Randomized Clinical Trial; SD = Standard Deviation; SPT = Standard Periodontal Treatment; SRP = Scaling and Root Planing; # = number; VLDL = Very Low Density Lipoprotein

Supplemental Table 2: Methodological quality scores of the selected studies

	Study reference / Criteria:	[20]	[17]	[26]	[27]	[23]	[21]	[15]	[25]	[24]	[16] a	[16] b	[28]	[22]	[18]	[19]
Internal validity	Random allocation*	+	+	+	+	-	+	+	+	+	+	?	+	+	+	+
	Allocation concealment	+	+	?	?	NA	+	?	?	?	?	?	?	+	+	-
	Blinded to patient*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Blinded to examiner*	?	+	+	?	?	+	?	?	?	?	?	?	+	+	?
	Blinding during statistical analysis	?	?	?	?	?	?	?	?	?	?	?	?	+	+	+
	Balanced experimental groups *	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Reported loss to follow up*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	# (%) of drop-outs	0 (0%)	8 (6%)	7 (12%)	5 (3%)	0 (0%)	3 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (5%)	0 (0%)	0 (0%)
	Treatment identical, except for intervention*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
External validity	Representative population group	+	±	±	±	±	+	±	±	±	+	±	±	+	+	+
	Eligibility criteria defined*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Statistical validity	Sample size calculation and power	-	+	+	+	?	+	?	?	?	?	?	?	+	+	?
	Point estimates	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
	Measures of variability presented for the primary outcome	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
	Include a per protocol analysis	+	+	-	-	+	-	+	+	+	+	+	+	-	-	+
	Include an intention- to-treat analysis	-	-	+	+	-	+	-	-	-	-	-	-	+	+	-
	Authors estimated risk of bias	Mod	Low	Low	Low	High	Low	Mod	Mod	Mod	Mod	High	Mod	Low	Low	Mod

Abbreviations: ? = not specified/unclear; + = yes; - = no; * = reporting criteria for estimating the potential risk of bias; NA = Not Applicable; Mod = Moderate

Supplemental Table 3A: Overview of selected trials investigating CRP levels for treatment and non-treatment group.

Type	Trial reference	Group	Mean ± SD			Significant difference
			Baseline	End	Difference	
C-reactive protein (mg/L)	[20]	PT NPT	2.3 ± 0.7 2.3 ± 0.7	1.8 ± 0.6 2.4 ± 0.7	-0.5 ± 0.7 ◊ 0.1 ± 0.7 ◊	YES NO
	[17]	IPT PT NPT	3.2 ± 4.5 3.1 ± 4.6 2.8 ± 4.1	1.6 ± 1.3 1.5 ± 1.3 3.2 ± 5.5	-1.6 ± 4.0 ◊ -1.6 ± 4.2 ◊ 0.4 ± 4.9 ◊	YES YES NO
	[26]	PT NPT	2.5 ± 2.3 2.4 ± 2.1	2.2 ± 2.4 2.4 ± 2.4	-0.3 ± 2.7 -0.1 ± 2.4	NO NO
	[27]	PT NPT	4.4 ± 3.1 4.4 ± 3.2	3.6 ± 3.8 3.4 ± 3.1	-0.8 ± 3.5 ◊ -1.0 ± 3.1 ◊	YES YES
	[23]	PT NPT	12.5 ± 15.9 6.1 ± 10.6	3.7 ± 7.9 8.5 ± 11.8	-8.8 ± 13.7 ◊ -2.4 ± 4.3 ◊	YES YES
	[21]	PT NPT	2.1 ± 2.4 3.0 ± 3.7	2.4 ± 2.8 2.1 ± 3.4	0.3 ± 2.3 -0.9 ± 2.3	NO NO
	[15] (Trial 1)	PT NPT	3.3 ± 0.6 3.3 ± 0.6	2.3 ± 0.4 3.1 ± 0.5	-0.9 ± 0.5 ◊ -0.2 ± 0.6 ◊	YES NO
	[15] (Trial 2)	PT NPT	4.9 ± 1.0 5.1 ± 1.1	4.2 ± 0.9 5.2 ± 1.2	-0.7 ± 1.0 ◊ 0.2 ± 1.1 ◊	YES NO
	[15] (Trial 3)	PT NPT	6.7 ± 1.2 6.6 ± 1.2	5.7 ± 1.1 6.4 ± 1.3	-1.0 ± 1.2 ◊ -0.2 ± 1.3 ◊	YES NO
	[25]	PT NPT	1.9 ± 2.5 2.3 ± 2.6	1.5 ± 1.6 2.1 ± 3.2	-0.3 ± 0.4 -0.1 ± 0.2	NO NO
	[24]	PT NPT	2.7 ± 1.9 2.6 ± 2.2	1.8 ± 0.9 2.5 ± 2.1	-0.9 ± 1.7 ◊ -0.1 ± 2.2 ◊	YES NO
	[16] (Trial 1)	PT NPT	2.1 ± 1.9 2.0 ± 2.0	1.3 ± 1.2 2.1 ± 2.3	-0.8 ± 1.7 ◊ 0.1 ± 2.2 ◊	YES NO
	[16] (Trial 2)	PT NPT	2.4 ± 2.2 2.2 ± 2.3	1.4 ± 1.2 2.1 ± 2.0	-1.0 ± 1.9 ◊ -0.1 ± 2.2 ◊	YES NO
	[22]	PT NPT	2.5 ± 2.7 3.8 ± 5.3	2.7 ± 4.9 3.0 ± 3.4	0.2 ± 3.9 -0.8 ± 4.0	NO NO
	[18]	PT PTA NPT	2.9 ± 2.2 2.0 ± 1.1 2.4 ± 1.6	2.9 ± 2.3 1.6 ± 0.9 2.5 ± 1.7	0.0 ± 2.3 ◊ -0.4 ± 1.0 ◊ 0.1 ± 1.7 ◊	NR NR NR
	[19]	PT NPT	1.0 ± 0.8 0.8 ± 1.0	1.0 ± 1.0 0.8 ± 0.9	0.1 ± 0.9 ◊ -0.0 ± 1.0 ◊	NO NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; PTA = Periodontal Therapy with Antibiotics; NR = Not Reported; ◊ = values were calculated

Supplemental Table 3B: Overview of selected trials investigating IL-6 levels for treatment and non-treatment group .

Type	Trial reference	Group	Mean ± SD			Significant difference
			Baseline	End	Difference	
Interleukin-6 (ng/L)	[15] (Trial 1)	PT NPT	5.0 ± 1.3 5.0 ± 1.4	4.0 ± 1.1 5.2 ± 1.4	-1.1 ± 1.3 ◊ 0.2 ± 1.4 ◊	YES NO
	[15] (Trial 2)	PT NPT	7.2 ± 1.6 7.3 ± 1.6	5.9 ± 1.3 7.1 ± 1.4	-1.4 ± 1.5 ◊ -0.2 ± 1.5 ◊	YES NO
	[15] (Trial 3)	PT NPT	11.3 ± 2.9 11.4 ± 2.9	10.8 ± 2.6 11.7 ± 2.7	-0.4 ± 2.7 ◊ 0.2 ± 2.8 ◊	NO NO
	[24]	PT NPT	2.6 ± 3.4 2.7 ± 3.9	1.6 ± 2.6 2.6 ± 4.4	-1.0 ± 3.1 ◊ -0.1 ± 4.2 ◊	YES NO
	[16] (Trial 1)	PT NPT	2.3 ± 3.9 2.6 ± 3.8	1.5 ± 2.2 2.7 ± 4.0	-0.8 ± 3.4 ◊ 0.1 ± 3.9 ◊	YES NO
	[16] (Trial 2)	PT NPT	2.8 ± 4.4 2.5 ± 4.2	1.7 ± 2.5 2.6 ± 4.3	-1.1 ± 3.8 ◊ 0.1 ± 4.3 ◊	YES NO
	[22]	PT NPT	2.4 ± 5.4 2.0 ± 3.9	2.4 ± 5.7 1.7 ± 3.4	0.1 ± 2.1 -0.3 ± 1.7	NO NO
	[18]	PT PTA NPT	2.0 ± 1.1 1.5 ± 0.9 1.9 ± 1.0	1.9 ± 1.1 1.0 ± 0.6 1.7 ± 0.3	-0.1 ± 1.1 ◊ -0.4 ± 0.8 ◊ -0.2 ± 0.9 ◊	NO YES NO
	[19]	PT NPT	1.7 ± 0.8 2.9 ± 3.6	1.5 ± 1.4 1.6 ± 1.5	-0.2 ± 1.2 ◊ -1.3 ± 3.1 ◊	NO NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; PTA = Periodontal Therapy with Antibiotics; ◊ = values were calculated

Supplemental Table 3C: Overview of selected trials investigating TNF-α levels for treatment and non-treatment group .

Type	Trial reference	Group	Mean ± SD			Significant difference
			Baseline	End	Difference	
Tumor Necrosis Factor-α (pg/mL)	[17]	IPT	11.2 ± 4.0	10.8 ± 3.0	-0.4 ± 3.6 ◊	NO
		PT	12.4 ± 15.6	11.0 ± 3.8	-1.4 ± 14.1 ◊	NO
		NPT	10.5 ± 3.4	11.0 ± 2.4	0.5 ± 3.0 ◊	NO
	[15] (Trial 1)	PT	6.7 ± 1.2	5.3 ± 1.2	-1.4 ± 1.2 ◊	YES
		NPT	6.8 ± 1.2	6.6 ± 1.4	-0.1 ± 1.3 ◊	NO
	[15](Trial 2)	PT	8.8 ± 2.4	7.3 ± 2.0	-1.5 ± 2.2 ◊	YES
		NPT	8.8 ± 2.3	8.6 ± 2.3	-0.2 ± 2.3 ◊	NO
	[15] (Trial 3)	PT	12.3 ± 2.2	11.6 ± 2.4	-0.7 ± 2.3 ◊	NO
		NPT	12.5 ± 2.2	12.6 ± 2.3	0.1 ± 2.3 ◊	NO
	[19]	PT	2.2 ± 0.5	2.2 ± 0.5	-0.0 ± 0.5 ◊	NO
		NPT	2.0 ± 0.5	1.8 ± 0.5	-0.2 ± 0.5 ◊	NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; ◊ = values were calculated

Supplemental Table 3D: Overview of selected trials investigating triglyceride levels for treatment and non-treatment group .

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
Triglycerides (mmol/L)	[20]	PT	1.2 ± 0.4	1.2 ± 0.4	0.0 ± 0.4 ◊	NO
		NPT	1.2 ± 0.3	1.2 ± 0.3	0.0 ± 0.3 ◊	NO
	[17]	IPT	5.9 ± 1.2	5.6 ± 1.4	-0.3 ± 1.3 ◊	NO
		PT	6.0 ± 1.6	5.3 ± 1.4	-0.8 ± 1.5 ◊	YES
		NPT	6.4 ± 1.9	5.8 ± 1.6	-0.6 ± 1.8 ◊	NO
	[27]	PT	2.0 ± 1.1	2.0 ± 1.6	0.0 ± 1.5 ◊	NO
		NPT	1.8 ± 1.3	1.9 ± 1.2	0.1 ± 1.3 ◊	NO
	[24]	PT	1.3 ± 0.8	1.3 ± 0.7	-0.1 ± 0.7 ◊	NO
		NPT	1.3 ± 0.7	1.3 ± 0.6	-0.0 ± 0.7 ◊	NO
	[16] (Trial 1)	PT	1.3 ± 0.7	1.2 ± 0.7	-0.0 ± 0.7 ◊	NO
		NPT	1.2 ± 0.6	1.2 ± 0.6	-0.0 ± 0.6 ◊	NO
	[16] (Trial 2)	PT	1.2 ± 0.8	1.2 ± 0.6	0.0 ± 0.7 ◊	NO
		NPT	1.3 ± 0.7	1.3 ± 0.6	-0.0 ± 0.7 ◊	NO
	[28]	PT	1.8 ± 0.9	1.8 ± 1.4	0.0 ± 1.3 ◊	NO
		NPT	1.8 ± 0.5	2.0 ± 0.7	0.2 ± 0.7 ◊	YES
	[22]	PT	1.4 ± 1.0	1.3 ± 0.8	-0.1 ± 0.6	NO
		NPT	1.3 ± 1.1	1.3 ± 0.8	-0.1 ± 0.5	NO
	[18]	PT	1.7 ± 1.1	1.6 ± 1.0	-0.1 ± 1.1 ◊	NO
		PTA	1.4 ± 1.1	1.3 ± 0.8	-0.1 ± 1.0 ◊	NO
		NPT	1.7 ± 1.2	1.4 ± 0.9	-0.3 ± 1.1 ◊	NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; PTA = Periodontal Therapy with Antibiotics; ◊ = values were calculated

Supplemental Table 3E: Overview of selected trials investigating total cholesterol levels for treatment and non-treatment group .

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
Total Cholesterol (mmol/L)	[20]	PT	5.4 ± 0.4	5.2 ± 0.6	-0.2 ± 0.5 ◊	NO
		NPT	5.5 ± 0.5	5.3 ± 0.4	-0.1 ± 0.5 ◊	NO
	[17]	IPT	2.1 ± 1.2	1.9 ± 1.1	-0.2 ± 1.1 ◊	NO
		PT	2.6 ± 1.3	2.2 ± 1.9	-0.5 ± 1.7 ◊	NO
		NPT	2.4 ± 1.8	2.3 ± 2.0	-0.1 ± 1.9 ◊	NO
	[27]	PT	5.4 ± 1.2	5.5 ± 1.1	0.1 ± 1.2 ◊	NO
		NPT	5.6 ± 1.3	5.5 ± 1.0	-0.1 ± 1.2 ◊	NO
	[24]	PT	5.0 ± 1.3	4.9 ± 1.3	-0.1 ± 1.3 ◊	NO
		NPT	4.8 ± 1.2	4.8 ± 1.7	-0.0 ± 1.5 ◊	NO
	[16] (Trial 1)	PT	5.0 ± 0.7	4.6 ± 0.7	-0.4 ± 0.7 ◊	NO
		NPT	4.6 ± 0.7	4.6 ± 0.7	-0.0 ± 0.7 ◊	NO
	[16] (Trial 2)	PT	4.6 ± 0.7	4.6 ± 0.8	-0.0 ± 0.8 ◊	NO
		NPT	4.7 ± 0.9	4.7 ± 0.9	0.0 ± 0.9 ◊	NO
	[28]	PT	2.5 ± 0.2	2.1 ± 0.3	-0.3 ± 0.3 ◊	YES
		NPT	2.4 ± 0.2	2.3 ± 0.3	-0.1 ± 0.3 ◊	NO
	[22]	PT	5.2 ± 1.0	5.1 ± 0.9	-0.1 ± 0.6	NO
		NPT	5.3 ± 1.2	5.3 ± 1.0	0.0 ± 0.6	NO
	[18]	PT	5.3 ± 0.7	5.4 ± 0.9	0.1 ± 0.8 ◊	NO
		PTA	5.5 ± 0.7	5.2 ± 0.7	-0.3 ± 0.7 ◊	YES
		NPT	5.4 ± 0.7	5.3 ± 0.8	-0.1 ± 0.8 ◊	NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; PTA = Periodontal Therapy with Antibiotics; ◊ = values were calculated

Supplemental Table 3F: Overview of selected trials investigating HDL levels for treatment and non-treatment group .

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
HDL (mmol/L)	[20]	PT	1.5 ± 0.3	1.4 ± 0.2	-0.1 ± 0.3 ◊	NO
		NPT	1.4 ± 0.2	1.4 ± 0.2	0.0 ± 0.2 ◊	NO
	[17]	IPT	1.3 ± 0.4	1.2 ± 0.3	-0.1 ± 0.4 ◊	YES
		PT	1.3 ± 0.5	1.1 ± 0.4	-0.2 ± 0.4 ◊	YES
		NPT	1.4 ± 0.5	1.3 ± 0.5	-0.2 ± 0.5 ◊	YES
	[27]	PT	1.3 ± 0.3	1.3 ± 0.3	-0.0 ± 0.3 ◊	NO
		NPT	1.3 ± 0.4	1.3 ± 0.4	-0.0 ± 0.4 ◊	NO
	[24]	PT	1.2 ± 0.6	1.2 ± 0.6	-0.0 ± 0.6 ◊	NO
		NPT	1.2 ± 0.7	1.2 ± 0.6	-0.0 ± 0.7 ◊	NO
	[16] (Trial 1)	PT	1.2 ± 0.5	0.2 ± 0.6	0.0 ± 0.5 ◊	NO
		NPT	1.2 ± 0.5	1.2 ± 0.5	-0.0 ± 0.5 ◊	NO
	[16] (Trial 2)	PT	1.2 ± 0.6	1.2 ± 0.4	-0.0 ± 0.6 ◊	NO
		NPT	1.2 ± 0.6	1.2 ± 0.4	0.0 ± 0.6 ◊	NO
	[28]	PT	0.5 ± 0.1	0.5 ± 0.1	0.0 ± 0.1 ◊	NO
		NPT	0.5 ± 0.1	0.5 ± 0.1	-0.0 ± 0.1 ◊	YES
	[22]	PT	1.5 ± 0.4	1.5 ± 0.4	0.1 ± 0.2	NO
		NPT	1.5 ± 0.4	1.5 ± 0.4	0.1 ± 0.2	NO
	[18]	PT	1.3 ± 0.5	1.4 ± 0.5	0.1 ± 0.5 ◊	NO
		PTA	1.5 ± 0.5	1.4 ± 0.4	-0.1 ± 0.5 ◊	NO
		NPT	1.3 ± 0.5	1.3 ± 0.4	0.0 ± 0.5 ◊	NO

Abbreviations: SD = Standard Deviation; HDL = High Density Lipoprotein; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; PTA = Periodontal Therapy with Antibiotics; ◊ = values were calculated

Supplemental Table 3G: Overview of selected trials investigating LDL levels for treatment and non-treatment group.

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
LDL (mmol/L)	[20]	PT	3.4 ± 0.5	3.1 ± 0.6	-0.3 ± 0.6 ◊	NO
		NPT	3.4 ± 0.5	3.5 ± 0.4	0.1 ± 0.5 ◊	NO
	[17]	IPT	3.6 ± 1.0	3.3 ± 0.9	-0.4 ± 0.9 ◊	YES
		PT	3.5 ± 1.3	3.0 ± 1.2	-0.5 ± 1.3 ◊	YES
		NPT	3.8 ± 1.5	3.3 ± 1.3	-0.5 ± 1.4 ◊	YES
	[27]	PT	3.2 ± 1.1	3.2 ± 0.9	0.0 ± 1.1 ◊	NO
		NPT	3.4 ± 1.0	3.4 ± 0.9	0.0 ± 1.0 ◊	NO
	[24]	PT	2.9 ± 1.1	2.8 ± 0.9	-0.1 ± 1.0 ◊	NO
		NPT	2.9 ± 1.0	2.9 ± 1.0	-0.0 ± 1.0 ◊	NO
	[16] (Trial 1)	PT	2.6 ± 0.7	2.6 ± 0.7	-0.0 ± 0.7 ◊	NO
		NPT	2.6 ± 0.7	2.5 ± 0.7	-0.0 ± 0.7 ◊	NO
	[16] (Trial 2)	PT	2.4 ± 0.7	2.4 ± 0.6	0.0 ± 0.7 ◊	NO
		NPT	2.7 ± 0.7	2.6 ± 0.5	-0.1 ± 0.7 ◊	NO
	[28]	PT	1.6 ± 0.2	1.2 ± 0.4	-0.4 ± 0.3 ◊	YES
		NPT	1.5 ± 0.2	1.4 ± 0.2	-0.0 ± 0.2 ◊	NO
	[22]	PT	3.1 ± 0.9	3.0 ± 0.8	-0.1 ± 0.5	NO
		NPT	3.1 ± 1.0	3.1 ± 0.9	-0.0 ± 0.5	NO
	[18]	PT	3.2 ± 0.6	3.4 ± 0.9	0.2 ± 0.7 ◊	NO
		PTA	3.4 ± 0.6	3.2 ± 0.6	-0.2 ± 0.8 ◊	NO
		NPT	3.2 ± 0.6	3.2 ± 0.7	0.0 ± 0.8 ◊	NO

Abbreviations: SD = Standard Deviation; LDL = Low Density Lipoprotein; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; PTA = Periodontal Therapy with Antibiotics; ◊ = values were calculated

Supplemental Table 3H: Overview of selected trials investigating HbA_{1c} levels for treatment and non-treatment group .

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
HbA_{1c} (%)	[17]	IPT	7.3 ± 1.2	7.1 ± 1.3	-0.2 ± 1.3 ◊	NO
		PT	7.3 ± 1.6	6.9 ± 1.1	-0.4 ± 1.4 ◊	YES
		NPT	7.3 ± 1.5	7.4 ± 1.6	0.1 ± 1.5 ◊	NO
	[26]	PT	7.9 ± 0.7	NR	-0.7 ± 0.9	YES
		NPT	7.6 ± 0.7	NR	-0.1 ± 0.5	NO
	[15] (Trial 2,3)	PT	7.1 ± 0.3	6.6 ± 0.4	-0.6 ± 0.4 ◊	YES
		NPT	7.1 ± 0.3	7.2 ± 0.3	0.1 ± 0.3 ◊	NO
	[25]	PT	7.2 ± 0.9	NR	-0.1 ± 0.6	NO
		NPT	6.9 ± 0.9	NR	-0.1 ± 0.6	NO

Abbreviations: SD = Standard Deviation; HbA_{1c} = Glycated Hemoglobin; PT = Periodontal Therapy; NPT = No Periodontal Therapy; IPT = Intensive Periodontal Treatment; NR = Not Reported; ◊ = values were calculated

Supplemental Table 3I: Overview of selected trials investigating systolic and diastolic blood pressure for treatment and non-treatment group.

Type	Trial reference	Groups	Mean ± SD			Significant difference
			Baseline	End	Difference	
Systolic blood pressure (mm Hg)	[27]	PT NPT	149.0 ± 6.9 147.0 ± 6.8	148.0 ± 7.0 146.0 ± 7.1	-1.0 ± 6.9 ◊ -1.0 ± 7.0 ◊	NO NO
	[24]	PT NPT	141.3 ± 20.2 141.3 ± 19.8	140.3 ± 19.3 140.8 ± 19.1	-1.0 ± 19.8 ◊ -0.5 ± 19.5 ◊	NO NO
	[16] (Trial 1)	PT NPT	115.1 ± 10.9 114.9 ± 11.1	114.6 ± 10.4 113.8 ± 10.7	-0.5 ± 10.7 ◊ -1.1 ± 10.9 ◊	NO NO
	[16] (Trial 2)	PT NPT	140.1 ± 20.3 141.2 ± 20.1	141.3 ± 21.4 140.0 ± 19.3	1.2 ± 20.9 ◊ -1.2 ± 19.7 ◊	NO NO
	[22]	PT NPT	125.7 ± 16.6 125.2 ± 18.3	122.6 ± 14.3 122.9 ± 15.5	-3.0 ± 11.3 -2.3 ± 9.3	NO NO
Diastolic blood pressure (mm Hg)	[27]	PT NPT	97.0 ± 4.1 98.0 ± 3.8	96.8 ± 3.8 96.8 ± 4.2	-0.2 ± 4.0 ◊ -1.2 ± 4.0 ◊	NO NO
	[24]	PT NPT	82.5 ± 13.3 82.3 ± 12.7	80.7 ± 12.9 81.9 ± 12.1	-1.8 ± 13.1 ◊ -0.4 ± 12.4 ◊	NO NO
	[16] (Trial 1)	PT NPT	66.1 ± 7.4 66.2 ± 7.6	67.4 ± 7.9 97.1 ± 8.1	1.3 ± 7.7 ◊ 0.9 ± 7.9 ◊	NO NO
	[16] (Trial 2)	PT NPT	89.2 ± 14.1 90.2 ± 13.8	88.7 ± 13.7 89.9 ± 13.3	-0.5 ± 13.9 ◊ -0.3 ± 13.6 ◊	NO NO
	[22]	PT NPT	80.5 ± 11.4 79.3 ± 11.2	77.8 ± 9.0 77.5 ± 10.1	-2.7 ± 7.5 -1.8 ± 6.2	NO NO

Abbreviations: SD = Standard Deviation; PT = Periodontal Therapy; NPT = No Periodontal Therapy; ◊ = values were calculated

Supplemental Table 4: Descriptive summary of statistical significance of the comparisons between the intervention and comparison

	Trial reference	Intervention	Inflammatory markers			Lipid and glucose metabolism					Blood pressure		
			CRP	IL-6	TNF-α	TG	TC	HDL	LDL	HbA_{1c}	Systolic	Diastolic	Comparison
Systemically healthy	[20]	PT	?	□	□	?	?	?	?	□	□	□	NPT
	[21]	PT	0	□	□	□	□	□	□	□	□	□	NPT
	[16] (Trial 1)	PT	0	0	□	0	0	0	0	□	0	0	NPT
	[22]	SRP	0	0	□	0	0	0	0	□	0	0	NPT
	[18]	PT PTA	? ?	0 ?	□ □	0 0	0 0	0 0	0 0	□	□	□	NPT
	[19]	PT	0	0	0	□	□	□	□	□	□	□	NPT
Systemically diseased	[17]	IPT PT	+ +	□ □	0 0	0 0	0 0	0 0	0 0	0 0	□ □	□ □	NPT
	[26]	PT	0	□	□	□	□	□	□	+	□	□	NPT
	[27]	PT	0	□	□	0	0	0	0	□	0	0	NPT
	[23]	PT	?	□	□	□	□	□	□	□	□	□	NPT
	[15] (Trial 1)	PT	+	+	+	□	□	□	□	□	□	□	NPT
	[15] (Trial 2)	PT	+	+	+	□	□	□	□	+	□	□	NPT
	[15] (Trial 3)	PT	+	0	0	□	□	□	□		□	□	NPT
	[25]	PT	+	□	□	□	□	□	□	0	□	□	NPT
	[24]	PT	0	0	□	?	?	?	?	□	0	0	NPT
	[16] (Trial 2)	PT	0	0	□	0	0	0	0	□	0	0	NPT
	[28]	PT	□	□	□	0	+	0	+	□	□	□	NPT

Abbreviations: CRP = C-Reactive Protein; IL-6= Interleukin-6; TNF-α = Tumor Necrosis Factor-α; TG = Tryglycerides; TC = Total Cholesterol; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; HbA_{1c} = Glycated Hemoglobin; PT = Periodontal Treatment; IPT = Intensive Periodontal Treatment; PTA = Periodontal Treatment with Antibiotics; NPT = No Periodontal Treatment; + = significant difference in favor of intervention, 0 =no significant difference, - = significant difference in favor of comparison, □ = no data available, ?= inconclusive data which does not allow to draw conclusions concerning statistical significance.

Supplemental Table 5. Meta-analyses for study duration in diseased populations

Parameter of interest	Group	References for included trials	Model[1]	# subjects		WMD	95% CI	Test for overall effect (*p*-value)	Test for heterogeneity	
				I	C				p-value	I^2 -value (%)
CRP	**< 6 months**	[15, 23]	F	93	93	-0.81	[-1.06 ; -0.56]	<0.00001	0.50	0
	≥ 6 months	[16-17, 24-27]	R	270	204	-0.24	[-0.47 ; -0.01]	0.04	0.40	3
IL-6	**< 6 months**	[15]	F	78	78	-1.16	[-1.66 ; -0.66]	<0.00001	0.78	0
	≥ 6 months	[16, 24]	F	41	33	-0.98	[-2.74 ; 0.77]	0.27	0.88	0

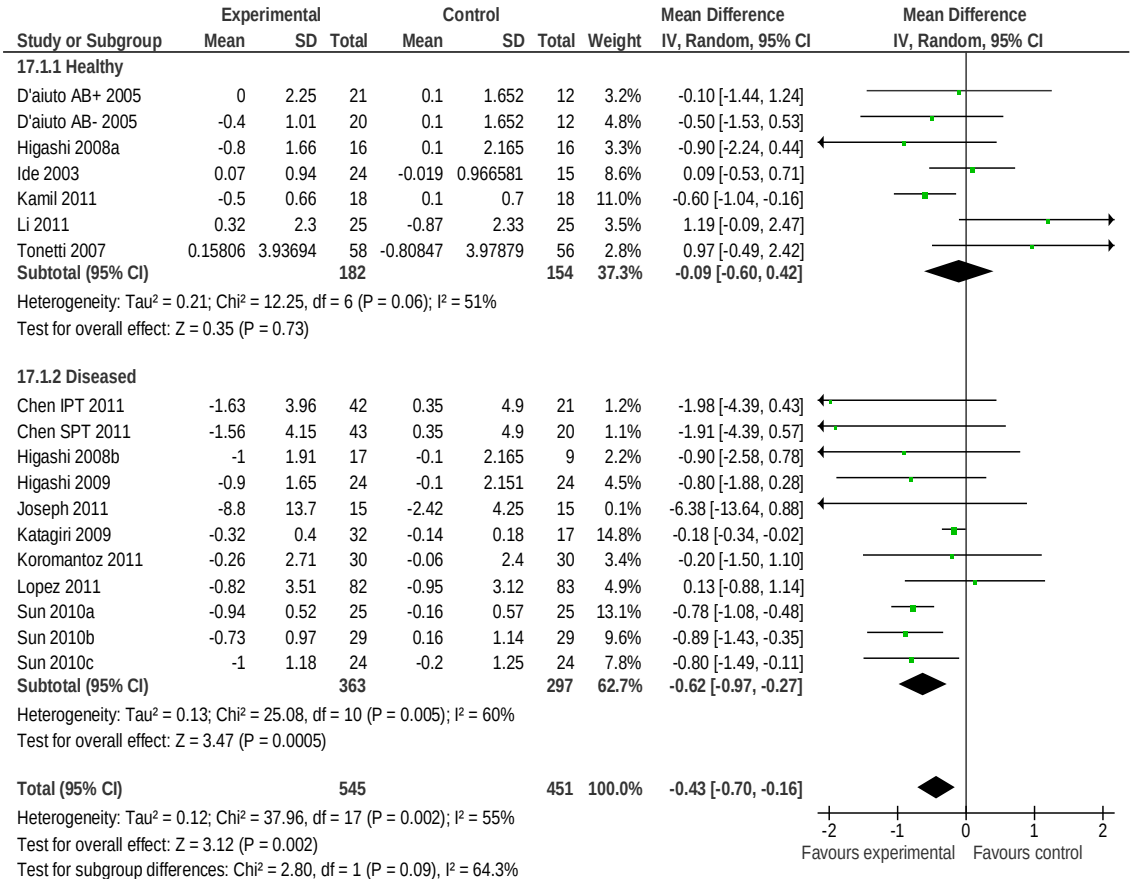
[1] A random effect model was used if at least 5 trials per analysis could be included. Otherwise a fixed effect model was performed.

Abbreviations: C = Non-intervention group; CI = Confidence Interval; CRP = C-Reactive Protein; F= Fixed; I = Intervention group; IL-6 = Interleukin-6; R= Random; WMD = Weighted Mean Difference

Supplemental Table 6: Evidence profile for impact of periodontal treatment compared no treatment from the presented systematic review.

	Systemic inflammation	**Lipid and glucose metabolism**	**Vascular function**
Risk of bias	Moderate	Moderate	Moderate
Consistency	Good	Moderate	Good
Directness	Good	Moderate	Moderate
Precision	Good	Moderate	Moderate
Publication bias	Possible	Possible	Possible
Quality body of evidence	***High***	***Moderate***	***Moderate***

A



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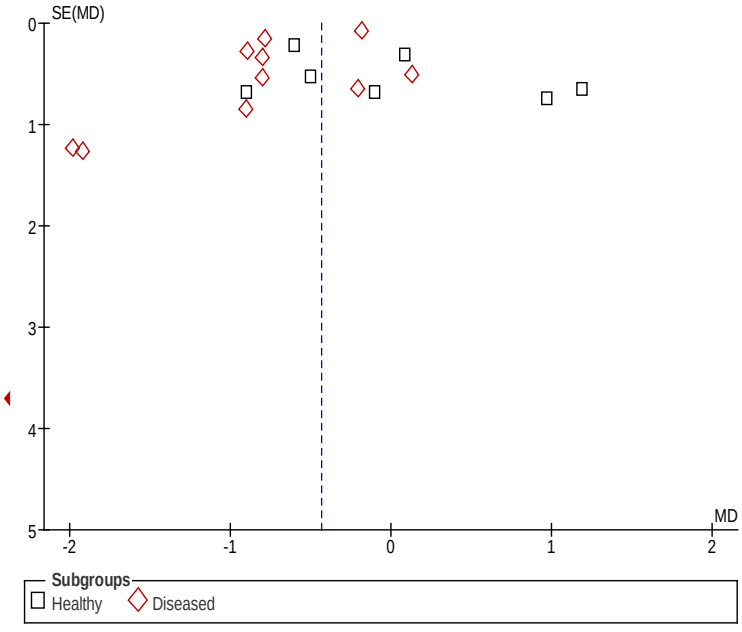
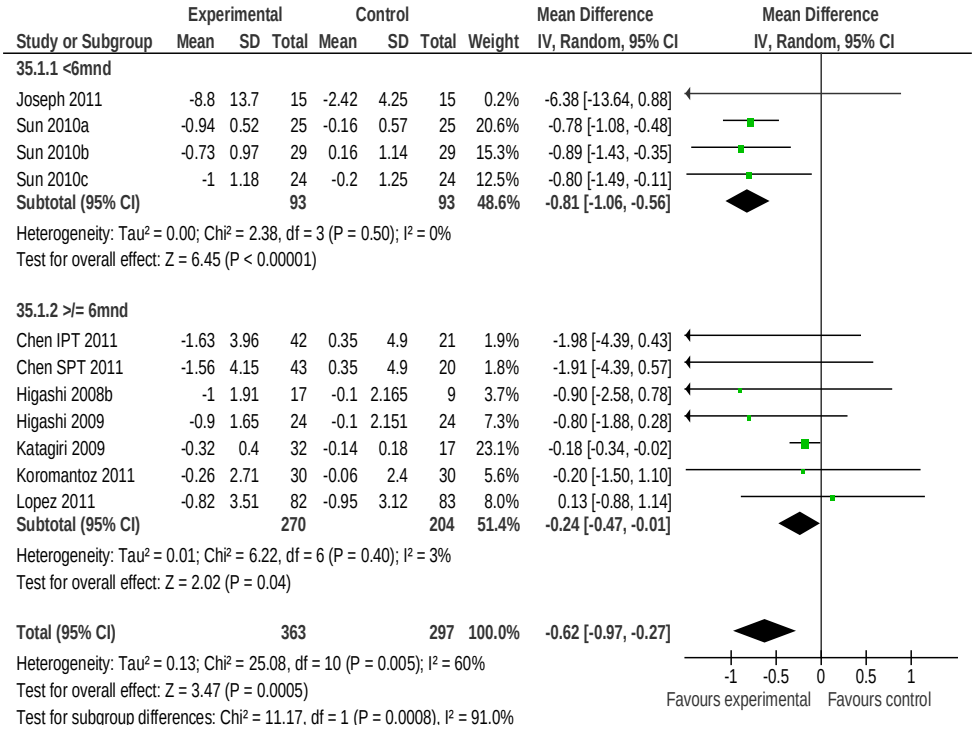
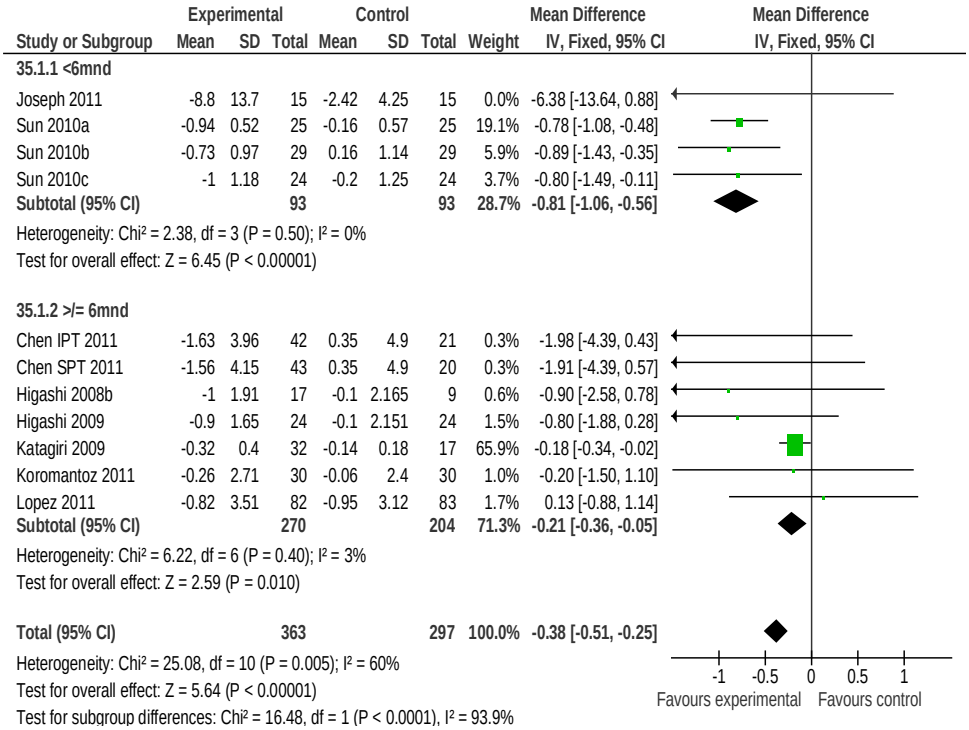


Supplemental Figure 1. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end CRP levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using CRP as an outcome parameter.

A (Random effect model)



(Fixed effect model)



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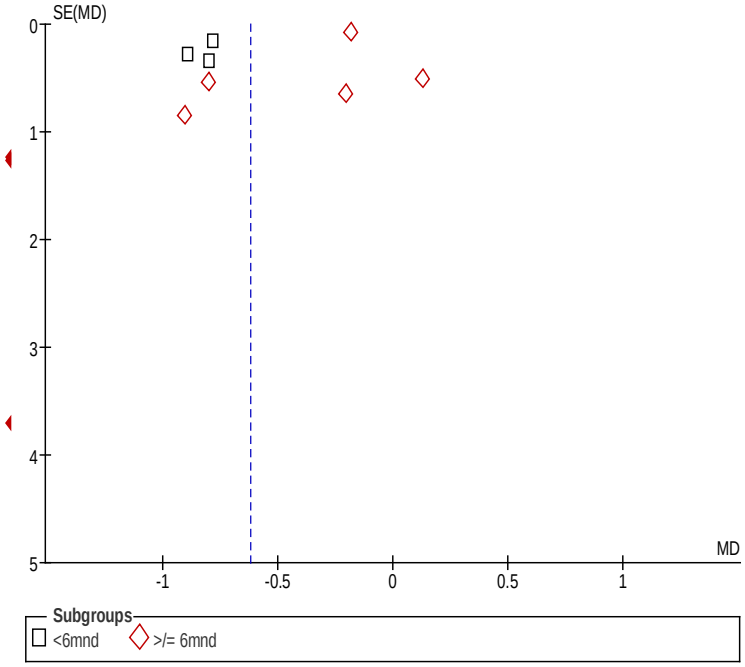
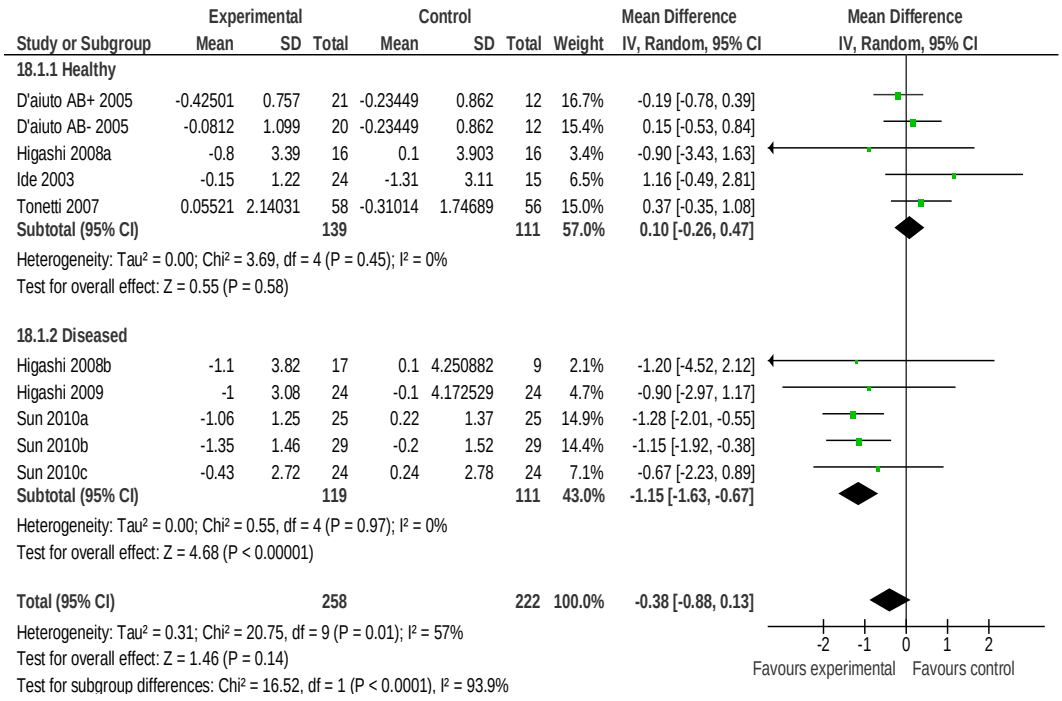


Supplemental Figure 2. (A) Forest plots, based on study duration, presenting WMD of baseline-end CRP levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random and fixed effect model. (**B)** Funnel plot of studies using CRP as an outcome parameter.

A



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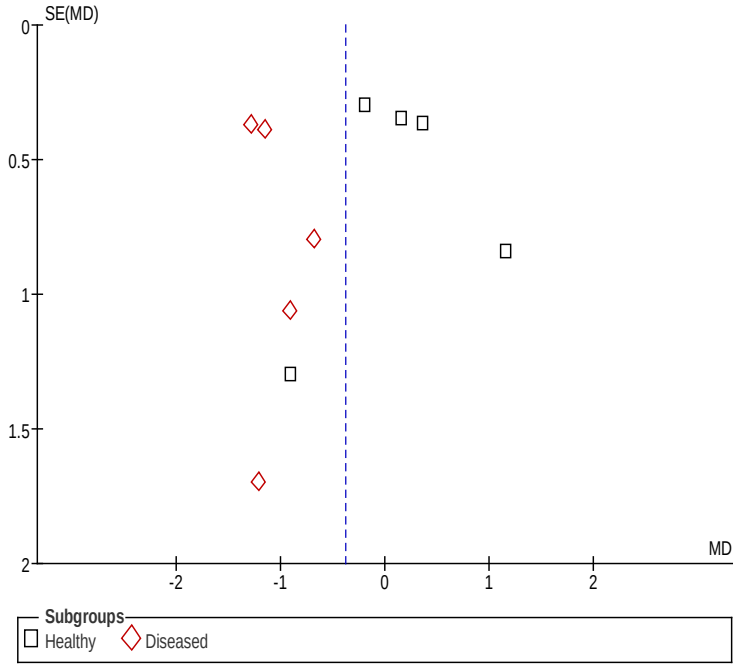
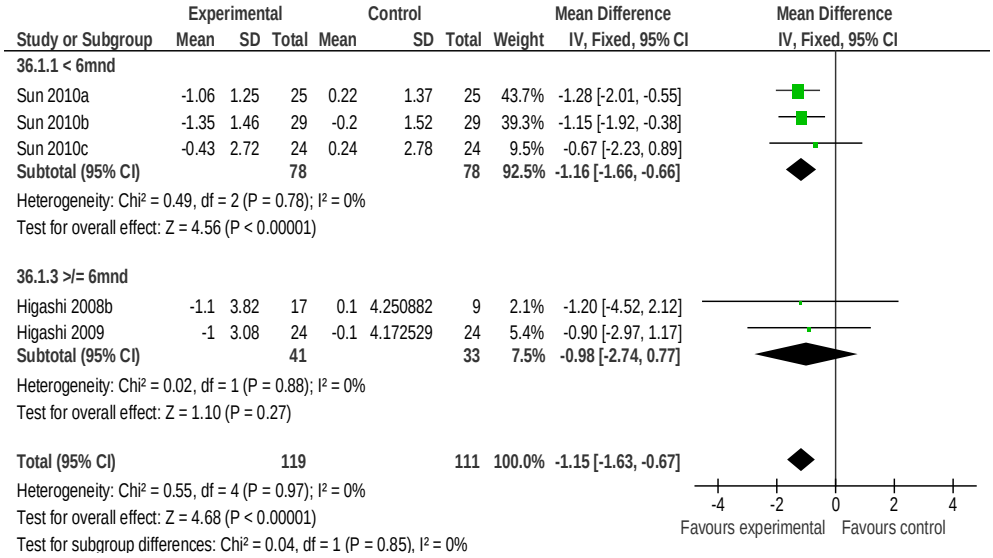


Supplemental Figure 3. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end IL-6 levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. **(B)** Funnel plot of studies using IL-6 as an outcome parameter.

A



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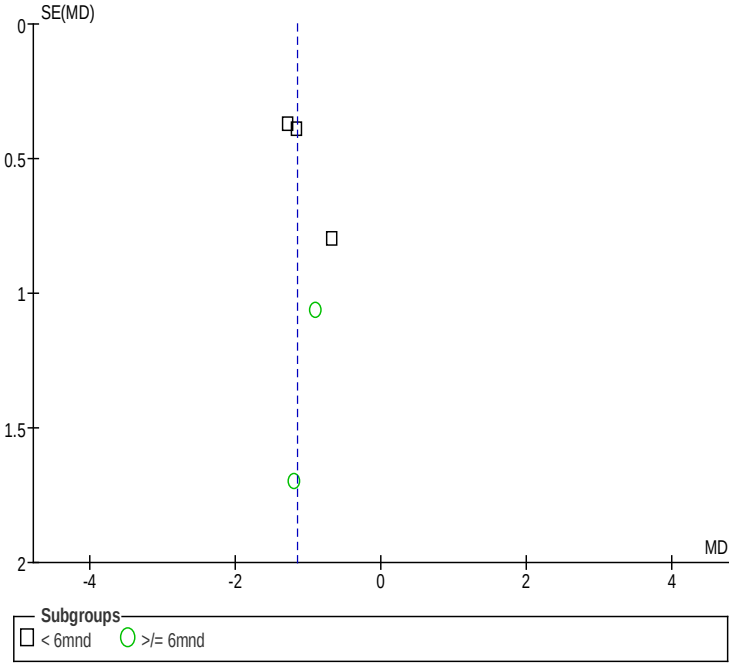


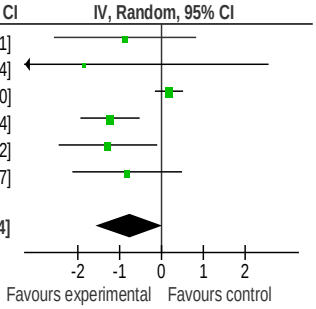
Supplemental Figure 4. (A) Forest plot, based on study duration, presenting WMD of baseline-end IL-6 levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a fixed effect model. (**B)** Funnel plot of studies using IL-6 as an outcome parameter.

A

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Chen IPT 2011	-0.41	3.58	42	0.46	3.01	21	12.7%	-0.87 [-2.55, 0.81]
Chen SPT 2011	-1.4	14.05	43	0.46	3.01	20	3.0%	-1.86 [-6.26, 2.54]
Ide 2003	-0.01	0.5	24	-0.19	0.49	15	26.9%	0.18 [-0.14, 0.50]
Sun 2010a	-1.37	1.19	25	-0.14	1.3	25	23.3%	-1.23 [-1.92, -0.54]
Sun 2010b	-1.45	2.21	29	-0.17	2.3	29	17.8%	-1.28 [-2.44, -0.12]
Sun 2010c	-0.72	2.32	24	0.1	2.25	24	16.3%	-0.82 [-2.11, 0.47]
Total (95% CI)			**187**			**134**	**100.0%**	**-0.77 [-1.57, 0.04]**

Heterogeneity: Tau² = 0.60; Chi² = 19.37, df = 5 (P = 0.002); I² = 74%

Test for overall effect: Z = 1.86 (P = 0.06)



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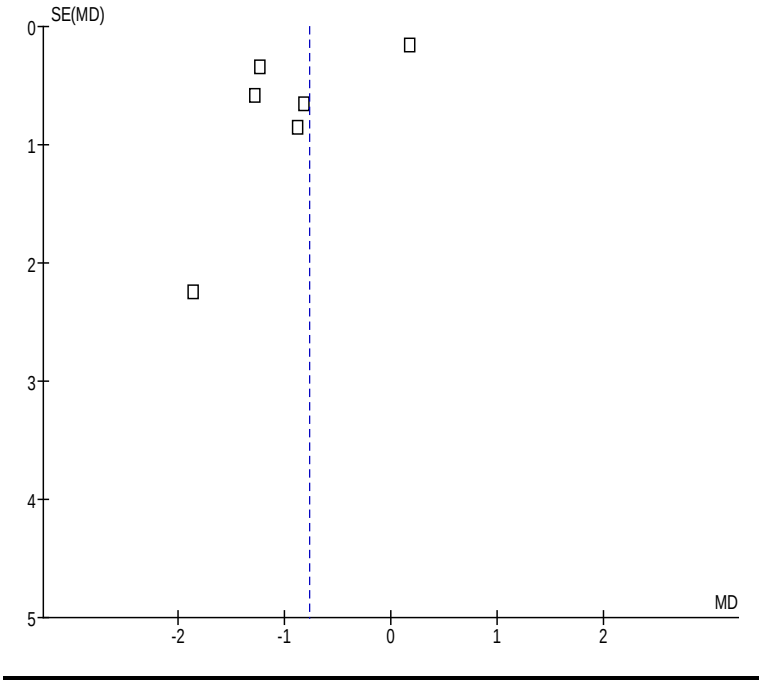
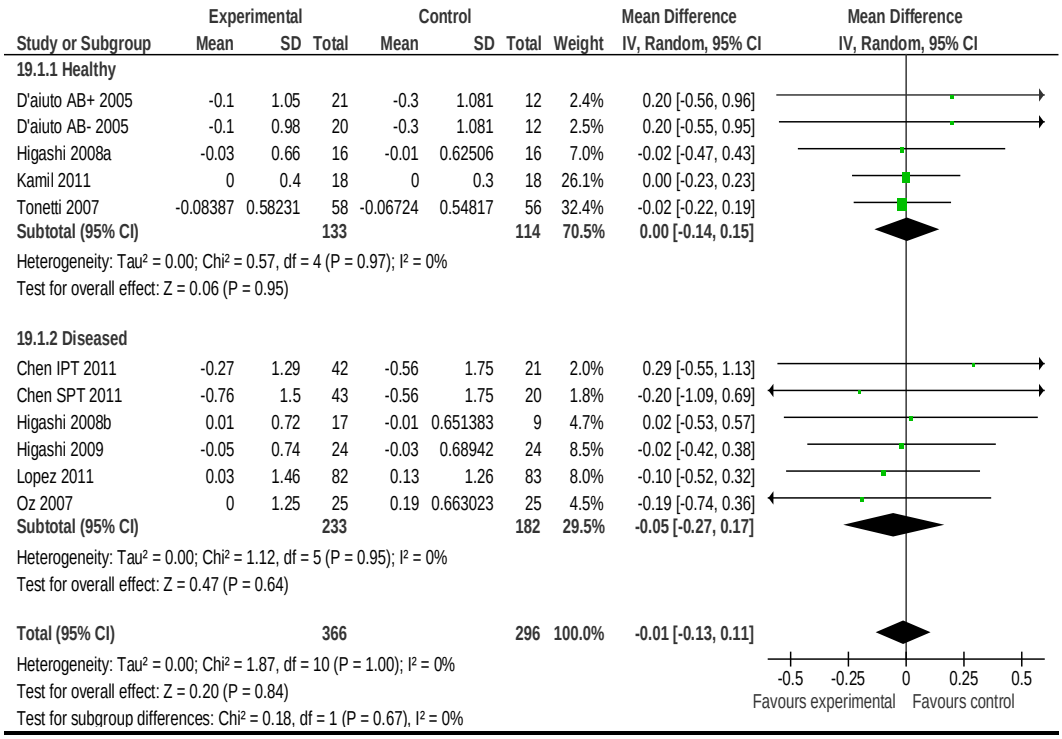


Supplemental Figure 5. (A) Forest plot presenting WMD of baseline-end TNF-α levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using TNF-α as an outcome parameter.

A



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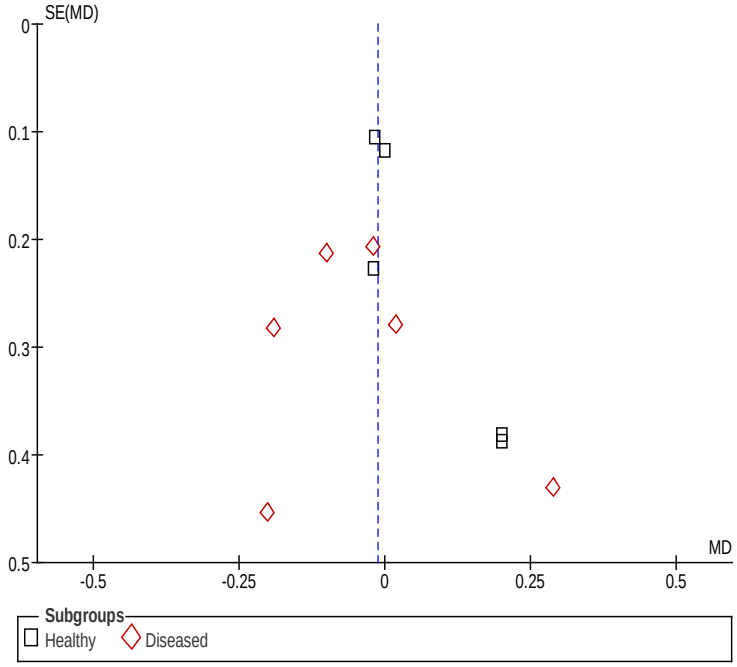
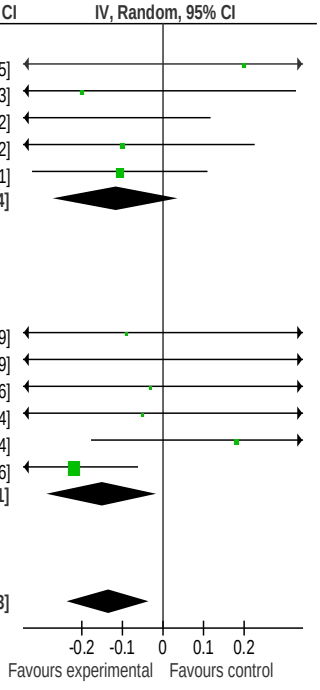


Supplemental Figure 6. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end triglycerides levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using triglycerides as an outcome parameter.

A

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
20.1.1 healthy								
D'aiuto AB+ 2005	0.1	0.82	21	-0.1	0.754983	12	3.4%	0.20 [-0.35, 0.75]
D'aiuto AB- 2005	-0.3	0.7	20	-0.1	0.754983	12	3.7%	-0.20 [-0.73, 0.33]
Higashi 2008a	-0.39	0.71	16	-0.01	0.720208	16	4.2%	-0.38 [-0.88, 0.12]
Kamil 2011	-0.2	0.53	18	-0.1	0.46	18	9.8%	-0.10 [-0.42, 0.22]
Tonetti 2007	-0.10484	0.55969	58	0.00169	0.60614	56	22.5%	-0.11 [-0.32, 0.11]
Subtotal (95% CI)			**133**			**114**	**43.6%**	**-0.12 [-0.27, 0.04]**
Heterogeneity: Tau² = 0.00; Chi² = 2.46, df = 4 (P = 0.65); I² = 0%								
Test for overall effect: Z = 1.47 (P = 0.14)								
20.1.2 Diseased								
Chen IPT 2011	-0.19	1.14	42	-0.1	1.89	21	1.3%	-0.09 [-0.97, 0.79]
Chen SPT 2011	-0.48	1.71	43	-0.1	1.89	20	1.1%	-0.38 [-1.35, 0.59]
Higashi 2008b	-0.01	0.75	17	0.02	0.910165	9	2.1%	-0.03 [-0.72, 0.66]
Higashi 2009	-0.07	1.28	24	-0.02	1.501	24	1.7%	-0.05 [-0.84, 0.74]
Lopez 2011	0.13	1.16	82	-0.05	1.17	83	8.2%	0.18 [-0.18, 0.54]
Oz 2007	-0.31	0.29	25	-0.09	0.274955	25	42.0%	-0.22 [-0.38, -0.06]
Subtotal (95% CI)			**233**			**182**	**56.4%**	**-0.15 [-0.29, -0.01]**
Heterogeneity: Tau² = 0.00; Chi² = 4.49, df = 5 (P = 0.48); I² = 0%								
Test for overall effect: Z = 2.17 (P = 0.03)								
Total (95% CI)			**366**			**296**	**100.0%**	**-0.14 [-0.24, -0.03]**
Heterogeneity: Tau² = 0.00; Chi² = 7.05, df = 10 (P = 0.72); I² = 0%								
Test for overall effect: Z = 2.61 (P = 0.009)								
Test for subgroup differences: Chi² = 0.11, df = 1 (P = 0.74), I² = 0%								



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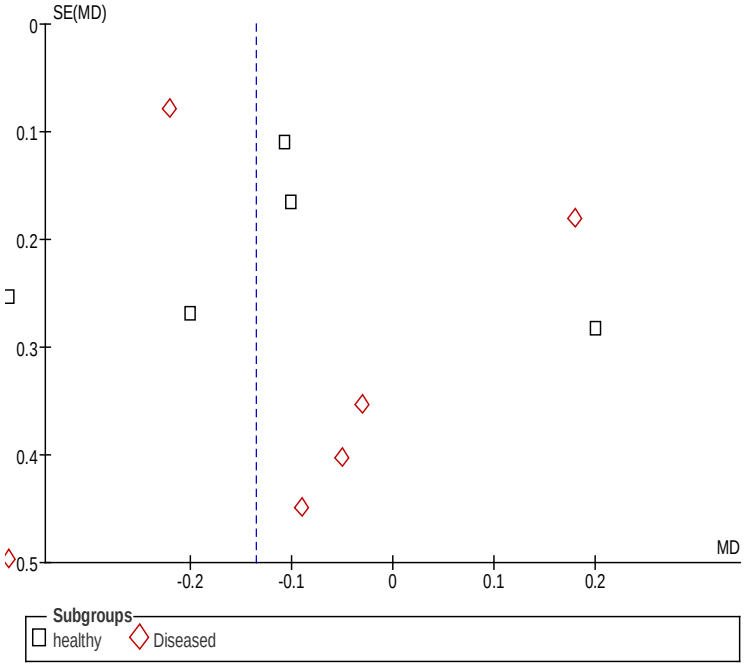
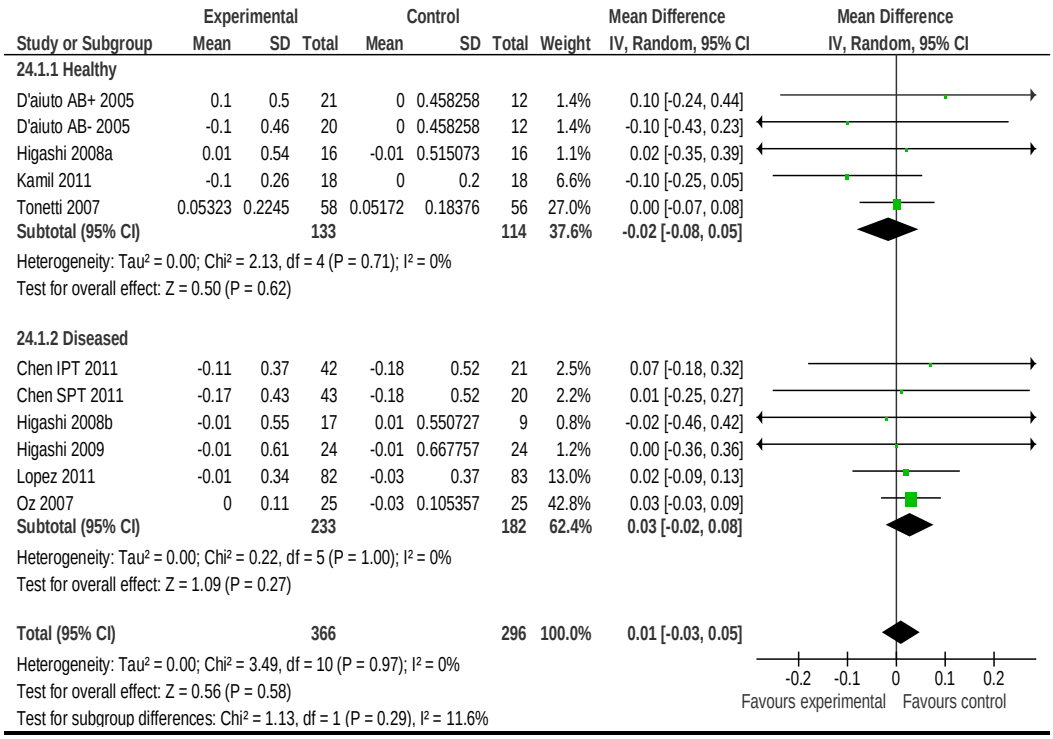


Supplemental Figure 7. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end total cholesterol levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using total cholesterol as an outcome parameter.

A



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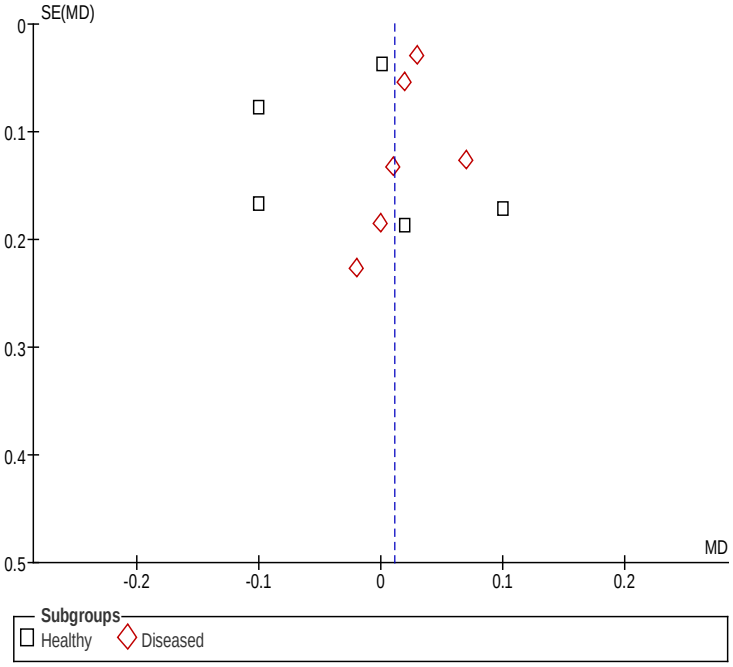
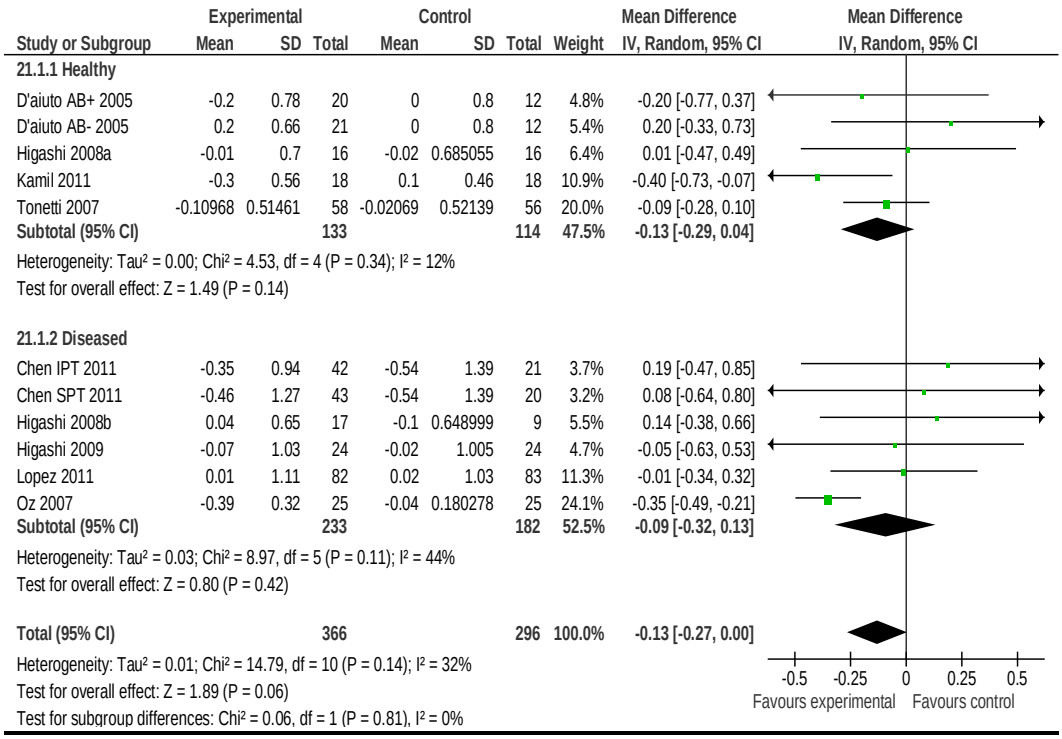


Supplemental Figure 8. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end HDL levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using HDL as an outcome parameter.

A



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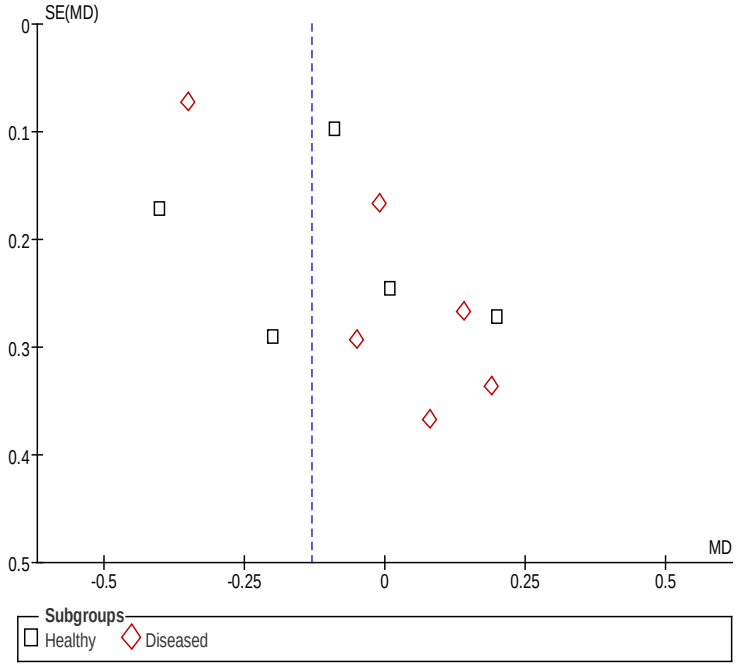
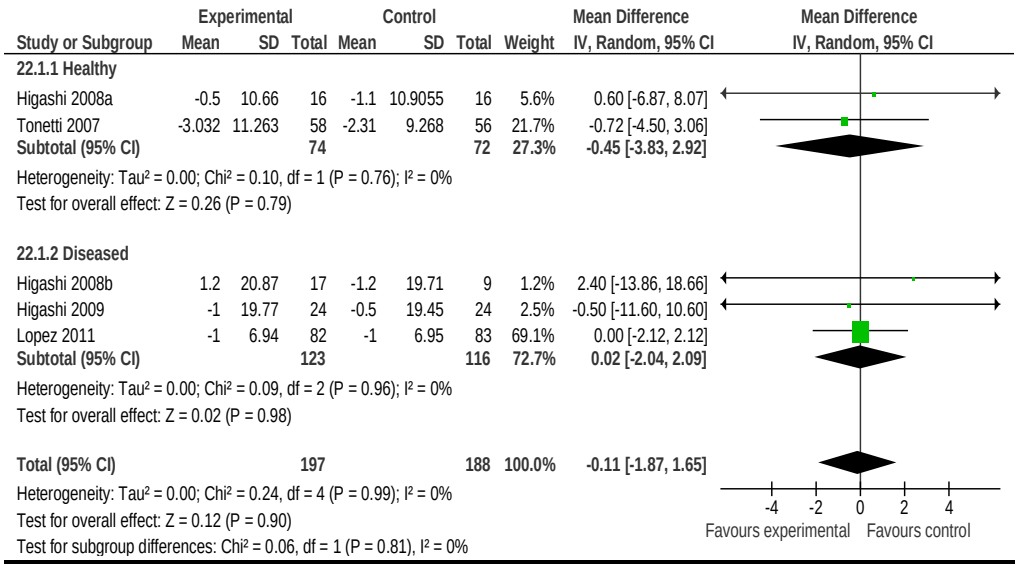
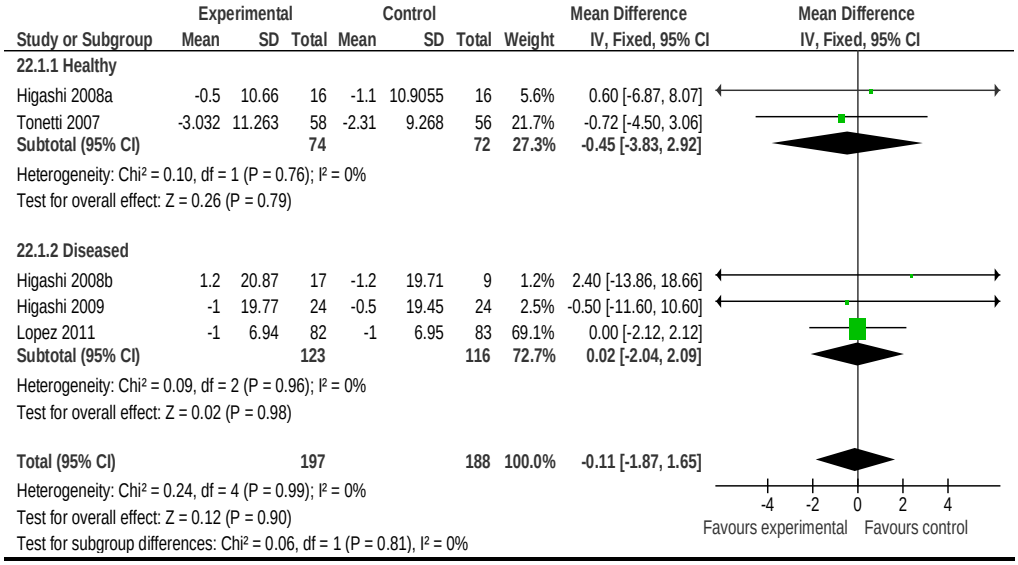


Supplemental Figure 9. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end LDL levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using LDL as an outcome parameter.

A (Random effect model)



(Fixed effect model)



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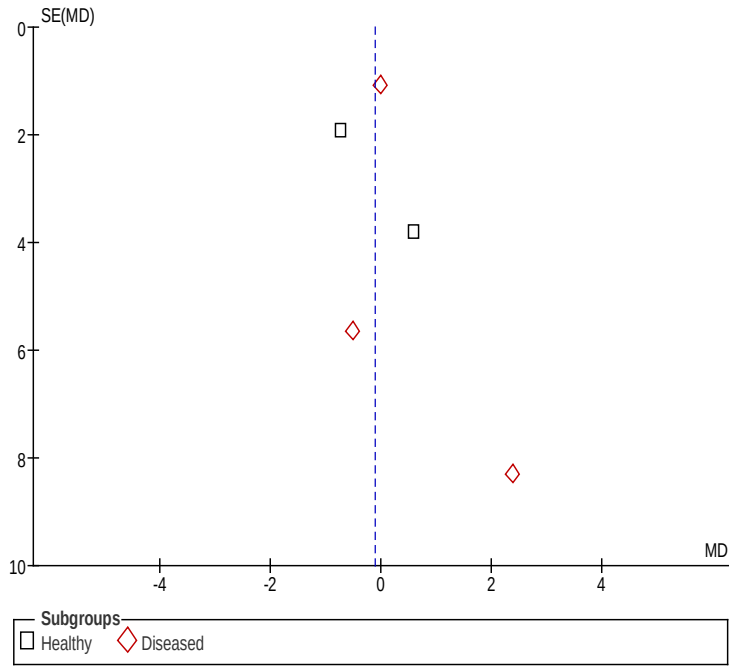
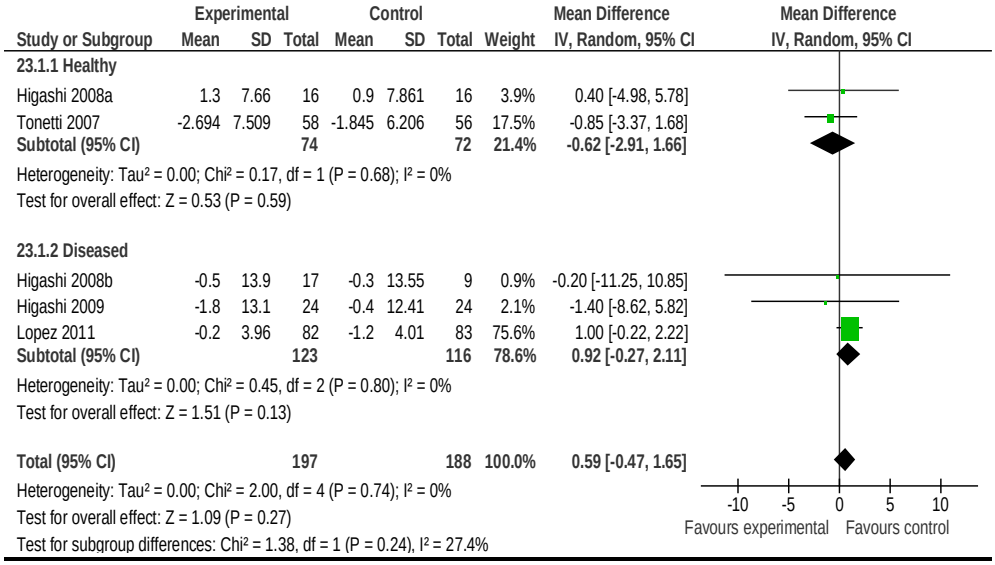
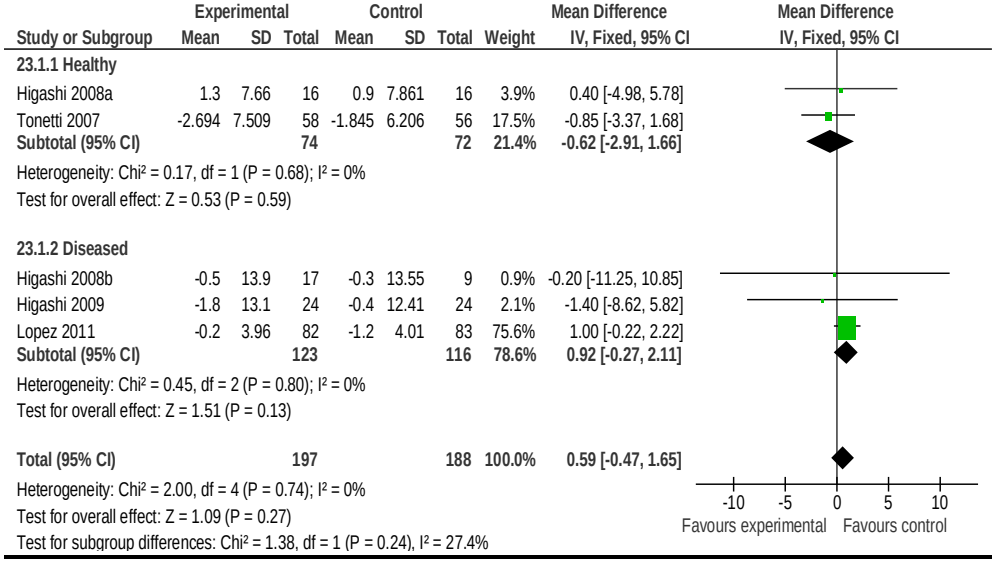


Supplemental Figure 10. (A) Forest plots, based on the systemic health of the study population, presenting WMD of baseline-end systolic blood pressure between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random and fixed effect model. (**B)** Funnel plot of studies using systolic blood pressure as an outcome parameter.

A (Random effect model)



(Fixed effect model)



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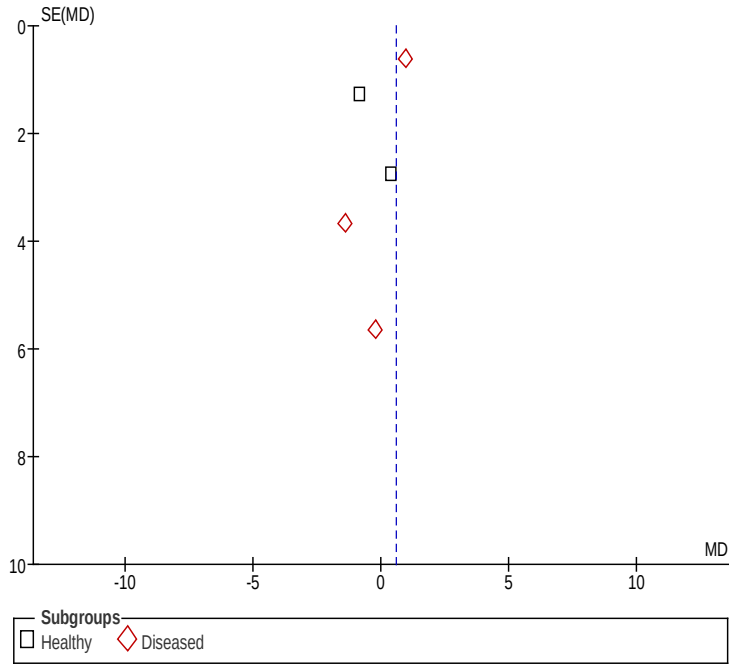
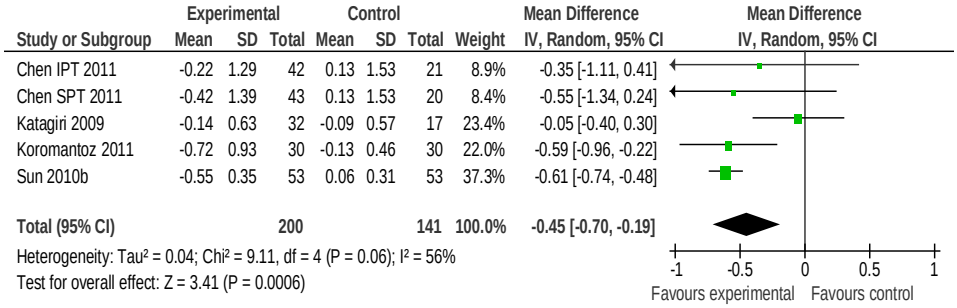
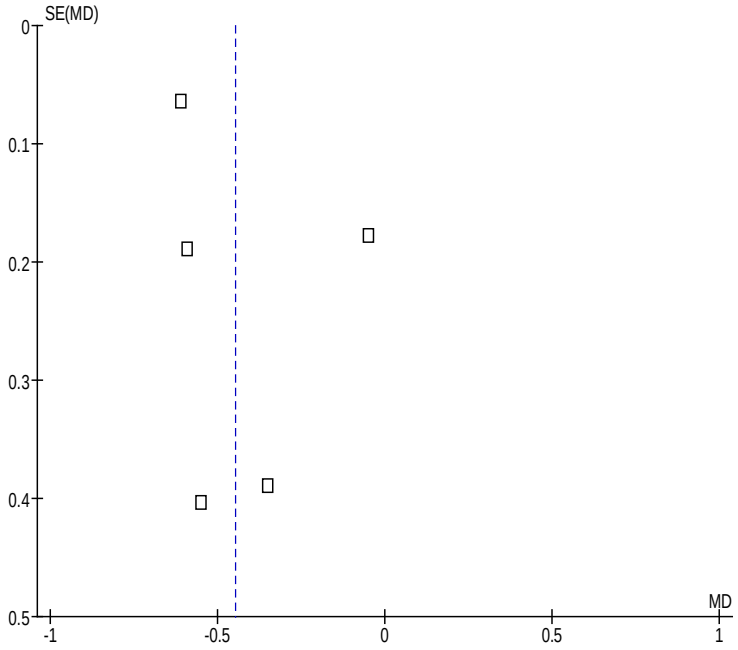


Supplemental Figure 11. (A) Forest plots, based on the systemic health of the study population, presenting WMD of baseline-end diastolic blood pressure between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random and fixed effect model. (**B)** Funnel plot of studies using diastolic blood pressure as an outcome parameter.

A



B



Supplemental Figure 12. (A) Forest plot, based on the systemic health of the study population, presenting WMD of baseline-end HbA_{1c} levels between the treatment groups (experimental) and non-treatment groups (control) including a systemically healthy and systemically diseased study population with periodontitis, heterogeneity and overall effect for treatment studies; a random effect model. (**B)** Funnel plot of studies using HbA_{1c} as an outcome parameter.

Discussion

The current review aimed to answer the question as to whether periodontal treatment reduces systemic levels of important risk markers for atherosclerosis. The vast majority of trials (18 out of 19) have investigated hsCRP levels before and after periodontal intervention. The main finding of the current study was that hsCRP was significantly reduced in all intervention trials combined into one meta-analysis. Interestingly, when we restricted the trials into including patients who were suffering from CVD and/or a metabolic disease (e.g. diabetes mellitus, metabolic syndrome, hyperlipidemia) hsCRP was significantly reduced, while this was not found in the intervention trials enrolling otherwise healthy periodontitis patients. In the systemically diseased population (11 trials), a total of 358 patients in the treatment group showed significantly more reduction of hsCRP compared to the 290 patients in the non-intervention group (ΔhsCRP -0.62 mg/L, CI: -0.97;-0.27; p=0.0005). This reduction in hsCRP is in our opinion clinically relevant; hsCRP levels exhibit a continuous association with the risk of coronary heart disease, ischemic stroke and vascular mortality,[29] suggesting that every decrease in hsCRP level may be beneficial for reducing the risk for CVD. It should be considered that baseline hsCRP values ranged from 0.8 ± 1.0 mg/L to 12.5 ± 15.9 mg/L.

Somewhat to our surprise and in contrast with previous meta-analyses[30-31] we did not observe significant reductions of hsCRP after treatment in periodontitis patients, who were systemically healthy (ΔCRP: -0.09 mg/L, CI: -0.60 ; 0.42; p=0.73). Perhaps, this could be explained by the Hawthorn effect in the non-intervention group. Another explanation is that hsCRP is not a good marker for successful periodontal therapy. More recently, clinical trials on periodontal intervention include vascular function parameters. All 5 trials showed a significant improvement in vascular function after periodontal therapy as measured by flow mediated dilation, acetylcholine-dependent vasodilatation or a decrease in $CD34^+$ cells. These results on markers of improved vascular function were observed in both systemically healthy and systemically diseased study populations. However, these results should be interpreted cautiously, because different methods for assessment of endothelial function were used and no proper meta-analysis could be performed.

A second major finding of this study was that periodontal treatment resulted in improvement of metabolic control in diabetic patients as shown by a significant reduction in HbA_{1c} levels. In the current meta-analysis, we observed a significant change in HbA_{1c} of 0.45% for the treatment group compared to the non-treatment group. This was found in 5 trials of which only 1 overlapped with our previous study on the effect of periodontal therapy on metabolic control;[13] in the latter publication we found a very comparable and significant improvement also (ΔHbA_{1c}: -0.40%). In the current analysis, metabolic diseased patients were included, since it is known that patients with elevated levels of HbA_{1c} run a higher risk for CVD.

A third important finding of the current study was, that after periodontal treatment, total cholesterol (TC) was also significantly reduced in periodontitis patients with CVD and/or metabolic disease compared to the non-treatment group. Similar to hsCRP and IL-6, this was not observed in otherwise healthy subjects with periodontitis. Elevated TC levels are strongly associated with increased risk for CVD.[32]

In all included trials of this systematic review, only surrogate markers were used to evaluate the effect of periodontal treatment on CVD. No hard clinical outcomes (e.g. secondary myocardial infarction, stroke, death, etc.) have been investigated. However, this latter is impossible to investigate, since it is unethical to leave patients with periodontitis untreated for a follow-up of at least 5-10 years to record a substantial number of clinical events. The current systematic review shows clearly that patients with known CVD or metabolic disease benefit from periodontal therapy, which could be present up to 12 months.

It has been widely accepted that low grade systemic inflammation contributes to an elevated risk for CVD. In this respect, elevated hsCRP levels are strongly associated with increased risk.[33] The elevated levels of hsCRP and other inflammatory markers in periodontitis (e.g. IL-6 and TNF-α)[34] might be related to the chronicity of periodontitis and the concomitant periodontal inflamed surface area (PISA[35]). The PISA in patients with severe periodontitis may be as large as 39 cm^2.[35] This breach in epithelial lining opens up the possibility for oral pathogens to enter the circulation and induce systemic inflammation, metabolic discontrol and vascular dysfunction. Periodontal therapy reduces PISA and the current meta-

analyses are highly suggestive that this reduction results in lower levels of systemic inflammation and improvement of metabolic control.

Some issues regarding the current study need discussion. First, although we combined trials in meta-analyses, we noted quite some heterogeneity between trials (Range I^2: 0%-74%, Table 1). Ideally, I^2 should be ≤40% and χ^2-test should result in $p \geq 0.1$. Furthermore, systematic reviews and meta-analyses run the risk that included studies may have some form of bias. Third, the follow-up of the included trials ranged between 4 weeks and 12 months and it should be questioned which follow-up time is appropriate to be able to draw clinical relevant conclusions about the effect of periodontal therapy on the biochemical risk markers of CVD. The issue of reporting surrogate outcomes is already discussed above.

A strong positive aspect of the current study is that we made a very broad search of the literature and that we performed a sub-analysis based on the systemic health of the subjects in the selected trials. This is the first study that indicates that especially those suffering from CVD and/or metabolic diseases, benefit from periodontal therapy with regard to biomarkers of atherosclerotic diseases.

Thus, we conclude from the current systematic review, based on strict inclusion criteria and quality control of literature, that periodontal treatment might reduce the risk for CVD by reducing plasma levels of inflammatory (CRP, IL-6) and metabolic (TC, HbA_{1c}) markers. This improvement was especially observed in periodontitis patients also suffering from systemic diseases, like CVD and/or diabetes mellitus, and this improvement was still present 6 month after periodontal intervention. This emphasizes the effectiveness and need for periodontal diagnosis and periodontal therapy in atherosclerotic and diabetic individuals to improve their systemic health. We recommend that cardiologists and diabetologists ask their patients to be screened for the presence of periodontal disease and if so, to undergo periodontal therapy as we have shown here that this will reduce systemic inflammation and will improve metabolic control and vascular function.

Acknowledgements

We thank Dr. M. Ide, Dr. S. Katagiri, Dr. P.A. Koromantzos, Dr. N.J. López, Dr. W. Sun for their contribution of providing additional data included in the meta-analyses. The department of Periodontology ACTA is supported in part by a grant of the University of Amsterdam for its participation in the focal point of “Oral infections and inflammation”. Dr. F. D’Aiuto holds a Clinical Senior Lectureship Award supported by the UK Clinical Research Collaboration and works at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

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Chapter 6

General Discussion

An increasing number of studies have demonstrated an association between periodontal disease and several systemic conditions and diseases such as diabetes mellitus type 2 (DM2) [1-10], rheumatoid arthritis (RA) [11-22], cardiovascular diseases (CVD) [23-35], adverse pregnancy outcome [36-43], osteoporosis[44-47] and respiratory diseases [48-56]. However the associations reported differ in magnitude between studies, which may be due to differences in either populations studied, severity of systemic diseases or the way in which periodontitis is operationalized. In case of Diabetes Mellitus type 2 (DM2) for example, periodontitis has been categorized as mild, moderate or severe on the basis of a variety of cut off points for pocket depth (PD), bleeding on probing (BOP), clinical attachment loss (CAL), bone loss (BL) and tooth mobility [4, 5, 7, 57]. Only one of those studies did not show a significant difference in prevalence and severity of periodontitis between DM2 and healthy controls [57]. One study operationalized periodontitis according to the community periodontal index of treatment need (CPITN) did not find significant differences in prevalence and severity of periodontitis between patients with DM2 and healthy controls [58].

Thus, different methods to define periodontitis may influence not only the strength of the associations between a given disease and periodontitis, but also whether an association is observed at all. In addition to DM2, in research linking periodontitis to preterm low birth weight, 13 different methods have been used to operationalize periodontitis. Depending on the method used, either an association [59, 60] or no association [43] between periodontitis and preterm low birth weight was observed. Like DM2 and preterm low birth weight, associations between periodontitis and RA may be influenced by periodontitis operationalization. Therefore, whether the method of operationalizing periodontitis affects the results of studies on the association between periodontitis and systemic disease was investigated in the present thesis.

To study possible associations, the commonly used and above mentioned methods to operationalize periodontitis prevalence and severity were used. In addition, a recently introduced measure to quantify periodontitis, the Periodontal Inflamed Surface Area (PISA) [61] was applied. Other indicators of inflammatory burden in patients with DM2 or RA were measured, such as erythrocyte sedimentation (ESR), serum levels of c-reactive proteins (CRP), rheumatoid factor and cyclic citrullinated antibodies (ACPA's).

PISA

PISA was introduced by Nesse et al (2008)[61] in order to quantify the amount of inflamed periodontal tissue. Commonly applied other methods use continuous variables e.g. mean probing pocket depth (PPD) and mean clinical attachment levels (CAL) or certain cut off points to classify patients as being either unaffected or affected by mild, moderate or severe/advanced periodontitis [62]. Nesse et al. (2008) stated in their landmark paper that these outcome measures do not necessarily quantify the amount of inflamed periodontal tissue. Therefore, they added a second dimension by calculating the inflamed (bleeding) pocket surface area in periodontitis, to quantify the inflammatory burden produced by periodontitis. PISA is calculated using CAL, PPD, recession and bleeding on probing (BOP). Although PISA seems to be a promising tool to better assess the inflammatory burden of periodontal disease than, e.g. PPD, CAL and BOP do, PISA has also some shortcomings. These shortcomings include measurement errors in assessing the various variables (similar to PPD, CAL, BOP etc.) and the use of population based mean values for root surface areas and root lengths. Furthermore, the extend and composition of the inflammatory infiltrate and underlying inflamed surface area are not assessed by PISA. Finally PISA, like all other variables used to operationalize periodontal disease thus far, does not provide information on the periodontal pathogenic flora and serum inflammatory mediators. Despite these shortcomings, PISA has demonstrated a dose response relationship between control of blood glucose levels over time as expressed by HbA1c in Afro-American DM2 patients from Curacao[9]. The observed association was independent of confounding factors as sex, oral hygiene, SES, BMI, smoking and the number of years since DM2 was diagnosed.

In the present thesis, the prevalence and severity of periodontitis in Indonesians with DM2 was investigated. PISA, but also traditional parameters as PPD, CAL and BOP were able to demonstrate a higher prevalence and severity of periodontitis in DM2 patients compared to controls in this ethnic different population, indicating the robust association between periodontitis and DM2. It also indicates that these methods, including PISA, can be applied in different ethnic populations. Furthermore, PISA together with CRP emerged as predictor of HbA1c in controls indicating that periodontitis may pose an inflammatory burden that contributes to developing DM2 (**chapter 2**). In Indonesian DM2 patients, however, the assumed association between PISA and HbA1c levels could not be confirmed .

The latter may be due to the antidiabetic drug regimes used in Indonesian DM2 patients (many of them used insulin derivates) as well as substantial lower body mass index (BMI) scores in Indonesia as compared to individuals in the Curacao study of Nesse et al (2009) who were mainly on oral antidiabetics or just a diet.

Thus, periodontitis (expressed as PISA) may pose an inflammatory burden that impairs blood glucose regulation (expressed as HbA1c), however this effect may be masked by use of antidiabetic drugs and modified by BMI. Furtehermore, based on our data it is suggested that subjects with a high PISA score as predictor for HbA1c but not yet known with DM2 should be tested for DM2.

Inflammatory markers

Additional information on the presence of inflammatory markers, e.g., CRPs, interleukins IL 1 and IL6, and tumor necrosis factor alpha (TNF**α)** and the periodontal pathogenic flora should be considered as indicators of the impact of periodontitis as an inflammatory burden and risk factor for systemic diseases and disorders. Our studies in Indonesia, presented in this thesis, indeed indicated that periodontitis may cause an infectious and inflammatory burden as evidenced by increased CRP levels, which may impair blood glucose control [63]. In **chapter 4** we were able to demonstrate that the presence of periodontitis in RA patients was associated with an increased inflammatory state as indicated by higher CRP and ESR levels. The increased levels of inflammatory markers in patients with RA may be explained by several factors. First, periodontitis may aggravate RA. Since periodontitis is accompanied by higher CRP levels [64-68] , long standing untreated periodontitis may contribute to a proinflammatory state in RA. Secondly, RA may aggravate periodontitis. Since more severe RA is also accompanied by higher CRP levels, [69, 70] higher CRP levels may be a reflection of active RA, which may contribute to a proinflammatory state in periodontitis. The same explanations may apply to higher ESR in patients with RA and periodontitis [12, 15] . Interestingly, studies reporting on the effect of periodontal treatment in RA patients not only show a reduction in ESR levels,[14, 18, 22, 71] but also a reduction in CRP levels after periodontal therapy [22] . These findings support periodontitis as contributer to a higher disease activity in RA. It should be noted that these are preliminary results of just a few intervention studies with small samples size. The existence and nature of an association between RA and periodontal disease needs further research in which

proper attention is paid to specific confounders like maintenance of proper oral hygiene.

RA affects the wrist and the small joints of the hand impairing handfunction of RA patients. As a result maintenance of proper oral hygiene may be impaired, thus posing a riskfactor for periodontitis [17] [21]. Although RA patients had significantly higher plaque scores than non-RA controls, thus supporting the previous hypothesis, RA patients with periodontitis had lower mean plaque scores than RA patients without periodontitis. Therefore, impaired maintenance of oral hygiene in RA patients probably does not appear toconfound the association between periodontitis and increased disease activity in RA.. Smoking and duration of RA may also serve as confounding factors. Although smoking is a risk factor for both RA [72] and periodontitis[73] and duration of RA is also associated with severity of periodontal disease, both smoking and duration of RA did not appear to confound the association between periodontitis and higher ESR and CRP levels in RA patients (**chapter 4**).

Treatment studies

Recently, it was demonstrated in a systematic review that treatment of periodontitis in RA patients reduces RA disease activity with fair evidence on reduction of the Disease Activity Score for Rheumatoid Arthritis DAS28 scores [74]. DAS28 is a composite, numerical score combining key components as tender and swollen joints, ESR, CRP and the patients global assessment of general health, measured on a visual analogue scale (VAS) [75].Regarding DM2, a meta-analysis showed that periodontal treatment led to improvement of glycaemic control probably due to reduction of CRP levels and inflammatory cytokines [10]. Thus, meta-analyses have shown that a reduction in inflammatory burden, as evidenced by a reduction in CRP levels, after periodontal treatment appears beneficial to patients with RA and DM2. CRP levels also play a key role in the risk for atherosclerotic diseases. Consequently, in the systemic review described in **chapter 5** the effect of periodontal treatment on the reduction of the levels of biomarkers of atherosclerotic disease was investigated. Significant reductions could be demonstrated of inflammatory markers (CRP, Il-6 levels) and metabolic markers (total cholesterol and HbA1c) after periodontal treatment, especially in periodontitis patients already suffering from cardiovascular and/or metabolic diseases. This finding demonstrates that periodontal treatment reduces the inflammatory burden caused by periodontitis.

Conclusion and future perspectives

Our studies on the association between periodontitis and DM2 and RA have shown that Indonesians patients with DM2 had more prevalent and severe periodontitis than controls. Furthermore, it appeared that PISA and CRP were predictors of glycaemic control of Indonesian subjects not on antidiabetics . On the contrary, in Indonesian patients with RA the prevalence and severity of periodontitis appeared was not higher than in healthy controls. However, within the group of RA patients, periodontitis presence was associated with an increased inflammatory state. CRP appears to play a major role in these associations as CRP levels can be reduced by periodontal treatment, thereby improving DM2 and RA conditions and reducing the risk for CVD. In future research the role of CRP's in different forms of periodontal diseases and the reduction induced by different therapeutic protocols should be investigated. Furthermore, diagnosis and treatment of periodontal disease should be an integral part of medical treatment of DM2 and RA and also deserves attention in the prevention of CVD. Periodontitis appears to cause a proinflammatory and procoagulatory state. Further research into the magnitude and nature of the contribution of periodontitis to the initiation and deterioration of systemic diseases is necessary. This may well lead to insights that further improve health of patients with periodontitis and systemic diseases.

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Chapter 7

Summary

Many studies suggest that periodontitis is associated with systemic diseases such as, diabetes mellitus (DM), rheumatoid arthritis (RA), cardiovascular diseases, bacterial pneumonia, adverse pregnancy outcome, osteoporosis and cerebrovascular disease. The association between periodontitis and certain systemic diseases is bidirectional. Not only may systemic disease aggravate periodontitis, but similarly periodontitis may aggravate systemic disease.

In **chapter 1** the theoretical background of the bidirectional association between periodontitis and systemic diseases is explained. Systemic diseases may affect periodontitis through a systemic inflammatory response by releasing systemic inflammation markers such as C-reactive protein (CRP). This systemic inflammation marker may amplify the local inflammatory response in periodontitis. On the other hand, periodontitis may aggravate systemic disease by direct translocation of bacteria or bacterial products as well as through a systemic inflammatory response by production of CRP. The assumed association between periodontitis and systemic diseases can also be demonstrated by the evidence that periodontal treatment is accompanied by a reduction of, e.g., serum CRP level, which in turn is involved in a reduction of the activity of systemic disease. However, both in periodontitis and systemic disease other confounding and/or modifying risk factors have to be considered such as age, sex, and smoking.

In **chapter 1** it is also explained why the various studies were conducted in Indonesia. The main reason was the still conflicting results amongst studies reported in the literature assessing the association between periodontitis and systemic diseases as well as that to date only few studies have been conducted in Asia. As a consequence, it is uncertain whether the results of the studies reported in the international literature to date may be generalized to Asian populations such as Indonesians. Finally, the application of different methods in defining periodontitis may have contributed to the different asscociations found between periodontitis and systemic diseases. Therefore, the aim of the PhD research described in this thesis was to investigate associations between periodontitis and systemic diseases as diabetes mellitus type 2 (DM2) and rheumatoid arthritis (RA) in Indonesia. In addition, it was systematically reviewed whether patients suffering from atherosclerotic diseases might also benefit from treatment of periodontitis.

In **chapter 2** the prevalence of periodontitis in patients with DM2 in an Indonesian population is reported. In addition, this study investigated the association between periodontitis and DM2 using a variety of methods to operationalize periodontitis. The use of different methods to operationalize periodontitis prevalence and severity has shown to influence the strength of the association of, e.g., in the linking of periodontitis and preterm birth. The same influence may be true for the association of periodontitis and DM2. In total 78 DM2 and 65 healthy subjects underwent full mouth periodontal screening including assessment of probing pocket depth, gingival recession, plaque index and bleeding on probing. Using these data the periodontal inflammatory surface area (PISA) was calculated. The results of the study presented in **chapter 2** showed differences in periodontitis prevalence and severity between patients with DM2 and healthy subjects, with odds ratios of 5.0 and 6.1, respectively. Univariate analyses showed a significantly higher prevalence and severity of periodontitis in patients with DM2 . With regard to the risk factors for periodontitis, only age and hypertension were found significantly higher in DM2 as compared to controls. In regression analyses only DM2 and age were predictors for periodontitis. It was concluded that Indonesian patients with DM2 had more prevalent and more severe periodontitis than Indonesian controls , independent of confounding factors or the methods used to operationalize periodontitis.

In **chapter 3** a study is described in which it was shown that periodontitis may raise an infectious and inflammatory burden to DM2 as evidenced by increased CRP. This burden may impair blood glucose control as monitored by HbA1c. In 132 Indonesian controls and 101 Indonesians treated for DM2, a full mouth periodontal examination was performed. HbA1c and CRP were measured. Regression analyses were applied to assess whether periodontitis severity and CRP predict HbA1c when controlling for confounding and effect modification. The results of these analyses showed that periodontitis severity and CRP were predictors of HbA1c levels in a group of healthy Indonesians. Such an association could not be demonstrated in a group of Indonesians treated for DM2. While in Indonesian controls , PISA predicted HbA1c together with CRP, age, sex and smoking, this was not the case in Indonesians treated for DM2. It was assumed that the potential effect of periodontitis on glucose

control in patients with DM2 might be masked by treatment of DM2 in these patients, and probably will be present in untreated and non-diagnosed DM2 subjects.

In **chapter 4** a study is described assessing the prevalence and severity of periodontitis and its potential association with systemic inflammation in Indonesians with RA. In total 132 Indonesian controls and 78 Indonesians with RA were investigated. Full mouth periodontal examination was performed. Serum levels of CRP, ESR, rheumatoid factor and anti cyclic citrullinated antibodies (ACPAs) were measured. No significant differences in periodontitis prevalence and severity were observed between RA patients and controls. It could, however, be shown that ESR and CRP were higher in RA patients with periodontitis compared to RA patients without periodontitis. It was concluded that, although periodontitis prevalence and severity were not higher in Indonesian RA patients than in Indonesians controls, periodontitis apparently is associated with an increased systemic inflammation in RA patients.

In **chapter 5** a systemic review and meta-analyses is described reporting on the robustness of observations that periodontal treatment reduces the levels of biomarkers of atherosclerotic diseases. A literature search was performed based on original investigations, in which effects of periodontal intervention were compared relative to no periodontal treatment. Periodontal treatment was the predictor and inflammatory, metabolic and/or vascular markers were outcomes. Meta-analyses including all available studies, demonstrated significant differences in weighted means for CRP, IL-6, total cholesterol and HbA1c favoring periodontal intervention especially in patients suffering from cardiovascular and/or metabolic diseases like DM2. This reduction of biomarkers emphasizes the effectiveness and need of periodontal therapy in these individuals to improve their health.

In **chapter 6** the main research findings are discussed and future perspectives are given. In general, also in Indonesia periodontal disease may have a considerable impact on general health, amongst others in DM2 and RA subjects. It was also concluded that CRPs may play a key role in the systemic effects of periodontal diseases and that CRP serum levels can be reduced by periodontal treatment. Moreover, improved periodontal health is accompanied by better

glycaemic control, reduction of RA disease activity and a reduced risk for atherosclerotic diseases. Periodontal disease, diagnosis and treatment should, therefore, be an integral part of health care thereby not only improving oral health, but also general health and quality of life.

Chapter 8

Samenvatting/Rangkuman

Parodontitis lijkt te zijn geassocieerd met ziektes zoals diabetes mellitus, reumatoïde artritis, hart en vaatziekten, bacteriële pneumonie, zwangerschapscomplicatie, osteoporose en cerebrovasculaire accidenten. De samenhang tussen parodontitis en bepaalde aandoeningen kan beide kanten opgaan ofwel is bidirectioneel. Niet alleen kunnen ziekten parodontitis verergeren, maar kan parodontitis ook aandoeningen verergeren.

In **hoofdstuk 1** wordt het tweerichtingsverkeer tussen parodontitis en systeemziekten uitgelegd. Systeemziekten kunnen parodontitis beïnvloeden door een systemische ontstekingsreactie waarbij systemische ontstekingsfactoren vrijkomen zoals c-reatieve eiwitten (CRP). Deze systemische ontstekingsfactor kan de lokale ontstekingsreactie bij parodontitis verergeren. Aan de andere kant kan parodontitis systemische aandoeningen verergeren door directe verplaatsing (translocatie) van bacteriën of bacteriële producten of door een systemische ontstekingsreactie (productie van CRP). Het veronderstelde verband tussen parodontitis en systemische aandoeningen blijkt ook uit de observatie dat behandeling van parodontitis vergezeld wordt door een afname van bijvoorbeeld serum CRP. Het gevolg hiervan is dat de ziekteactiviteit van de systemische aandoening ook afneemt. Men moet hierbij bedenken dat bij parodontitis en systeemziektes voor een deel dezelfde oorzakelijke factoren hebben en dat confounders (verstorende variabelen) en/of risicofactoren een rol kunnen spelen zoals leeftijd, geslacht en roken bij de tot nu toe gevonden resultaten. In hoofdstuk 1 wordt ook verklaard waarom het onderzoek in Indonesië werd uitgevoerd. De belangrijkste reden daarvoor was dat in de literatuur tegenstrijdige resultaten worden gerapporteerd met betrekking tot het verband tussen parodontitis en andere aandoeningen. Daarnaast zijn nog maar weinig studies verricht in Azië. Het gevolg daarvan is dat het onzeker is of de in de huidige internationale literatuur beschreven resultaten ook toepasbaar zijn voor Aziatische populaties zoals Indonesië. Tenslotte, kunnen ook de verschillende manieren van definiëren van parodontitis in de verschillende studies hebben bijgedragen aan het niet eensluidende of moeilijk te vergelijken resultaat van het onderzoek naar de relatie tussen parodontitis en andere aandoeningen. Daarom werd in dit proefschrift de associatie tussen parodontitis en systeemziektes zoals diabetes mellitus type 2 (DM2) en reumatoïde artritis (RA) in Indonesië onderzocht. Bovendien werd in de

literatuur door middel van een systemic review onderzocht of atherosclerose patiënten baat zouden kunnen hebben bij behandeling van parodontitis.

In **hoofdstuk 2** wordt het vóórkomen van parodontitis in een groep Indonesische DM2 patiënten gerapporteerd. In dit onderzoek werd bovendien de relatie tussen parodontitis en DM2 aan de hand van een groot aantal in de literatuur beschreven methoden om parodontitis vast te stellen onderzocht. Bij. het vaststellen van het verband tussen parodontitis en vroeggeboorte is gebleken dat de wijze waarop parodontitis werd gedefinieerd van grote invloed is op de uitkomst van dit onderzoek. Hetzelfde zou kunnen gelden voor het verband tussen parodontitis en DM2. Bij 78 DM2 patiënten en 65 controle personen werd een volledig parodontaal onderzoek uitgevoerd inclusief bepaling van de pocketdiepte, gingivarecessie, plaque index en bloeding na sonderen. Op basis van deze gegevens werd de oppervlakte van het parodontaal ontstoken weefsel (PISA) berekend. De resultaten van het onderzoek in dit hoofdstuk beschreven onderzoek laten verschillen in vóórkomen en ernst van parodontitis zien tussen DM2 patiënten en gezonde individuen met Odds Ratio's van respectievelijk 5.0 en 6.1. Met univariatie analyses kon worden aangetoond dat parodontitis significant vaker voorkomt en ernstiger is in DM2 patiënten. Met betrekking tot risicofactoren voor parodontitis bleken leeftijd en hoge bloeddruk als significante factoren aanwezig bij DM2 patiënten vergeleken met controle personen. In regressie analyses bleken DM2 en leeftijd een voorspellende waarde voor parodontitis te hebben. De conclusie was dat Indonesische DM2 patiënten vaker en ernstiger parodontitis hebben dan gezonde Indonesische individuen, onafhankelijk van andere verstorende variabelen (confounders) en de wijze van vaststellen van parodontitis.

In **hoofdstuk 3** wordt beschreven dat parodontitis kan bijdragen aan de infectie en ontstekingsdruk voor DM2 blijkens een verhoogd CRP. Hierdoor kan de regulatie van de bloedglucose, zoals dat zijn weerslag heeft in HbA1c waarden, verstoord raken. Volledig parodontaal onderzoek werd uitgevoerd bij 132 controle Indonesiërs en 101 Indonesiërs die behandeld werden voor DM2 en tevens werden HbA1c en CRP gemeten.
Regressie analyses werden uitgevoerd om vast te stellen of de ernst van parodontitis en de CRP waardes het HbA1c kunnen voorspellen waarbij werd gecorrigeerd voor

confounders en effect modificatie. Uit de analyse bleek dat de ernst van parodontitis en de hoeveelheid CRP een voorspellende waarde hadden voor het HbA1c nivo in de groep van gezonde Indonesiërs die waren behandeld voor DM2. Een dergelijke relatie kon niet worden aangetoond in de DM2 patiënten. Verondersteld werd dat het potentiële effect van parodontitis op de bloed glucose instelling in DM2 patiënten werd gemaskeerd door de behandeling van DM2 en waarschijnlijk wel aanwezig zou zijn in onbehandelde en/of niet gediagnosticeerde DM2 patiënten.

In **hoofdstuk 4** wordt het onderzoek beschreven naar het vóórkomen en de ernst van parodontitis en de mogelijke samenhang met systemische ontsteking in een groep Indonesiërs met reumatoïde artritis (RA). In totaal werden 132 controle Indonesiërs en 78 Indonesiërs met RA onderzocht. Beide groepen werden volledig parodontaal onderzocht en CRP, de BSE (bloedbezinking), reumafactor en anticyclische gecitrullineerde antistoffen (ACPA's) werden gemeten. Er werden geen significante verschillen in vóórkomen en ernst van parodontitis geconstateerd tussen RA patiënten en de gezonde controle groep. Daarentegen kon wel worden aangetoond dat de ESR en CRP verhoogd waren in RA patiënten met parodontitis vergeleken met RA patiënten zonder parodontitis. Hieruit werd geconcludeerd dat hoewel het vóórkomen en de ernst van parodontitis niet hoger waren in Indonesische RA patiënten versus de gezonde controle groep, het kennelijk zo is dat parodontitis is geassocieerd met een verhoogde inflammatoire status in RA patiënten.

In **hoofdstuk 5** wordt een systematische review en meta-analyse beschreven betreffende de robuustheid van waarnemingen dat parodontale behandeling de biomakers voor atherosclerotische aandoeningen reduceert. De bestaande wetenschappelijke literatuur werd doorzocht op studies die het effect van parodontale behandeling vergeleken met geen behandeling. Parodontale behandeling was de voorspeller en ontstekings-, metabole- en of vasculaire markers de uitkomstfactoren. Meta-analyse van alle beschikbare studies liet zien dat parodontale behandeling een verlaging gaf van ontstekingsfactoen (CRP, Il-6) en metabole markers (totaal cholesterol en HbA1c). Dit effect was het sterkst in patiënten lijdend aan cardiovasculaire en of metabole aandoeningen zoals DM2. Deze reductie van biomakers onderschrijft het belang en de effectiviteit van parodontale behandeling aan bij deze patiënten om hun algehele gezondheid te verbeteren.

In **hoofdstuk 6** worden de voornaamste onderzoeksbevindingen bediscussieerd en worden suggesties voor verder onderzoek gegeven. Uit de bevindingen komt naar voren dat ook in een Indonesische populatie parodontitis een aanzienlijke invloed kan hebben op de algehele gezondheid, met name bij DM2 en RA patiënten. De conclusie was dat CRPs een rol spelen in het systemisch effect van parodontitis en dat CRP serum spiegels kunnen dalen door parodontale behandeling. Daarnaast gaat een verbeterde parodontale conditie gepaard met een betere glycaemische instelling, reductie van RA activiteit en een verlaagd risico voor atherosclerotische aandoeningen. De diagnostiek en behandeling van parodontale aandoeningen zou daarom integraal onderdeel van de gezondheidszorg moeten zijn, niet alleen om de mondgezondheid te verbeteren maar ook de algehele gezondheid en kwaliteit van leven

Banyak penelitian menunjukkan bahwa periodontitis berhubungan dengan penyakit sistemik seperti, diabetes mellitus, rheumatoid arthritis, penyakit kardiovaskular, pneumonia bakterial, kehamilan, osteoporosis dan penyakit serebrovaskular. Hubungan antara periodontitis dan penyakit sistemik kemungkinan merupakan hubungan dua arah. Penyakit sistemik tidak hanya memperparah periodontitis, tapi periodontitis juga dapat memperburuk penyakit sistemik. Dalam bab 1 dijelaskan latar belakang teoritis hubungan dua arah antara periodontitis dan penyakit sistemik. Penyakit sistemik dapat mempengaruhi periodontitis melalui respon inflamasi sistemik dengan melepaskan penanda inflamasi sistemik seperti C-reaktif protein (CRP). Pelepasan CRP sebagai penanda inflamasi sistemik dapat meningkatkan respon inflamasi lokal pada periodontitis. Di sisi lain, periodontitis dapat memperburuk penyakit sistemik dengan cara translokasi langsung bakteri atau produk bakteri serta melalui respon inflamasi sistemik berupa produksi CRP. Hubungan antara periodontitis dan penyakit sistemik juga dapat ditunjukkan oleh bukti bahwa adanya perawatan periodontal dapat disertai dengan penurunan CRP serum, yang kemudian dapat menurunkan keparahan penyakit sistemik. Namun, dalam hubungan periodontitis dan penyakit sistemik, perlu mempertimbangkan faktor risiko dan/atau faktor yang memodifikasi hubungan tersebut seperti usia, jenis kelamin, dan merokok. Dalam bab 1 ini juga dijelaskan beberapa alasan penelitian dilakukan di Indonesia. Alasan utama adalah masih adanya pertentangan antara berbagai hasil penelitian mengenai hubungan antara periodontitis dan penyakit sistemik serta baru sedikit bukti yang menjelaskan adanya hubungan periodontitis dan penyakit sistemik di Asia demikian juga sampai saat ini baru sedikit penelitian telah dilakukan di Asia. sehingga, masih ada ketidakpastian apakah hasil penelitian di negara lain yang telah diterbitkan dalam literatur internasional sampai saat ini dapat diekstrapolasi ke populasi Asia seperti Indonesia. Alasan terakhir adalah penerapan metode yang berbeda dalam mendefinisikan periodontitis mungkin telah berkontribusi terhadap perbedaan hasil dalam penelitian-penelitian tentang hubungan periodontitis dan penyakit sistemik. Oleh karena itu, tujuan dari penelitian PhD dalam tesis ini adalah untuk mengetahui hubungan antara periodontitis dan penyakit sistemik seperti diabetes mellitus tipe 2 (DM2) dan rheumatoid arthritis (RA) di Indonesia. Selain itu, kajian secara sistematik tentang

manfaat perawatan periodontitis terhadap pasien atherosklerotik seperti penyakit kardiovaskular.

Di dalam bab 2 dilaporkan prevalensi periodontitis pada pasien DM2 pada populasi orang Indonesia. Selain itu, penelitian ini juga mengkaji hubungan antara periodontitis dan DM2 menggunakan berbagai metode dalam mengoperasionalkan periodontitis. Penggunaan metode yang berbeda untuk mengoperasionalkan prevalensi dan keparahan periodontitis telah diketahui berpengaruh terhadap kekuatan hubungan tersebut, misalnya, dalam hubungan periodontitis dan kelahiran bayi prematur. Perbedaan metode dalam mengoperasionalkan periodontitis juga mungkin berpengaruh terhadap hubungan antara periodontitis dan DM2. Pemeriksaan periodontal secara menyeluruh terdiri atas kedalaman poket, resesi gingiva, indeks plak dan perdarahan saat probing dilakukan pada subyek penelitian yang terdiri atas 78 DM2 dan 76 orang sehat . Berdasarkan data pemeriksaan periodontal tersebut dilakukan perhitungan luas permukaan periodontal yang terinflamasi (PISA). Hasil penelitian pada bab ini menunjukkan perbedaan prevalensi dan keparahan periodontitis antara DM2 dan orang sehat, dengan rasio kemungkinan masing-masing 5.0 dan 6.1. Analisis univariat menunjukkan prevalensi dan tingkat keparahan periodontitis lebih tinggi secara signifikan pada subyek DM2. Dalam analisis juga menunjukkan bahwa faktor risiko terhadap periodontitis yaitu umur dan hipertensi diketahui lebih tinggi secara signifikan pada DM2 dibandingkan dengan orang sehat yang dalam analisis regresi hanya DM2 dan usia yang terbukti menjadi prediktor untuk periodontitis. Dapat disimpulkan bahwa orang Indonesia yang menderita DM2 memiliki periodontitis lebih banyak dan lebih parah dibandingkan orang Indonesia sehat, terlepas dari faktor pengganggu atau metode yang digunakan dalam mengoperasionalkan periodontitis.

Di dalam bab 3 dijelaskan hasil penelitian yang menunjukkan bahwa periodontitis dapat meningkatkan beban risiko inflamasi dan infeksi bagi DM2 yang dibuktikan dengan peningkatan CRP serum. Peningkatan inflamasi ini dapat mengganggu kontrol glukosa darah yang diukur dengan adanya HbA1c dalam darah. Pemeriksaan periodontal secara menyeluruh, HbA1c dan CRP darah dilakukan pada 132 orang Indonesia yang sehat dan 101 orang pasien DM2. Analisis regresi dilakukan untuk menilai apakah keparahan periodontitis dan CRP dapat sebagai prediktor untuk HbA1c setelah mengendalikan faktor yang dapat memodifikasi

hubungan tersebut dan faktor pengganggu. Hasil analisis ini menunjukkan bahwa keparahan periodontitis dan CRP merupakan prediktor untuk HbA1c pada kelompok orang Indonesia sehat. Hubungan seperti yang ditemukan pada orang Indonesia sehat tersebut tidak dapat ditunjukkan dalam kelompok pasien DM2. Sementara pada kelompok orang Indonesia sehat, PISA, CRP juga usia, jenis kelamin dan merokok menjadi prediktor untuk HbA1c. Namun tidak untuk pasien DM2. Diasumsikan bahwa efek periodontitis pada kontrol glukosa darah pada pasien DM2 mungkin tertutupi oleh pengobatan pada pasien DM2, dan mungkin akan terlihat pada DM2 yang tidak terdiagnosis dan tidak terawat.

Di dalam bab 4 dijelaskan penelitian yang bertujuanuntuk mengetahui prevalensi dan keparahan periodontitis dan hubungannya dengan inflamasi sistemik pada pasien RA. Pemeriksaan periodontal secara menyeluruh dilakukan pada 132 orang Indonesia yang sehat dan 78 pasien RA. Pemeriksaan hsCRP, LED, faktor rheumatoid dan anti antibodi citrullinated siklik (ACPAs) serum juga dilakukan. Adapun hasil penelitian menunjukkan tidak ada perbedaan yang signifikan dalam prevalensi dan keparahan periodontitis yang ditemukan pada pasien RA dan orang sehat. Meskipun demikian, pada penelitian ini menunjukkan bahwa ESR dan hsCRP serum lebih tinggi pada pasien RA dengan periodontitis dibandingkan dengan pasien RA tanpa periodontitis. Dapat disimpulkan bahwa, meskipun prevalensi dan keparahan periodontitis tidak lebih tinggi pada pasien RA daripada kelompok kontrol sehat, periodontitis tampaknya berhubungan dengan peningkatan respon inflamasi pada pasien RA.

Dalam bab 5 dijelaskan review sistemik dan meta-analisis yang melaporkan kekuatan hasil penelitian bahwa perawatan periodontal menurunkan penanda penyakit aterosklerotik. Sebuah pencarian literatur dilakukan berdasarkan penelitian yang asli tentang perawatan periodontal dibandingkan tanpa perawatan periodontal. Perawatan periodontal adalah prediktor dan penanda inflamasi, metabolik dan / atau darah. Meta-analisis pada semua studi yang terseleksi, menunjukkan perbedaan yang signifikan pada CRP, IL-6, total kolesterol dan HbA1c pada perawatan periodontal terutama pada pasien yang menderita penyakit jantung dan / atau metabolik seperti DM2. Penurunan penanda-penanda tersebut menekankan efektivitas dan kebutuhan terapi periodontal pada individu-individu untuk meningkatkan kesehatan mereka.

Dalam bab 6 dibahas hasil penelitian utama dan perspektif penelitian yang akan datang. Secara umum, di Indonesia penyakit periodontal mungkin memiliki dampak besar terhadap kesehatan umum, antara lain pada pasien DM2 dan RA juga dapat disimpulkan bahwa CRP mungkin memiliki peran kunci terhadap efek sistemik penyakit periodontal dan CRP serum dapat menurun oleh perawatan periodontal. Selain itu, peningkatan kesehatan periodontal dapat berpengaruh pada kontrol glukosa darah pada pasien DM2 yang lebih baik, penurunan aktivitas penyakit RA dan mengurangi risiko penyakit kardiovaskular. Diagnosis dan perawatan penyakit periodontal harus dilakukan, dan menjadi bagian integral dari pelayanan kesehatan sehingga tidak hanya meningkatkan kesehatan mulut, tetapi juga kesehatan umum dan kualitas hidup.

Acknowledgement

This thesis arose in part out years of research that has been done since I joined Professor dr. Frank Abbas's research group. By the time, I have worked with a great number of people whose contribution in assorted ways to the research and the making of the thesis deserved special mention. It is pleasure to convey my gratitude to them all in my humble acknowledgment.

First of all, I would like to express my deepest gratitude to my first promotor Professor dr. Frank Abbas for his supervision, advice, discussion, support in the process of writing this thesis and patient guidance from the very early stage of my study as well as giving me extraordinary experiences throughout my study and enrich my growth as a student, a researcher and a lecturer want to be. Above all, he is the one who initiated this research group for my PhD program with the financial support by University Medical Center Groningen, the Netherlands (Bernouille Bursary) and also supported by the Directorate of Higher Education, Ministry of Education, Republic of Indonesia. Pak Frank, you and your families always provided me warmth hospitality as family member, give me unflinching encouragement, give me solution for the problem arose during my study and support in various ways throughtout my study specially consider my family as an important part during my study.

I gratefully acknowledge second promotor Professor dr. Arjan Vissink for his supervision, valuable advice and discussion, crucial contribution, critical comments for my manuscripts and thesis which made him a truly scientist intitution. Pak Vissink, I am gratefull in every possible way and hope to keep up our collaboration in many research in oral medicine in the furture.

I am thankful to Professor dr. Pieter Ubele Dijkstra for his supervision, discussion, comments, generous assistance during my study. Pieter, you teached me many things about research methodology and statistics. You always give a solution for my statistic problem for my manuscript and also for my thesis. You and your families kindly treated me very well during my time in Groningen.

I would like to express my gratitude to Dr. drg. Dewi Agustina, MDSc who gave me support during my study. Bu Dewi, thank you for your advice, patience and valuable discussion for my manuscript and thesis. As local supervisor you always gave your

patience assistance to my research. As my senior in Department of Oral Medicine you always want me to do my job carefully.

I am deeply indebted to Dr. Willem Nesse for his assistance, supervision, advice, friendship, guidance, valuable discussion, critical comments and contribution for our manuscript and thesis. Brother Muslim Willem, you always provided your time for me to discuss our manuscripts and everything about life, introduce me Groningen and Muslim life in Groningen also always try to find out the way for me to learn our research topic. You always encourage me during my research, and teach me how to be a good researcher and writer. You and your families are kindly treating me as part of family member as well.

My special thanks to Professor Dr. drg. Widowati Siswomiharjo, thank you for her support, advice, and motivation during my study. Bu Bundi, you are the kindly person who introduce me to Professor Frank Abbas and make the chance to study abroad for me come true. You always remind me to keep be a low profile person and everything has to be well organized.

I am also pleased to record my gratitude to the former and the present Dean of faculty of Dentistry Universitas Gadjah Mada, Yogyakarta, Professor Dr. drg. Munakhir Ms. S.U. Sp.RKG and Professor Dr. drg. Iwa Sutardjo, RS, S.U. Sp. KGA(K). Next to that I would like to express my sincere thanks to the former and present Head Department of Oral Medicine, drg. Sri Hadiati, S.U. and drg. Goeno Subagyo, Sp.OPath for their support during my study.

It is pleasure to express my appreciation to the reading committee. Prof. dr. W.M. Molenaar, Prof. dr. U. van der Velden, Prof. dr. A.J. van Winkelhoff . Thank you for your efforts in judging my thesis.

It is a pleasure to pay tribute also to to Dr. Wijnand Teeuw, Prof. Bruno Loos, for giving me a chance to collaborate in systematic review and meta analysis for my thesis and for a nice period working in Periodontology Department of ACTA Amsterdam with staff and also for other co authors Dagmar E. Slot, Victor E.A. Gerdes, Francesco D'Aiuto , John J.P. Kastelein, thank you for sharing knowledge in the systematic review and meta analysis.

I would like to thank colleagues and staff of W.J. Kolff Insitute (formerly BMSA), especially Prof. H. J. Busscher, Pak Henk you are the first who initiated the cooperation between my faculty and UMCG, RuG. I would not have had the opportunity to follow a PhD program without your involvement.

I would like to acknowledge Yvonne Huijser van Reenen, Evelien Hoedemaker, and their families for helping me in research in Yogyakarta and spending time together, city touring, and sailing with their families and kind hospitality in their houses during my time in Netherland.

Thanks to Bu Teatske and all colleagues and staff in the Center for Dentistry and Oral Hygiene, UMCG, University of Groningen for work experience, sharing knowledge and kindly cooperation during my period in Groningen. Thanks also to Mr. Henk Holtjer for arranging my financial affairs.

My special thanks to Indonesian Rheumatologist Dr. Nyoman Kertia, Dr. Joewono Suroso, dr. Rawan Broto, dr. Dedy Nur Wahid for advice, discussion, assistance and provide me Rheumatoid Arthritis patients during my study.

I would like to thank to my Indonesian friends in Groningen, Alex den Engelsmen, Nila and families, Indonesian muslim Student community of Groningen (De Gromiest), PPI Groningen, Fadjar Hari Mardiansjah families and Adhi Dharma Wibawa families for a kind hospitality during my period in Groningen. Personally, I would like to thank to Adhi Darma Wibawa and drg. Dessy Rachmawati for being my paranimfs in my defense

Collective and Individual acknowledgments are also owed to my colleagues at Oral Medicine Department, Periodontology Department Faculty of Dentistry, Prof. Soedomo Dental Hospital Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Rheumatology clinic of Internal Medicine Department, Clinical Pathology Laboratory, all nurses of Dental clinic of Dr. Sardjito Hospital Yogyakarta, Prodia Laboratory Yogyakarta, Jogjakarta International Hospital Yogyakarta, Dental clinic and Rheumatology clinic of PKU Muhammadiyah Hospital Yogyakarta and all patients or volunteers who participated in my researcch.

I also acknowledge mbak Anjar, drg. Ninung, drg. Amber, drg. Bertha Aulia, drg Nurina, drg. Wanda for their help during my study in Yogyakarta and Surabaya and special thank to drg. Margareta Rinastiti, PhD and drg. Rosa Amalia who always share their experience and knowledge to me.

I take this opportunity to express my profound gratitude to my beloved parents and parents in law. My father and my mother who sincerely raised me with their caring and gently love always pray for me. My big brother Agok and my little brother Iik thank for being supportive. Especially in memories of my father in law who always inspired me in my intellectual thinking. My grand mother and big families in Sumenep and Yogyakarta deserve special mention for their inseparable support and prayers.

Words fail me to express my appreciation to my wife NANA whose dedication, love and persistent confidence in me. I owe her for being unselfishly let her intelligence, passions, and take care our sons KAKA and VERRILL during my study with our nanny mbak Siti.

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Curriculum Vitae

Hendri Susanto was born in Sumenep, Madura, East Java province, Indonesia at September 2nd, 1976. He got his bachelor degree in Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia in 2000 and graduated as a dentist in 2002. He married with Ratnawati on 2003. Now they have two sons: Kaka and Verril and live at Dayu Permai c 10, Jaban, Sinduharjo, Ngaglik, Sleman, Yogyakarta. His email address is drghendri@ugm.ac.id. He studied for Master degree in Health Science at the Graduate School, Universitas Gadjah Mada, Yogyakarta, Indonesia which funded by Directorate of Higher Education, Ministry of Education, Republic of Indonesia and obtained his master degree in 2005. He started his academic career at the Faculty of Dentistry, Universitas Gadjah Mada, in 2005 as a lecturer at Department of Oral Medicine until now. As one of Oral Medicine Department Staff, he is a tutor and works lecturer in the undergraduate program. He is also researcher in the Department of Oral Medicine and work as a dentist at the Prof. Soedomo Dental Hospital at Faculty of Dentistry, University Gadjah Mada, Yogyakarta, Indonesia. He also maintains a private dental practitioner in Yogyakarta. In 2007, he started a sandwich PhD program at the Center for Dentistry and Oral Hygiene, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.