Periodontal therapy and treatment of hypertension – alternative to the pharmacological approach. A systematic review and meta-analysis.

Short running title: Periodontal treatment and hypertension

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Abstract

Aim: Quantitative comparison of the effects of intensive (IPT) or conventional (CPT) periodontal treatment on arterial blood pressure, endothelial function and inflammatory/metabolic biomarkers.

Materials and Methods: A systematic search was conducted to identify randomized controlled trials (RCT) of IPT (supra and subgingival instrumentation). Eight RCTs were included in the meta-analysis. Difference in change of systolic blood pressure (SBP) and diastolic blood pressure (DBP) before and after IPT or CPT were the primary outcomes. The secondary outcomes included: endothelial function and selected inflammatory/anti-inflammatory (CRP, IL-6, IL-10, IFN-γ) and metabolic biomarkers (HDL, LDL, TGs).

Results: The overall effect estimates (pooled Weighted Mean Difference (WMD)) of the primary outcome for SBP and DBP was -4.3 mmHg [95%CI: -9.10-0.48], p=0.08 and -3.16 mmHg [95%CI: -6.51-0.19], p=0.06 respectively. These studies were characterised by high heterogeneity. Therefore, random effects model for meta-analysis was performed. Sub-group analyses confirmed statistically significant reduction in SBP [WMD =-11.41mmHg (95%CI: -13.66, -9.15) *P*<0.00001] and DBP [WMD=-8.43 mmHg (95%CI: -10.96,-5.91)*P*<0.00001] after IPT vs CPT among prehypertensive/hypertensive patients, while this was not observed in normotensive individuals. The meta-analyses showed significant reductions in CRP and improvement of endothelial function following IPT at all analysed timepoints.

Conclusions: IPT leads to improvement of the cardiovascular health in hypertensive and prehypertensive individuals.

Keywords: Periodontitis, hypertension, endothelial dysfunction, inflammation, LDL, CRP

Conflict of Interest Statement:

None of the authors declare conflict of interests in relation to the date of the submitted article.

1.INTRODUCTION

Hypertension, defined as chronically increased blood pressure above 140/90 mmHg, affects more than 30% of adults of the world population becoming a leading risk factor for heart attack, stroke, chronic kidney disease, heart failure, cognitive impairment and dementia (Williams et al., 2018) . According to the World Health Organisation, hypertension is the main contributor to human mortality and accounts for 51% of deaths from strokes and 45% of

overall mortality from cardiovascular diseases (Williams et al., 2018, Muñoz Aguilera et al., 2020). The pathogenesis of hypertension is complex involving several organs such as: heart, vasculature, brain with sympathetic nervous system and kidney (Drummond et al., 2019, Guzik et al., 2007b). For years, the focus of research has been mostly placed on two key systems of blood pressure regulation: sympathetic nervous system (SNS) and the renin–angiotensin– aldosterone system (RAAS) (Drummond et al., 2019). A majority of current treatment strategies for hypertension target these BP regulatory mechanisms. Nevertheless, lowering blood pressure to the target is unsuccessful in almost 40% of patients with hypertension (Drummond et al., 2019) . This fact is partially explained by poor treatment adherence and indicates that additional mechanisms may regulate blood pressure. Over the past decade, the role of systemic activation of the immune system in hypertension has been uncovered (Guzik et al., 2007a, Harrison et al., 2011, Higaki et al., 2019). An overarching concept postulates that activated immune cells may accumulate in blood vessels, kidneys and brain and promote local chronic inflammatory response that disrupts the blood pressure-regulating functions of these organs, leading to hypertension(Guzik et al., 2007b, Guzik et al., 2017). However, the mechanisms of immune cells activation that interfere with blood pressure regulation are not fully elucidated. There are a few hypotheses regarding T cells activation ranging from the search of neoantigens to the concept of other chronic inflammatory diseases, such as periodontal diseases and rheumatoid arthritis, being the trigger of immune cells pre-activation. Accumulating body of evidence from mice model experiments, clinical trials, mendelian randomization and meta-analytical approaches documents periodontal disease as a new risk factor for hypertension (Czesnikiewicz-Guzik et al., 2019a, Czesnikiewicz-Guzik et al., 2019b, Zhou et al., 2017, Muñoz Aguilera et al., 2020). All these studies demonstrate a significant contribution of periodontal disease to the development of hypertension. In this context, periodontal treatment appears as a new, attractive non-pharmacological treatment of hypertension. Unfortunately, randomized clinical trials available that address this question are relatively small (Czesnikiewicz-Guzik et al., 2019b, D'Aiuto et al., 2006, Zhou et al., 2017).To address this question in a more robust statistical manner, we performed a systematic review and meta-analysis.

Herein, we conducted a critical appraisal and synthesis of the evidence on the impact of IPT on blood pressure, vascular function and immunometabolic parameters in comparison to CPT. The specific research design was based on the following PICO elements: Population: normotensive, hypertensive and pre-hypertensive individuals with moderate or severe periodontitis; Intervention: Non-surgical periodontal therapy with both supra and subgingival

instrumentation (intensive periodontal therapy - IPT); Comparison: No intervention group or supragingival debridement only (control periodontal therapy – CPT); Outcome: Changes in mean SBP and DBP levels following periodontal therapy

2.MATERIALS AND METHODS

2.1 Protocol registration

The systematic review protocol was registered in PROSPERO (International prospective register of systematic reviews) with ID CRD42020173133. The PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist was used (Shamseer et al., 2015) **(Appendix S1).**

2.2 Primary and secondary outcomes

The primary outcomes of interest in this meta-analysis were the differences in change in blood pressure (SBP and DBP) between patients undergoing IPT and control patients with no treatment or supragingival scaling alone.

The secondary outcomes included:

1) the difference in change in the endothelial function as measured by the flow mediated dilatation (FMD)

2) the difference in change in systemic levels of CRP as well as pro-inflammatory (IL-6, IFN-γ) and anti-inflammatory (IL-10) cytokines.

3) the difference in change in levels of lipids (HDL, LDL and triglycerides)

between patients undergoing IPT and CPT

2.3 Inclusion/exclusion criteria

Randomized clinical trials/studies using intensive and control periodontal therapy in subjects with diagnosis of generalized moderate and severe periodontitis in which periodontal status and SBP/DBP values were reported at the beginning and at the end of the follow-up period were included. **(Appendix S2a.)**

2.4 Types of periodontal interventions

Intervention groups undergoing non-surgical periodontal therapy in the form of supra- and subgingival instrumentation were compared with either no periodontal treatment or supra-gingival scaling in the control group.

In all analysed studies, authors used variety of treatment protocols from single RSD (root surface debridement) through RSD combined with local or systemic antibiotics/antimicrobials, to many subsequent RSDs during 4,8,12 weeks. Additionally, the strategy of plaque control after finalising the treatment varied significantly from follow up visits with oral hygiene instruction only or with performing another RSD or both OHI and RSD, one study carried out surgical treatment if necessary. The frequency of the follow up visits depends on length of the study. However, there were three studies 6 months long without any follow up visits. (summary in table **Appendix S2b.**)

2.5 Search methods for identification of the studies

The detailed search strategy with search terms appropriate for each database is provided in **Appendix S3**. Five electronic databases MEDLINE(OVID), EMBASE (OVID), Cochrane CENTRAL, Web of Science and CINAHL were searched up to 23 March 2020 without any restriction for the time frame. Reference lists from the included studies were hand searched to retrieve relevant studies not identified through other search methods. We employed no language restrictions in our search.

2.6 Data extraction and management

The process of data extraction and analysis was performed based on the methods described in the Cochrane handbook for systematic reviews of interventions (Higgins et al., 2019). Study selection was based on a two-step approach: (1) screening of titles and abstracts; and (2) full-text analysis. All the retrieved papers were evaluated by two independent reviewers (MCZ and SS). The first stage involved eligibility assessment of the studies by screening the titles and abstracts based on the pre-specified inclusion and exclusion criteria. The studies not meeting the inclusion criteria were excluded. If the agreement could not be reached, third reviewer was consulted (TG). After full text screening, eight randomized controlled studies met the criteria for inclusion in the meta-analysis. The list of excluded studies is provided in **Appendix S4**.

The study data were assembled in evidence tables according to study characteristics, population characteristics, type of interventions, impact of intervention on the periodontal condition and the blood pressure (**Appendix S5**). The outcomes were reported individually as pre-intervention and post intervention values at different time-points. All the included studies reported mean SBP/DBP values for the intervention and control groups at baseline and follow-up periods. The data extracted are reported as change in SBP/DBP from baseline for

each group as well as the standard deviation of the change (SD). Standard deviation of the change was calculated using the formula previously described (Teeuw et al., 2010). Secondary outcomes were reported in some of the studies.

2.7 Assessment of the bias of the individual studies

Quality assessment of all included studies was undertaken independently and in duplicate by two reviewers (SS, MCZ) as part of the data extraction process. The Cochrane recommended Review Manager, [RevMan] version 5.3 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014 was used to assess individual studies. For bias assessment of randomized controlled trials, we used the revised Cochrane tool (ROB-2.0 tool) (Whiting et al., 2016).

2.8 Data synthesis

All the statistical analyses included in the Meta-analysis were done using Review Manager, [RevMan] version 5.3 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014 or R version 3.6.2. with the packages Metafor version 2.4-0 and mixmeta version 1.0.8 (Viechtbauer, 2010). Meta-analyses were conducted for both the primary and the secondary outcomes. The continuous outcome data for each study were entered as Mean difference (M.D.), Standard deviations (SD) and Sample sizes for both the comparison groups (Intervention vs Control).

Where available, change from baseline in mean SBP and DBP were extracted along with the standard deviation of the change. Where this information was not available, mean and standard deviation of SBP and DBP at baseline and all follow-up time points were extracted. For those studies, the change in mean SBP/DBP from baseline was calculated by subtracting the baseline data from the follow-up data. The standard deviation of the change from baseline was calculated assuming a correlation of 0.3 between baseline and follow-up measurements. Secondary outcome data was extracted in the same way. Other correlations between baseline and follow-up measurements were assumed as sensitivity analyses.

3. RESULTS

3.1 Study selection

The combined search of the five databases resulted in the identification of a total of 729 potentially eligible studies and subsequently 506 papers, after removal of duplicates. 506

studies were then screened for relevance by examining the titles and abstracts. This screening resulted in 33 studies eligible for full text assessment. After a reading of the full text, 25 studies were excluded. This left eight randomized controlled trials which met the inclusion criteria and were included for data extraction and meta-analysis **(Figure 1)**.

3.2 Study characteristics

This systematic review included randomized controlled trials conducted in different countries involving varied population groups. Out of eight studies, two were conducted in the UK (F. D'Aiuto et al., 2018; Tonetti et al., 2007), one in Poland (Czesnikiewicz-Guzik, Osmenda, et al., 2019), two in Japan (Higashi et al., 2009; Higashi et al., 2008) and one each in India (Hada, Garg, Ramteke, & Ratre, 2015), China (Zhou et al., 2017) and Australia (Taylor et al., 2010). The studies involved in total 798 individuals (403 in the intervention group and 395 in the control group after accounting for missing or lost to follow-up participants). The duration of follow-up in the included trials ranged from 1 month to 6 months, with one study (F. D'Aiuto et al., 2018) including a 12-month follow-up. In terms of sample selection, five of the trials included normotensive individuals while the other three had hypertensive populations. The blood pressure change was the primary outcome measure in only two of the trials (Czesnikiewicz-Guzik, Osmenda, et al., 2019; Zhou et al., 2017). The method of blood pressure (BP) monitoring varied across studies. The investigators in one study used 24-Hr ambulatory blood pressure monitoring (ABPM) (Czesnikiewicz-Guzik, Osmenda, et al., 2019) whereas all the other trials used a routine average of two or three in office BP readings. The criteria for diagnosing a periodontal condition in most of the studies were similar and they included individuals with moderate to severe chronic periodontitis (Czesnikiewicz-Guzik, Osmenda, et al., 2019; F. D'Aiuto et al., 2018; Taylor et al., 2010; Tonetti et al., 2007; Zhou et al., 2017). One study included patients with mild periodontitis (Hada et al., 2015) while other trials used a self- reported questionnaire confirmed by clinical examination (Higashi et al., 2009; Higashi et al., 2008).

All the studies randomly assigned their participants into intervention and control groups. In these trials (Czesnikiewicz-Guzik, Osmenda, et al., 2019; F. D'Aiuto et al., 2018; Hada et al., 2015; Higashi et al., 2009; Higashi et al., 2008; (Taylor et al., 2010); Tonetti et al., 2007; Zhou et al., 2017), both the groups were similar in their disease characteristics and differed only in terms of the interventions received. All subjects in the intervention group received non-surgical periodontal therapy in the form of supra and subgingival scaling with or without topical 0.2% chlorhexidine gel (Czesnikiewicz-Guzik, Osmenda, et al., 2019) or locally

delivered minocycline (Tonetti et al., 2007). Subsequently, the control group either received no treatment (Hada et al., 2015; Higashi et al., 2009; Higashi et al., 2008; Taylor et al., 2010) or only supragingival scaling (Czesnikiewicz-Guzik, Osmenda, et al., 2019; F. D'Aiuto et al., 2018; Tonetti et al., 2007; Zhou et al., 2017).

3.2 Impact of periodontal treatment on clinical periodontal parameters

All the reported studies demonstrated significant improvement in the clinical measures of periodontitis such as probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) assessment after treatment in the intervention group compared to the controls **(Appendix S9).** One of the most important criteria in this review was the documentation of the improvement in periodontal status. Most of the studies published in the past did not report periodontal outcomes following periodontal therapy, focusing mainly on the cardiovascular parameters.

3.4 Synthesis of results (Meta-analysis)

Primary outcome

In this review, eight RCTs were subjected to meta-analysis and the overall effect of IPT on change in SBP/DBP between the intervention and the control groups was estimated. **Figure 2** shows that the overall effect on reduction of SBP and DBP has the trend towards favouring the intervention group, although it was not statistically significant. The amount of heterogeneity, I^2 in our meta-analyses was 76% and 73% respectively, representing considerable heterogeneity between the included studies. Therefore, the results of the random effects model are presented. The funnel plot for these analyses shows some degree of asymmetry **(Appendix S7),** in particular for SBP.

In 3/8 analysed studies, 212 patients randomized were hypertensive or pre-hypertensive, while in remaining 5 studies, 586 subjects were normotensive. Accordingly, we next analysed the effects of IPT on blood pressure in subgroups of hypertensive/pre-hypertensive and normotensive patients. This analysis demonstrated a statistically significant reduction in mean SBP and DBP changes in the intervention group when compared to the controls in studies with hypertensive/pre-hypertensive patients **(Figure 3)**. In studies with normotensive

patients the reduction in SBP and DBP was not significant. The test for subgroup differences indicates that there is a statistically significant subgroup effect (P<0.00001).

A second key element suspected to add heterogeneity was different length of follow up. Summarizing together the results which were obtained 2,3,6 and 12 months after treatment did not give biological plausibility. Therefore, the studies were categorized into time-point 1 (follow-up up to 3 months), time-point 2 (values at 6 months) and time-point 3 (values at 12 months follow-up). **Appendix S10** shows the results obtained when analysed separately by time point. Within each time point, there remained considerable heterogeneity (I^2=0.78 at time 1 and 2). To further investigate this, a mixed effects meta-regression model predicting the difference in change from baseline in blood pressure between the intervention and the control group from hypertension at baseline, time point of follow-up (as a continuous variable) and the interaction of both was used. The interaction of time of follow-up and hypertension was significant for both SBP and DBP (p=0.0002 and p=0.0014 respectively). While there was little difference in blood pressure change between the intervention and the control group in the studies in normotensive participants across all time points. The difference in blood pressure change between the intervention and the control group in studies in hypertensive participants was found to increase over time. **Figure 4** shows the pooled effect estimates for SBP and DBP by time point and hypertension at baseline.

Secondary outcomes

Effect of periodontal treatment on endothelial function:

The pooled estimate of the treatment effect on the endothelial function (measured by % FMD) was 1.49% (95% CI 0.9 to 2.08, p<0.0001) favouring the intervention group **(Figure 5).**

Effect on circulating proinflammatory mediators and cardiometabolic markers:

Our meta-analyses confirmed that IPT is associated with significant reductions in the serum levels of CRP at all timepoints as shown in **(Figure 6 A)**. However, no significant differences in change of the levels of other pro-inflammatory cytokines IL-6 and IFN-γ were observed **(Figure 6 B, D)**. Additionally, no significant differences in change of anti-inflammatory cytokine IL-10 levels were found **(Figure 6 C)**. Lipids (HDL-C, LDL-C and TGs) showed no statistically significant differences in change between the intervention and control groups at all investigated timepoints **(Figure 6 E-G)**.

3.6 Sensitivity analyses in relation to correlation assumptions

Sensitivity analyses have been carried out to assess the effect of the assumption made about the correlation between baseline and follow-up measurement when calculating the standard deviation of the change from baseline. Three alternative assumptions have been considered: (1) a correlation of 0.1 between baseline and follow-up measurements, (2) a correlation of 0.5 between baseline and follow-up measurements and (3) using the measurement at follow-up instead of change from baseline. **Appendix S11** shows the resulting pooled intervention effect estimates. The effect of the amount of correlation assumed is only minimal and does not affect any of the conclusions. When using the measurement at follow-up only, there is no longer a significant intervention effect on DBP. All other conclusions remain unchanged.

3.7 Risk of bias within studies

All the included trials described their random sequence generation and allocation concealment methods and were classified as low risk/unclear risk (**Appendix S6)**. Most studies used computer generated permuted block technique for randomization and opaque envelope method for allocation concealment. The information regarding randomization technique was not clear in two studies (Higashi et al., 2008, Tonetti et al., 2007) and two trials were unclear about their method of allocation concealment (Higashi et al., 2009,

Higashi et al., 2008). These were categorized as unclear risk of bias. Only in one trial, the statistician was masked (Hada et al., 2015). The main reasons for high risk of bias were due to blinding of participants and outcome assessment.

3.8 Strength of Recommendation

Evidence from the RCTs on the impact of IPT on blood pressure regulation were vigorously assessed for a SORT recommendation. The final recommendation, assessed on three essential elements of the individual studies: quality, quantity and consistency of evidence, was grade C(SORT C) based on the disease-oriented evidence (**Appendix S8)**. (Newman et al., 2007).

Discussion

Link between periodontitis and hypertension has been postulated for many years based on observational evidence, however cause-effect association between these two diseases is difficult to study due to existence of many confounding factors. Recently, more clinical and experimental studies supported the role of inflammation and the immune system in the pathogenesis of HTN (Drummond et al., 2019). This has stimulated interest in the relationship between periodontitis-driven chronic inflammatory disease and the development of high blood pressure. Since, the activation of the cells of the immune system appears to play a role in the pathogenesis of hypertension (Guzik et al., 2007a) , it is important to consider potential new prevention strategies and new therapies for hypertension by reducing inflammatory burden. The evidence in support of periodontal treatment to lower blood pressure is still limited as interventional studies are lacking.

Two previous meta-analyses in this field have investigated the link between PD and HTN (Martin-Cabezas et al., 2016, Muñoz Aguilera et al., 2020). Munoz-Aguilera et al. reported the most recent evidence from cross-sectional and case-control studies about odds ratio of association between periodontitis and hypertension. However, to the best of our knowledge,

the current study is the first systematic review with meta-analysis involving only the RCTs, which examined the effect of the periodontitis treatment on blood pressure in patients with moderate to severe periodontitis.

The overall summary from our qualitative and quantitative analyses indicates that IPT decreases SBP and DBP. The pooled effect estimates of all eight trials for SBP and DBP reduction after periodontal treatment is 4.31 mmHg with 95% CI [-9.10 to 0.48] mmHg and 3.16 with 95% CI [-6.51 to 0.19] mmHg respectively. Even though not statistically significant, this could be of clinical significance (Law et al., 2009). The reason for lack of statistical significance of the analysis might be associated with the huge heterogeneity of available studies which differ in length of the post-treatment observation, in type of the investigated cohorts in terms of cardiological status and in the genetic background of investigated patients.

Eight studies in the current review were conducted in different geographic locations (cohorts from Europe, Asia and Australia) and therefore might have presented considerable ethnic variability. The general health status of the study participants varied and included subjects with hypertension, pre-hypertension, coronary artery disease, type 2 diabetes mellitus, stable coronary heart disease and normotensives. Moreover, the blood pressure measurement methods used in each study were quite diverse from single to triplicate office blood pressure measurement to 24-hour blood pressure monitoring. The substantial disadvantage of office blood pressure is a potential cofounding by white coat effect – especially when the measurements are taken in the dental surgery. According to the recent European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) 2018 Guidelines, Ambulatory BP Monitoring (ABPM) has more clinical and prognostic value (Williams et al., 2018). However, this method is also criticized as giving huge variation in the blood pressure levels during 24-hour observation. The new method recently introduced is unsupervised

office blood pressure measurement which is done by the patients without presence of the supervising physician. Nevertheless, none of the published study used this method so far. In the included studies, only one trial by Czesnikiewicz-Guzik et al used 24-hour ABPM. However, it is expensive and has limited availability. Other diversity comes from the fact that control group was either completely untreated or had supragingival scaling done. Supragingival scaling did not destroy anaerobic microbiome in the pocket area but still interfere with the balance of the bacterial load. This diversity comes from the fact that in many countries leaving patients untreated for months of the observation with diagnosis of periodontitis creates significant ethical issues. This remains a challenging issue in designing the studies and needs to be addressed, so that clear benefit of the intervention is observed.

One of the prerequisite conditions of the study assessing the impact of PT on BP is to monitor if periodontal therapy improved the periodontal status. Some studies in the past, have neglected this aspect, showing no influence of the periodontal therapy on blood pressure without documenting the fact that periodontal therapy was successful. Without curing inflammation in periodontal tissue, one cannot expect any effect on the systemic health improvement. The most important periodontal indices for the assessment of periodontitis status are bleeding on probing (BOP) and probing pocket depth which reflects current status of inflammation. It has been previously shown that gingival bleeding was independently associated with greater odds (OR1⁄41.42; 95% CI1⁄41.19–1.68; P<0.001) of high/uncontrolled blood pressure in 5396 adults NHANES III cohort (Pietropaoli et al., 2020). Additionally, clinical attachment loss (CAL) is describing the advancement of the periodontal disease by indicating the amount of the bone loss (Claffey et al., 1990, Haffajee et al., 1991). All the included trials showed significant improvement in the clinical periodontal parameters. However, two of the trials (D'Aiuto et al., 2018, Tonetti et al., 2007), used presence of plaque and number of pockets deeper more than 4 mm as assessment

criteria. Presence of plaque may not be a reliable criterion as host inflammatory response to plaque is highly variable (Darcey and Ashley, 2011). Also, number of pockets deeper more than 4 mm is not fully describing the amount of inflammation. Another major challenge in assessing the link between PD and systemic diseases is the variability in the classification of periodontitis definitions. Even though standardized case definitions for periodontitis have been reported (Eke et al., 2012, Preshaw, 2009), we found lack of consistency among studies in defining the disease.

Another fact, which might influence the result is a small number of RCTs choosing BP monitoring as a primary outcome, making our analysis a mixture of primary and secondary outcomes studies. In the last 15 years (2006-2020), thirteen RCTs have examined the effect of PT on BP, the most recent in 2019 (Czesnikiewicz-Guzik et al., 2019b). However, only two trials reported changes in BP following PT as the primary outcome (Czesnikiewicz-Guzik et al., 2019b, Zhou et al., 2017) and demonstrated significant reduction in both SBP and DBP following the periodontal treatment. This lack of consistency in choosing BP change as the primary outcome can make its interpretation difficult, thereby, limiting its clinical usefulness (Choudhary and Garg, 2011, Meher and Alfirevic, 2014).

Additionally, the effect of periodontal treatment on hypertension reduction might be diminished due to the different mean age of the investigated cohort. SBP elevation is the most prevalent type of HTN in individuals with age 50 or above. The rise in SBP with ageing is associated with arterial stiffness (AS) (Pinto, 2007, Wu et al., 2019). In the trial by Czesnikiewicz-Guzik et al, a subgroup analysis showed that the beneficial effect of PT on SBP was found to be less discernible beyond the age of 58 years. This finding could be attributed to age-related increase in arterial stiffness. Possibly, when the studied group is younger with the less arterial stiffness developed, there is more room for improvement of blood pressure regulation due to successful treatment of periodontal disease.

Consequently, we did sub-group analysis to reduce variability of analysed studies. Interestingly, when we investigated only hypertensive and pre-hypertensive cohort of patients, we observed the statistically significant difference in BP change which was -11.41[-13.66, -9.15] mmHg and -8.43[-10.96, -5.91] mmHg for SBP and DBP respectively in favour of IPT. This was observed despite the fact that hypertensive cohort was not controlled for age and the duration of hypertension. The fact that, the longer the hypertension is uncontrolled the more difficult it is to treat dysregulated system with significant wall stiffening, has been already well demonstrated (Guzik and Touyz, 2017). The observation that blood pressure was reduced in pre-hypertensive and hypertensive individuals not in normotensive cohorts, provides important insight into design of future studies focusing on the effects of periodontal therapy on blood pressure. However, whether the beneficial effect of periodontal therapy is observed across the entire spectrum of blood pressure distribution, will require large scale population based studies."

The sub-group analysis in respect of duration of the follow up showed that BP reduction was more pronounced, however still not significant in the early (up to 3 months) follow-up period. The effect diminished with increase in follow-up duration. Similar findings were observed when comparing two intervention trials by Graziani et al with different follow-ups (6 months and 12 months) (Graziani et al., 2010a, Graziani et al., 2010b).They found no reduction in either SBP or DBP in the trial with longer duration of follow-up. This observation might be associated with the fact that the key to success of sustained periodontal treatment effect is effective personal oral hygiene and many patients fail to maintain pristine oral hygiene in the long term.

Another important novel question raised by our analysis is: "which of periodontal treatment protocol is most effective in improving blood pressure control?" Successful reduction of local periodontal tissue inflammation, manifested by the reduction of PPD is the main principle in order to reduce blood pressure. However, it is difficult to link the best blood pressure outcomes to one treatment protocol and/or supplementary follow up periodontal care therapy due to heterogeneity of protocols used in individual studies (**Appendix S2b**). Differences in

periodontal therapy and follow up supportive treatment can provide additional explanation for study heterogeneity."

In our study, we have also analysed influence of periodontal treatment on endothelial function. Data from the Framingham offspring cohort suggest that HTN is associated with endothelial disfunction (ED) (Tsao and Vasan, 2015). ED ensues even before the development of hypertension and is an early stage finding of atherogenesis (Davignon and Ganz, 2004, Gimbrone and García-Cardeña, 2016). Studies have demonstrated diminished endothelial function (EF) in patients with PD (Amar et al., 2003, Higashi et al., 2009, Holtfreter et al., 2013). Flow-mediated dilatation (FMD) is a reliable technique to assess endothelial function and correlates with the ED in the coronary artery (Broxterman et al., 2019, Teragawa et al., 2005). In this systematic review, we attempted to evaluate the change in endothelial function (EF) following PT. The meta-analysis findings indicate that the IPT significantly improves the EF. These findings are further corroborated by the results of the previous two meta-analyses (Orlandi et al., 2014, Teeuw et al., 2014). Our results have shown that IPT in the patients with moderate to severe periodontitis had increased FMD by 1.49% [95% CI 0.9, 2.08; $p<0.00001$]. However, it should be noted that EF was the primary outcome in only one of our trials (Tonetti et al., 2007) and the number of studies included was low. Despite low statistical heterogeneity (I^2 =0%) in our analysis, unexplained additional sources of heterogeneity include: the underlying general health conditions (e.g.: diabetes, hypertensives, healthy individuals), variable durations of follow-up ranging from 2 months-12 months and the different ethnic origin of the participants. Lastly, the inherent technical, methodological and physiological factors involved in the assessment of EF with FMD could have influenced the validity of results in our studies. Nevertheless, looking for the evidence supporting the beneficial effect of IPT on endothelial function is important as improvement in EF could reduce the risk of adverse cardiovascular events. Studies have

shown that with every 1% increase in FMD, future CVD risk decreases by 8-13% (Inaba et al., 2010, Ras et al., 2013).

A very significant effect of periodontal therapy on endothelial function provides important mechanistic insight suggesting that the vasoprotective effect may be critical for improvement of not only blood pressure profile but cardiovascular risk in general. The potential mechanisms of this effect may be related to direct effects of periodontal bacteria and their cell wall components or endotoxins on endothelium (Dietrich et al., 2017), indirect modulation through altered CRP or pro- and anti-inflammatory cytokine profiles (Czesnikiewicz-Guzik et al., 2020) or a possibility raise by our recent study that periodontitis leads to local dysfunctional activation of immune cells (including monocytes and T cells) that migrate to perivascular tissues, modulating nitric oxide biology and endothelial function. These aspects have been reviewed by us elsewhere (Czesnikiewicz-Guzik et al., 2019b, Muñoz Aguilera et al., 2020).

The association between PD and hypertension is hypothesised to stem from the activation of immune system with up-regulation of pro-inflammatory cytokines driving high blood pressure. If treatment of periodontal diseases is beneficial for the cardiovascular status, the reduction of systemic inflammation due to periodontal treatment must be documented. Therefore, we analysed the levels of cytokines and cardiometabolic markers, which were available in selected RCTs. Interestingly, we observed significant decrease in CRP levels in patients undergoing IPT at each point of our observation. This observation is in line with previous observational studies reporting beneficial influence of periodontal therapy on CRP level (Vidal et al., 2013, Hussain Bokhari et al., 2009, Lopez et al., 2012). Unfortunately, due to substantial data heterogeneity and low number of studies available we could not draw significant conclusions for other studied inflammatory cytokines and cardiometabolic markers. In our quantitative summary, there was no significant difference in change of IL-6,

Il-10, IFN-γ even though in the observational studies some authors were reporting significant decrease in IL-6 (Vidal et al., 2009, Vidal et al., 2013) and IFN-γ (Czesnikiewicz-Guzik et al., 2019b) levels after successful periodontal therapy. Also, analysis of metabolic markers such as HDL, LDL and TG did not show significant change after periodontal treatment, however only 5 studies reported lipid levels in the context of periodontal therapy, so much more evidence is needed to investigate this aspect in future.

Conclusions

This systematic review and meta-analysis make an important contribution to the available literature investigating causal association between periodontitis and hypertension. The results of this review show that the IPT improves endothelial function, a process leading to hypertension and its cardiovascular complications. While in overall analysis no significant effect of IPT on blood pressure lowering was observed, subgroup analysis demonstrated that in hypertensive and pre-hypertensive individuals a significant decrease of blood pressure was achieved. Additionally, we could observe a significant decrease in CRP level in successfully treated patients. Although other inflammatory and cardiometabolic markers did not change significantly following IPT, more research is needed to analyse these inflammation markers in detail. Overall, the results suggest that successful periodontal treatment could support anti-hypertensive therapy in hypertensive and pre-hypertensive patients who should be motivated to monitor and maintain periodontal health regularly to reduce cardiovascular risk.

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Figure legends:

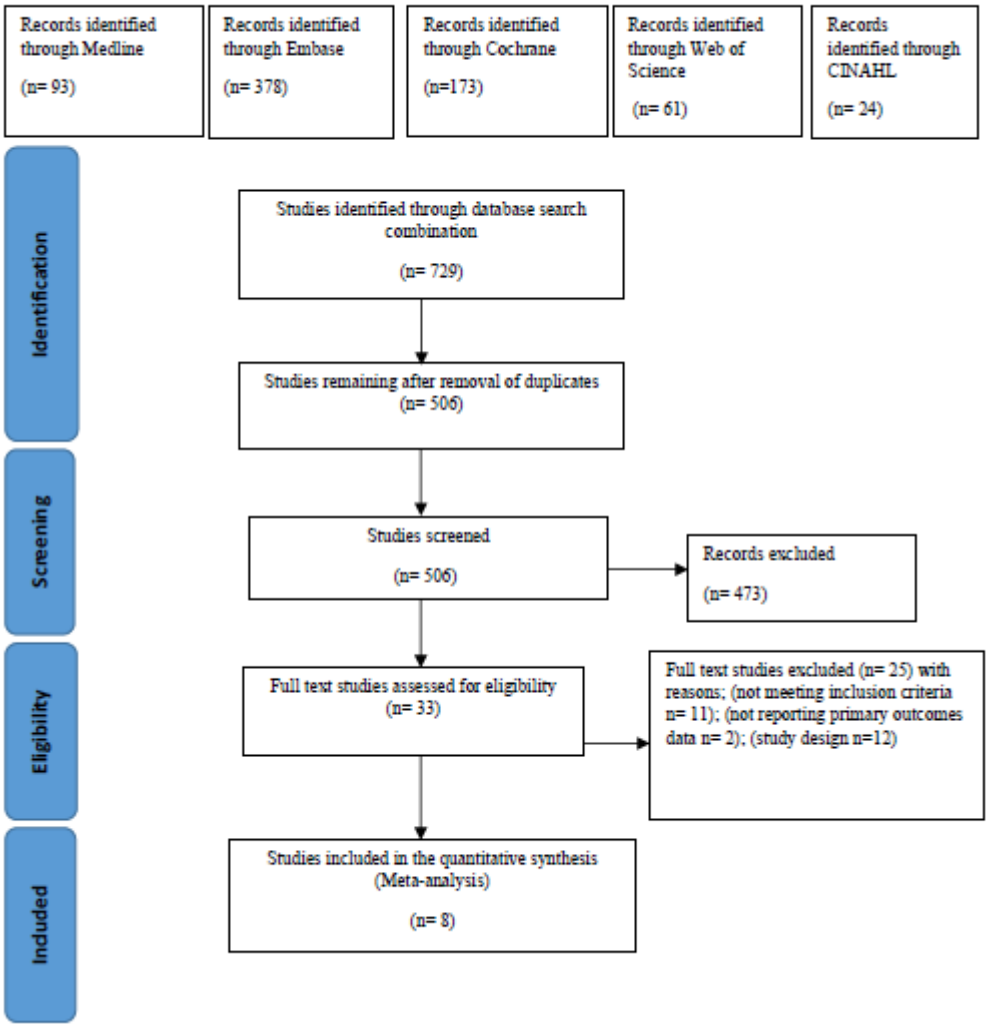


Figure 1. PRISMA flow diagram of the study selection process.

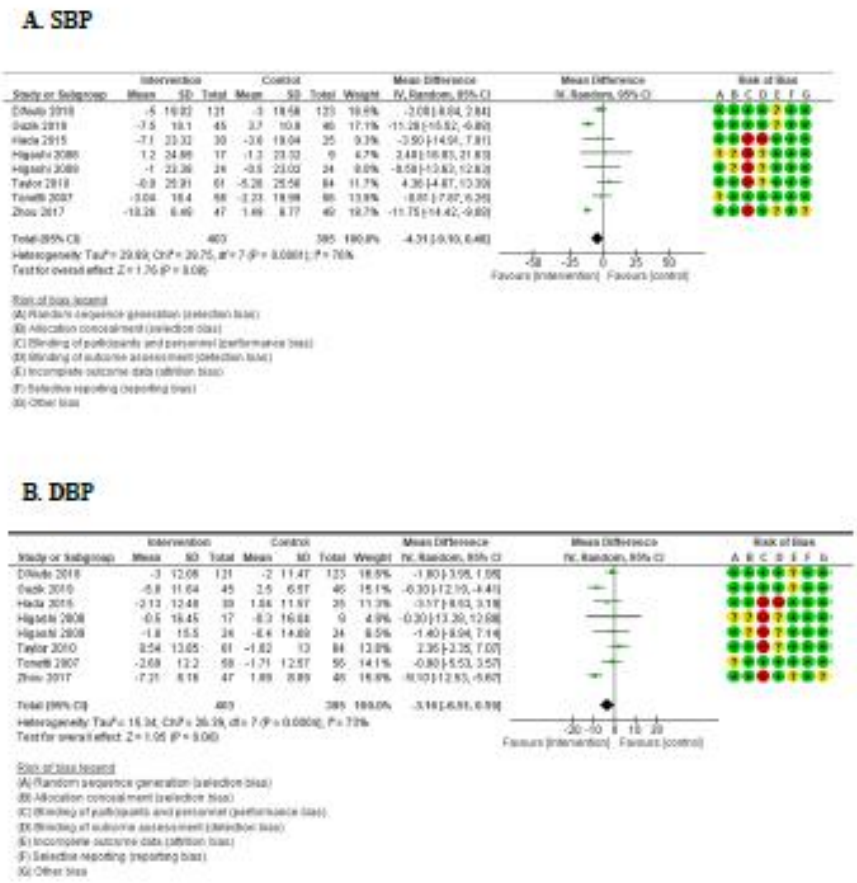


Figure 2. Effect of periodontitis treatment on **systolic blood pressure (SBP) (Panel A)** and **diastolic blood pressure (DBP) (Panel B) changes in all studies at the latest follow-up point available**. Summary forest plot for the change in BP post treatment in the intervention and the control groups. The random effects model was used. The results are presented as Weighted Mean Difference (WMD). SD, Standard déviation ; CI, Confidence Intervals ; IV, Inverse variance.

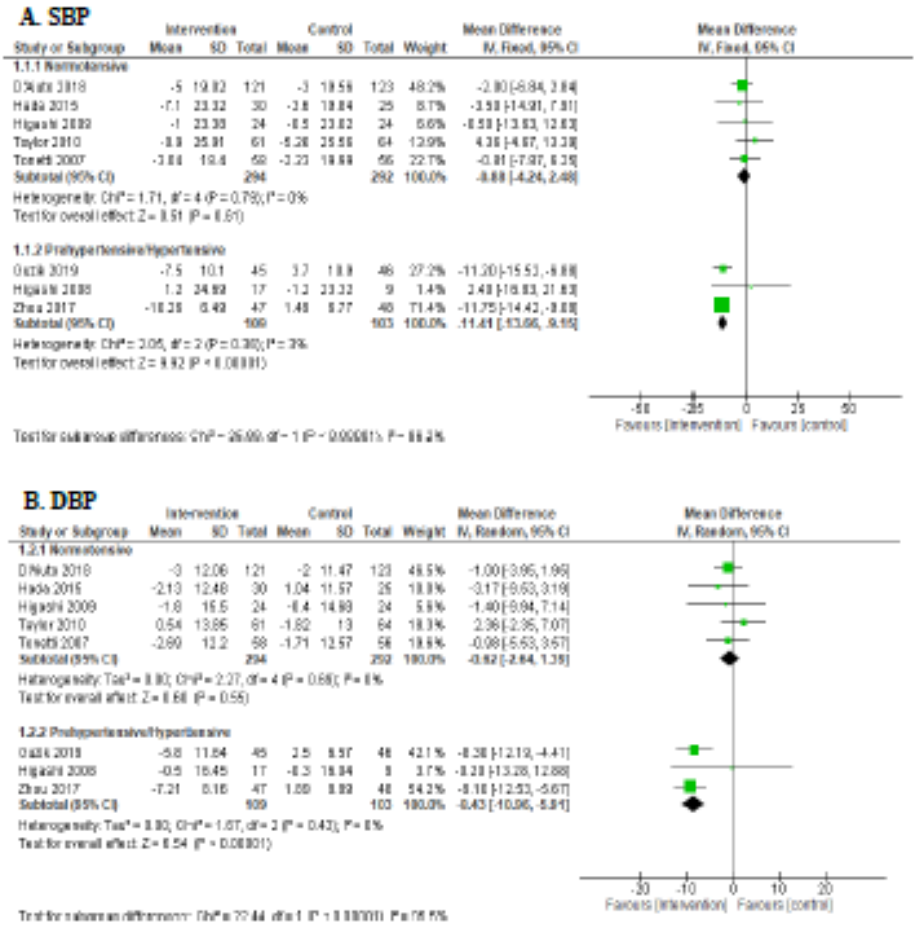
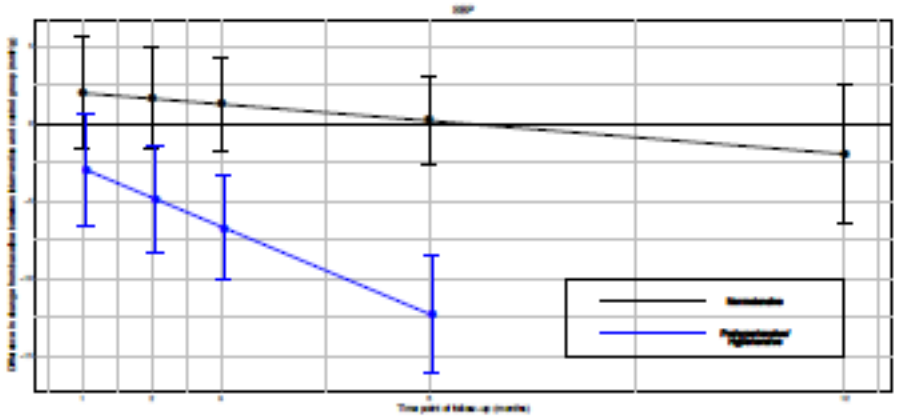


Figure 3. Subgroup meta-analyses of the effect of IPT on SBP **(Panel A)** and DBP **(Panel B)** using random effects model. The included studies were divided into two subgroups; normotensives and hypertensives based on the inclusion criteria in each study.

A.



B.

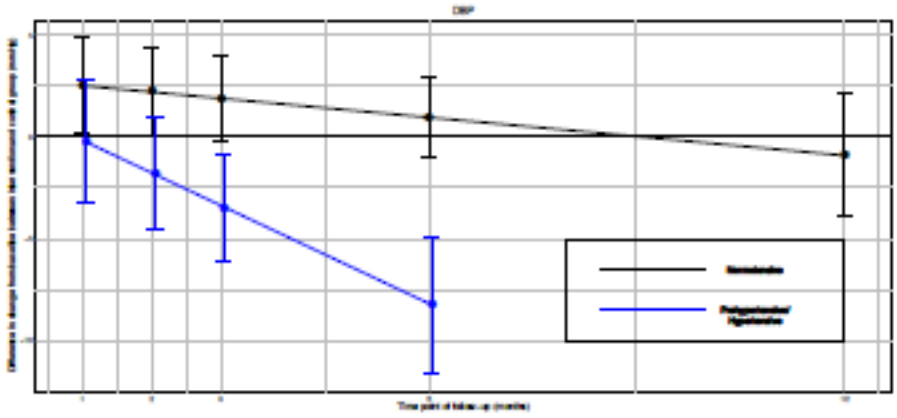


Figure 4. Pooled difference in change from baseline between intervention and control group by time point of follow-up in normotensive (black line) and in hypertensive/pre-hypertensive patients (blue line), estimated in a multivariate mixed-effects meta-regression predicting SBP (A) or DBP (B) from time point of follow-up and hypertension at baseline and the interaction of both (p value for interaction between time and hypertensive status for SBP p=0.0002 and for DBP p=0.0014)

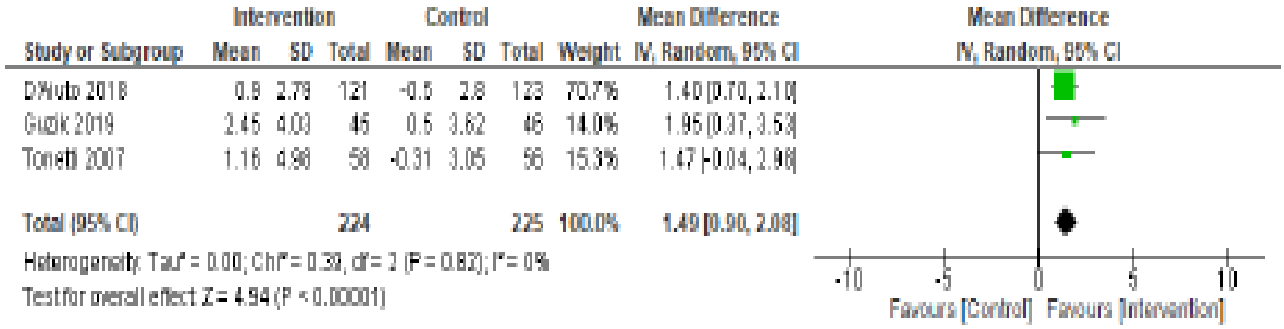


Figure 5. Effect of periodontitis treatment on endothelial function. Summary forest plot for the change in endothelial function post intervention in the two groups (intervention versus control).

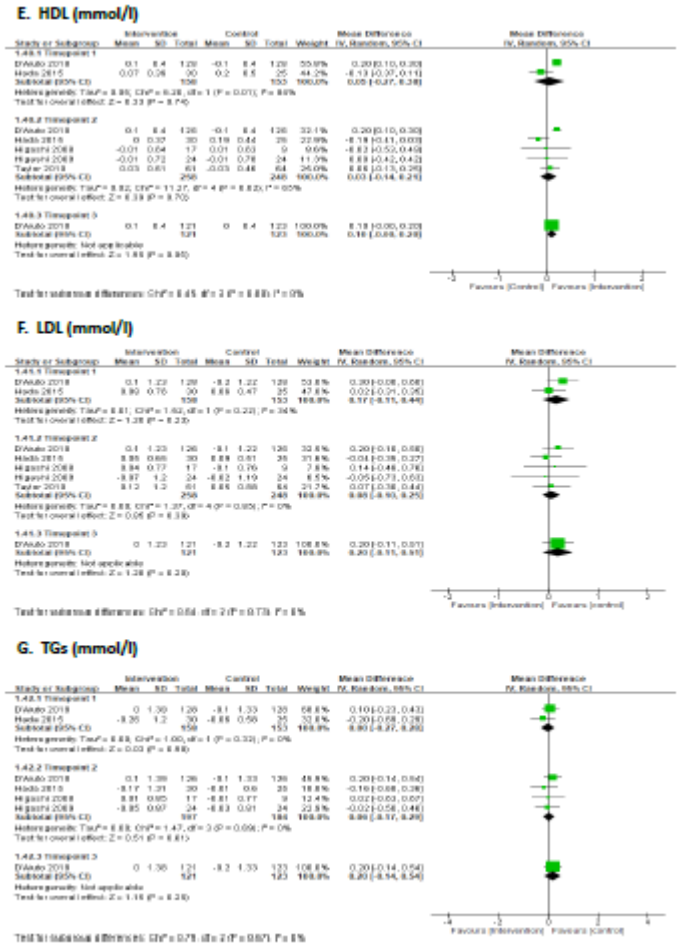


Figure 6. Effect of periodontal intervention on serum CRP, pro-inflammatory cytokines and metabolic markers of cardiovascular disease. Subgroup analyses Forest plots for the mean differences in serum levels of various markers in intervention versus control groups at different timepoints**.** Analysis for CRP, IL-6, IL-10 and IFN gamma (**A-D)**. Analysis for HDL-C, LDL-C, TGs **(E-G)**. The random effects model was used. In panel B - weighted standardized mean difference for IL-6 is reported due to variability in the units of measurement. (HDL -High density lipoproteins; LDL-C - Low density lipoproteins; TGs -Triglycerides)

Graphical Abstract

Visual abstract created with BioRender.com