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Efficacy of locally delivered statins as an adjunct to scaling and root planning in the treatment of periodontitis: a systematic review and meta-analysis

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Abstract. – OBJECTIVE: The aim of this systematic review is to assess the efficacy of locally delivered statins used in adjunct to scaling and root planing (SRP), compared with SRP alone.

MATERIALS AND METHODS: An electronic and hand search was carried out up to April 2020. Only randomized controlled trials (RCTs) were included. Clinical attachment level gain (CALgain) and probing depth reduction (PDred), modified sulcular bleeding index reduction (mSBIred), and intrabony defect reduction (IBDred) were the investigated outcomes. Meta-analysis was performed, and the power of the meta-analytic findings determined by trial sequential analysis (TSA). Studies were also sub-grouped based on the type of statin used. Statistical heterogeneity and publication bias were assessed.

RESULTS: Twenty RCTs were included (1212 patients, 1289 defects). An overall statistically significant effect size in favor of statins for CALgain and PDred was found. As opposed to atorvastatin and rosuvastatin, simvastatin did not reach statistical significance for these outcomes, as shown by the sub-group analysis.

CONCLUSIONS: Within the limits of the available studies, the local administration of statins (in particular, atorvastatin and rosuvastatin) in adjunct to SRP may result in additional significant improvement in terms of CALgain and PDred compared with SRP alone. The high heterogeneity of data and the high risk of bias found, however, impose caution. No approved preparations, moreover, exist, and further well-designed RCTs from independent research centers are needed to confirm the beneficial effects of the different statins and their mutual differences in the non-surgical periodontal treatment.

Key Words:
Statin, Periodontitis, Non-surgical periodontal therapy, Meta-analysis, Trial sequential analysis.

Introduction

Periodontitis is a chronic inflammatory, infectious disease of tooth-supporting tissues associated with complex multifactorial microbial interactions, host immune responses, harmful environmental changes, and genetic susceptibility. For these reasons, periodontitis is characterized by a wide range of microbiological, immunological and clinical manifestations[1].

This inflammatory condition may result from a localized or generalized dysbiosis within the dental plaque microbiota of the periodontal sulcus, which may lead to progressive loss of periodontal attachment and supporting bone, and, eventually, to tooth loss[2].

In almost all forms of periodontal diseases, both the periodontal microbiota and the host response play critical roles in the onset and progression of these diseases. It is now well established that tobacco use and systemic conditions (i.e., diabetes) are among the most important preventable risk factors in the incidence and progression of periodontal diseases[3].

The primary aim of periodontitis treatment is to resolve the inflammatory process and to arrest the disease progression. The traditional mechanical de-

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bridement of dental and root surfaces by scaling and root planing (SRP) still plays a central role in the non-surgical therapy of periodontitis[4]. However, such an approach alone may be poorly effective in inaccessible areas, such as deep periodontal pockets, furcations, and interproximal areas of misaligned teeth. Moreover, diabetic patients and smokers tend to respond to periodontal treatment less favorably than healthy patients[5]. Therefore, a combination of SRP along with selective elimination or inhibition of pathogenic microbes by systemic or locally delivered antimicrobial and host modulating agents has recently aroused considerable interest[6]. Such an approach allows to achieve maximum antibacterial concentration in selected periodontal sites with minimal systemic side effects and has demonstrated improved therapeutic outcomes.

Statins, such as simvastatin, atorvastatin, and rosuvastatin, have recently been introduced as adjunctive aids in periodontal therapy. Statins are mainly known for their property of reducing cholesterol levels by specifically inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a rate-limiting enzyme for cholesterol synthesis[7]. Apart from lipid-lowering properties, statins also express dynamic functions. The reduction in mevalonate pathways by statins is responsible for several pleiotropic effects, including anti-inflammatory modulation of vascular response, microvascular reperfusion, antimicrobial effect, and also improvement of wound healing processes[8].

Statins have also been shown to inhibit osteoclast differentiation and improve the production of bone anabolic factors, such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2), which promotes osteoblastic differentiation and bone formation[9].

Statins have immunomodulatory, antioxidant, and antithrombotic actions as well, and even help in inhibiting tumor growth and metastasis. They have direct anti-inflammatory and plaque-stabilizing effects through the inhibition of monocyte recruitment and adhesion to the endothelium, and the improvement of endothelial function[10].

Previous human studies[11,12] have shown that local delivery of statins may result in additional clinical benefits when associated with SRP.

This study aimed to systematically review randomized clinical trials (RCTs) concerning the effect of locally applied statins, in conjunction with SRP, in the non-surgical treatment of periodontitis.

Materials and Methods

The study protocol was registered in PROSPERO (CRD42020181742) and was undertaken following the Cochrane Handbook[13]. The search strategy used in this systematic review was based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (http://www.prisma-statement.org)[14].

Focused Question

The focused question for this systematic review was: "How does the local application of statins in adjunct to SRP affect clinical periodontal parameters compared to SRP alone in patients affected by periodontitis? Clinical questions were formulated according to the PICO framework for evidence-based practice[15] comprising: patients with chronic or aggressive periodontitis (P), non-surgical mechanical periodontal treatment with statins as intervention (I), compared to non-surgical mechanical treatment alone or with placebo as control (C), and probing pocket depth reduction and clinical attachment level gain as primary outcomes (O).

Search Strategy

A literature search was carried out in April 2020 by two independent and calibrated reviewers. PubMed (MEDLINE), EMBASE, ClinicalTrials.gov website, ResearchGate, and the Cochrane Central Register of Controlled Trials (CENTRAL) were searched. The authors used an ad-hoc search string: "(statin OR simvastatin OR atorvastatin OR rosuvastatin) AND (periodontitis OR periodontal disease OR chronic periodontitis OR aggressive periodontitis OR periodontal therapy OR periodontal treatment) AND (periodontal attachment loss OR probing depth OR periodontal pocket)". A hand search was conducted on the major international journal of periodontics (Journal of Periodontology, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research, International Journal of Periodontics and Restorative Dentistry). Grey literature was also searched (https://www.greylit.org/; http://www.opengrey.eu/). The references list of all eligible studies was scanned for possible additional studies. Two independent reviewers (A.P. and G.M.) screened the title and abstract of

studies identified by the search strategy. In case of disagreements, a consensus was achieved through discussion. For eligible studies, or when abstracts did not provide sufficient data, the full text was carefully read and analyzed for inclusion and data extraction. The inter-examiner agreement was verified by kappa coefficient. Any discrepancy was resolved *via* discussion.

Inclusion Criteria

- Study design: randomized controlled trials (RCTs) with both parallel-group and split-mouth design;
- RCTs with at least 3-month follow-up;
- patients with a diagnosis of chronic or aggressive periodontitis;
- studies that consider healthy patients and/or with systemic diseases that may influence the course of periodontitis and/or the response to treatment;
- intervention group should use any statin, as a sole adjunct to non-surgical mechanical periodontal treatment;
- application of the statin gel to the test group after SRP inside the periodontal pocket;
- the comparison group should comprise non-surgical mechanical periodontal therapy alone or associated with placebo;
- the outcome should include at least one clinical periodontal measurement, such as probing depth, clinical attachment level.

Exclusion Criteria

- Non-RCT studies;
- pregnant and lactating patients;
- patients younger than 18 years old;
- patients receiving systemic treatment with statins;
- statins used with any other drug/biomaterial in the same study group;
- statins used differently from the subgingival application;
- animal studies;
- studies without mechanical periodontal therapy.

No language or publication date restriction was applied. In case of doubtful or incomplete data, the corresponding author was contacted. After analysis of the selected studies, clinical data were extracted by two independent reviewers (A.P. and G.M.).

Primary outcomes were: clinical attachment level (CAL) gain and pocket depth (PD) reduction. Secondary outcomes were: changes in bleeding indices, changes in plaque indices, changes of intrabony defect depth.

Mean changes from the baseline for the measured outcomes and their standard deviations were extracted, when available. The following information for each study was also registered: study design, type of periodontitis, number of patients/sites, gender, smokers, age, study groups, type of tooth and defect, type of statin and method of administration, follow-up, outcomes evaluated, method of evaluation, and conclusions.

Risk of Bias (Quality) Assessment

The quality of the included studies was assessed by two independent calibrated examiners (A.P. and G.C.) using the Cochrane Risk of Bias Tool for RCTs (RoB 2) (updated on 22 August 2019)[16]. For each RCT five domains were considered: (1) bias arising from the randomization process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in the measurement of the outcome; (5) bias in the selection of the reported result. Each domain was judged at low, uncertain, or high risk. The overall risk of bias of the included studies was categorized as low if all criteria were judged at low risk; as high, if one or more criteria were at high risk, and at moderate risk if one or more criteria were unclear and none at high risk.

Strategy for Data Synthesis

When possible, a meta-analysis was performed on primary and secondary outcomes to compare treatment effects. Data were displayed as a difference in means, with 95% confidence intervals. The study-specific estimates were pooled using the random effects model if a significant heterogeneity was found. Forest plots were created to illustrate the effects of the different studies and global estimation. Subgroup analysis was conducted regarding the type of statin used. Comprehensive Meta-analysis software (Biostat, USA) was used to perform all analyses. Statistical significance was defined as a p-value $< .05$. The statistical heterogeneity among the included studies was evaluated using Cochrane's Q-test, with significance set at $p < 0.1$, and the I^2 test with a >75% value corresponding to high heterogeneity. If the meta-analysis contained sufficient trials to make a visual inspection of the plot meaningful (ten trials minimum), funnel plots were considered as a tool for assessment of publication bias.

Trial Sequential Analysis

Trial Sequential Analysis (TSA) was undertaken for the main outcomes (CAL gain and PD

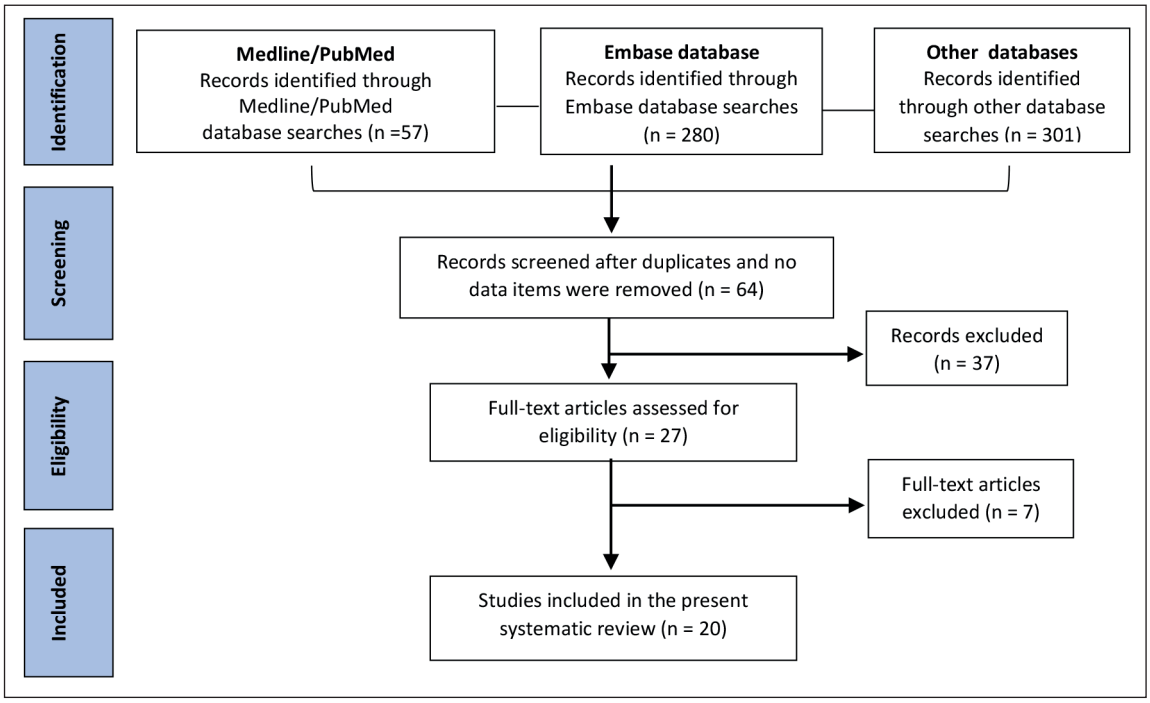

Figure 1. Flow diagram (PRISMA format) of the screening and selection process.

reduction), to adjust the results for types I and II errors, and to assess the power of the meta-analysis findings. The software TSA 0.9.5.10 Beta (Copenhagen Trial Unit Centre for Clinical Intervention Research Department, Copenhagen, Denmark) was used. A random-effects model was chosen for meta-analysis. The required information size (RIS) and alpha-spending monitoring boundaries were estimated by setting type I and type II errors at 5% and 20% (power of 80%), respectively. For RIS calculation, the incidence in both test (statins) and control arms was estimated according to the results of the meta-analysis, and no heterogeneity correction was applied. No subgroup analysis was performed for TSA. The graphical analysis was performed to see if the cumulative Z-curve (in blue) crosses the trial sequential monitoring threshold (horizontal red line), and the RIS threshold (vertical red line).

Results

Study Selection

Fifty-seven items in MEDLINE/PubMed, 280 items in Embase, and 301 in other sources were found after the initial search. After the removal of duplicates and items with no data available, 64 records remained. After the screening of titles and abstracts for inclusion/exclusion criteria, 37 studies were excluded, and 27 studies remained. After full text assessment, seven studies were excluded because they used systemically administered statins[17], toothpaste used as delivery vehicle[18], had a follow-up <3 months[19-22], or used statins in the surgical treatment of periodontitis[23]. Finally, 20 RCTs published between 2010 and 2019 were included in this systematic review (Figure 1). A high level of agreement was found between the reviewers at both screening stages (K=0.85).

Study Characteristics

The number of participants ranged from 20 patients[24,25] to 100 patients[26]. The age of participants ranged from 25 to 60 years old. All patients, male and female, suffered from chronic periodontitis except for 24 patients from one study[27], who suffered from aggressive periodontitis. All the RCTs were placebo-controlled studies.

Fifteen of the included studies had authors in common[20-34].

The effect of the local use of simvastatin gel was assessed in 8 studies[24,27,28,34,35,37,42,43], atorvastatin in 5 studies[25,36,39-41] statins have shown pleiotropic effects such as anti-inflammation and bone stimulation. The aim of the present study is to investigate the effectiveness of 1.2% ATV as an adjunct to scaling and root planing (SRP and ro-

suvastatin in 4 studies[26,32,33,38,41]. In addition, in 1 study[31] the efficacy of simvastatin was compared with atorvastatin and in 2 other studies[29,30], atorvastatin was compared with rosuvastatin.

All the examined studies included all types of teeth (incisors, canines, premolars, and molars) of the maxilla and mandible. In all studies, teeth with PD≥3 mm, CAL≥4 mm, intrabony defects, and vertical bone loss ≥3 mm were considered. Two studies[30,34] also included teeth with furcation defects. The main characteristics of selected studies are described in Tables I and II. In particular, design, characteristics of the study population, tooth types, type of statin, and method of administration are explained in Table I. Follow-up, outcome (clinical parameters), methods of evaluation of the use of statins in conjunction with SRP, and conclusions are described in Table II.

PD reduction, CAL gain, or relative attachment level (RAL) gain, were evaluated in all studies. In case of furcations, relative horizontal attachment level, RHAL, and relative vertical attachment level, RVAL or RVCAL was reported. Plaque index (PI), gingival index (GI), and modified sulcular bleeding index (mSBI) were evaluated in most studies. Radiographic intrabony defect (IBD) depth decrease or reduction (DDR) were measured in 15 studies[26-33,35,36,38-42]. The radiographic bone fill percentage was evaluated in two studies[37,43]. In one study[25] the intraosseous defect sites were measured at baseline and after 6 months. In particular, of the bone defect height [CEJ-BD (base of the defect)], the level of the alveolar crest [CEJ-AC (alveolar crest)], the bone defect depth (AC-BD), and the mesiodistal (MD) and buccolingual (BL) bone defect width were evaluated.

The changes from the baseline of all the clinical and radiographic parameters at each follow-up are reported in Supplementary Table I.

Risk of Bias and Power of Analysis

The overall risk of bias for each study included is presented in Figure 2. No study fulfilled all criteria with a low risk of bias. All the studies showed at least an unclear (some concerns) risk of bias, mainly concerning the randomization process (no details about the allocation concealment), deviations from the intended interventions (no details about the masking and lack of an appropriate analysis to estimate the effects of the assigned intervention), and selection of the reported results. 15% of the studies were considered at high risk due to possible bias in the outcome measurements, and 5% for bias due to missing outcome data.

Fifteen percent of the studies reported rx images which seriously hampered the correct interpretation and intra-group/inter-group comparison.

Out of all the included studies, 15% did not report information on sample size calculation and power analysis, and 15% were underpowered.

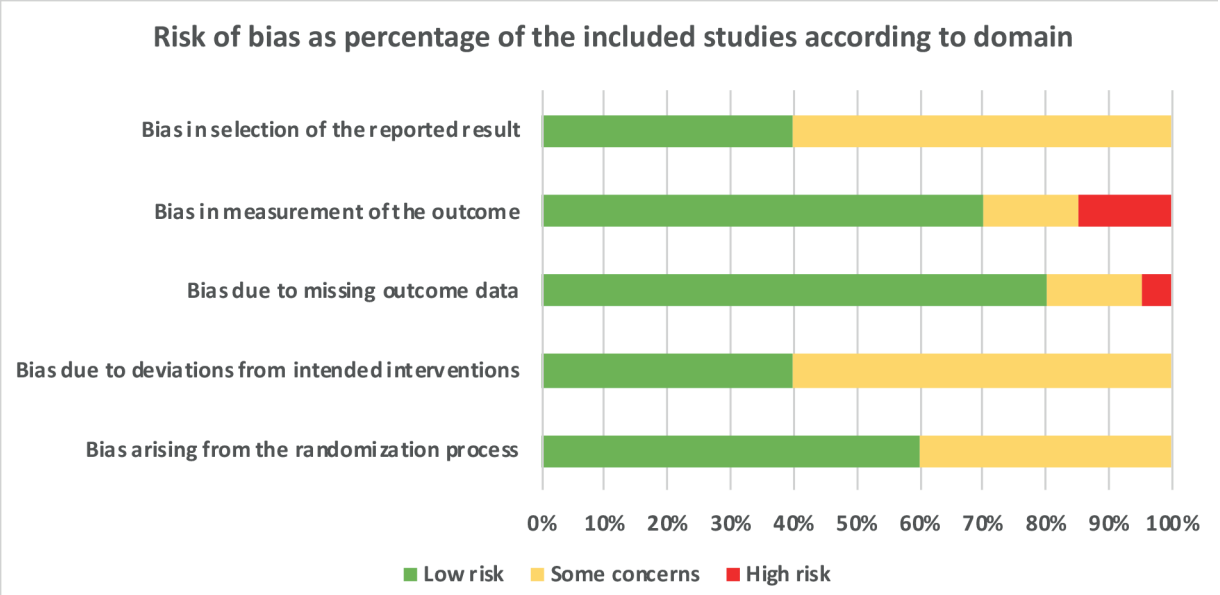

Figure 2. Over-all risk of bias of included study according to the Cochrane Risk of Bias Tool for randomized clinical trials (RoB 2) (updated on 22 August 2019).

Table I. Main characteristics of selected studies: study population.

Study	RCT design (PG or SM)	CP or AP	- N. patients/n. sites - Gender (M/F) - Smokers/Non smokers	Age (range and/or mean±SD)	Study groups (patients/defects)		Type of tooth and defect	Type of statin and method of administration
					Test	Ctr		
Pradeep et al, 2010[21]	PG	PG	- Patients/sites: 60/60 - M/F: 33/31 - Smokers: none	25 to 45 years (30.5±4.1)	30 sites (SRP+SMV)	30 sites (SRP+placebo)	moderate and deep pockets (PD>5 mm; CAL from 4 to 9 mm) and vertical BL>3 mm	SMV gel in methylcellulose gel, subgingival; locally delivered (1.2 mg/0.1 ml)
Pradeep et al, 2012[22]	PG	CP	- Patients/sites: 66/66 - M/F: 38/34 - Smokers: none	30 to 50 years	33 sites (SRP+SMV)	33 sites (SRP+placebo)	furcation defects (buccal, class II, mandibular 1st and 2nd molars), PD≥5 mm & horizontal PD≥3 mm	0.1 mL SMV gel in methylcellulose gel; subgingival, locally delivered (1.2 mg/0.1 ml)
Rath et al, 2012[35]	PG	CP	- Patients/sites: 60/60 - M/F: 33/27 - Smokers: none	25 to 45 years	30 sites (SRP+SMV gel)	30 sites (SRP+placebo gel)	intrabony defects, PD>5 mm & vertical BL≥3 mm	SMV gel 0.1 ml in methylcellulose gel; locally delivered subgingival (1.2 mg/0.1 ml)
Pradeep et al, 2013a[23]	PG	CP	- Patients/sites: 35/35 - M/F: 20/18. - Smokers: none	30 to 50 years	17 sites (SRP+SMV)	18 sites (SRP+placebo)	PD≥5 mm or CAL≥4 mm and vertical BL≥3 mm with ≥20 teeth	10 µl of SMV gel in methylcellulose gel; subgingival, locally delivered (1.2 mg/0.1 ml)
Pradeep et al, 2013b[24]	PG	CP	- Patients/sites: 60/60 - M/F: 35/32. - Smokers: none	30 to 50 years	30 sites (SRP+ATV)	30 sites (SRP+placebo)	PD≥5 mm or CAL≥4 mm and vertical BL≥3 mm	10 µl of 1,2% ATV gel in methylcellulose gel; sub-gingival, locally delivered (1.2 mg/0.1 ml)
Rao et al, 2013[25]	SM	CP	- Patients/sites: 35/67 - M/F: 35/0 - Smokers: everyone	30 to 50 years	33 sites (SRP+SMV)	34 sites (SRP+placebo)	PD≥5 mm or CAL≥4 mm and vertical BL≥3 mm	10 µl SMV gel in methylcellulose gel; subgingival, locally delivered (1.2 mg/0.1 ml)
Gaekwad et al, 2015[17]	SM	CP	- Patients/sites: 20/40 - M/F: 0/20 - Smokers: none	mean age 50±5 years	20 sites (SRP+SMV)	20 sites (SRP+placebo)	PD≥4 mm, CAL≥3 mm	Subgingivally delivered 1.2 mg simvastatin *in situ* gel
Pradeep et al, 2015[26]	PG	CP	- Patients/sites: 65/65 - M/F: 33/37. - Smokers: none	25 to 55 years	32 sites (SRP+ RSV)	33 sites (SRP+placebo)	PD 5 to 6 mm, CAL 4 to 6 mm, and vertical BL≥3 mm, with ≥20 teeth	0.1 mL RSV gel in methylcellulose gel; subgingival, locally delivered (1.2 mg/0.1 ml)
Agarwal et al, 2016[36]	SM	CP	- Patients/sites: 19/38 - M/F: 14/16. - Smokers: none	25 to 50 years	19 sites (SRP+SMV)	19 sites (SRP+placebo)	At least two periodontal pockets of PD ≥5mm and vertical BL ≥2mm (periapical Rx)	Subgingivally delivered SMV 1.2% in methylcellulose gel
Kumari et al, 2016[28]	PG	CP	- Patients/sites: 60/60 - M/F: 38/37. - Smokers: none	40 to 50 years	30 sites (SRP+ATV)	30 sites (SRP+placebo)	PD ≥5 mm, RAL ≥4 mm, and vertical BL≥3 mm (periapical Rx), no history of periodontal therapy and no history of use of antibiotics in the preceding 6 months, minimum of 20 teeth.	Local delivery of ATV 1.2% in methylcellulose gel into periodontal pockets

Table continued

Table I. *(Continued).* Main characteristics of selected studies: study population.

Study	RCT design (PG or SM)	CP or AP	- N. patients/n. sites - Gender (M/F) - Smokers/Non smokers	Age (range and/or mean±SD)	Study groups (patients/defects)		Type of tooth and defect	Type of statin and method of administration
					Test	Ctr		
Pradeep et al, 2017[29]	PG	CP	- Patients/sites: 90/90 - M/F: 53/51. - Smokers: none	30 to 50 years	30 sites (SRP+ATV) 30 sites (SRP+alendronate)	30 sites (SRP+placebo)	PDs≥5 mm or CALs≥4 to 6 mm and vertical BL≥3 mm on periapical Rx	1.2% ATV gel (1.2 mg/0.1 mL) into the periodontal pocket
Garg& Pradeep, 2017[31]	PG	CP	- Patients/sites: 90/90 - M/F: NR - Smokers: none	Mean age 36.3 years	30 sites (SRP+RSV) 30 sites (SRP+ATV)	30 sites (SRP+placebo)	PD≥5 mm and horizontal PD≥3 mm with a radiolucency in the furcation area on a periapical Rx	subgingivally delivered RSV and ATV 1.2% in methylcellulose gel
Martande et al, 2017[32]	PG	CP	- Patients/sites: 88/88 - M/F: 50/46. - Smokers: none	30 to 50 years	30 sites (SRP+ATV) 30 sites (SRP+SMV)	28 sites (SRP+-placebo)	PD≥5 mm, CAL≥4 mm, and vertical BL≥3 mm (periapical radiographs)	local drug delivery: 1.2% ATV gel (1.2 mg/0.1 mL); 1.2 % SMV gel (1.2 mg/0.1 mL)
Priyanka et al, 2017[20]	PG	AP	- Patients/sites: 21/60 - M/F: 14/10 - Smokers: none	30 to 50 years	11 patients/30 sites (SRP+SMV)	10 patients/30 sites (SRP+placebo)	PD≥5 mm, CAL≥5 mm, radiographic B ≥30% of root length, affecting >3 permanent teeth	local drug delivery SMV gel (1.2 mg/0.1 mL)
Pankaj et al, 2018[33]	PG	CP	- Patients/sites: 90/90 - M/F: 44/46 - Smokers: none	25 to 45 years (mean 34.32±5.09)	30 sites (SRP+RSV) 30 sites (SRP+MF)	30 sites (SRP+placebo)	PD>5 mm and CAL>3 mm	0.1 mL RSV gel in methylcellulose gel (1.2 mg/0.1 mL) and 1% MF gel (1 mg/0.1 mL); subgingival, locally delivered
Chatterjee et al, 2019[19]	PG	CP	- Patients/sites: 100/100 - M/F: 47/53 - Smokers: none	30 to 60 years	50 sites (SRP+RSV)	50 sites (SRP+placebo)	CAL≥3 mm; PD≥4 mm; vertical BL≥3 mm; ≥20 teeth	0.1 mL RSV gel in methylcellulose gel (Sodium methylparaben and sodium propylparaben were added to methylcellulose gel in the concentration of 0.1 and 0.02% as preservative) (1.2 mg/0.1 mL); locally delivered
Kanoriya et al, 2019[34]	PG	CP	- Patients/sites: 60/60 - M/F: 60/0. - Smokers: everyone	30 to 50 years	30 sites (SRP+RSV)	30 sites (SRP+placebo)	IBD depth≥3 mm, PD≥5 mm or CAL≥3 mm in an asymptomatic tooth	local drug delivery RSV gel (1.2 mg/0.1 mL)
Shirke et al, 2019[18]	SM	CP	- Patients/sites: 20/40 - M/F: 10/10 - Smokers: none	30 to 45 years	20 sites (SRP+ATV)	20 sites (SRP+placebo)	PD and CAL≥5 mm; at least one pair of similar intra osseous defects≥3 mm deep	local drug delivery of 1.2% ATV gel (1.2 mg/0.1 mL)

RCT: randomized clinical trial; PG: parallel group; SM: split mouth; CP: chronic periodontitis; AP: aggressive periodontitis; M: male; F: female; SD: standard deviation; SRP: scaling and root planing; SMV: simvastatin; PD: probing depth; CAL: clinical attachment level; BL: bone loss; ATV: atorvastatin; RSV: rosuvastatin; RAL: relative attachment level; NR: not reported; MF: metformin; IBD: intrabony defect

Table II. Main characteristics of selected studies: outcomes, parameters, methods of evaluation and conclusions.

Study	Follow up	Outcomes (Changes in clinical parameters)	Method of evaluation	Conclusions
Pradeep et al, 2010[21]	1, 2, 4, 6 months	PD, CAL, IBD, and mSBI	Periodontal probe, periapical Rx with a parallel-angle technique	There was a greater decrease in gingival index and PD and more CAL gain with significant IBD fill with locally delivered SMV in patients with chronic periodontitis. These observations may give new direction in the field of periodontal regeneration, to achieve the goal of regeneration without invasive procedures, thereby causing less discomfort to the patients. However, long-term studies, using different vehicles and concentrations of SMV, should be carried out to support the observations of our study.
Pradeep et al, 2012[22]	3, 6 months	PD, RVAL, RHAL, mSBI, GI and PI	Periodontal probe, periapical Rx with a parallel-angle technique	It can be concluded from the current study that there was a greater decrease in gingival index and PD and more RVAL and RHAL gain with significant bone fill with locally delivered SMV in patients with Class II furcation defects. These observations may give a new direction in the therapeutic management of furcation defects, without invasive procedures, thereby causing less discomfort to the patients. However, long-term, multicenter, longitudinal studies, using different vehicles and concentrations of SMV, should be performed to support the observations of our study.
Rath et al, 2012[35]	2, 3, 6 months	PD, CAL, and IBD (%)	Periodontal probe, periapical Rx with a parallel-angle technique	These observations may give new direction in the field of periodontal regeneration, to achieve the goal of regeneration without invasive procedures, thereby causing less discomfort to the patients. However, long-term studies, using different vehicles and concentrations of SMV, should be carried out to support the observations of our study.
Pradeep et al, 2013a[23]	3, 6, 9 months	PD, CAL, IBD (%), mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This clinical trial thus demonstrates that local delivery of 1.2% SMV into periodontal pockets of patients with type 2 diabetes stimulated a significant increase in PD reduction, CAL gain, and improved bone fill compared to placebo gel as an adjunct to SRP. However, long-term, multicenter, randomized, controlled clinical trials will be required to know its clinical, histologic, and radiographic effect over bone regeneration in patients with diabetes.
Pradeep et al, 2013b[24]	3, 6, 9 months	PD, CAL, IBD (%), mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This clinical trial demonstrates that local delivery of 1.2% ATV into IBDs in individuals with CP stimulates a significant increase in PD reduction, CAL gain, and improved bone fill in adjunct to SRP compared to placebo gel. This can provide a new direction in the management of IBDs in CP. However, a long-term, multicenter, randomized, controlled clinical trial will be required to confirm the findings of this study.
Rao et al, 2013[25]	3, 6, 9 months	PD, CAL, IBD (%), bone defect fill (%), mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This clinical trial thus demonstrates that local delivery of 1.2% SMV into periodontal pockets of smokers with chronic periodontitis stimulated a significant increase in PD reduction, CAL gain and improved bone fill as compared to placebo gel as an adjunct to SRP. This can provide a new direction in the field of periodontal healing in this special group of patients who are at greater risk for periodontal destruction. However, long-term, multicenter randomized, controlled clinical trials are required to ascertain the clinical, histological and radiographical effect on bone regeneration in smokers.
Gaekwad et al, 2015[17]	3, 6 months	PD, CAL, PI, GI, and BI	Periodontal probe	Simvastatin in situ gel showed improvements in clinical parameters and prolonged anti-inflammatory effects. The results encourage the use of simvastatin in situ gel for the predictable treatment of chronic periodontitis in postmenopausal women.
Pradeep et al, 2015[26]	1, 3, 4, 6 months	PD, CAL, IBD (%), and mSBI	Periodontal probe, periapical Rx with a parallel-angle technique	The authors conclude that 1.2% RSV in situ gel, when delivered subgingivally/locally, showed bone formation in IBD sites, thereby reducing PD and increasing CAL gain. The study also found an overall improvement in gingival health, with a decrease in mSBI. These findings may give new insight into the use of hydrophilic statins (pravastatin and RSV) in periodontal regenerative techniques. However, further longitudinal studies need to be carried out using RSV at differing concentrations and incorporating it in other vehicles to confirm the findings of the present study.
Agarwal et al, 2016[36]	1, 3, 6 months	GI, PI, PD, CAL, and IBD	Periodontal probe, periapical Rx with a parallel-angle technique	SMV gel in the treatment of intrabony defects has shown clinically favorable results as evident by reduction in PD and gain in CAL. Radiographic changes as well as significant bone fill were seen in the test group.
Kumari et al, 2016[28]	3, 6, 9 months	PD, RAL, mSBI, IBD, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This clinical trial demonstrates local delivery of 1.2% ATV into periodontal pockets of patients with t2DM stimulated significant improvement in clinical and radiographic parameters compared with placebo gel as an adjunct to SRP.
Pradeep et al, 2016[30]	6, 9 months	PD, CAL, DDR, mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	Adjunctive subgingival administration of statins is superior to mechanical periodontal therapy alone, with local drug delivery of 1.2% RSV resulting in significantly greater clinico-radiographic improvements compared with 1.2% ATV.

Table continued

Table II. (*Continued*). Main characteristics of selected studies: outcomes, parameters, methods of evaluation and conclusions.

Study	Follow up	Outcomes (Changes in clinical parameters)	Method of evaluation	Conclusions
Kumari et al, 2017[27]	3, 6, 9 months	PD, CAL, IBD (%), mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This clinical trial demonstrates that local delivery of 1.2% ATV into periodontal pockets in smokers with CP stimulated a significant improvement in clinical parameters compared to placebo gel as an adjunct to SRP. However, long-term, multicenter, randomized, controlled clinical trials will be required to understand its clinical, histological, and radiographical effects in bone regeneration in smokers.
Pradeep et al, 2017[29]	3, 6, 9 months	PD, CAL, IBD, DDR (%), mSBI and PI	Periodontal probe, periapical Rx with a parallel-angle technique	This RCT demonstrated that both ATV and ALN can be used as effective modes of treatment for CP patients. However, ALN was comparatively better than ATV in treatment of intrabony defect in CP patients. More long-term, multicenter comparative randomized control trials must be carried out to further evaluate the efficacy of the drugs.
Garg& Pradeep, 2017[31]	6, 9 months	PD, RVAL, RHAL, BDD, DDR (%), mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	There is improvement in clinical and radiographic parameters with use of RSV and ATV gel delivery as adjunct to SRP in patients with CP with mandibular Class II defects. Results with RSV gel delivery were significantly better than with ATV.
Martande et al, 2017[32]	3, 6, 9 months	PD, RAL, mSBI, IBD (%) and PI	Periodontal probe, periapical Rx with a parallel-angle technique	ATV resulted in greater improvements in clinical parameters with higher percentage of radiographic defect depth reduction as compared to SMV in the treatment of intrabony defects in CP subjects.
Priyanka et al, 2017[20]	3, 6 months	PD, CAL, IBD, mSBI, and PI	Periodontal probe, periapical Rx with a parallel-angle technique	Local delivery of 1.2 mg SMV into the periodontal pocket in group 2 patients stimulated a significant increase in PD reduction and CAL gain, and improved bone fill compared to group 1 patients. However, long-term, multicenter, longitudinal studies using different vehicles and concentrations of SMV should be carried out to support the observations of this study.
Pankaj et al, 2018[33]	6, 12 months	PD, CAL, mSBI, IBD, DDR (%) and PI	Periodontal probe, periapical Rx with a parallel-angle technique	RSV was comparatively better than MF. Further multicentric long-term clinical trials must be carried out to evaluate the efficacy of both drugs.
Chatterjee et al, 2019[19]	6 months	PD, CAL, IBD, mSBI, and PI	Periodontal probe, conventional periapical Rx, and CBCT	An increased knowledge of host immune inflammatory mediators in CP has increased the number of studies in host modulatory agents used as an adjunct therapy to periodontal therapy. The study concludes that locally administered 1.2% RSV has bone anabolic and anti-inflammatory effect and can emerge as a host modulatory agent. Further, RCTs are required to evaluate the antimicrobial and clinical efficacy of RSV at different concentrations to support the findings of the present study.
Kanoriya et al, 2019[34]	3, 6, 9 months	PD, CAL, IBD, and DDR (%), mSBI and PI	Periodontal probe, periapical Rx with a parallel-angle technique	It can be concluded that 1.2% RSV gel delivered locally into periodontal pockets of smokers with CP patient results in significantly improved clinical as well as radiographic parameters when compared to placebo as an adjunct to mechanical therapy. Hence, it may be a new approach to achieve periodontal regeneration in smokers who are otherwise more prone to periodontal destruction.
Shirke et al, 2019[18]	3, 6 months	PD, CAL, CEJ–BD, CEJ–AC, AC-BD, MD bone defect width, BL bone defect width, mSBI, and PI	Periodontal probe, CBCT	Within the limits of the study, the use of 1.2% ATV gel as an adjunct to SRP is more beneficial in achieving better results in terms of periodontal regeneration. Being a non-invasive procedure, it helps to treat periodontal intraosseous defects, leading to a functionally more stable masticatory apparatus.

PD: probing depth; CAL: clinical attachment level; IBD: intrabony defect; mSBI: modified sulcular bleeding index; PI: plaque index; SMV: simvastatin; RVAL: relative vertical attachment level; RHAL: relative horizontal attachment level; SRP: scaling and root planing; ATV: atorvastatin; CP: chronic periodontitis; GI: gingival index; BI: bleeding index; RSV: rosuvastatin; RAL: relative attachment level; t2DM: type 2 diabetes mellitus; DDR: defect depth reduction; RCT: randomized clinical trials; ALN: alendronate; BDD: bone defect depth; MF: metformin; CBCT: cone beam computed tomography; FMPS: full mouth plaque score; CEJ–BD: cementoenamel junction – base of defect; CEJ–AC: cementoenamel junction – alveolar crest; AC-BD alveolar crest – base of defect; MD: mesiodistal; BL: buccolingual

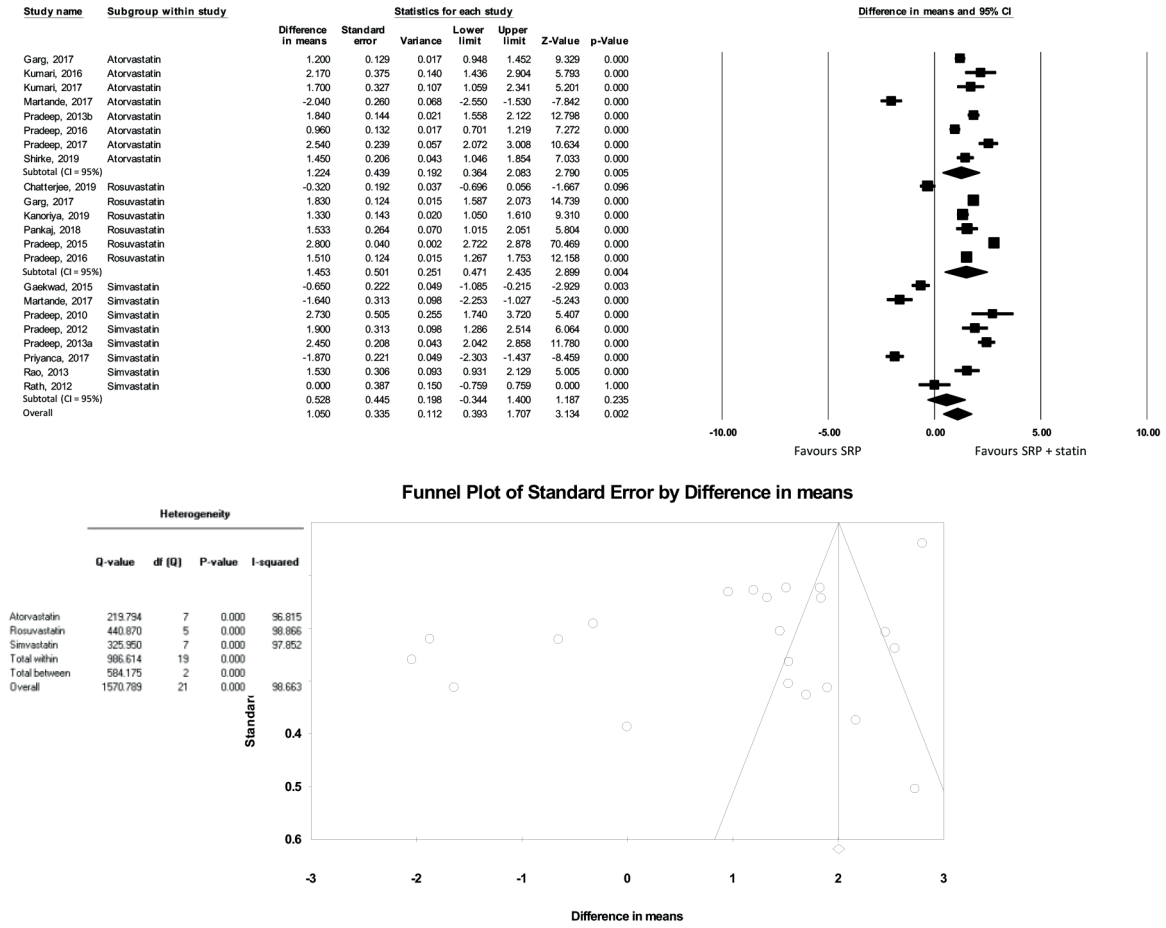

Figure 3. Forest plot, heterogeneity test and Funnel plot for RCTs assessing 6-month CAL gain using statins + SRP compared to SRP alone. Overall effect for all statins and sub-group analyses for each molecule is presented.

Meta-Analysis

Nineteen studies were included in the quantitative analysis of PD and CAL (including CAL, RAL and RVAL)[17-35], 17 in the meta-analysis of mSBI (excluding studies reporting GI or bleeding index (BI) instead of mSBI)[19-35] and 15 in the meta-analysis of IBD change[19-21,23,24,26-35].

One study was excluded due to the high risk of bias.

Three of the included studies[29-31] evaluated two different statins with the same placebo group. Therefore, 22 data set were available for PD and CAL meta-analysis, 20 for mSBI meta-analysis, and 18 for IBD meta-analysis. Only data at the 6-month follow-up were considered.

The overall effect size for all the subgingivally delivered statins in adjunct to SRP, irrespectively of the molecule used, was calculated. Furthermore, for each statin (atorvastatin, simvastatin, and rosuvastatin), a subgroup analysis was carried out. Considering the high data heterogeneity found, a random effect model was preferred in all cases for data meta-analysis.

Regarding the CAL gain (Figure 3), 16 data sets from 14 studies[25,28-30,32-41] showed a statistically significant effect size in favor of statins, 4 from 3 studies[24,27,31] in favor of the control group, and 2 studies showed no statistically significant inter-group difference[26,42]. The overall effect size in favor of statins was statistically significant ($p = 0.002$) with a difference in means of 1.05 mm (95% CI 0.393; 1.707). The subgroup analyses revealed a highly significant effect for atorvastatin ($p = 0.005$) and rosuvastatin ($p = 0.004$). Conversely, it was not significant for simvastatin ($p = 0.235$). Data heterogeneity was significant for both the overall and the sub-group analysis. Publication bias was graphically shown by Funnel plot (Figure 3).

All studies reporting PDred data at the 6-month follow-up showed a statistically significant

effect estimate (Figure 4). Seventeen data sets from 15 studies[25,28-30,32-42] were in favor of statins and 5 data set from 4 studies[24,26,27,31] in favor of the control group. There was a significant overall effect size in favor of statins (p = 0.000), with a difference in means of 1.063 mm (95% CI 0.513; 1.613). Subgroup analyses revealed a significant effect estimate in favor of the test group for rosuvastatin (p = 0.007), and atorvastatin (p = 0.029), whereas it was not significant for simvastatin (p = 0.075). Data heterogeneity was significant for both the overall and the sub-group analyses. Publication bias was graphically shown by Funnel plot (Figure 4).

Regarding mSBI reduction, 8 data sets from 7 studies showed an effect size in favor of statins[27-29,32,34,38,42], 10 from 8 studies favored the control group[30,31,33,35-37,39,40], whereas 2 studies revealed no significant effect[26,41]. The overall effect size showed a non-significant trend in favor of statins (p =.433), with a difference in means of 0.213 mm (95% CI -0.319; 0.744). The subgroup analysis showed no significant effects for any statin type. Data heterogeneity was significant for both the overall and the sub-group analyses. Publication bias was graphically shown by Funnel plot (Figure 5).

Regarding IBDred, 12 data sets (11 studies) showed an effect size in favor of statins[27,28,30,32,33,35,36,38-40,42], 5 (3 studies)[26,29,31] were in favor of the control group and 1 showed no between-group difference[41]. The overall effect size favored statins, although not significantly (p = 0.205) with a difference in means of 0.347 (95% -0.190; 0.884). The subgroup analysis showed no significant effects in favor of the test group (atorvastatin, p = 0.703; rosuvastatin, p = 0.520 and simvastatin, p = 0.211). Data heterogeneity was

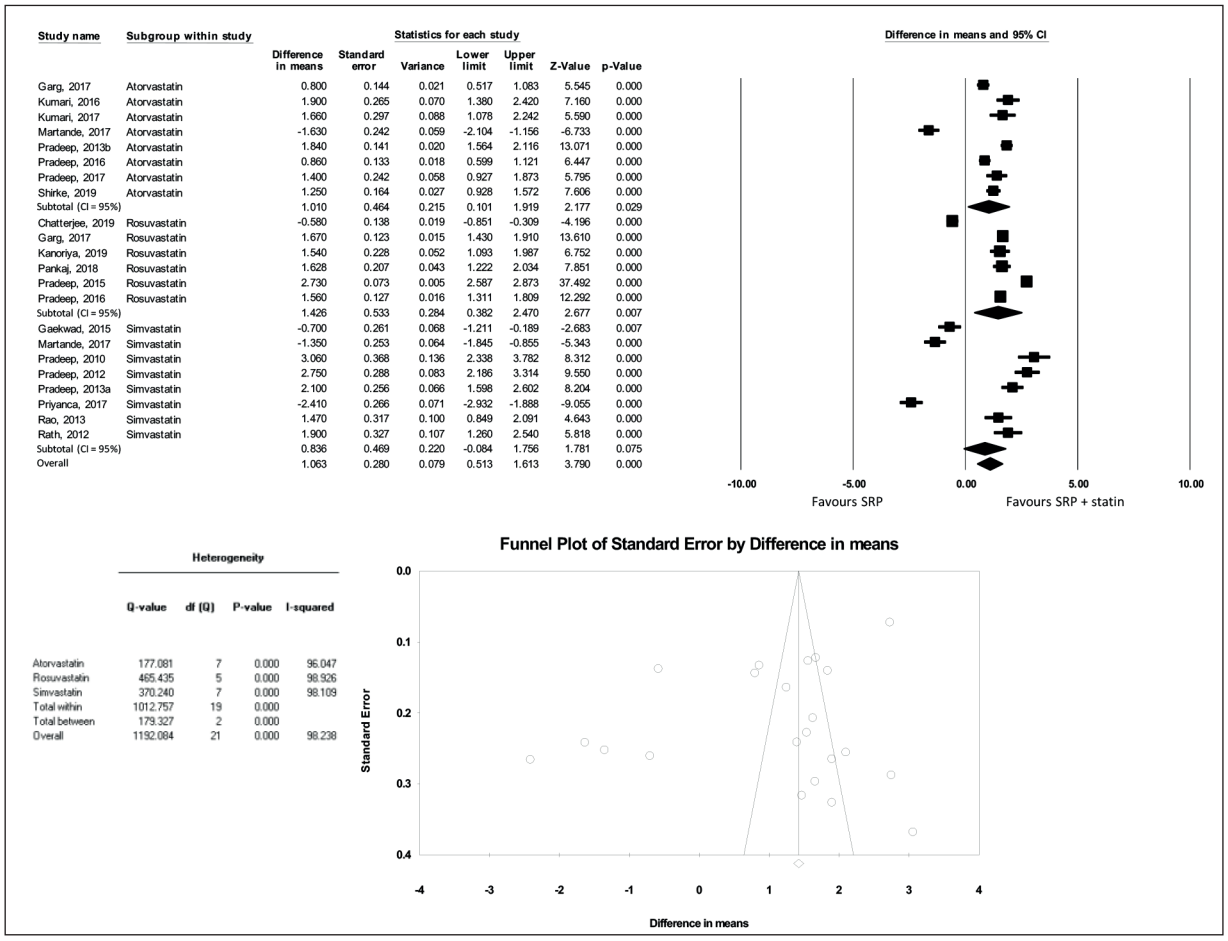

Figure 4. Forest plot, heterogeneity test and Funnel plot for RCTs assessing 6-month PD reduction using statins + SRP compared to SRP alone. Overall effect for all statins and sub-group analyses for each molecule is presented.

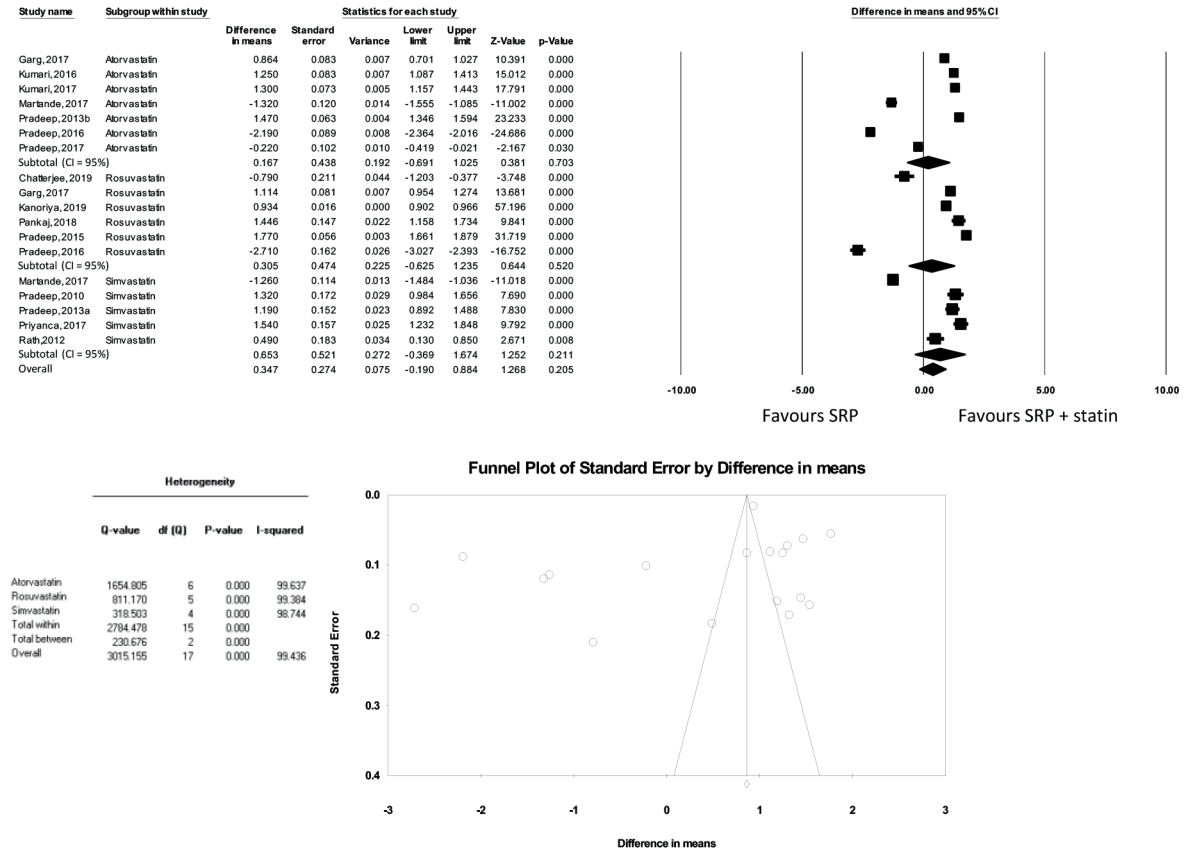

Figure 6. Forest plot, heterogeneity test and Funnel plot for RCTs assessing 6-month IBD reduction using statins + SRP compared to SRP alone. Overall effect for all statins and sub-group analyses for each molecule is presented.

significant for both the overall and the sub-group analyses. Publication bias was graphically shown by Funnel plot (Figure 6).

Trial Sequential Analysis

Both for CAL gain and PD reduction, TSA showed that the cumulative Z-curve maintains below the significance threshold in favor of statins (except for the study of Rath et al[42], that crossed the boundary in the CAL gain graph). In addition, the total sample size is above the required information size (n=930 for CAL gain and n=670 for PD reduction) (Figure 7A and B). This confirmed that the meta-analysis had sufficient power to detect the beneficial effect of statins over the control treatment.

Discussion

The present systematic review has assessed the efficacy of the use of locally delivered statins in combination with SRP compared with SRP alone, or in combination with placebo, in the treatment of periodontitis, based on the evaluation of existing RCTs.

Studies assessing the combination of statins with other biomaterials or with statins applied systemically were excluded in the present review. In one of these works[17], the authors concluded that the systemic administration of atorvastatin may be effective in the management of periodontal disease due to the beneficial effect of statins on alveolar bone metabolism. In another similar study[44] the authors found a significant decrease of interleukin (IL)-6 in the gingival crevicular fluid in the atorvastatin group. However, orally administered statins have a very low bioavailability (5 to 30% of the dose taken) due to their rapid absorption in the liver and more side effects (e.g., myopathy, rhabdomyolysis) compared to topical use. Conversely, local administration allows higher drug concentration in the target site (the periodontal pocket), better patient compliance, and fewer systemic side effects.

Beyond the conventional systemic use of statins in the treatment of hypercholesterolemia through the inhibition of the HMG-CoA reductase, the topical use of statins in periodontal treatment exhibits multiple effects. These include modulation of inflammatory-immune crosstalk, antibacterial activity, reduction of tissue destruction, and improvement of periodontal healing. In this review, three different statin types were tested: simvastatin, atorvastatin, and rosuvastatin. All were prepared in the form of gels and locally injected with a blunt cannula subgingivally in the periodontal defects. Statins were generally well tolerated without any complications, adverse reactions/side-effects, or allergic symptoms.

Statins have pleiotropic pharmacological effects besides their hypolipidemic effects, including anti-inflammatory and antioxidant properties, improvements in endothelial function, angiogenesis stimulation, and positive regulation of bone formation pathways[11,45,46]. Recent evidence indicates that statins may also reduce periodontal inflammation by increasing IL-10 levels and decreasing interleukin IL-1β in crevicular fluid of patients with periodontitis[47].

Simvastatin at 1.2% seems to have an anti-inflammatory effect when locally delivered by reducing the production of IL-6 and promoting alveolar bone metabolism by stimulating VEGF expression in bone tissue[26,27,38,48-50].

Atorvastatin at 1.2% is thought to have stronger antioxidant and anti-inflammatory potential as compared to simvastatin. Furthermore, atorvastatin therapy has been found to decrease tumor necrosis factor (TNF)-α production in lipopolysaccharides-activated monocytes and matrix metalloproteinases (MMP) in human fibroblasts[39,40].

Rosuvastatin at 1.2% is a synthetic sulphur containing hydrophilic statin. Rosuvastatin, unlike other lipophilic drugs, is actively transpor-

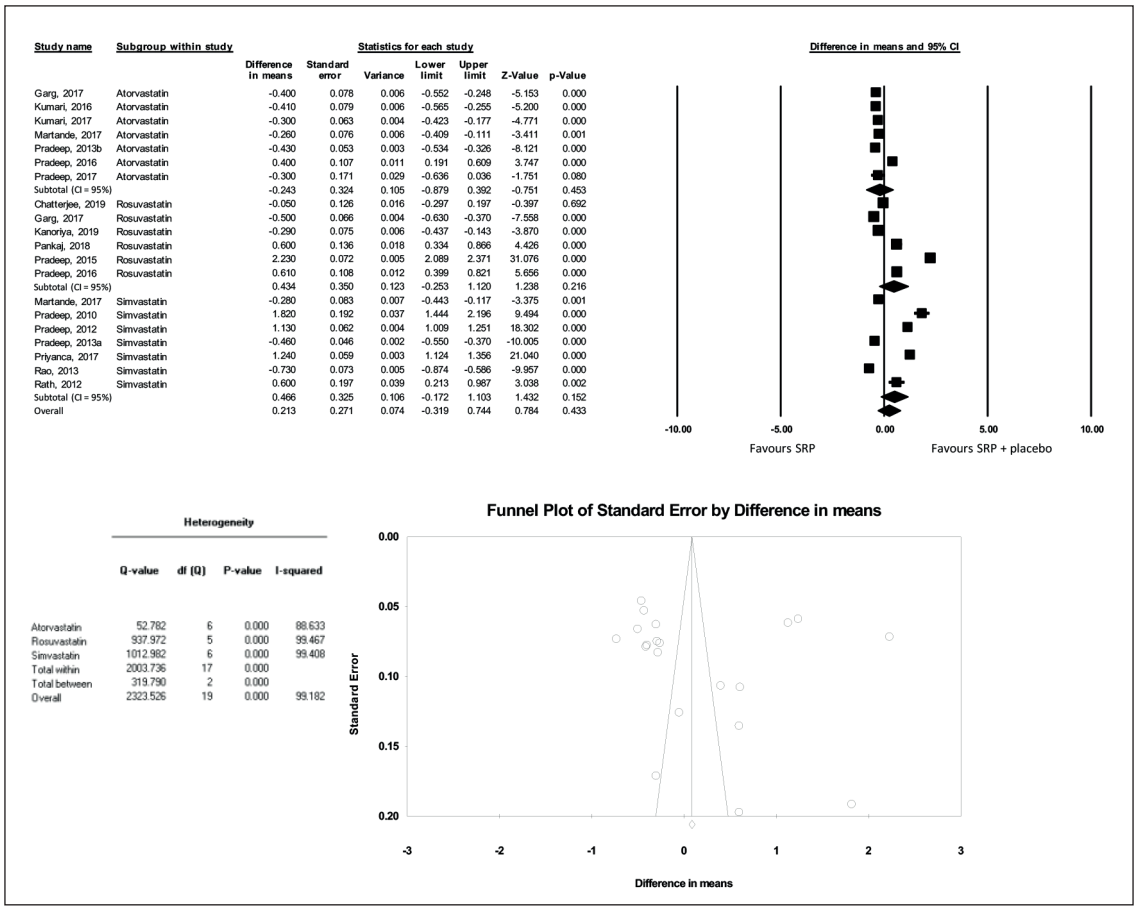

Figure 5. Forest plot, heterogeneity test and Funnel plot for RCTs assessing 6-month mSBI reduction using statins + SRP compared to SRP alone. Overall effect for all statins and sub-group analyses for each molecule is presented.

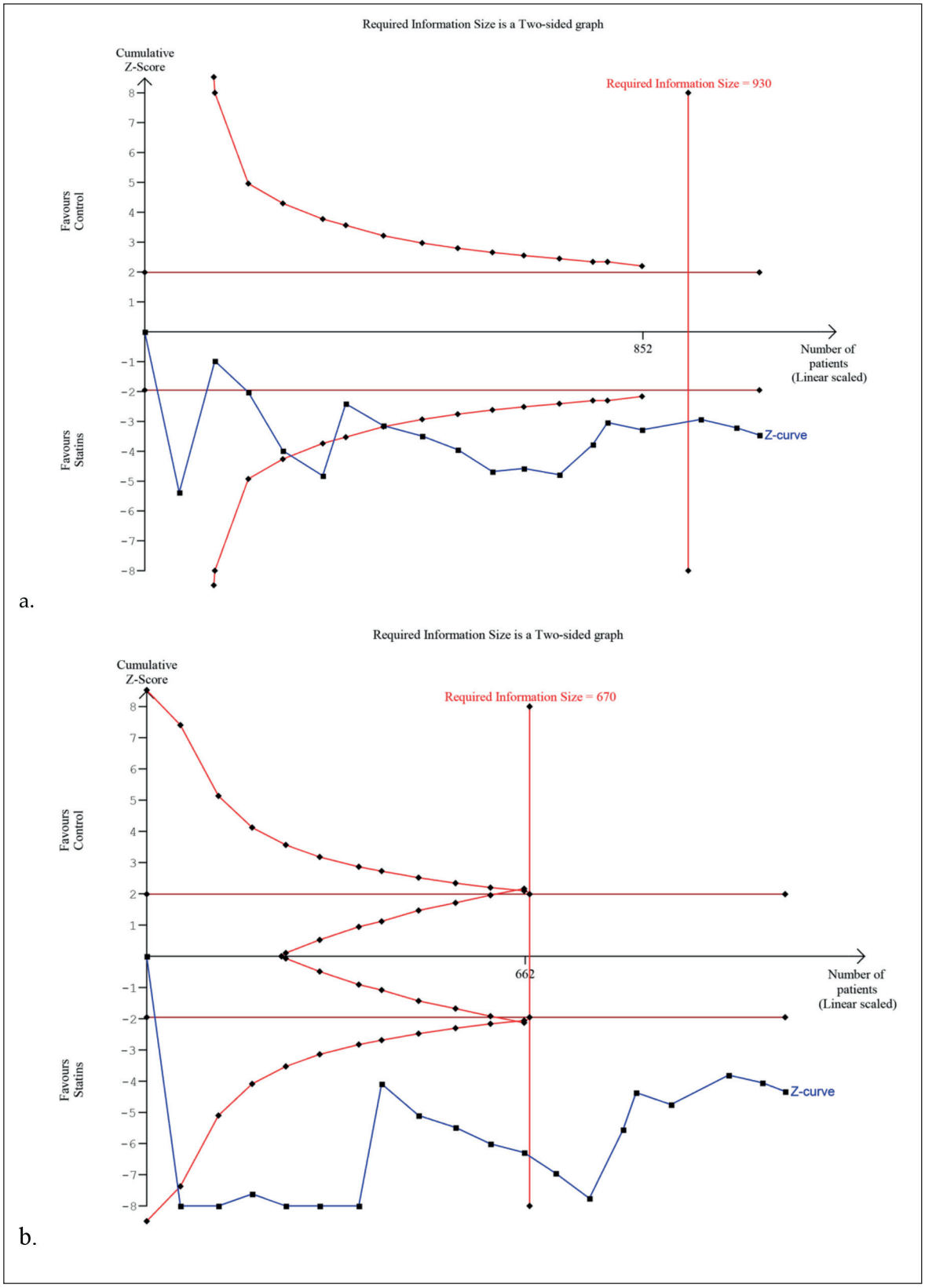

Figure 7. Trial sequential analysis for RCTs assessing 6-month CAL gain (a) and PD reduction (b) using statins + SRP compared to SRP alone. The cumulative z- curve crosses both alpha-spending boundaries and required information size threshold, revealing a high power for current evidence.

ted in osteoblastic cells and induces BMP 2 gene expression, secretion and increases alkaline phosphatase activity, thereby promoting osteoblastic differentiation[51,52]. It has potent anti-inflammatory action, shown by reduced levels of high-sensitivity C-reactive protein (a clinical marker of inflammation produced in response to pro-inflammatory cytokines such as IL-6). Furthermore, rosuvastatin has been shown to normalize reactive oxygen species production and antiplatelet aggregation[53].

The overall quality of the RCTs included in the present systematic review was low. 15% of the studies showed a high risk of bias in at least one domain, and all studies showed some concerns in at least one domain. Furthermore, the remarkable heterogeneity found in the selection criteria and study design (patient characteristics, tooth type and location, follow-up, type of statin used, and outcomes assessed), might have played a role in the various outcomes reported, so that caution in data interpretation is mandatory.

Among the analyzed studies, a balanced gender distribution was reported by most of the studies, two of them included only males[33,37], and one only females[24]. All the studies included patients with chronic periodontitis, but one[27], that treated patients with aggressive periodontitis. Two studies also included furcation defects[30,34]. CAL gain data from this study were included in the present meta-analysis considering only the vertical CAL gain of the treated furcations. Most of the studies also assessed IBD reduction, although it is not usually considered an evaluation outcome of non-surgical periodontal therapy. The evaluation period varied between 3 and 12 months among the studies. The 6-month follow-up was chosen for meta-analysis because it was reported in all the studies and is generally sufficient to properly evaluate clinical outcomes of non-surgical periodontal therapy. Statins were topically applied only once per site in all the studies, except one study[30] in which the statin was re-delivered to the same sites after 6 months.

Regarding patient systemic status and habits, some of the studies exclusively included smokers[33,37,39], postmenopausal women[24], or patients with type 2 diabetes[35,40]. Conversely, the majority of them (14 studies) treated systemically healthy, non-smoking periodontal patients. Data on all these categories of patients were pooled together on the basis of the comparable results obtained when separate analyses were performed.

The overall quantitative analysis indicates that the combination of SRP and statins may result in a significant additional clinical improvement in terms of CAL gain and PD reduction, compared with SRP alone or in combination with a placebo.

To assess the power of evidence of included studies, TSA was performed and RIS calculated. TSA, indeed, is a cumulative random-effects meta-analysis method that estimates a 'required information size' (i.e., required meta-analysis sample size), using the same framework as sample size calculation for an individual RCT, but additionally accounting for heterogeneity and multiple comparisons when new RCTs are added[54]. According to the results of the TSA, the performed meta-analysis showed high power to detect adjunctive effects of statins over the control treatment.

The subgroup analyses performed on the three different statins revealed significant effects in terms of PDred and CAL gain in favor of rosuvastatin and atorvastatin, whereas only a non-significant trend in favor of the simvastatin could be found for both the clinical outcomes. Thus, locally released rosuvastatin and atorvastatin have demonstrated the most evident clinical effects among those tested as adjuncts to SRP. However, the low number of studies available for each statin and the high heterogeneity detected among them contribute to limit the value of the subgroup analyses.

Some limitations affecting the primary studies included in the present review can be pointed out. For example, only one concentration of statins (1.2%) and one vehicle (methylcellulose) have been tested in the included studies. In addition, very few direct comparisons between different statins have been made, and no standardized formulations are still available for clinical use. Finally, most of the available studies were performed by the same research group, whereas the inclusion of similar RCTs from different study centers would be desirable. According to these limitations, the European Federation of Periodontology, in its S3 level clinical practice guidelines for the treatment of stage I-III periodontitis[55], provided a grade A recommendation to do not use local statins as adjuncts to subgingival instrumentation.

Other review articles[12,49,56-59] have focused on the use of statins as an adjunct to SRP in the treatment of periodontitis. The overall conclusion reached by the authors was similar to the present review, with the locally delivered statins conferring additional clinical benefits to non-surgical periodontal therapy. Nevertheless, the number of RCTs included in all previous studies is markedly lower compared to the present one, no sub-

group analysis for the different statins used was attempted, and no TSA analysis was performed. Some differences may be observed in the results of the meta-analysis in terms of significance of the outcomes assessed. This may be due to the different number of studies included and to the different selection criteria applied. Similarly, an overall high-moderate risk of bias in individual studies together with a high heterogeneity among the studies were found.

Conclusions

There is initial evidence that local release of statins in combination with SRP, in particular rosuvastatin and atorvastatin, may result in additional clinical improvements in terms of pocket depth and clinical attachment gain. The use of topical statins as a complement to conventional periodontal treatment might be a promising alternative to other locally delivered adjunctive agents. Such an approach might be particularly useful in areas of difficult access, such as deep periodontal pockets, furcations, and interproximal areas of misaligned teeth, and could also be useful in patients usually less responsive to periodontal treatment. However, the high heterogeneity of data and the high risk of bias found impose caution. Moreover, an approved and standardized formulation is currently not available. Further well-designed RCTs performed by independent research groups would be needed to confirm the beneficial effects of locally delivered statins, and to highlight mutual differences of each type of statin, in the non-surgical treatment of periodontitis.

Author Contributions

Conceptualization, L.G. and M.A.; study selection and data extraction, G.C., A.P., and G.M.; quality assessment, G.C. and A.P.; data analysis, G.C., A.P., M.D.F., and M.A.; writing – original draft preparation, G.C., A.P., and G.M; writing – review and editing, G.C., A.P., G.M, M.D.F., M.A., and L.G; supervision, M.A. and L.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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