Journal of Dentistry 155 (2025) 105613

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Review article

Adjunctive use of hyaluronic acid in non-surgical periodontal therapy: A systematic review and meta-analysis

Deema Dababseh [a,b], Roa Altell [c], Jing Kang [d], Jiangyue Lu [a], Zainab Malaki [e], Petros Mylonas [f], Emily Ming-Chieh Lu [a,*]

[a] Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral and Craniofacial Sciences, King's College London, London SE1 9RT, UK
[b] University of Jordan, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Oral Medicine and Periodontology, Amman, Jordan
[c] Columbia University, College of Dental medicine, New York NY 10032, United States
[d] Centre for Clinical Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, Kings College London, London SE1 9RT, UK
[e] Department of Periodontology, Guy's and St Thomas' NHS Foundation Trust, London, SE1, UK
[f] Cardiff University, School of Dentistry, University Dental Hospital Wales, Cardiff, UK

ARTICLE INFO

Keywords:
Hyaluronic acid
Non-surgical periodontal therapy
Periodontitis
Probing depth
Clinical attachment level
Bleeding on probing
Systematic review
Meta-analysis

ABSTRACT

Introduction and objectives: Clinical studies have shown that adjunctive use of hyaluronic acid (HA) as part of non-surgical periodontal treatment (NSPT) has led to favourable clinical outcomes. However, no systematic review and meta-analysis has been carried out recently and technical and patient factors have not been previously explored. Therefore, the aim of this systematic review and meta-analysis is to evaluate the clinical effects of topical- HA as an adjunct to NSPT on probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BoP) in periodontitis patients.

Study selection and sources: Systematic literature searches using the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) approach identified clinical studies involving randomized controlled trials (RCTs) and split-mouth designs involving adult periodontitis. The searches were performed across six databases (PubMed, Embase, Medline, Cochrane, Web of Science, Scopus, and Google Scholar).

Data: Of the 1479 articles identified from the initial searches, a total of 23 were included in this systematic review, and 12 studies were included in the meta-analysis and sub-group analyses. Based on the included studies, HA adjunctive therapy showed improvements in PD reduction (WMD of -0.46, 95 % Confidence Interval CI:0.89 to -0.04, $P = 0.04$), CAL gain (WMD of -0.35, 95 % CI:0.61 to -0.09, $P = 0.01$), and BoP reduction (WMD of -0.38, 95 % CI:0.78 to 0.01, $P = 0.06$). However, due to the heterogeneity of the included studies, further evidence were needed to support the improvement of HA adjunctive therapy outcomes due to wider prediction intervals (PD reduction 95 % prediction interval, PI:1.95 to 1.03; CAL enhancement 95 % PI -1.11 to 0.42; BoP reduction 95 % PI -1.35 to 0.59)Higher HA concentrations (0.8 %)showed more pronounced PD reduction. The overall quality of the included studies were moderate using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) assessment tool.

Conclusion: Although topical HA application may provide additional benefits when used with NSPT, the limited number of studies, risk of bias, heterogeneity and moderate quality of evidence indicate that further research is warranted to confirm these findings and establish more definitive clinical guidelines.

Clinical Significance: HA showed promise as an adjunctive treatment in enhancing the clinical outcomes following NSPT.

1. Introduction

Hyaluronan is a natural high-molecular weight glycosaminoglycan (GAG) found in various bodily fluids, including synovial fluid, serum, saliva, and gingival crevicular fluid (GCF) [1–3]. The term "hyaluronan" encompasses both its acid form; hyaluronic acid (HA) which is a non-sulfated GAG, as well as its salt forms such as sodium or potassium hyaluronate. As a primary constituent of the extracellular matrix in

* Corresponding author.
E-mail address: Emily.lu@kcl.ac.uk (E.M.-C. Lu).

https://doi.org/10.1016/j.jdent.2025.105613
Received 28 September 2024; Received in revised form 30 January 2025; Accepted 2 February 2025
Available online 8 February 2025

mineralized and non-mineralized tissues, notably within the periodontium, hyaluronan is synthesized by hyaluronan synthase enzymes [4], expressed in various cells including fibroblasts, keratinocytes, chondrocytes, and osteoblasts [5,6].

HA exhibits anti-inflammatory [7,8], bacteriostatic [9], anti-edematous [10], osteoinductive [11], and pro-angiogenetic properties [12], making HA an optimal biologic for promoting wound healing [13]. A prominent characteristic of HA is its viscoelasticity and hygroscopicity [14], thus it is integral in maintenance of tissue resilience, hydrodynamics, and volume preservation [15]. Consequently, HA is widely used therapeutically in dermatology to restore lost skin volume and reduce wrinkles [16], as well as in osteoarthritis to ease pain, enhance lubrication, stimulate chondrocyte synthesis, protect cartilage, promote regeneration, and reduce inflammation [17,18].

Clinically, the topical use of HA has been shown to promote periodontal wound healing [19,6]. Adjunctive use of HA with nonsurgical periodontal therapy (NSPT) in periodontitis patients has resulted in favourable clinical outcomes including reduction in plaque index (PI) and bleeding index (BI) in gingivitis patients [20]; and improvements in clinical outcomes such as bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), as well as improvement in the bacterial profile compared to NSPT alone [21–27]. However, other studies failed to find statistically significant differences when HA was used subgingivally in addition to NSPT in periodontitis patients [28,29].

A 2019 meta-analysis suggested additional clinical benefits following the use of adjunctive HA in non-surgical and surgical periodontal treatment [30]. The authors proposed that exogenous HA reduces production of prostaglandins, metalloproteinases, and other inflammatory cytokines molecules, all of which contributes to its anti-inflammatory properties [30]. Another systematic review explored the use of HA in NSPT and surgical periodontal therapy; they concluded that the adjunctive use of HA can reduce the need for prescribing nonsteroidal anti-inflammatory drugs and improve clinical outcomes, although their work did not include a meta-analysis [31]. Hence, the current meta-analysis will include new clinical studies and explore subgroup analyses such as frequency of application, HA concentration, and patient factors, which have not been previously investigated.

Periodontitis treatment aligns with established guidelines from the European Federation of Periodontology (EFP), which recommends evidence-based interventions to control disease progression [32]. However, while the EFP guidelines highlight various adjunctive therapies, they do not explicitly address the use of HA in NSPT. This gap underscores the need for further investigation, as our systematic review and meta-analysis aim to evaluate the evidence to date, regarding the clinical outcomes following adjunctive HA application in NSPT

2. Materials and methods

2.1. Protocol registration

The protocol for the systematic review was developed according to Preferred Reporting Items for Systematic Review and Meta Analysis, PRISMA Statement 15. The investigative group included a chief investigator (EL) and two reviewers (DD, RA). The review was registered in PROSPERO, an international prospective register of systematic reviews (CRD42024508182), that addressed the following focused question: What is the effect of HA application on clinical parameters in conjunction with NSPT?

PICO:

Population: Adult with periodontitis (excluding as a manifestation of systemic or necrotizing disease)

Intervention: Hyaluronic acid applied as adjunct to non-surgical therapy.

Comparison: non-surgical therapy alone

Outcomes: probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP).

2.2. Search method

The identification of studies using HA as an adjunct to NSPT involved an extensive electronic search across PubMed, Embase, Medline, Cochrane, Web of Science, Scopus, and Google Scholar, from the earliest date to March 2024. The search used MeSH terms and free text, including "periodontics," "periodontal disease," "periodontitis," "periodontal," "operative," and "periodontal therapy," paired with "hyaluronic acid," "hyaluronan," or "hyaluronate." Manual searches of references in eligible articles were also conducted. The detailed search strategy is available in Appendix 1.

Two authors, DD and RA, independently selected and evaluated articles throughout the entire review process to ensure a thorough and unbiased assessment. In addition to this, DD and RA independently extracted the relevant data from these articles. This extracted data was subsequently verified against the full manuscript by EL to ensure accuracy and consistency. Any discrepancies or disagreements between the authors regarding the selection, evaluation, or data extraction were addressed and resolved by the chief investigator, EL. Articles with full texts available were included. For articles with restricted access, requests were submitted to the Interlending and Document Delivery service at King's College London library was contacted to attempt retrieval of the full text.

2.3. Eligibility criteria

Inclusion Criteria

1. Hyaluronic acid applied to NSPT
2. Human studies
3. Study design: randomized controlled trials (RCT): Parallel-arm and split-mouth clinical studies
4. Full-text available
5. English language
6. No year restriction
7. Studies reporting on PD, CAL and BOP as outcomes

Exclusion Criteria:

No HA formulations combined with biomaterials or other agents, including chlorhexidine, polynucleotides, sodium hypochlorite gel and others.

Type of Outcome measurement

What is the effect of HA when used as an adjunctive to NSPT on the following:

- Primary outcome measure: PD
- Secondary outcome measures: CAL and BoP

2.4. Risk of bias in individual studies

The quality of the enrolled studies was evaluated using the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2). The ROB 2 tool is used to evaluate the risk of bias in individual trial results that compare two interventions or strategies [33].

Three authors (JL, ZM, PM) independently assessed ROB based on the five mandatory domains that address potential sources of bias: (1) bias from the randomization process, (2) bias due to deviations from intended interventions, (3) bias from missing outcome data, (4) bias in outcome measurement, and (5) bias in selection of reported results [34]. A traffic light system for visualising the risk of bias was created (Fig. 2).

2.5. Data analysis

The study design, sample size, mean age of participants, type of intervention provided, and follow-up period were documented, along with measurements of PD, BoP, and CAL if these metrics were included

at baseline and follow-up visits. The meta-analysis assessed changes in PD, CAL and BoP following NSPT for periodontal pockets from baseline and at 3 months. The effect sizes were calculated as the weighted mean difference (WMD) with 95 % confidence intervals (CIs) and 95 % prediction interval (PI) for each clinical parameter. The effect size was depicted in forest plots using a random-effects model to quantify the extent of PD, CAL, and BoP reduction. Subgroup analysis was conducted for each clinical parameter, with subgroups based on the type of study (parallel-arms vs. split-mouth), concentration used (0.2 %, 0.8 %, and other formulations), interval of application (baseline only vs. multiple applications), and whether smokers were included. Additionally, a funnel plot was utilized to explore potential small-study effects and to assess whether the studies included in the meta-analysis fell within acceptable pseudo-confidence intervals for heterogeneity. The Hartung-Knapp-Sidik-Jonkman method was applied to adjust the standard error. . The relative statistical heterogeneity was quantified using the τ^2 (Tau-squared) and I^2 (I-squared) accompanied by a 95 % CI, which was calculated using R with the 'metafor' package. Inverse Variance meta-analysis and generation of forest plots and funnel plots was performed with STATA/MP 18.0.. 2.0. Statistical significance was defined at $p < 0.05$

2.6. Certainty of evidence

The GRADE approach was adopted to assess the level of certainty for each outcome measure, which were rated as high, moderate, low or very low using the GRADEpro Guideline Development Tool (GDT) software (gradepro.org, McMaster University 2021). The GRADE assessments examined 5 domains: risk of bias, inconsistency, indirectness imprecision, and publication bias.

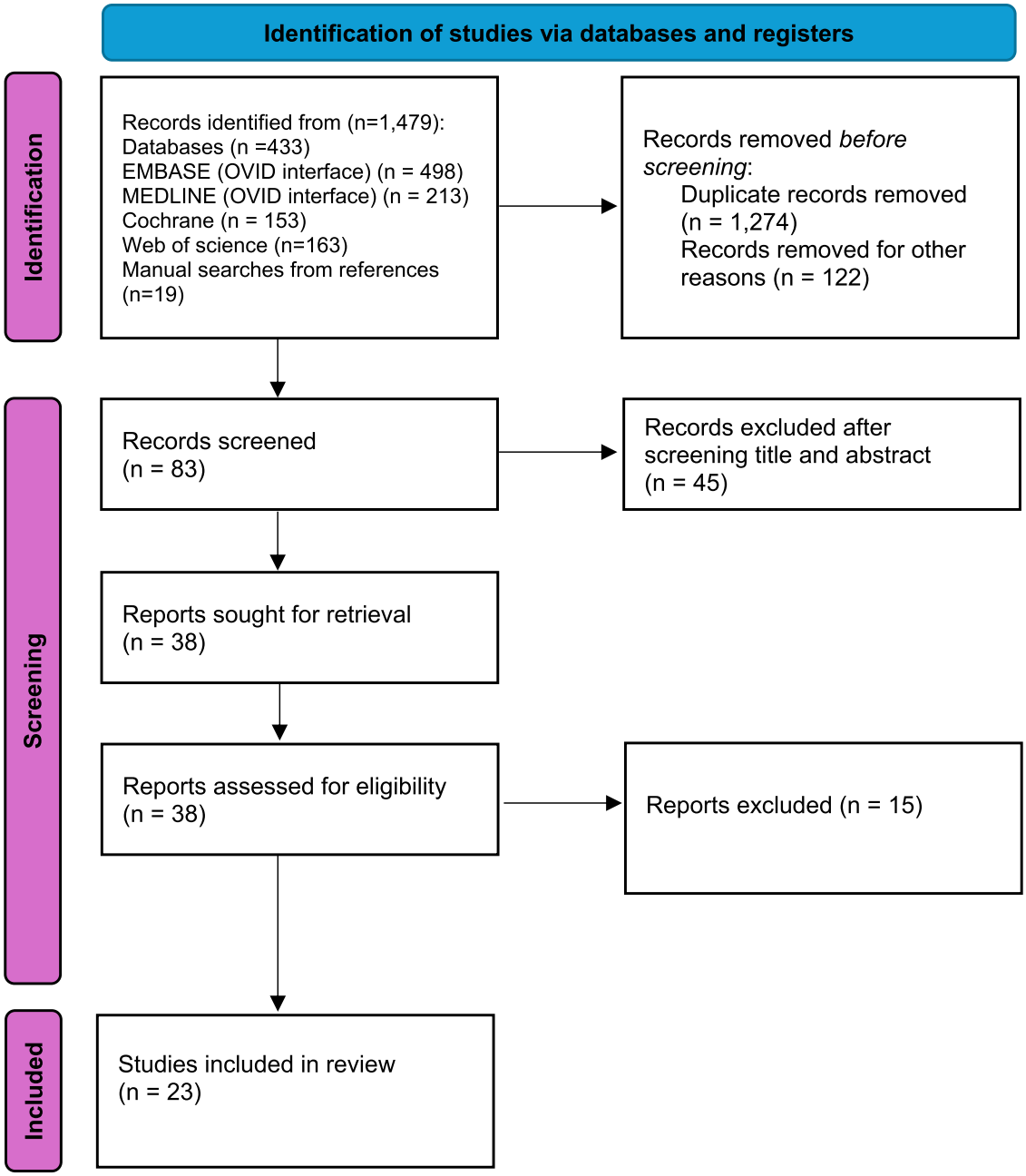

Fig. 1. PRISMA 2020 flow diagram describing the searches and selected studies.

3. Results

3.1. Study selection and characteristics

A comprehensive search across six databases yielded a total of 1479 studies. After removing duplicates, a preliminary screening based on the titles of the studies was conducted, resulting in 83 studies selected for further assessment. Following the review of the title and abstracts, further 45 studies were excluded. Subsequently, 38 full-text publications underwent a thorough eligibility assessment, after which, further 15 studies and the surgical part of one study (Engstrom 2001) were excluded **(Supplementary File, Table s1)**, ultimately leading to the inclusion of 23 studies in this review (Fig. 1).

Among the 23 included studies, 15 were split-mouth clinical trials examining the effects of HA in NSPT in patients diagnosed with periodontitis, while seven were parallel-arm randomized clinical trials. Out of the 15 split-mouth studies, 14 employed randomized methodologies. However, Kandil (2017), did not clearly specify the randomization method [35]. (Table 1).

The concentration of HA varied across studies, with 9 using 0.2 % HA, 8 using 0.8 % HA, and 1 study (Eick et al. 2013), employed a dual-application of both concentrations, where the clinician administered 0.8 % HA, and the patient applied 0.2 % HA at home. The remaining 5 studies used other concentrations and formulations, including sodium hyaluronate at 2.0 mg/mL and 16.0 mg/mL [36], a mix of 16 mg/mL cross-linked and 2 mg/mL non-cross-linked HA [37], 14 mg of sodium hyaluronate, 8.5 mg of sodium chloride, 0.28 mg of disodium hydrogen phosphate dihydrate, and 0.04 mg of sodium dihydrogen phosphate [38], benzylic ester of HA [24], and 0.3 % non-crosslinked [39].

In addition, the frequency of HA application varied considerably. Seven studies applied HA exclusively at baseline, immediately following instrumentation. In contrast, 16 studies implemented multiple HA application intervals. Moreover, the inclusion of smokers in the studies was relatively limited, with only four studies including smokers in their analyses. Notably, [22] conducted separate analyses for smokers and non-smokers, providing insights into the differential effects of HA in these subpopulations.

3.2. Meta-Analysis and heterogeneity testing

Following preliminary analysis and heterogeneity testing, Polepalle (2014) lied outside the 95 % pseudo-confidence interval of the Funnel plot. The inclusion of Polepalle (2014) resulted in extremely high heterogeneity (I^2 = 97.3 %, CI 94.65 % to 98.96 %; τ^2 = 1.88, CI 0.94 to 5.06) for PD, suggesting significant variability in effect sizes. Excluding it reduced the heterogeneity substantially (I^2 = 89 %, CI 76.70 % to 96.04 %, τ^2 = 0.41, CI 0.17 to 1.23), indicating that Polepalle (2014) differed markedly from other studies. This study was excluded from the primary meta-analysis due to methodological differences, which may affect the generalizability and comparability of results **(Supplemental file, Fig s 2.1 and Fig s2.2).** Similarly, for CAL, Polepalle (2014) in the analysis resulted in extremely high heterogeneity (I^2 = 93.8 %, CI 87.43 % to 97.88 %, τ^2 = 0.82, CI 0.3703 to 2.46), suggesting significant variability in effect sizes. Excluding it reduced the heterogeneity substantially (I^2 = 64.4 %, CI 21.3785 % to 87.8862 %, τ^2 = 0.09, CI 0.0143 to 0.3811), indicating that Polepalle (2014) is an outlier. Even after excluding Polepalle (2014), considerable heterogeneity remained (I^2 = 62 %) **(Supplementary File, Fig s3.1 and Fig s3.2).**

Therefore, 12 studies were included in the meta-analysis. Forest plots for and PD, CAL and BoP were conducted, with subgroup analyses on (1) type of study (parallel- arm vs split-mouth RCT), (2) interval of HA application (at baseline only vs multiple applications of HA, (3) smoking, and (4) concentration (0.2 % vs 0.8 %). There was significant variability in the bleeding indices used. For instance, [23,28,40,41] used gingival index, while [22,29,25,21,42,39] used percentage of sites with BoP. Therefore, only the studies using BoP were included in the meta-analysis. Vajawat (2022) presented separate results for smokers and non-smokers, therefore, in the meta-analysis they were analyzed separately [22]. Some studies conducted their analyses at the patient level, while others focused on the tooth level. This meta-analysis incorporates the level of analysis used in each individual study. However, patient-level analyses were also performed separately, and the findings were consistent with the overall results.

Probing Depth (PD)

Based on the included studies, HA adjunctive therapy showed improvements in PD reduction (WMD of −0.46, 95 % Confidence Interval CI: −0.89 to −0.04, P = 0.04) (Fig. 2.1). However, due to the high heterogeneity of the included studies (I^2 = 89 %, CI 76.70 % to 96.04 %, τ^2 = 0.42, CI 0.17 to 1.23), the wide prediction interval (95 % prediction interval, PI: −1.95 to 1.03) indicate that further evidence is needed to support the significance of effect size.

Clinical Attachment Level (CAL)

Based on the included studies, HA adjunctive therapy showed improvements in CAL gain (WMD of −0.35, 95 % CI: −0.61 to −0.09, P = 0.01) (Fig. 2.2). However, due to the high heterogeneity of the included studies (I^2 = 65.7 %, CI 21.37 % to 87.88 %, τ^2 = 0.09, CI 0.014 to 0.381), the wide prediction interval (95 % PI: −1.11 to 0.42) indicate that further evidence is needed to support the significance of effect size.

Bleeding on probing (BoP)

Based on the included studies, HA adjunctive therapy showed improvements in BoP reduction (WMD of −0.38, 95 % CI: −0.78 to 0.01, P = 0.06) (Fig. 2.3). However, due to the high heterogeneity of the included studies (I^2 = 72.9 %, CI 41.039 % - 94.120 %, τ^2 = 0.12, CI 0.030 - 0.6898), the wide prediction interval (95 % PI −1.35 to 0.59) indicate that further evidence is needed to support the significance of effect size.

3.2.1. Subgroup analysis

Probing depth (PD)

For PD subgroup analysis by the type of study indicated that parallel-arm RCT subgroup had a statistically non-significant overall effect size of −0.15 (95 % CI:−1.13 to 0.83, P = 0.65), while the split-mouth RCT subgroup had a statistically significant overall effect size of −0.60 (95 % CI: −1.16 to −0.0.04, P = 0.04). Subgroup analysis by concentration indicated that the 0.8 % subgroup had a statistically significant overall effect size of –0.55 (95 % CI: −1.08 to −0.01, P = 0.047), while 0.2 % subgroup had a statistically non-significant overall effect size of –0.46 (95 % CI:−2.07 to 1.15, , P = 0.43). Subgroup analysis by the interval of HA application revealed that multiple applications resulted in a statistically significant negative effect size of −0.64 favoring HA treatment (95 % CI: −1.17 to −0.11, P = 0.02), while single application at baseline had a statistically non-significant overall effect size of 0.02 (95 % CI: −0.85 to 0.90, , P = 0.94). However, due to the high heterogeneity of the included studies, the wide prediction intervals for subgroups pertaining to split mouth RCT (95 % PI: −2.29 to 1.08), 0.8 % HA concentration (95 % PI: −1.96 to 0.87) and multiple applications of HA (95 % PI: −2.27 to 0.98) meant that further evidence is needed to support the significance of the respective effect sizes. Exclusion or inclusion of smokers from the studies did not yield statistically significant differences **(Supplementary File, Fig. s4).**

Clinical attachment level (CAL)

For CAL subgroup analysis by study type showed that split-mouth RCT subgroup had a statistically significant effect size of −0.39(95 % CI:−0.72 to −0.06, P = 0.03), compared to parallel-arm RCT subgroup which had a statistically non-significant overall effect size of - 0.22 (95 % CI: −1.23 to 0.79, P = 0.45). By concentration, Subgroup analysis by concentration indicated that the 0.2 % subgroup had a statistically non-significant effect size of −0.10 (95 % CI: −0.41 to 0.21, P = 0.38) while the 0.8 % subgroup had a statistically significant effect size of −0.47 (95 % CI: −0.92 to −0.02, P = 0.04). Eick (2013) showed the highest statistically significant effect size of −0.75 (95 % CI: −1.45 to −0.06, P = 0.03) with a combination of 0.8 % and 0.2 %. For HA application

Table 1
Characteristics of included studies.

Author (year) place of study	Study design	Sample	Intervention	Follow-up	outcome measured	Findings
Engstrom (2001)	Randomized split-mouth	9 individuals (mean 48 years)	Control: NSPT. Test: NSPT and hyaluronan was administered 3 times with an interval of 1 week in the test pockets. (3X).	2 weeks, 1, 3, 6, and 12 months	PD, BOP, and PI	**PD:** BL, 6 months and 12 months: Control: (6.8 ± 1.5, 4.2 ± 1.4, 3.7 ± 1.5). Test: (6.4 ± 1.3, 3.9 ± 1.2, 3.9 ± 1.4). **BOP:** BOP was found to be ≤25 % for both test and control teeth on mesial, buccal, distal, and lingual sides
Xu (2004)	Randomized split-mouth	20 patients (mean 48.6 years)	Control: NSPT. Test: 0.2 % hyaluronic acid gel/quadrant was administered subgingivally in all selected test sites once a week starting at baseline, and at weeks 1, 2, 3, 4, 5, and 6 (7X)	6 weeks and 12 weeks	BOP, CAL, and PD	**PD:** BL, 6 weeks and 12 weeks: control: (5.2 ± 1.62, 4.3 ± 1.55, 4.2 ± 1.57), test: (5.3 ± 1.61, 4.3 ± 1.48, 4.3 ± 1.46) **BOP:** BL, 6 weeks and 12 weeks: control: (72 %, 27 %, 23 %), test: (78 %, 21 %, 19 %) **CAL:** BL, 6 weeks and 12 weeks: control: (5.4 ± 1.97, 4.6 ± 1.85, 4.5 ± 1.90), test: (5.5 ±.1.79, 4.5 ± 1.70, 4.5 ± 1.68)
Johannsen (2009)	Randomized split-mouth	12 patients (42 to 63 years)	Control: NSPT. Test: 0.8 % HA at BL after 1 week. (2X)	1, 4, and 12 weeks	BOP, CAL, PD, and PI	**PD:** BL and 12 weeks: control: (4.2 (3.6 - 4.7), 3.4 (2.9 −3.8)), test: (4.2 (3.7 - 4.7), 3.2 (2.6 - 3.7)). **BOP:** Significantly lower bleeding on probing scores were observed in the test group compared to control at 12 weeks ($P < 0.05$). **CAL:** BL and 12 weeks, control: (4.5 (4.2 - 4.7), 4.4 (4.1 - 4.6)) test: (4.4 (4.1 - 4.8), 4.4 (4.0 - 4.7))
Pilloni (2011)	Randomized split-mouth	19 patients (mean 41.9 ± 15.1 years)	Control: OHI. Test: NSPT and HA in test and then placed by patient daily for 3 weeks (multiple applications)	1, 2, and 3 weeks	BOP, CAL, PPD, PI, and GI	**PD:** BL, 1 week, 2 weeks, 3 weeks: control: (3.3 ± 0.6, 3.1 ± 0.6, 2.8 ± 0.9, 3.0 ± 0.7), test: (3.3 ± 0.6, 2.8 ± 0.6, 2.4 ± 0.7, 2.5 ± 0.7). **BOP:** BL, 1 week, 2 weeks, 3 weeks: control: (31.1 ± 21.2, 22.9 ± 16.3, 11.6 ± 10.5, 7.1 ± 6.8), test: (39.6 ± 29.6, 20.8 ± 16.7, 5.2 ± 4.1, 2.9 ± 4.3). **CAL:** BL, 1 week, 2 weeks, 3 weeks: control: (2.0 ± 0.5, 2.0 ± 0.5, 2.0 ± 0.5, 2.0 ± 0.4), test: (2.2 ± 0.7, 2.1 ± 0.7, 2.0 ± 0.8, 1.9 ± 0.8)
Gontiya (2012)	Parallel-arm RCT	26 patients (25–55 years)	Control: NSPT. Test: 0.2 % HA gel subgingivally at BL, 1st, 2nd, and 3rd week (4X)	4, 6, and 12 weeks	PD, CAL, and GI	**PD:** BL, 4 weeks, 6 weeks, 12 weeks: control: (6.42 ± 0.44, 5.56 ± 0.4, 5.10 ± 0.33, 4.94 ± 0.26), test: (6.57 ± 0.45, 5.41 ± 0.46, 5.02 ± 0.41, 4.82 ± 0.32). **GI:** BL, 4 weeks, 6 weeks, 12 weeks: control: (2.02 ± 0.07, 1.27 ± 0.29, 1.21 ± 0.26, 1.19 ± 0.24), test: (2.04 ± 0.09, 1.07 ± 0.23, 0.93 ± 0.23, 0.89 ± 0.21). **CAL:** BL, 4 weeks, 6 weeks, 12 weeks: control: (8.56 ± 0.41, 8.02 ± 0.56, 7.75 ± 0.54, 7.64 ± 0.53), test: (8.91 ± 0.41, 7.92 ± 0.44, 7.73 ± 0.58, 7.6 ± 0.59).
Eick (2013)	Parallel-arm RCT	34 participants (mean 54 years)	Control: NSPT. Test group ($n = 17$), 0.8 % HA applied on BL and 1 week later. Then twice daily for 2 weeks 0.2 % HA gel (multiple applications)	3 and 6 months	PD, BOP, CAL, and PI	**PD:** BL, 3 months and 6 months: control: (4.1 ± 0.4, 3.36 ± 0.4, 3.28 ± 0.36), test: (4.2 ± 0.4, 3.02 ± 0.3, 3.13 ± 0.36) **BOP:** BL, 3 months and 6 months: control: (18.8 ± 11.1, 17.81 ± 12.02, 13.62 ± 19.33), test: (16.3 ± 8.7, 16.28 ± 9.42, 8.84 ± 24.73) **CAL:** BL, 3 months and 6 months: control: (5.7 ± 0.6, 4.7 ± 0.62, 4.36 ± 0.57), test: (5.5 ± 0.9, 4.23 ± 0.63, 4.26 ± 0.58)
Rajan (2014)	Randomized split-mouth	33 subjects	Control: NSPT. Test: NSPT and hyaluronon gel (0.2 %) and 1 week post therapy (2X)	4 and 12 weeks	GI, PI, BOP, PPD, and CAL	**PD:** BL, 4 weeks and 12 weeks: control: (6.09 ± 1.26, 4.09 ± 1.38, 4.36 ± 1.29), test: (6.33 ± 0.99, 3.21 ± 0.65, 2.49 ± 0.51). **BOP:** BL, 4 weeks and 12 weeks: control: (1.00 ± 0.00, 0.52 ± 0.51, 0.48 ± 0.51), test: (1.00 ± 0.00, 0.09 ± 0.29, 0.06 ± 0.24). **CAL:** BL, 4 weeks and 12 weeks control: (9.12 ± 1.67, 7.76 ± 1.80, 7.48 ± 1.51), (10.18 ± 2.08, 7.24 ± 1.25, 6.91 ± 1.16).

(*continued on next page*)

Table 1 (*continued*)

Author (year) place of study	Study design	Sample	Intervention	Follow-up	outcome measured	Findings
Polepalle (2014)	Randomized split-mouth	18 patients (mean 45 years)	Control: NSPT. Test: NSPT and 0.8 % hyaluronan gel at BL and 1 week later (2X)	1, 4, and 12 weeks	PD, BOP, CAL, and PI	**PD:** BL and 12 weeks: control: (5.21 ± 0.54, 4.49 ± 0.47), test: (4.99 ± 0.34, 2.45 ± 0.31) **BOP:** BL and 12 weeks: control: (1.00 ± 0.00, 0.80 ± 0.11), test: (1.00 ± 0.00, 0.22 ± 0.07) **CAL:** BL and 12 weeks: control: (5.41 ± 0.65, 4.71 ± 0.64), test: (5.4 ± 0.71, 2.68 ± 0.57).
Shah (2016)	Randomized split-mouth	9 patients (100 sites) (30–60 years)	Control: NSPT. Test: NSPT and 0.8 % hyaluronan gel and 1-week later (2X)	4 and 12 weeks	GI, PI, PD, and CAL	**PD:** at BL, 4 weeks and 12 weeks control: (5.37 ± 0.56, 3.90 ± 0.80, 3.33 ± 0.92), test: (5.37 ± 0.56, 3.30 ± 0.91, 2.37 ± 0.61). **BOP:** at BL, 4 weeks and 12 weeks control: (2.28 ± 0.20, 1.77 ± 0.32, 1.76 ± 0.92), test: (2.36 ± 0.18, 1.72 ± 0.30, 1.74 ± 0.32). **CAL:** at BL, 4 weeks and 12 weeks control: (9.10 ± 2.50, 8.07 ± 2.48, 7.50 ± 2.58), test: (9.17 ± 2.04, 7.50 ± 1.66, 6.70 ± 1.64).
Mallikarjun (2016)	Randomized split-mouth	20 patients (80 sites) (20–60 years)	Control: NSPT. Test: NSPT and 0.2 % at BL (1X)	6 and 12 weeks	PI, PD, and CAL	**PD:** BL and 6 weeks: control: (5.90 ± 1.07, 3.25 ± 0.79), test: (6.00 ± 1.03, 3.10 ± 1.07). **CAL:** BL and 6 weeks: control: (4.85 ± 1.09, 3.05 ± 1.00), test: (5.05 ± 1.15, 2.80 ± 1.15).
Omer (2018)	Randomized split-mouth	33 patients	Control: NSPT. Test: NSPT and 0.2 % HA at BL (1X)	6 weeks	PI, PD, CAL, BOP	**PD:** BL and 6 weeks: control: (5.45 ± 0.97,5.33 ± 0.99), test: (5.64 ± 1.29, 4.15 ± 1.92). **BOP**: BL and 6 weeks: control: (1.48 ± 0.57, 0.88 ± 0.55), test: (1.67 ± 0.54, 0.73 ± 0.52) **CAL:** BL and 6 s: control: (5.73 ± 1.13, 5.42 ± 0.97), test (5.91 ± 0.91 ,4.88 ± 1.08)
Kandil (2017)	Split mouth study	20 patients (40 sites) (35–55 years)	Control: NSPT. Test: 1 mL of 0.8 % HA gel was applied subgingivally after NSPT at baseline and 1 week post therapy. (2X)	6 and 12 weeks	PD, PI, and CAL	**PD:** BL, 6 weeks, 12 weeks control:(3.5 ± 0.7, 3.8 ± 0.6, 4 (3–5)), test: (5.5 ± 0.7, 2.4 ± 0.8, 2(1–4)). **CAL:** BL, 6 weeks, 12 weeks control: (3.3 ± 0.7, 2(0–2), 2(1–3)), test: (3.5 ± 0.7, 0 (1–3), 0(0–3))
Al-Shammari (2018)	Randomized split-mouth	24 participants (48 sites) (24–57 years)	Control: NSPT. Test: NSPT and 0.8 % hyaluronan gel was applied subgingivally after NSPT at baseline and 1 week post therapy. (2X)	6 and 12 weeks	PD, PI, and CAL	**PD**: control: 4.86 ± 0.87 at BL and 3.5 ± 0.99, test: 4.92 ± 1.12 at BL to 3.05 ± 1.05. **CAL:** There was no significant difference between control and test site in median of CAL in all durations (baseline, 6 weeks, and 12 weeks) with p-values 0.201, 0.543, and 0.116 respectively.
Lobato (2019)	Randomized split-mouth	16 patients (mean 55 years)	Control: NSPT. Test: NSPT and 0.8 % hyaluronan gel at BL (1X)	6 and 12 weeks	GI, PD, PI, and CAL	**PD:** control (3.9 ± 0.6, 3.3 ± 0.6), test: (3.8 ± 0.6, 3.2 ± 0.5). **BOP:** test compared to the control sides (9.4 ± 4.0 vs. 14.9 ± 8.9) **CAL:** control: CAL (4.8 ± 1.2, 4.3 ± 1.0), Test (4.9 ± 1.1, 4.2 ± 1.0).
Aydinyurt (2020)	Parallel-arm RCT	96 patients (mean 34.6 years)	0.2 % HA: Group 1 (control): NSPT and application of saline. -Group 2 (NSPT + HAgel): NSPT + intrasulculary HA gingival gel. -Group 3 (NSPT + HAmo): NSPT + intrasulcular irrigation with HA hydrogel mouth rinse. -Group 4 (NSPT + HAmo+HAgel): NSPT + irrigation with HA hydrogel mouth rinse + intrasulcular HAgingival gel	4 weeks	BOP, PI, PD, and CAL	**PD**: at week 4 (group 1: 3.02 ± 0.79, group 2: 2.56 ± 0.74 group 3: 2.61 ± 0.92, group 4: 2.82 ± 1.39). **CAL** at week 4 (group 1: 3.21 ± 0.96, group 2: 3.04 ± 0.67, group 3: 2.63 ± 0.90, group 4: 2.97 ± 1.56)
Nguyen (2021)	Randomized split-mouth	28 patients (733 sites)	Control: NSPT. Test: NSPT and 1 ml 0.2 % HA gel into each pocket immediately after NSPT, and then after 1, 2 , and 3 weeks. (4X)	6 weeks	BOP, PI, PD, and CAL	**PD:** Control (3.18 ± 1.59 and 2.41 ± 1.32), test: 3.31 ± 1.81 and 2.33 ± 1.27. **CAL:** Control (3.68 ± 1.69 and 2.97 ± 1.69) Test (3.78 ± 1.96 and 2.88 ± 1.49).
Olszewska-Czyz (2021)	Parallel-arm RCT	100 patients (mean 52 years)	Control: NSPT. Test: NSPT and HA 16 mg/mL of cross-linked and 2 mg/mL of non-cross-linked HA at BL and 6 weeks (2X)	12 weeks	PD, BOP, and CAL	**PD:** Bl and 12 weeks, control: (4.25 (4–4.5), 3.5 (2.8–3.8)), test: (4.75 (4.4–5), 3.5 (2.75–3.75)). **BOP:** BL and 12 weeks, control: (31 % (22.8–40.3), 20.5 % (15–25)), Test: (33.5 % (23.8–42), 13 % (9.5–18.25))

(*continued on next page*)

Table 1 (*continued*)

Author (year) place of study	Study design	Sample	Intervention	Follow-up	outcome measured	Findings
						CAL: BL and 12 weeks, Control: (4 (3–4) and 3 (2–3)), test: (4 (3.5–4), 1.63 (1–2)) Median (IQR)
Pilloni (2021)	Parallel-arm RCT	126 patients (mean 50.1 years)	Control: NSPT+ placebo. Test:NSPT with two rounds of application of HA, at Bl and 3 months (2X)	3, 6, 9, 12 months	BOP, PI, PD, and CAL	**PD**: BL, 3 months, 6 months, 9 months, and 12 months: control: (6 (5–7), 5 (4–6), 4 (3–5), 4 (3–5), 4 (3–4.5)), test: (6 (5–7), 5 (4–5), 4 (3–5), 4 (3–5), 4 (3–5)) expressed as median (IQR) **BOP:** BL, 3 months, 6 months, 9 months, and 12 months: control: (67.2 %, 51.6 %, 46.9 %, 25.4 %, 23.8 %), test: (77.4 %, 53.2 %, 40.3 %, 31.1 %, 37.7 %) **CAL:** BL, 3 months, 6 months, 9 months, and 12 months: control: (6 (5–8), 5 (4–6), 5 (3–6), 5 (3–6), 5 (3–5.5)), test: (7 (6–8), 5 (4–6.7), 5 (3–6), 5 (3–6), 4 (3–6)) expressed as median (IQR)
Vajawat (2022)	Randomized split-mouth	24 patients (48 sites) (35–65 years)	Control: NSPT. Test: 0.8 % HA gel whereas the control site received placebo gel once after NSPT (1X)	6 and 12 weeks	PI, GI, BI, PPD, and CAL	**PD:** S: BL, 4 weeks and 12 weeks control: (7.00 ± 1.673, 5.27 ± 1.737, 5.36 ± 1.567), Test: (6.55 ± 1.036, 3.91 ± 1.221,4.36 ± 1.362). NS: control: (5.73 ± 1.00, 3.27 ± 1.191, 3.55 ± 1.440), Test: (6.45 ± 1.128, 2.73 ± 1.104,4.27 ± 1.272). **CAL**: S: BL, 4 weeks and 12 weeks control: (4.36 ± 1.629, 3.73 ± 1.191, 3.82 ± 1.168), Test: (4.18 ± 1.401, 2.27 ± 1.009, 2.64 ± 0.809). NS: control (2.45 ± 1.036, 2.00 ± 0.775, 2.27 ± 0.905), Test: (3.27 ± 1.168, 1.64 ± 1.027, 1.91 ± 0.831).
Gangadhar (2022)	Parallel-arm RCT	120 sites (25–55 years)	Control: NSPT. Test: NSPT and 0.2 % HA (1X)	4 and 6 weeks	PI, GI, PD, and CAL	**PD:** BL, 4 weeks and 6 weeks: control: (5.5 ± 0.53, 5.12 ± 0.64, 4.75 ± 0.46), test: (5.6 ± 0.51, 4.12 ± 0.35, 3.5 ± 0.53). **CAL:** BL, 4 weeks and 6 weeks: control: (5.37 ± 0.51, 5.0 ± 0.75, 4.75 ± 0.46), test: (5.5 ± 0.75, 4.0 ± 0.75, 3.5 ± 0.53).
Ariel (2022)	Randomized split-mouth	34 patients (272 sites)	Control: NSPT. Test: NSPT and 0.8 % HA at BL and 1 month later (2X)	3 and 6 months.	BOP, PI, PD, and CAL	**PD:** BL, 3 months, 6 months: control: (7.46±0.98, 6.24±0.88, 5.96±0.70), test: (7.39±0.91, 5.41±0.70, 4.69±0.55). **BOP:** BL, 3 months, 6 months: control: (46.84 ± 7.71, 18.21 ± 14.17 , 17.66 ± 12.68), Test: (48.07 ± 7.19, 7.05 ± 6.67, 3.03 ± 4.04) **CAL:** BL, 3 months, 6 months: control: (8.59 ± 1.51, 7.45 ± 1.47 ,7.23±1.47), test: (8.49 ± 1.71, 6.22 ± 1.41, and 5.22 ± 0.88).
El Emam (2024)	Parallel-arm RCT	28 patients	Control: NSPT. Test group received NSPT with 0.2 % at BL (1X)	4 weeks, 12 weeks	PI, BI, CAL, PD	**PD:** BL and 12 weeks: control:(5.07 ± 0.27, 3.43 ± 0.51), test: (5.21 ± 0.43, 3.64 ± 0.50). **BOP**: BL and 12 weeks: control (1.43 ± 0.65, 0.21 ± 0.43), test: (1.50 ± 0.94, 0.36 ± 0.63) **CAL:** BL and 12 weeks: control:(5.79 ± 0.89, 3.86 ± 0.95), test: (5.71 ± 0.83, 3.93 ± 0.92)
Bertle (2024)	Parallel-arm RCT	56 patients (221 sites)	Control: NSPT. Test: NSPT and 0.3 % HA at BL and 3 months (2X)	12 and 48 weeks.	PD, BOP, CAL, and PI	**PD**: BL, 12 weeks, 48 weeks control: (5.1 ± 0.4, 4.4 ± 0.7, 4.5 ± 0.8), test: (5.1 ± 0.4, 4.6 ± 0.7, 4.2 ± 0.9) **BOP**: 12 weeks, 48 weeks control: (50 ±48.5, 30±29.4), test: (52±44.1, 39 ±33.1)

BOP bleeding on probing, PD probing depth, CAL clinical attachment level, PI plaque index, HA hyaluronic acid, NSPT non-surgical periodontal therapy, GI gingival index, BL baseline, S Smokers, NS Non-smokers.

intervals, a single application at baseline had a statistically non-significant effect of −0.37 (95 % CI:−1.23 to 0.49, $P = 0.26$), whereas multiple applications showed a statistically significant effect size of −0.34, (95 % CI:−0.67 to −0.01, $P = 0.045$). The non-smokers subgroup had a statistically significant effect size of −0.26(95 % CI:−0.51 to −0.01, , $P = 0.047$), whereas when smokers where included it showed statistically non-significant effect size of −0.63 (95 % CI: −2.10 to 0.84, , $P = 0.21$) **(Supplemental File, Fig s5).** However, due to the high heterogeneity of the included studies, the wide prediction intervals for subgroups pertaining to split mouth RCT (95 % PI: −1.26 to 0.48), 0.8 % HA concentration (95 % PI: −1.57 to 0.62), multiple applications of HA (95 % PI: −1.19 to 0.50) and non-smokers (95 % PI: 0.81 to 0.30) meant

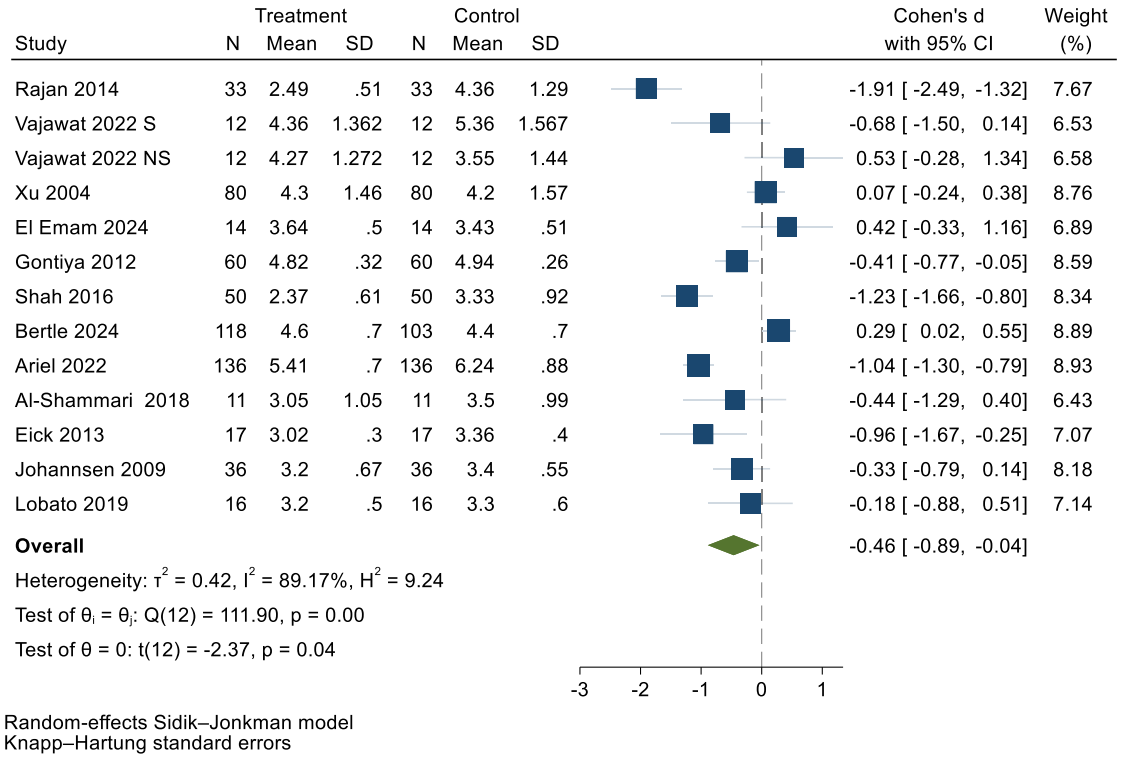

Fig. 2.1. Forest plot for PD reduction following non- surgical therapy after 3 months.

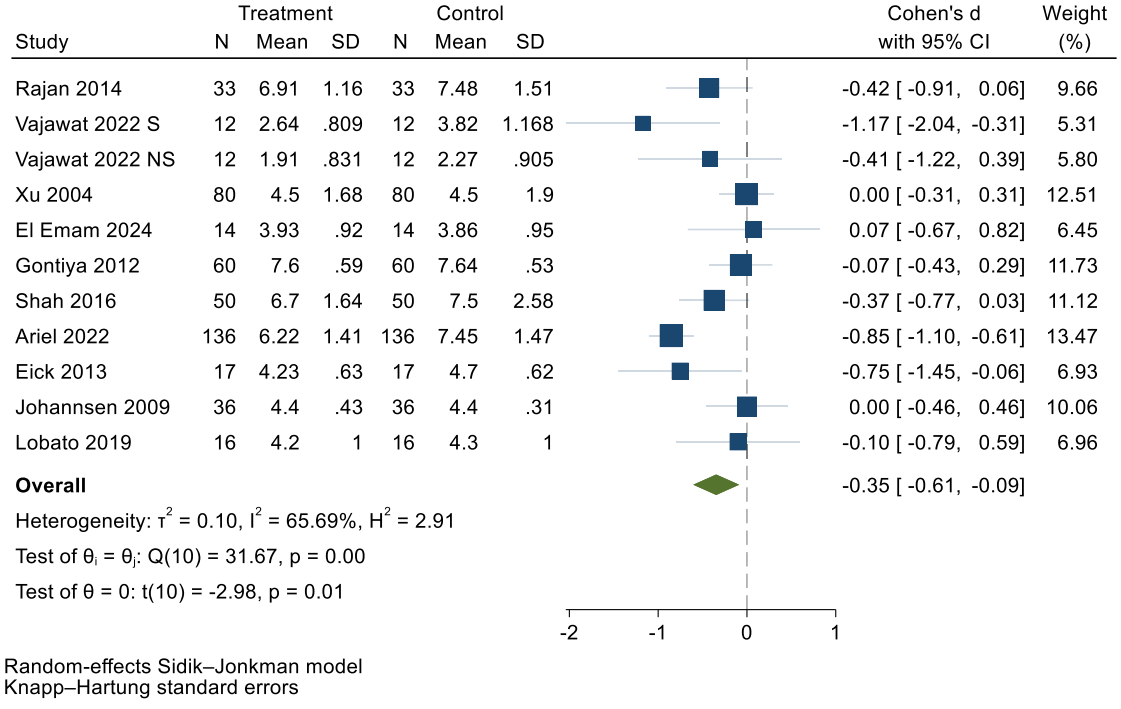

Fig. 2.2. Forest plot for CAL reduction following non - surgical therapy after 3 months.

that further evidence is needed to support the significance of their respective effect sizes.

Bleeding on probing (BoP)

Finally, for BoP subgroup analysis showed split-mouth RCT subgroup had a statistically significant effect size of −0.56 (95 % CI: −1.06 to −0.06, $P = 0.04$), whereas parallel-arm RCT subgroup had a statistically non-significant overall effect size of 0.03 (95 % CI: −0.54 to 0.59, $P = 0.66$). However, due to the high heterogeneity of the included studies, the wide prediction intervals for the split mouth RCT subgroup (95 % PI: −1.71 to 0.59), meant that further evidence is needed to support the significance of the effect size.

For concentration of HA used, the 0.8 % subgroup had a statistically

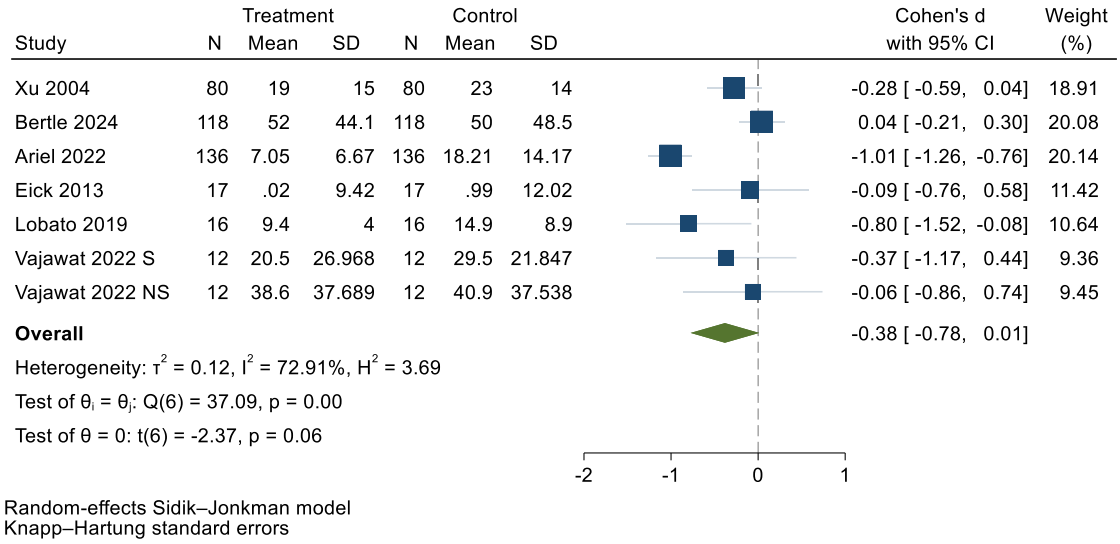

Fig. 2.3. Forest plot for BoP reduction following non- surgical therapy after 3.

non- significant effect size of −0.67 (95 % CI: −1.35 to 0.01, $P = 0.054$), additionally the 0.2 % subgroup had a statistically non-significant effect size of −0.27 (95 % CI: −0.59 to 0.04, $P = 0.08$). Subgroup analysis by the interval of HA application revealed that a single application at baseline had a statistically non-significant effect of −0.43 (95 % CI:−1.36 to 0.50, $P = 0.19$), similarly multiple applications resulted in a statistically non-significant effect of −0.36 (95 % CI: −1.13 to 0.41, $P = 0.24$). Finally, subgroup analysis by smoking indicated that the non-smokers subgroup had a statistically non-significant negative effect size of −0.30 (95 % CI: −0.76 to 0.16, $P = 0.13$), additionally the subgroup including smokers had a statistically non-significant effect size of −0.45 (95 % CI:−1.85 to 0.94, 95 % PI: −8.1 to 7.19, $P = 0.30$) **(Supplemental File, Fig s6).**

3.3. Risk of bias and certainty of evidence

A total of 23 studies were evaluated for ROB across the five domains. Among these studies, 10 (43.5 %) were found to have a high overall risk of bias [38,29,27,24,23,26,40–43]. These studies exhibited significant issues in multiple domains, particularly in domains which pertain to the randomization process and deviations from intended interventions. Another 7 studies (30.4 %) were categorized as having some concerns regarding bias. Lastly, 6 studies (26.1 %) were evaluated as having a low overall risk of bias. Twelve studies included in the meta-analysis provided data on PD, CAL, and BOP at both baseline and 3 months. The remaining studies were excluded from the meta-analysis due to the absence of 3-month data, requiring a consistent approach. Of the 12 studies, 7 were at high risk of bias, 3 at low risk, and 2 had some concerns (Fig. 3). A GRADE assessment demonstrated a moderate certainty of evidence for PPD, CAL and BoP **(Supplementary File, Fig. s7).** A sensitivity analysis excluding high-risk studies showed a PD reduction with an effect size of −0.28 (95 % CI: −0.80 to 0.23, $P = 0.28$) compared to −0.46 (95 % CI: −0.89 to −0.04, $P = 0.04$) in the main analysis. For CAL, the sensitivity analysis showed an effect size of −0.83 (95 % CI: −1.05 to −0.61, $P < 0.001$) compared to −0.35 (95 % CI: −0.61 to −0.08) in the main analysis.

4. Discussion

HA has several properties that contribute to its effectiveness in periodontal therapy. These include its anti-inflammatory effects, which can alleviate gingival inflammation [31,15], the retention of moisture and enhanced tissue healing [44]. When applied locally as adjunctive to NSPT, HA may promote tissue regeneration [6]. The culmination of these biological effects has been associated with reduced pocket depths and improved clinical attachment levels [45,21,46].

The methodological rigor of included studies varied, particularly concerning randomization protocols and participant selection criteria. While most studies employed a form of randomized methods, some did not adequately specify their randomization strategy, with 4 studies scoring high risk in Domain 1 (bias from the randomization process) reinforcing the importance of carefully interpreting the outcomes of HA therapy in periodontitis treatment [47].

The control group, which consisted of NSPT only, experienced a mean PD reduction of 1.28 mm after 3 months, closely aligning with the 1.4 mm mean PD reduction reported by Suvan et al. achieved after 6–8 months [48]. The aim of Suvan et al.'s study was to assess the clinical outcomes of NSPT in patients with periodontitis over a 6–8 month period; this indicates that the quality of NSPT in the studies included in the meta-analysis meets recognized standards. In contrast, the test group, employing the adjunctive HA treatment with NSPT, achieved a mean PD reduction of 1.8 mm, suggesting that the addition of HA resulted in further reduction in PD.

A recent meta-analysis by Eliezer et al. reported that adding HA to NSPT reduced PD by a weighted mean difference (WMD) of −0.36 mm (95 % CI: −0.54 to −0.19 mm; $p < 0.0001$), compared to our findings of a − 0.46 mm reduction (95 % CI: −0.89 to −0.04 mm; $p = 0.04$). Eliezer et al. also showed an improvement in CAL of 0.73 mm (95 % CI: 0.28 to 1.17 mm; $p < 0.0001$), while our results indicated a reduction of −0.35 mm (95 % CI: −0.61 to −0.09, $p = 0.01$). Additionally, Eliezer et al. reported a 15 % reduction in BoP (95 % CI: −22 % to −8 %; $p < 0.001$), compared to our observed 38 % reduction (95 % CI: −0.77 to 0.01; $p = 0.06$), both studies were measured three months after treatment, compared to NSPT alone [30].. However, Eliezer's meta-analysis (2019) included studies by Polepalle (2014) and Bevilacque et al. (2012) which were excluded in this review, with reasons for their exclusion documented earlier. The present review builds on Eliezer's work by incorporating new clinical studies conducted since 2019, adding an additional five studies to the analysis [39,22,28,25,42]. This inclusion of more recent research provides a broader and more current understanding of the topic. Furthermore, we reported the 95 % prediction interval, which was not included in Elizer's study. The prediction interval incorporates both the uncertainty about estimating the summary treatment effect and the statistical heterogeneity. Due to the heterogeneity of the included studies, further evidence is needed to support the improvement of HA adjunctive therapy outcomes due to wider

Fig. 3. Risk of Bias analysis traffic lights for included studies and also presented as a percentage of included studies per domain.

prediction intervals (PD reduction 95 % prediction interval, PI: −1.95 to 1.03; CAL gain 95 % PI −1.11 to 0.42; BoP reduction 95 % PI −1.35 to 0.59)

Our meta-analysis reported a WMD in PD of 0.46 mm at 3 months. This compares favourably with the WMD for systemic antibiotic use, which was 0.45 mm [49], while that for local antibiotic application was 0.37 mm [50], both measured at 6-months following NSPT as discussed in the recent EFP S3 guidelines [32]. Meanwhile, this meta-analysis reported a PD reduction of 0.46 mm at 3 months. However, it remains to be seen whether further improvements with adjunctive HA in NSPT are possible beyond 3 months. Understanding these dynamics is crucial for assessing the potential benefits of incorporating HA as an adjunct to NSPT to enhance PD reduction; this approach could be particularly beneficial after a second round of NSPT if initial improvements plateau.

Our meta- analysis included many studies employing a split-mouth design. This design uses the same patient as their own control, with one side receiving treatment and the other side receiving control intervention [51], in contrast to traditional parallel-arm RCTs, which have more variability between participants, potentially masking treatment effects. Statistically significant differences in PD and CAL reductions between split-mouth and whole mouth designs suggest that individual variations influence the clinical response [52]. However,

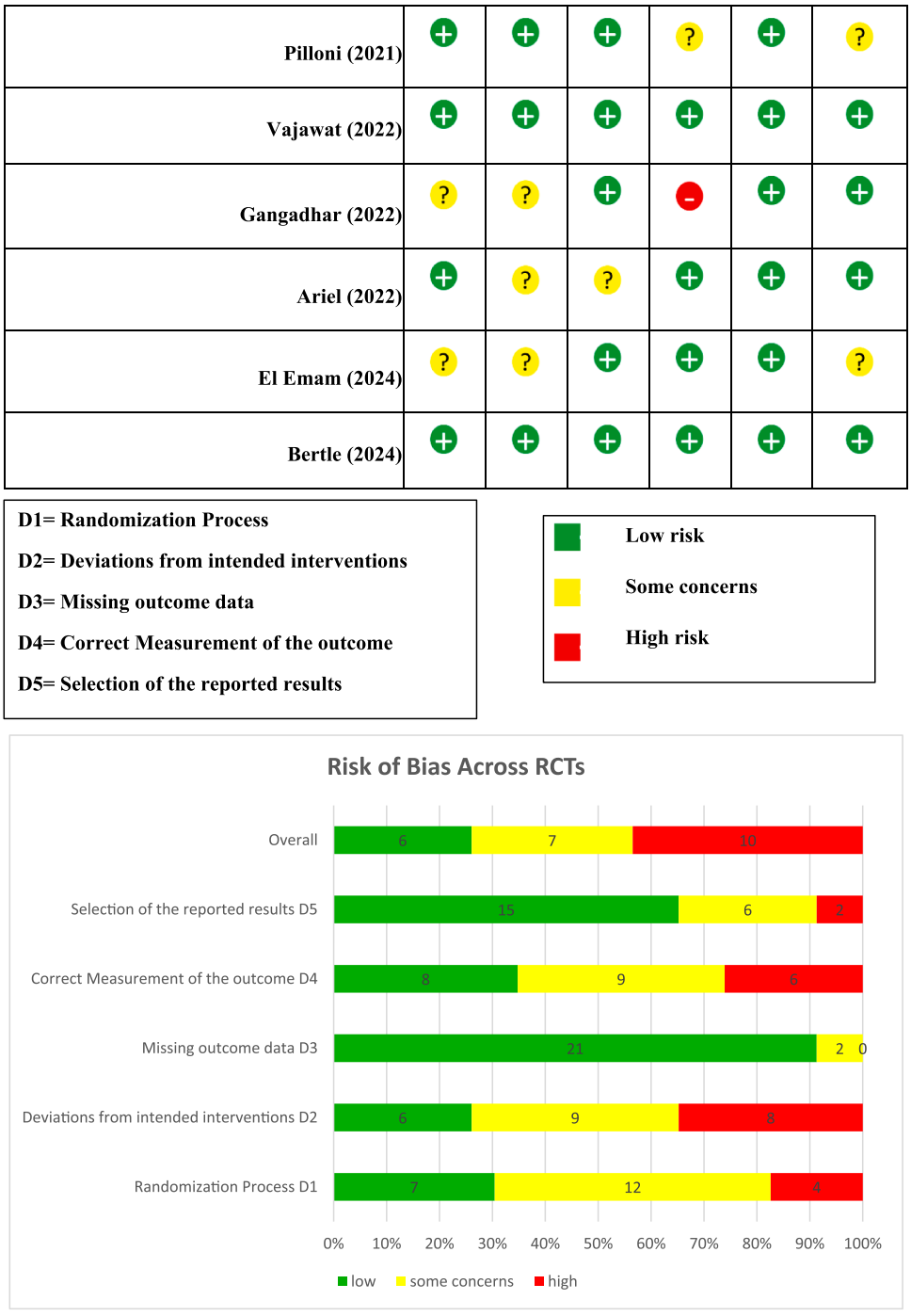

Fig. 3. (*continued*).

whole-mouth designs are more efficient than split-mouth designs, especially when accounting for the initial pocket depth stratification as periodontitis is unevenly distributed within the patient [53]. Additionally, they present challenges, including correlated measurements and potential cross-contamination, which can lead to inaccurate analyses if not properly addressed, especially in meta-analyses where correlation coefficients are often underreported [54].

In addition, many of the clinical trials referenced in our meta-analysis, and indeed, in previous systematic reviews were found to have a high risk of bias and high heterogeneity amongst the studies, indicating significant variability in their methodologies and outcomes, which further complicates the interpretation of the overall findings [30, 31]. We conducted sensitivity analyses, showing that favorable outcomes persisted even after excluding high-risk studies when assessing CAL. However, for PD, the sensitivity analysis yielded a WMD of −0.28 (vs. −0.46 in the main analysis), indicating bias in high-risk studies.

The group using a 0.8 % concentration of HA demonstrated statistically significant reductionin PD compared to the group using 0.2 % concentration. The higher concentration likely influences its therapeutic efficacy; and thus provides a substantial barrier effect, enhancing its ability to coat periodontal tissues and thus augmenting its anti-inflammatory and tissue-regenerative properties [26]. This can result in enhanced tissue integrity, reduce inflammation, and therefore account for the significant improvements in pocket depth and CAL compared to lower concentrations (0.2 %).

An *in-vitro* study suggested that high molecular weight (MW) HAs, especially the cross-linked formulation (CHA), exhibited significant anti-biofilm properties, and modulated immune responses by decreasing the pro-inflammatory cytokine IL-1β and increasing the anti-inflammatory cytokine IL-10 levels in biofilm-stimulated immune cells [13]. Additionally, higher concentrations (up to 40 mg/ml) were noted in previous studies to inhibit growth against certain bacterial species such as β-hemolytic streptococci, Staphylococcus aureus, and S. *epidermidis* [55].

Compared to a single administration of HA, periodic administration of HA facilitates the maintenance a therapeutic levels of HA in the pockets over time, and therefore promotes sustained anti-inflammatory effects, encourages tissue hydration, and wound healing. Consequently, this approach is expected to yield cumulative benefits, such as furtherreductions in PD, enhancements in CAL, and overall improvements in periodontal health compared to a single application with NSPT. However, due to the significant variability in application intervals; and the application of HA either as adjunct to NSPT by the clinician or supplementary to routine homecare by patients demonstrated in the studies, it was not possible to determine the precise number of intervals required following NSPT for maximum beneficial effect (Table 1).

The less favorable outcomes of HA therapy in smokers can be attributed to multiple underlying mechanisms. Smoking is known to compromise periodontal health by impairing immune responses, reducing blood flow to the gums [56], and impairing tissue healing processes through inhibiting collagen production and fibroblast activity [57]. These factors create a less favorable environment for the beneficial effects of HA [58]. Additionally, nicotine and other chemicals in tobacco smoke cause vasoconstriction, limiting blood flow to the gums and impairing the delivery of essential nutrients and oxygen necessary for tissue healing and regeneration [59]. Moreover, it increases oxidative stress in the periodontal tissues, leading to cellular damage and exacerbating inflammation, which can counteract the anti-inflammatory effects of HA [60]. Furthermore, smoking alters the oral microbiome, promoting the growth of pathogenic bacteria associated with periodontal disease and potentially undermining HA's ability to restore microbial balance [61]. Collectively, these factors cultivate an environment less conducive for optimal healing, resulting in unfavourable clinical outcomes [62].

While the present data suggests the adjunctive use of HA to NSPT results in favourable clinical outcomes, several limitations must be acknowledged. These include the heterogeneity in study designs, variations in HA formulations and protocols, potential biases introduced by inadequate randomization, the limited number of studies, risk of bias, and the quality of evidence, not to mention, whether magnitude of clinical improvement would be clinically relevant. In particular, the statistical heterogeneity has resulted in the wide predictional intervals for our outcome measures. As such, we emphasize the need for cautious interpretation of the subgroup analysis results. Furthermore, we recommend that future studies should aim to incorporate individual patient data to enable more nuanced analyses that could clarify the impact of specific characteristics on treatment outcomes. Future research should focus on standardizing HA treatment protocols, clarifying optimal concentrations and application intervals, and addressing methodological inconsistencies. Long-term RCTs with larger sample sizes and diverse patient populations, including smokers, are warranted to further elucidate the effectiveness of HA in the non-surgical management of periodontitis.

5. Conclusion

Although topical HA application may provide additional benefits when used with NSPT, the limited number of studies, risk of bias, heterogeneity and moderate quality of evidence indicate that further research is warranted to confirm these findings and establish more definitive clinical guidelines.

Ethical approval

Not applicable.

Informed consent

Not applicable.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

CRediT authorship contribution statement

Deema Dababseh: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Roa Altell:** Writing – review & editing, Formal analysis, Data curation. **Jing Kang:** Writing – review & editing, Supervision, Methodology. **Jiangyue Lu:** Formal analysis. **Zainab Malaki:** Writing – review & editing, Formal analysis. **Petros Mylonas:** Writing – review & editing, Formal analysis, Conceptualization. **Emily Ming-Chieh Lu:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare no competing or financial interests.

Appendix 1

Detailed search terms used in search strategy.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2025.105613.

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