Clinical Oral Investigations (2025) 29:375
https://doi.org/10.1007/s00784-025-06451-w

REVIEW

Clinical performance of self-adhesive resin composite direct restorations in permanent teeth: a systematic review and meta-analysis

João Felipe Besegato[1] · Andrea Freire[1] · Joissi Ferarri Zaniboni[2] · Guilherme Loubet Melo[1] · Aryvelto Miranda Silva[3]

Received: 19 March 2025 / Accepted: 30 June 2025

Abstract

Objective This systematic review and meta-analysis compared the clinical performance of direct restorations using conventional or bulk-fill resin composites (RC) with self-adhesive RCs in permanent teeth, regardless of cavity type or adhesive strategy employed. PICOS question was "Do direct restorations using self-adhesive RC exhibit clinical performance comparable to that of conventional or bulk-fill RCs?"

Methods Search strategy was applied across Medline/PubMed, Web of Science, Scopus, Embase, LILACS, The Cochrane Library, Base, Google Scholar, and OpenGray databases on January 17th, 2024 and updated on May 5th, 2025. Randomized clinical trials that evaluated the clinical performance of direct restorations with self-adhesive RC compared to conventional or bulk-fill RC in permanent teeth were included. Risk of bias was assessed using the RoB 2 tool. All analyses were performed using RevMan 5 with Risk Difference (RD) and corresponding 95% confidence intervals (CIs). Random-effects models and the Mantel-Haenszel method were applied.

Results 1206 articles were identified. 12 studies (15 reports) were included in qualitative and quantitative analyzes. A total of 356 participants aged 6 to 79 years received 794 restorations, of which 396 were performed with conventional or bulk-fill RCs and 398 with self-adhesive RCs. The follow-up period ranged from immediately after restoration (baseline) to 60 months. Meta-analyses revealed that no significant differences were found between the RCs across all the evaluated outcomes: marginal staining ($P \geq 0.13$), color stability ($P \geq 0.27$), fracture/retention ($P \geq 0.45$), and marginal adaptation ($P \geq 0.08$) regardless of the follow-up periods. For wear, postoperative sensitivity, and recurrence of caries, erosion, or abrasion, effects estimates were not possible. From 12 included studies, 6 of them showed a high overall risk of bias. Certainty of evidence was considered low or very low across all the evaluated criteria mainly due to imprecision and risk of bias.

Conclusion The low certainty of evidence suggests that self-adhesive RC direct restorations demonstrate clinical performance similar to conventional or bulk-fill RCs across all cavity types over a follow-up period of 6 to 48 months.

Clinical significance

This systematic review and meta-analysis indicates that self-adhesive resin composites are a clinically viable material for direct restorations, as they exhibit comparable performance to conventional or bulk-fill resin composites over a 6- to 48-month follow-up period.

Keywords Self-adhesive resin composite · Dental restoration · Systematic review · Clinical performance

Extended author information available on the last page of the article

Published online: 08 July 2025

Introduction

Resin composite (RC) is widely used for direct restorations due to its clinical versatility and adequate physical, mechanical, biological, and esthetic properties [1, 2]. However, RC direct restoration is a technique-dependent and sensitive procedure, where the operator's skill plays a significant role in the clinical success and longevity [3]. Key challenges undergoing the restorative procedure include proper acid etching, dentin moisture control, complete solvent removal, correct adhesive system application, and the formation of a stable and homogeneous hybrid layer. These factors are crucial for achieving optimal adhesion between RC and tooth structure [1, 4–7].

To reduce technique sensitivity and clinical time, self-adhesive RCs have been developed. These materials eliminate the need for an adhesive system application as a separate step, thereby simplifying the restorative procedure [8]. This innovation is enabled by the incorporation of acidic monomers– such as 10-Methacryloyloxydecyl Dihydrogen Phosphate (10-MDP), glycerol phosphate dimethacrylate (GPDM), carboxylic methacrylates (4-MET), and bis(methacryloyloxyethyl) phosphate (BMEP)– which allow for self-etching of the dental substrate. Additionally, hydrophilic monomers like hydroxyethyl methacrylate (HEMA) improve resin infiltration and surface wettability, enhancing adhesion to dentin. The bonding mechanism of self-adhesive RCs involves both chemical interactions, through the bonding of acidic monomers to hydroxyapatite, and micromechanical retention, as the resin penetrates the collagen fibrils and dentin smear layer [9].

The first commercially available self-adhesive RC, Vertise Flow (Kerr), combined phosphate ester methacrylate and GPDM as functional monomers [10]. These materials are available in various viscosities, with higher filler content resulting in increased viscosity, improved mechanical properties, and greater wear resistance. Conversely, low-viscosity (flowable) RCs offer superior cavity adaptation and are particularly useful for small restorations [11]. Self-adhesive RCs are also available in bulk-fill formulations, which differ from conventional RCs by allowing single-increment placement of up to 4–5 mm due to increased translucency and enhanced depth of cure [12–14].

Despite their advantages, the clinical performance of self-adhesive RCs remains questionable. Limitations in bond strength and microleakage compared to conventional RCs is reported [15]. A systematic review found that conventional flowable RCs applied with an etch-and-rinse adhesive system showed superior marginal adaptation compared to self-adhesive flowable RCs [16]. Additionally, bulk-fill self-adhesive RCs have shown inferior esthetic outcomes, including reduced surface gloss, color stability, translucency, and increased marginal discoloration [13, 14, 17]. Some studies have also reported mild, reversible alterations in the dentin-pulp complex following placement of self-adhesive RCs [18]. Postoperative sensitivity– particularly within the first 15 days– has been reported, though symptoms typically resolve over time [6]. Nonetheless, clinical studies indicate that the overall performance of self-adhesive RCs is comparable to conventional RCs, supporting their use as a viable alternative for direct restorations in permanent teeth [9, 13, 14, 16, 19].

Overall, modern RCs exhibit appropriate mechanical and biological properties for clinical use. However, in situation involving high occlusal stress, restorations may be more prone to fractures and wear over time [1]. Therefore, restorative materials must demonstrate long-term durability. While in vitro studies offer valuable information on physical and mechanical properties, clinical trials remain essential for assessing long-term performance under intraoral conditions [20]. Thus, evaluating the clinical behavior of self-adhesive RCs is crucial to guide dental clinicians in selecting materials that provide reliable esthetic, functional, and biological outcomes for safe and effective patient care.

This systematic review and meta-analysis compared the clinical performance of direct restorations using self-adhesive RCs with conventional or bulk-fill RCs in permanent teeth, regardless of cavity type or adhesive strategy used.

Materials and methods

Protocol and registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [21] and following the recommendations provided in the Cochrane Handbook for Systematic Reviews of Interventions. The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42024502834.

Eligibility criteria

The PICOS (Population, Intervention, Comparison, Outcome, Study Design) framework was used to formulate the research question: "Do direct restorations with self-adhesive resin composites exhibit superior clinical performance compared to conventional or bulk-fill resin composites?"

The components of the PICOS question were defined as follows: Population (P): Patients requiring direct RC restorations in permanent teeth, regardless of cavity location; Intervention (I): Direct restorations using self-adhesive RC;

Comparison (C): Direct restorations using conventional or bulk-fill RC, regardless of the adhesive strategy used; Outcome (O): Clinical performance evaluated using the criteria established by the World Dental Federation (FDI) or the United States Public Health Service (USPHS); Study Design (S): Randomized clinical trials (RCTs).

The exclusion criteria included non-randomized clinical trials, observational studies, case reports, pilot studies, laboratory and animal studies, conference abstracts and consensus papers. Additionally, studies evaluating primary teeth, self-adhesive resin cements, and RC used for pit and fissure sealants or orthodontic bracket bonding were excluded.

Databases and search strategy

A comprehensive literature search was conducted across the following databases: PubMed, Web of Science, Scopus, Embase, The Cochrane Library, and LILACS. Gray literature was also searched using Google Scholar, BASE, and OpenGray. The search was independently performed by two reviewers (JFB and AF) without restrictions on language or publication year.

Search strategies were developed using keywords relevant to the research question. The final search strategy was exported, and duplicates were removed using the reference management tool Rayyan QCRI.

Table 1 shows the search strategy applied to each database and the total number of studies retrieved before duplicate removal.

Article selection process

References retrieved through the search strategy across different databases were managed using Rayyan QCRI. After removing duplicates, two independent reviewers (JFB and AF) screened the titles and abstracts of each study to select those meeting the inclusion and exclusion criteria for full-text review. The selected studies were then read in full to determine their eligibility for inclusion in qualitative and quantitative analyses. In cases of disagreements between the primary reviewers (JFB and AF), a third reviewer (JFZ) was consulted, and consensus was reached through discussion. Reasons for excluding studies after full-text review were documented.

Data collection process

Data extraction from the included studies was performed by three authors (JFB, AF, and GLM) using an Excel spreadsheet specifically designed for this review. Any discrepancies in data collection were resolved through consensus. Extracted data included: author/year; country; journal; study design; type of self-adhesive and conventional or bulk-fill RC; number of patients and cavities evaluated; cavity type; follow-up periods; method of operative field isolation; curing time and irradiance of the light-curing unit; criteria for clinical performance evaluation; study conclusions; decision regarding inclusion in the meta-analysis; and other relevant observations.

Clinical performance was assessed based on failures related to esthetic (marginal staining, color stability), functional (fracture/retention, marginal adaptation, wear) and biological outcomes (postoperative sensitivity, recurrence of caries, erosion, and abrasion).

All included studies clinically evaluated the restorations using either the FDI or USPHS criteria. The FDI system assesses the quality of direct and indirect restorations using 5 grades (1 to 5) for each criterion. Scores from 1 to 3 indicated clinically acceptable restorations, while scores of 4 and 5 reflect clinically unacceptable situations, indicating the need for repair (score 4) or replacement (score 5).

In contrast, the USPHS criteria classify restorations as Alpha, Bravo or Charlie for each criterion. Alpha indicates a clinically acceptable restorations, Bravo denotes a restoration that is clinically unacceptable but reparable (excluding secondary caries), and Charlie indicates a restoration that requires replacement.

Given the differing scoring approaches, the data were unified into a binary outcome for meta-analysis. Restorations scored as 4 and 5 (FDI) or as Bravo or Charlie (USPHS) were considered as failures, while those scored as 1, 2, 3, or Alpha were considered successful.

Meta-analysis

Data extracted from eligible studies were dichotomous. The effect measure used to summarize the results was the Risk Difference (RD) with corresponding 95% confidence intervals (CIs). Random-effects models and the Mantel-Haenszel method were applied. Heterogeneity was assessed using Cochran's Q test and I^2 statistics. Subgroup analyses were conducted based on different follow-up periods, and sensitivity analyses were performed to explore potential sources of high heterogeneity when identified. All analyses were performed using RevMan 5 (Review Manager version 5.4.1, The Cochrane Collaboration, Copenhagen, Denmark).

Risk of bias and certainty of evidence assessment

Two independent reviewers (JFB and AF) evaluated the risk of bias using the Revised Cochrane Risk-of-Bias Tool for Randomized Trials (RoB 2). The five domains assessed were: bias arising from the randomization process; deviations from intended interventions; missing outcome data;

Table 1 Search strategy conducted in each database

Database	Search strategy	Results
PubMed	#1 ("composite resins"[MeSH Terms] OR resin composite restoration OR composite resin restoration OR direct resin composite restoration OR direct composite resin restoration OR restoration OR dental caries OR tooth decay OR dental decay OR caries OR carious) #2 ("self adhesive" OR "self bond*" OR self-adhesive restorative material OR self-adhesive flowable composite OR self-adhering flowable composite OR self-adhering flowable resin composite OR self-bonding resin composite OR self-bonding composite OR self-adhesive bulk-fill composite OR self-adhesive bulkfill composite) #3 (secondary caries OR postoperative sensitivity OR retention OR marginal discoloration OR marginal staining OR marginal adaptation OR anatomic form OR anatomical form OR anatomic contour OR surface texture OR surface luster OR surface lustre OR surface staining OR color match OR fracture) #4 ("clinical trial" OR "randomized clinical trial" OR randomized split-mouth design controlled study OR "randomized controlled trial" OR "controlled clinical trial" OR "RCT" OR "clinical study") #1 AND #2 AND #3 AND #4	536
Web of Science	#1 TS= (composite resin OR resin composite restoration OR composite resin restoration OR direct resin composite restoration OR direct composite resin restoration OR restoration OR dental caries OR tooth decay OR dental decay OR caries OR carious) #2 TS= (self-adhesive OR self-adhesive restorative material OR self-adhesive flowable composite OR self-adhering flowable composite OR self-adhering flowable resin composite OR self-bonding resin composite OR self-bonding composite OR self-adhesive bulk-fill composite OR self-adhesive bulkfill composite) #3 TS= (secondary caries OR postoperative sensitivity OR retention OR marginal discoloration OR marginal staining OR marginal adaptation OR anatomic form OR anatomical form OR anatomic contour OR surface texture OR surface luster OR surface lustre OR surface staining OR color match OR fracture) #4 TS=(clinical trial OR randomized clinical trial OR randomized split-mouth design controlled study OR randomized controlled trial OR controlled clinical trial OR RCT OR clinical study) #1 AND #2 AND #3 AND #4	203
The Cochrane Library	IDSearchHits #1MeSH descriptor: [Composite Resins] explode all trees2553 #2(composite resin restoration): ti, ab, kw1846 #3(direct composite resin restoration): ti, ab, kw236 #4(restoration): ti, ab, kw10921 #5(dental caries): ti, ab, kw7853 #6(tooth decay): ti, ab, kw635 #7(dental decay): ti, ab, kw617 #8(caries): ti, ab, kw9607 #9(carious): ti, ab, kw2599 #10#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #920,008 #11(self-adhesive restorative material): ti, ab, kw26 #12(self-adhesive flowable composite): ti, ab, kw16 #13(self-adhering flowable composite): ti, ab, kw24 #14(self-adhering flowable resin composite): ti, ab, kw19 #15(self-adhesive bulkfill composite): ti, ab, kw12 #16#11 OR #12 OR #13 OR #14 OR #1569 #17(secondary caries): ti, ab, kw2735 #18(postoperative sensitivity): ti, ab, kw3803 #19(retention): ti, ab, kw28318 #20(marginal discoloration): ti, ab, kw608 #21(marginal staining): ti, ab, kw276 #22(marginal adaptation): ti, ab, kw1189 #23(anatomic form): ti, ab, kw535 #24(anatomical form): ti, ab, kw809 #25(anatomic contour): ti, ab, kw49 #26(surface texture): ti, ab, kw413 #27(surface luster): ti, ab, kw61 #28(surface staining): ti, ab, kw1749 #29(color match): ti, ab, kw505 #30(fracture): ti, ab, kw24422 #31#17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #3,061,318 #32#10 AND #16 AND #3130	30

Table 1 (continued)

Database	Search strategy	Results
SCOPUS	#1 (TITLE-ABS-KEY (composite resin OR resin composite restoration OR composite resin restoration OR direct resin composite restoration OR direct composite resin restoration OR restoration OR dental caries OR tooth decay OR dental decay OR caries OR carious)) #2 (TITLE-ABS-KEY "self-adhesive" OR "self-adhesive restorative material" OR "self-adhesive flowable composite" OR "self-adhering flowable composite" OR "self-adhering flowable resin composite" OR "self-bonding resin composite" OR "self-bonding composite" OR "self-adhesive bulk-fill composite") #3 (TITLE-ABS-KEY ("secondary caries" OR "postoperative sensitivity" OR retention OR "marginal discoloration" OR "marginal staining" OR "marginal adaptation" OR "anatomic form" OR "anatomical form" OR "anatomic contour" OR "surface texture" OR "surface luster" OR "surface lustre" OR "surface staining" OR "color match" OR fracture)) #4 (TITLE-ABS-KEY ("clinical trial" OR "randomized clinical trial" OR "randomized split-mouth design controlled study" OR "randomized controlled trial" OR "controlled clinical trial" OR "RCT" OR "clinical study")) #1 AND #2 AND #3 AND #4	27
LILACS (BVS)	("composite resins" OR "resin composite restoration" OR "composite resin restoration" OR "direct resin composite restoration" OR "direct composite resin restoration" OR "restoration" OR "dental caries" OR "tooth decay" OR "dental decay" OR "caries" OR "carious") AND ("self adhesive" OR "self bonding" OR "self-adhesive restorative material" OR "self-adhesive flowable composite" OR "self-adhering flowable composite" OR "self-adhering flowable resin composite" OR "self-bonding resin composite" OR "self-bonding composite" OR "self-adhesive bulk-fill composite" OR "self-adhesive bulkfill composite") AND ("secondary caries" OR "postoperative sensitivity" OR "retention" OR "marginal discoloration" OR "marginal staining" OR "marginal adaptation" OR "anatomic form" OR "anatomical form" OR "anatomic contour" OR "surface texture" OR "surface luster" OR "surface lustre" OR "surface staining" OR "color match" OR "fracture") AND ("clinical trial" OR "randomized clinical trial" OR "randomized split-mouth design controlled study" OR "randomized controlled trial" OR "controlled clinical trial" OR "RCT" OR "clinical study")	4
EMBASE	#1 'resin'/exp OR 'resin' OR 'resin composite restoration' OR 'composite resin restoration' OR 'direct resin composite restoration' OR 'direct composite resin restoration' OR 'restoration' OR 'dental caries' OR 'tooth decay' OR 'dental decay' OR 'caries' OR 'carious' #2 'self adhesive' OR 'self bonding' OR 'self-adhesive restorative material' OR 'self-adhesive flowable composite' OR 'self-adhering flowable composite' OR 'self-adhering flowable resin composite' OR 'self-bonding resin composite' OR 'self-bonding composite' OR 'self-adhesive bulk-fill composite' OR 'self-adhesive bulkfill composite' #3 'secondary caries' OR 'postoperative sensitivity' OR 'retention' OR 'marginal discoloration' OR 'marginal staining' OR 'marginal adaptation' OR 'anatomic form' OR 'anatomical form' OR 'anatomic contour' OR 'surface texture' OR 'surface luster' OR 'surface lustre' OR 'surface staining' OR 'color match' OR 'fracture' #4 'clinical trial' OR 'randomized clinical trial' OR 'randomized split-mouth design controlled study' OR 'randomized controlled trial' OR 'controlled clinical trial' OR 'rct' OR 'clinical study' #1 AND #2 AND #3	406
Google Scholar	"resin" AND "self adhesive" AND "clinical trial" filetype: pdf	100
Open Gray	"resin" AND "self adhesive"	49
Base	resin composite AND self adhesive AND clinical trial	143

*All searches were conducted on January 17th, 2024 and updated on May 5th, 2025

measurement of the outcome; selection of the reported result.

The certainty of the evidence was assessed using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach through the online tool GradePRO (https://gdt.gradepro.org/). For randomized clinical trials, GRADE begins with high-certainty evidence, considering five factors that may downgrade the level of certainty: risk of bias, inconsistency, indirect evidence, imprecision, and publication bias [22]. Each criterion was categorized as "no limitation," "serious limitation," or "very serious limitation," leading to evidence being rated as high, moderate, low, or very low certainty.

Uncertain or high risk of bias and imprecise effect estimates or high heterogeneity led to downgrading the certainty of evidence for risk of bias and inconsistency, respectively. Additionally, overlapping confidence intervals crossing the line of null effect and a sample size < 300 resulted in downgrading for imprecision [23].

Results

Identification and selection of studies

The database searches yielded a total of 1498 articles, distributed as follows: Medline/PubMed ($n=536$), Web of Science ($n=203$), Scopus ($n=27$), Embase ($n=406$), LILACS ($n=4$), The Cochrane Library ($n=30$), Base ($n=143$), Google Scholar ($n=100$), and OpenGray ($n=49$). After

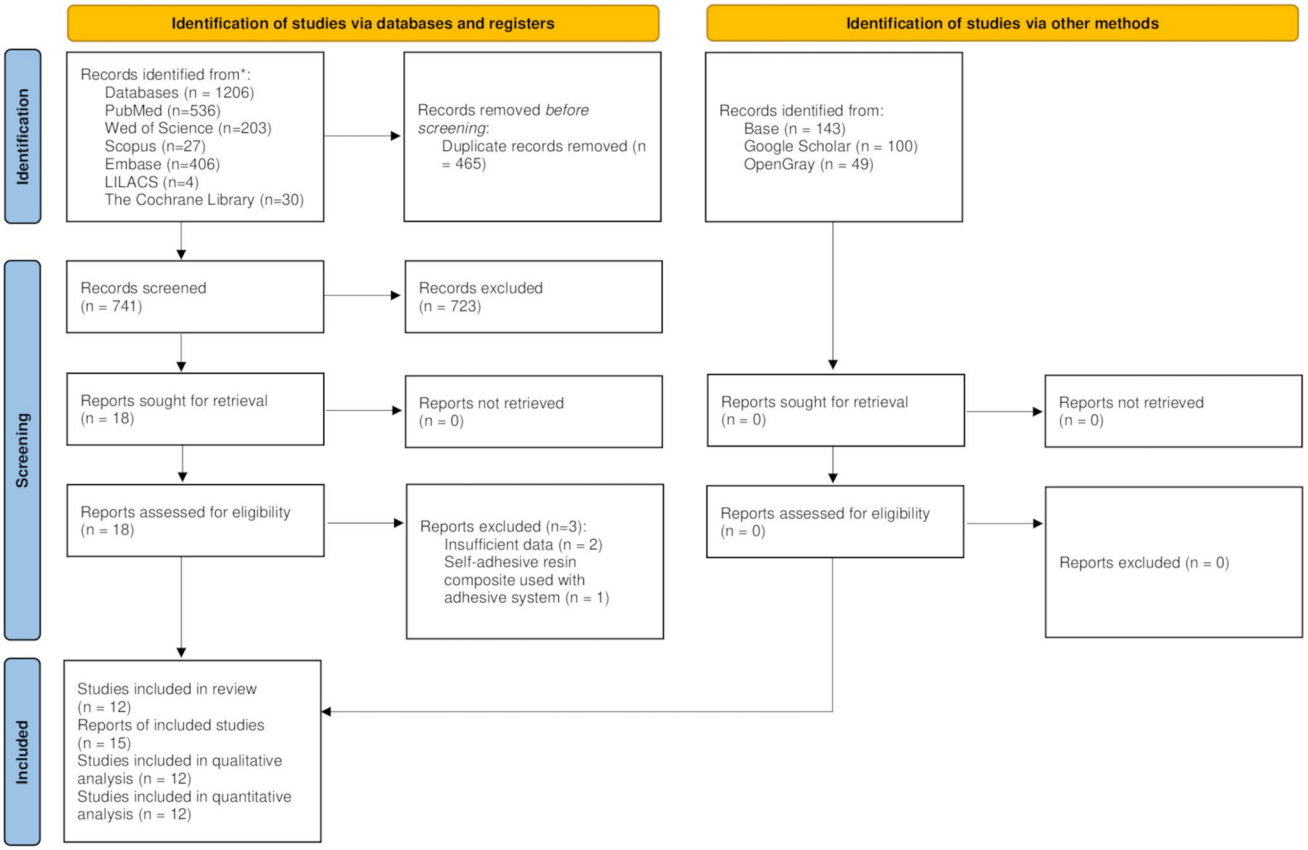


Fig. 1 Flowchart describing the identification, screening, and inclusion processes

Table 2 Excluded studies (and their reasons) after full-text review

Ref#	Study	Title	Reasons for exclusion
24	Kalola et al., 2022	Comparative clinical evaluation of a self-adhering flowable composite with conventional flowable composite in Class I cavity: An in vivo study	Insufficient data: outcomes displayed in charts hindered data extraction.
25	Peskersoy et al., 2022	The effect of flowable composite resins on periodontal health, cytokine levels, and immunoglobulins	Study design: the study evaluated the self-adhesive resin composite associated with an adhesive system.
26	Maj et al., 2020	A comparative clinical study of the self-adhering flowable composite resin Vertise Flow and the traditional flowable composite resin Premise Flowable	Insufficient data: study did not report the total number of restorations at each follow-up period, hindering data extraction

removing duplicates, 741 articles remained for title and abstract screening. Among these, 18 articles were selected for full-text review (Fig. 1).

Following full-text assessment, three studies were excluded for the following reasons: did not present all data in the article, as results were displayed using line graphs, making data extraction and analysis impossible [24]; self-adhesive RC were associated with an adhesive system [25]; lack of reported total restorations at each follow-up, hindering data extraction [26] (Table 2).

Characteristics of included studies

A total of 12 studies (15 reports) [9, 13, 14, 19, 20, 27–36] were included in both qualitative and quantitative analyses, all of which were randomized clinical trials (RCTs). Table 3 summarizes the characteristics of the included studies after full-text review.

The earliest study was published in 2015, while the most recent ones were from 2025. Regarding authors' country of origin, 10 studies were conducted by researchers from Asia and/or Africa, and one study originated from Europe. Additionally, three studies had authors from multiple countries.

Table 3 Characteristics of the included studies after full-text review (12 studies; 15 reports)

Ref	Authors, year	Country	Self-adhesive resin composite (intervention)	Conventional or bulk-fill resin composite (control)	Number of patients and cavities	Cavity design	Follow-up periods	Isolation of the operative field	Light-curing time; irradiance	Clinical criteria
32	Albelasy et al., 2024	Egypt and USA	Surefil One™ (Dentsply Sirona)	Tetric® PowerFill (Ivoclar Vivadent)	32 pat. 64 cav.	Class I and II	7 days; 6 months; 12 months; 24 months	Abs.	20–30 s.	FDI
34	AlHumaid et al., 2018	Saudi Arabia and Egypt	Fusio™ (Pentron)	Tetric® Flow (Ivoclar Vivadent)	20 pat. 40 cav.	Class V	Baseline; 1 week; 6 months; 12 months; 18 months	Abs.	Not reported	USPHS
20	Çelik et al., 2015	Turkey	Fusio™ Liquid Dentin (Pentron)	G-ænial (GC)	19 pat. 80 cav.	Class V	Baseline; 6 months	Rel.	1000 mW/cm²	FDI
14	Cieplik et al., 2022 (1)	Germany	Experimental	Filtek™ One Bulk Fill (3 M/Solventum)	30 pat. 60 cav.	Class II	Baseline; 6 months; 12 months	Abs. or rel.	20 s; 1250 mW/cm²	FDI
13	Cieplik et al., 2022 (2)	Germany	Experimental	Filtek™ One Bulk Fill (3 M/Solventum)	30 pat. 60 cav.	Class II	24 months; 36 months	Abs. or rel.	20 s; 1250 mW/cm²	FDI
31	Ellithy et al., 2024	Egypt	Surefil One™ (Dentsply Sirona)	Filtek™ One Bulk Fill (3 M/Solventum)	32 pat. 64 cav.	Class II	Baseline; 6 months; 12 months	Abs.	40 s; 1250 mW/cm²	FDI
35	El-Shazly et al., 2025	Egypt	Surefil One™ (Dentsply Sirona)	NeoSpectra ST HV (Dentsply Sirona)	15 pat. 54 cav.	Class V (carious lesions)	Baseline; 6 months; 12 months	Abs.	20–40 s; 1200 mW/cm².	USPHS
27	Ibrahim et al., 2023	India and Egypt	Fusio™ Liquid Dentin (Pentron)	Filtek™ Bulk Fill Posterior (3 M/Solventum)	20 pat. 40 cav.	Class I	Baseline; 6 months; 12 months; 18 months	Abs.	850–1200 mW/cm²	USPHS
33	Maghaireh et al., 2023	Jordan	Surefil One™ (Dentsply Sirona)	Filtek™ Bulk-Fill Posterior Restorative (3 M/Solventum)	83 pat. 166 cav.	Class I and II	24 horas; 7 days; 30 days	Abs.	20 s.; 1200 mW/cm²	USPHS
19	Oz et al., 2020	Turkey	Vertise™ Flow (Kerr)	Luxa Flow (DMG)	25 pat. 65 cav.	Class I	7 days; 12 months; 24 months; 36 months; 48 months; 60 months	Rel.	1200 mW/cm²	FDI
28	Oz et al., 2021	Turkey	Constic (DMG)	G-ænial Universal Flo (GC)	28 pat. 57 cav.	Class I	Baseline; 6 months; 12 months; 24 months	Rel.	20 s.; 1200 mW/cm²	USPHS

Table 3 (continued)

Ref	Authors, year	Country	Self-adhesive resin composite (intervention)	Conventional or bulk-fill resin composite (control)	Number of patients and cavities	Cavity design	Follow-up periods	Isolation of the operative field	Light-curing time; irradiance	Clinical criteria
29	Sabbagh et al., 2017	Lebanon	Vertise™ Flow (Kerr)	Premise™ Flowable (Kerr)	34 pat. 68 cav.	Class I	7 days; 6 months; 12 months; 24 months	Abs. or rel.	20 s.; 800 mW/cm²	USPHS
30	Shaalan et al., 2018	Egypt	Vertise™ Flow (Kerr)	Filtek™ Z350 XT Flowable (3 M/Solventum)	18 pat. 36 cav.	Class I	7 days; 6 months	Abs.	20 s.	USPHS
9	Shaalan et al., 2021	Egypt	Vertise™ Flow (Kerr)	Filtek™ Z350XT Flowable (3 M/Solventum)	18 pat. 36 cav.	Class I	7 days; 24 months	Abs.	20 s.	USPHS
36	Schenke et al., 2025	Germany	Experimental	Filtek™ One Bulk Fill (3 M/Solventum)	30 pat. 60 cav.	Class II	Baseline; 48 months; 60 months	Abs. or rel.	20 s; 1250 mW/cm²	FDI

Abbreviations: pat– patients; cav– cavities; abs– absolut; rel– relative; sec– seconds; FDI– World Dental Federation; USPHS– United States Public Health Service

In total, 794 cavities were evaluated, with 396 restored using conventional or bulk-fill RC and 398 restored using self-adhesive RC in 356 participants aged 6 to 79 years. The follow-up period ranged from baseline (immediately after restoration) to 60 months (5 years).

Five studies evaluated Class I restorations [9, 19, 27–30]. Two studies assessed Class II restorations [13, 14, 31, 36]. Two studies included both Class I and II restorations [32, 33]. Three studies analyzed Class V restorations [20, 34, 35].

The majority of studies [9, 27, 30–35] performed the restorative procedure exclusively under rubber dam isolation, while three studies used relative isolation [19, 20, 28]. The remaining studies applied relative isolation only when rubber dam placement was not feasible [13, 14, 29, 36].

The most frequently evaluated self-adhesive RC was Vertise™ Flow (Kerr) [9, 19, 29, 30] and Surefil One™ (Dentsply Sirona) [31–33, 35], while Fusio™ Liquid Dentin (Pentron) was evaluated in three studies [20, 27, 34]. Constic (DMG) was investigated in a single study [28]. Three reports did not disclose the brand, since they used experimental RCs [13, 14, 36].

For the control group, conventional RC were used in eight studies [9, 19, 20, 28–30, 34, 36], while bulk-fill RC were used in seven studies [13, 14, 27, 31–33, 36]. In terms of viscosity, nine were high-viscosity RC [13, 14, 20, 27, 31–33, 35, 36] and six were low-viscosity (flowable) RC [9, 19, 28–30, 34].

Regarding light-curing time and irradiance, only eight studies reported both parameters, with curing times ranging from 20 to 40 s and irradiance values between 800 and 1250 mW/cm². One study did not provide any information on light-curing time or irradiance [34].

All RCTs followed manufacturer recommendations for applying restorative materials to ensure standardization of the clinical procedure and prevent technique-related discrepancies.

For the clinical performance assessment, seven reports used the FDI criteria [13, 14, 19, 20, 31, 32, 36] while eight reports used the USPHS criteria [9, 27–30, 33–35].

Meta-analysis

Failures were observed in both conventional or bulk-fill RC and self-adhesive RC over time. These failures were categorized into three distinct groups: aesthetic failures (marginal staining and color stability); functional failures (fracture/retention, marginal adaptation, and wear); biological failures (postoperative sensitivity and recurrence of caries, erosion, and abrasion).

Overall, the meta-analysis showed that no significant difference was found between cavities restored with conventional/bulk-fill RC or self-adhesive RC ($P \geq 0.08$).

Marginal staining outcome

No significant differences between materials over time were observed at 6 months (risk difference: 0.11; 95% CI: -0.03–0.25; $I^2 = 61\%$; $Chi^2 = 5.17$; $P = 0.13$), 12 months (risk difference: 0.12; 95% CI: -0.10–0.33; $I^2 = 89\%$; $Chi^2 = 37.64$; $P = 00.29$), and 18 months (risk difference: 0.07; 95% CI: -0.39–0.53; $I^2 = 87\%$; $Chi^2 = 7.58$; $P = 0.78$) (Fig. 2).

Effects estimates were not possible for 1 week, 24-, 36-, 48-, and 60-month follow-up periods due to the absence of in both materials.

Color stability outcome

No significant differences were found between the materials regardless of the follow-up periods ($P \geq 0$ 0.27) (Fig. 3). The results for each follow-up period were as follows: 6 months (risk difference: 0.07; 95% CI: -0.06–0.20; $I^2 = 77\%$; $Chi^2 = 13.12$; $P = 0.30$), 12 months (risk difference: 0.18; 95% CI: -0.14–0.49; $I^2 = 88\%$; $Chi^2 = 17.27$; $P = 0.27$), 18 months (risk difference: 0.09; 95% CI: -0.37–0.54; $I^2 = 88\%$; $Chi^2 = 8.69$; $P = 0.70$).

Effects estimates were not possible for 1 week, 24-, 36-, 48-, and 60-month follow-up periods due to no failures for both materials.

Fracture/retention outcome

No significant differences were observed between the materials ($P > 0.45$) at 24-month (risk difference: 0.03; 95% CI: -0.05–0.12; $I^2 = 0\%$; $Chi^2 = 0.01$; $P = 0.45$) and at 48 months (risk difference: -0.02; 95% CI: -0.09–0.05; $I^2 = 0\%$; $Chi^2 = 0.28$; $P = 0.59$) (Fig. 4).

Effects estimates were not possible for 1 week, 6-, 12-, 18-, 36-, and 60-month follow-up periods due to no failures for both materials.

Marginal adaptation outcome

Regarding marginal adaptation, no significant differences were observed between the materials at 6-month (risk difference: 0.43; 95% CI: -0.05–0.91; $I^2 = 94\%$; $Chi^2 = 17.93$; $P = 0.08$) and at 24-month follow-up periods (risk difference: 0.03; 95% CI: -0.05–0.12; $I^2 = 0\%$; $Chi^2 = 0.01$; $P = 0.45$) (Fig. 5).

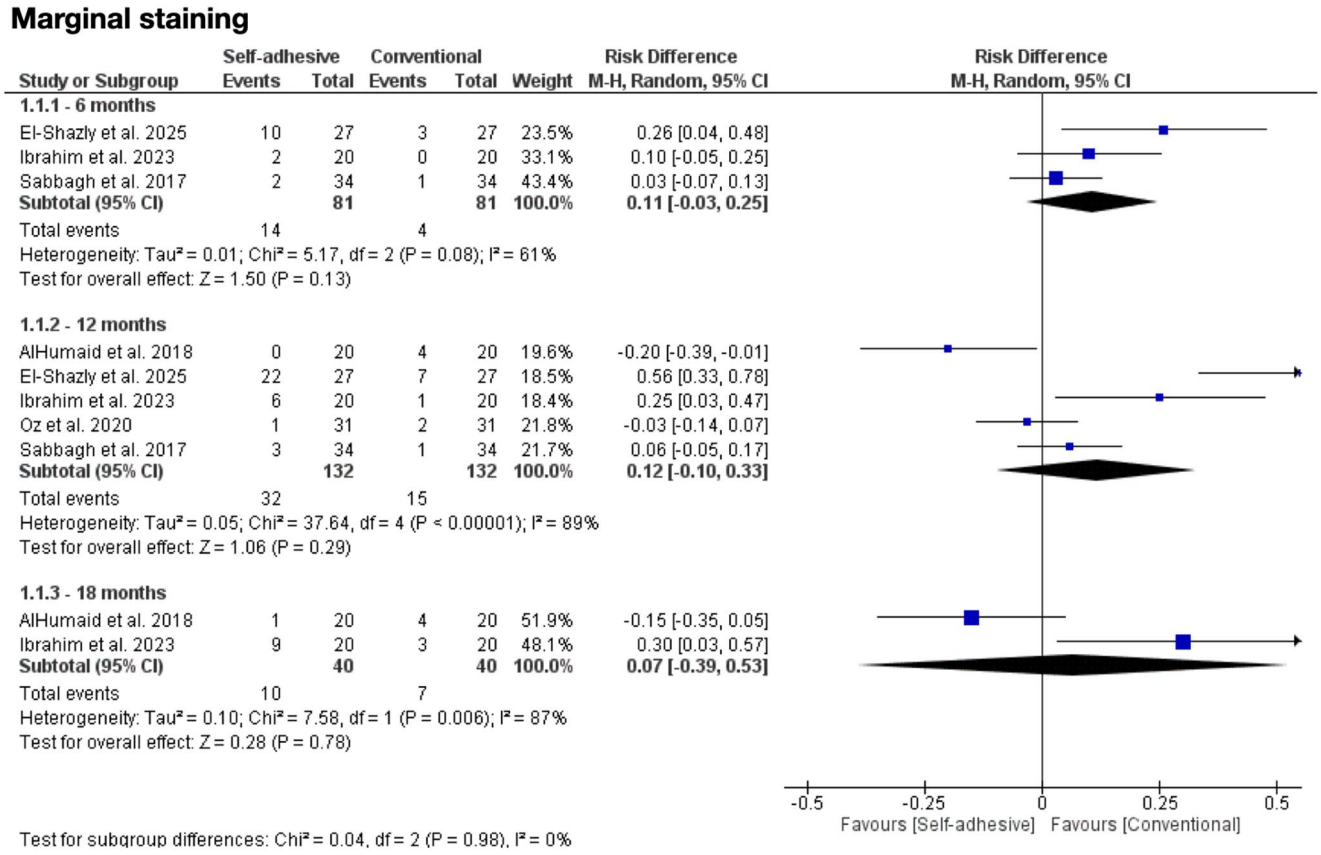


Fig. 2 Forest plot for marginal staining outcome

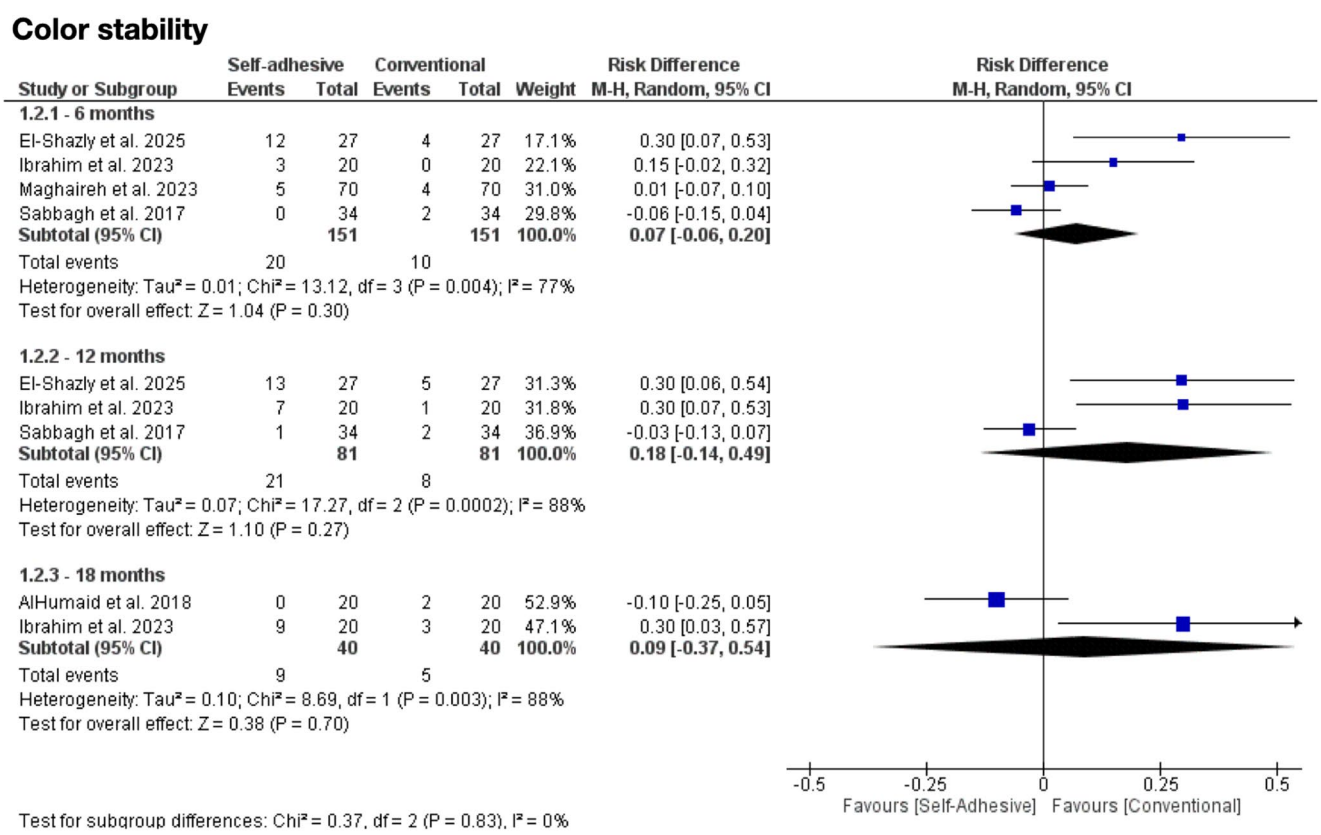


Fig. 3 Forest plot for color stability outcome

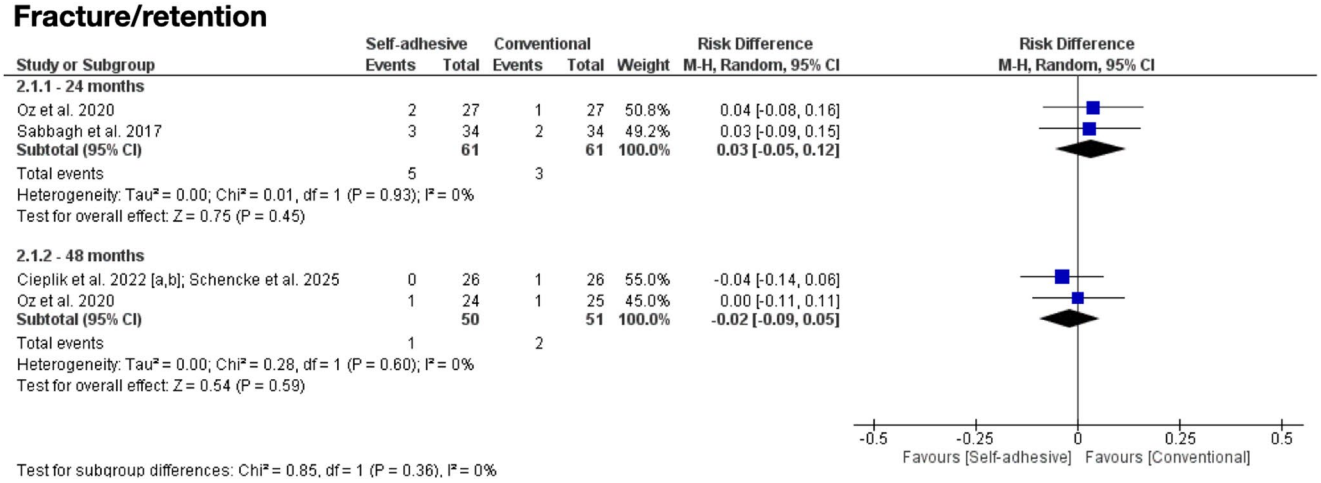


Fig. 4 Forest plot for fracture/retention outcome

Effects estimates were not possible for 1 week, 12-, 18-, 36-, 48-, and 60-month follow-up periods due to no failures for both materials.

Wear outcome

Effect estimates could not be calculated due to an insufficient number of studies reporting failures related to wear for either material, regardless of follow-up period.

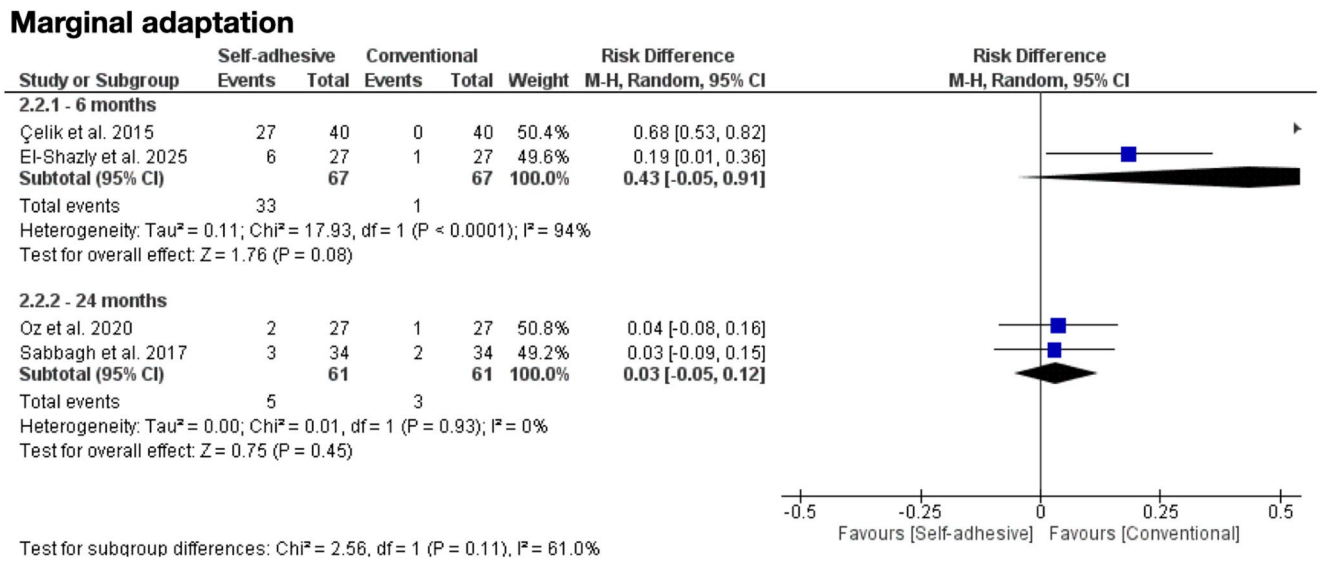


Fig. 5 Forest plot for marginal adaptation outcome

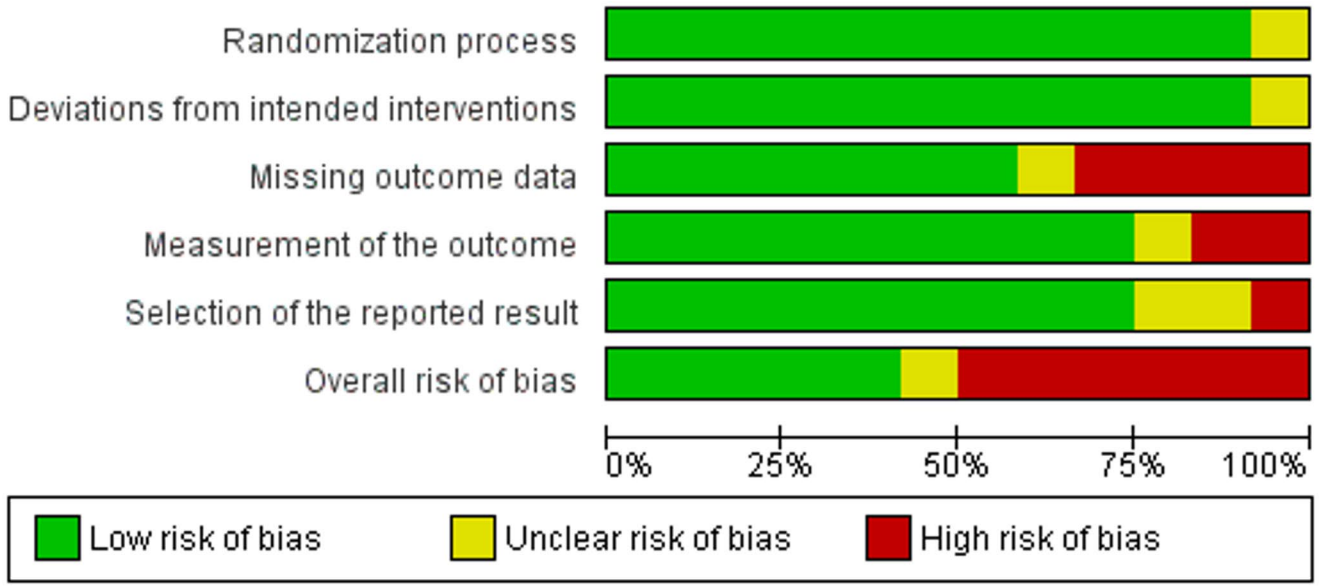


Fig. 6 Risk of bias assessment for included studies according to the *Revised Cochrane risk-of-bias tool for randomized trials* (RoB 2)

Postoperative sensitivity outcome

No events of postoperative sensitivity were reported for either material, precluding the estimation of effect sizes for this outcome.

Recurrence of caries, erosion, and abrasion outcome

No failures related to recurrence of caries, erosion, and abrasion were reported, regardless of follow-up periods. Thus, effect estimates could not be calculated.

Risk of bias and certainty of evidence

The overall risk of bias revealed that from 12 RCTs, 6 showed high risk for the analyzed outcomes [13, 14, 20, 27–29, 33, 36]. High risk of bias was more frequently in the domain related to missing outcome data [27–29], while low risk of bias was more common in the domains of randomization process and deviations from intended interventions (Figs. 6 and 7).

Six studies demonstrated low risk of bias across all domains [9, 19, 30–32, 35]. However, one RCT was classified as having uncertain risk of bias in four out of the five domains analyzed [34] (Fig. 7).

Fig. 7 Percentage of risk of bias for included studies in each domain according to the author's judgment

Table 4 displays the analysis of the certainty of evidence according to the GRADE tool for each outcome, follow-up periods and their explanations. Overall, the certainty of evidence was considered low or very low for all outcomes. The main factors lowering the certainty level were imprecision and risk of bias.

Discussion

The results of this systematic review and meta-analysis showed that the clinical performance of self-adhesive RC restorations was comparable to conventional or bulk-fill RC restorations, regardless of the follow-up period or evaluated criteria.

Among the criteria used to clinically assess the performance and effectiveness of a restorative treatment, the FDI (World Dental Federation) and USPHS (United States Public Health Service) criteria are the most commonly employed. However, the FDI criterion is recognized for its sensitivity in detecting restoration failures, making it more suitable than the USPHS criterion and appropriate for use in current studies [37].

In general, the FDI criterion evaluates restorations based on 16 parameters, while the USPHS evaluates them based on 8. However, not all parameters are mandatory in either criterion, and some can be selected depending on the study's needs [37]. For this review, the 7 most relevant and significant parameters were selected to assess the clinical performance of a restoration: marginal staining, color stability, fracture/retention, marginal adaptation, wear, postoperative sensitivity, and recurrence of caries, erosion, and abrasion. Failures in these parameters can predict adhesive failure between the restorative material and the tooth structure, significantly compromising restoration quality and potentially indicating its replacement [20].

Although RCTs require methodological rigor and substantial time and financial investment, the follow-up period is relevant to infer the longevity of materials/treatments. In this systematic review, only two studies exceeded the 3-year follow-up period, with 5 years of follow-up [19, 36], which should be considered a limitation. These studies exhibited similar clinical performance between self-adhesive and conventional RCs after 60 months of follow-up in Class I and II cavities, corroborating with previous studies [9, 27–30, 32].

Tooth-related factors, including cavity type, size, location, and the number of restored surfaces, play a crucial role in the longevity of RC restorations [38, 39]. In our review, most failures involving self-adhesive RC were associated with class V cavities [20, 34, 35], inferring that cavity design and location may affect restoration survival. Moreover, cavity margins located within dentin exhibited a higher failure rate compared to those within enamel [20, 35]. These clinical failures may be attributed to the lack of macroretention in class V cavities, the structural characteristics of dentin, and weak bonding resulting from the hydrolytic instability of the functional monomer (4-MET) and the inherently lower etching capacity of self-adhesive RC [20, 27]. Thus, the overall performance of self-adhesive RC restorations may be improved when placed in cavities exhibiting favorable macromechanical retention like class I cavities [27].

In our review, the vast majority of studies evaluated retentive cavities (Class I (O), and small Class II cavities) [13, 14, 19, 27–33, 36], which hinders the validation of self-adhesive RC bonding to tooth structure, since restorative materials without enamel and dentin bonding characteristics show adequate retention in these types of cavities [40]. A considerable number of failures were reported when self-adhesive RCs were placed in non-retentive cavities [20, 34, 35]. El-Shazly et al. [35] observed clinical signs of degradation and failure after a one-year follow-up, likely due to the formation of a weak and unstable adhesive interface, which is more susceptible to gap formation, marginal staining, loss of retention, reduced surface luster, and lower resistance to abrasion [15]. Therefore, more studies assessing

Table 4 GRADE (Grading of recommendations assessment, development and Evaluation) summary of the included studies

Outcomes	№ of participants (studies)	Certainty of the evidence (GRADE)
Marginal Staining follow-up: 1 weeks	173 (3 RCTs)	⨁⨁◯◯ Low[a]
Marginal Staining follow-up: 6 months	672 (11 RCTs)	⨁⨁◯◯ Low[b]
Marginal Staining follow-up: 12 months	497 (9 RCTs)	⨁⨁◯◯ Low[b]
Marginal Staining follow-up: 18 months	80 (2 RCTs)	⨁◯◯◯ Very low[c, d]
Marginal Staining follow-up: 24 months	319 (6 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal Staining follow-up: 36 months	108 (2 RCTs)	⨁⨁◯◯ Low[d, f]
Marginal Staining follow-up: 48 months	100 (2 RCTs)	⨁⨁◯◯ Low[b, e]
Marginal Staining follow-up: 60 months	96 (2 RCTs)	⨁⨁◯◯ Low[b, e]
Color Stability follow-up: 1 weeks	173 (3 RCTs)	⨁⨁◯◯ Low[b, e]
Color Stability follow-up: 6 months	610 (10 RCTs)	⨁⨁◯◯ Low[b, e]
Color Stability follow-up: 12 months	439 (8 RCTs)	⨁⨁◯◯ Low[b, e]
Color Stability follow-up: 18 months	80 (2 RCTs)	⨁◯◯◯ Very low[c, d]
Color Stability follow-up: 24 months	265 (5 RCTs)	⨁⨁◯◯ Low[a, e]
Color Stability follow-up: 36 months	108 (2 RCTs)	⨁⨁◯◯ Low[b, d]
Color Stability follow-up: 48 months	101 (2 RCTs)	⨁⨁◯◯ Low[b, e]
Color Stability follow-up: 60 months	96 (2 RCTs)	⨁⨁◯◯ Low[b, e]
Fractutes/retention follow-up: 1 weeks	173 (3 RCTs)	⨁⨁◯◯ Low[b, e]
Fractures/retention follow-up: 6 months	699 (11 RCTs)	⨁◯◯◯ Very low[a, g,h]
Fractures/retention follow-up: 12 months	497 (9 RCTs)	⨁⨁◯◯ Low[b, e]
Fractures/retention follow-up: 18 months	80 (2 RCTs)	⨁⨁◯◯ Low[c, e]
Fractures/retention follow-up: 24 months	319 (6 RCTs)	⨁⨁◯◯ Low[a, e]
Fractures/retention follow-up: 36 months	108 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Fractures/retention follow-up: 48 months	101 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Fractures/retention follow-up: 60 months	96 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal adaptation follow-up: 1 weeks	173 (3 RCTs)	⨁⨁◯◯ Low[b, e]
Marginal adaptation follow-up: 6 months	699 (11 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal adaptation follow-up: 12 months	498 (9 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal adaptation follow-up: 18 months	80 (2 RCTs)	⨁◯◯◯ Very low[c, e,i]

Table 4 (continued)

Outcomes	№ of participants (studies)	Certainty of the evidence (GRADE)
Marginal adaptation follow-up: 24 months	319 (6 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal adaptation follow-up: 36 months	108 (2 RCTs)	⨁◯◯◯ Very low[a, e,f]
Marginal adaptation follow-up: 48 months	99 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Marginal adaptation follow-up: 60 months	95 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Wear follow-up: 6 months	177 (3 RCTs)	⨁⨁◯◯ Low[a, e]
Wear follow-up: 12 months	124 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 1 weeks	231 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 6 months	370 (7 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 12 months	335 (6 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 24 months	251 (5 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 36 months	108 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 48 months	99 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Post-operative sensitivity follow-up: 60 months	95 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Recurrence of caries, erosion, abrasion follow-up: 6 months	528 (8 RCTs)	⨁⨁◯◯ Low[a, e]
Recurrence of caries, erosion, abrasion follow-up: 12 months	389 (7 RCTs)	⨁⨁◯◯ Low[a, e]
Recurrence of caries, erosion, abrasion follow-up: 24 months	251 (5 RCTs)	⨁⨁◯◯ Low[a, e]
Recurrence of caries, erosion, abrasion follow-up: 36 months	108 (2 RCTs)	⨁◯◯◯ Very low[a, e,f]
Recurrence of caries, erosion, abrasion follow-up: 48 months	99 (2 RCTs)	⨁⨁◯◯ Low[a, e]
Recurrence of caries, erosion, abrasion follow-up: 60 months	93 (2 RCTs)	⨁⨁◯◯ Low[a, e]

Explanations: (a) Some studies are at high risk of bias; (b) The majority of the studies are at unclear or high risk of bias; (c) All studies are at unclear or high risk of bias; (d) Evidence is limited by the scarcity of studies; (e) The 95%CI crosses the threshold of the minimally important difference; (f) Effect estimates are not similar; (g) High heterogeneity; (h) Wide 95% confidence interval; (i) High heterogeneity and effect estimates are not similar

non-retentive cavities are needed to confirm the appropriate bonding of self-adhesive RC.

Clinical studies observed failures of self-adhesive RCs in terms of marginal adaptation and marginal discoloration over time compared to baseline results immediately after restoration placement. This outcome can be explained by the fact that low-viscosity self-adhesive RCs (Vertise™ Flow with GPDM monomer, Fusio™ Liquid Dentin with 4-MET monomer, Constic with MDP monomer, SABF bulk fill with phosphoric-acid functionalized methacrylate, and Surefil One™ with bifunctional acrylate (BADEP) and acrylic acid monomer) are vulnerable to hydrolysis due to additional water absorption at the interface between the resin matrix and filler particle, which may increase its degradation in the oral environment [9, 13, 14, 27, 32].

The functional monomer present in self-adhesive RCs can directly affect the longevity of the restoration. The MDP monomer [28] tends to promote adequate long-term clinical performance, strong chemical bond with hydroxyapatite [41], insufficient demineralization [42] and forms long and hydrophobic chains [43]. In contrast, the 4-META and GPDM monomers have worse chemical bonding to

hydroxyapatite [43, 44]. The tags formed in dentin with the Fusio Liquid Dentin and Vertise Flow resins were thin and sparse [18]. Surefil One appears to be a promising self-adhesive RC [45], since its formula contains a modified polyacid (MOPOS) that bonds with hydroxyapatite and a bifunctional monomer, BADEP, which provides a cohesive and stable molecular network, strengthening bonds with the dental substrate [46].

Color changes of RCs over time are multifactorial, depending on the size and distribution of filler particles. In this regard, larger particles increase the susceptibility to color change due to hydrolysis at the filler-matrix interface [25]. Furthermore, nanoscale particles of amorphous silica and glass in self-adhesive RCs can make their surface smoother, resulting in better finishing after polishing [34]. However, superior aesthetic properties were observed in conventional bulk-fill RCs compared to self-adhesive RCs due to slight degradation at the adhesive interface, leading to small imperfections, cracks, and pigment accumulation within marginal defects [13, 14]. Similarly, our findings showed frequent failures related to color stability and marginal staining of self-adhesive RC restorations, likely attributable to microleakage and microgaps resulting from inadequate dentin demineralization, deficient bonding, and compromised marginal sealing [27, 35]. Thus, the lack of phosphoric acid etching and adhesive system application on the tooth structure [31] may result in weaker adhesion and microgaps at the tooth/restoration interface, leading to greater susceptibility to marginal staining [47].

Regarding postoperative sensitivity, it was shown that cavities restored with self-adhesive RCs exhibited similar responses to those restored with conventional RCs associated with self-etching and conventional adhesive systems [6, 26]. In general, postoperative sensitivity was reported during shorter follow-up periods, vanishing over time.

Several limitations must be addressed in this systematic review and meta-analysis. Factors such as heterogeneity in cavity design, variability in operator technique and skill, operative field isolation protocols, lack of standardization in reporting light curing parameters, variable follow-up periods, and the differences in product brands may influence inter-study comparisons. Moreover, the limited number of high-quality long-term RCTs available and potential bias related to small sample sizes and limited geographic representation should be considered in the extrapolations of our results.

For future research, it is recommended to conduct well-designed long-term RCTs with standardized outcome reporting (preferably using FDI criteria), more robust cavity classification controls, and direct head-to-head comparisons of specific brands and viscosities of self-adhesive RCs. Studies focusing on non-retentive cavity designs, particularly in anterior or cervical areas, would be valuable to better assess bonding efficacy in the absence of macromechanical retention. Moreover, investigating patient-reported outcomes such as sensitivity and aesthetic satisfaction could further strengthen the clinical relevance of such studies.

Conclusion

Within the limitations of this systematic review and meta-analysis, we concluded that the clinical performance of direct restorations placed with self-adhesive RCs is comparable to that of conventional or bulk-fill RCs, regardless of follow-up duration (6 to 48 months), cavity type or location, and outcome assessed. However, both materials showed degradation or failure over time. The certainty of evidence was considered low or very low for most evaluated criteria, indicating the need for well-designed long-term clinical studies to validate these findings.

Author contributions João Felipe Besegato: Writing– original draft, Methodology, Formal analysis, Data curation and Writing– review & editing. Andrea Freire: Writing– original draft, Methodology, Formal analysis, Data curation and Writing– review & editing. Joissi Ferrari Zaniboni: Formal analysis and Writing– review & editing. Guilherme Loubet Melo: Writing– original draft and Methodology. Aryvelto Miranda Silva: Writing– review & editing, Validation and Supervision.

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

Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval Not applicable.

Informed consent Not applicable.

Competing interests The authors declare no competing interests.

References

1. Ferracane JL (2011) Resin composite—state of the Art. Dent Mater 27:29–38. https://doi.org/10.1016/j.dental.2010.10.020
2. Ilie N, Hilton TJ, Heintze SD, Hickel R, Watts DC, Silikas N et al (2017) Academy of dental materials guidance—Resin composites: part I—Mechanical properties. Dent Mater 33:880–894. https://doi.org/10.1016/j.dental.2017.04.013
3. Demarco FF, Corrêa MB, Cenci MS, Moraes RR, Opdam NJ (2012) Longevity of posterior composite restorations: not only a matter of materials. Dent Mater 28:87–101. https://doi.org/10.1016/j.dental.2011.09.003
4. de Menezes FC, da Silva SB, Valentino TA, Oliveira MA, Rastelli AN, Gonçalves LS (2013) Evaluation of bond strength and thickness of adhesive layer according to the techniques of applying

adhesives in composite resin restorations. Quintessence Int 44:9–15. https://doi.org/10.3290/j.qi.a28737
5. Giachetti L, Scaminaci Russo D, Bambi C, Nieri M, Bertini F (2008) Influence of operator skill on microleakage of total-etch and self-etch bonding systems. J Dent 36:49–53. https://doi.org/10.1016/j.jdent.2007.10.011
6. de Oliveira NG, Lima ASLC, da Silveira MT, de Souza Araújo PR, de Melo Monteiro GQ, de Vasconcelos Carvalho M (2020) Evaluation of postoperative sensitivity in restorations with self-adhesive resin: a randomized split-mouth design controlled study. Clin Oral Investig 24:1829–1835. https://doi.org/10.1007/s00784-019-03046-0
7. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L et al (2018) Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. Dent Mater 34:78–96. https://doi.org/10.1016/j.dental.2017.11.005
8. Sachdeva P, Goswami M, Singh D (2016) Comparative evaluation of shear bond strength and Nanoleakage of conventional and self-adhering flowable composites to primary teeth dentin. Contemp Clin Dent 7:326–331. https://doi.org/10.4103/0976-237X.188549
9. Shaalan OO, Abou-Auf E (2021) A 24-month evaluation of self-adhering flowable composite compared to conventional flowable composite in Conservative simple occlusal restorations: a randomized clinical trial. Contemp Clin Dent 12:368–375. https://doi.org/10.4103/ccd.ccd_600_20
10. Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y, Peumans M (2020) From buonocore's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. J Adhes Dent 22:7–34. https://doi.org/10.3290/j.jad.a43994
11. Baroudi K, Rodrigues JC (2015) Flowable resin composites: A systematic review and clinical considerations. J Clin Diagn Res 9:ZE18–ZE24. https://doi.org/10.7860/JCDR/2015/12294.6129
12. Van Ende A, De Munck J, Lise DP, Van Meerbeek B (2017) Bulk-Fill composites: A review of the current literature. J Adhes Dent 19:95–109. https://doi.org/10.3290/j.jad.a38141
13. Cieplik F, Hiller KA, Buchalla W, Federlin M, Scholz KJ (2022) Randomized clinical split-mouth study on a novel self-adhesive bulk-fill restorative vs. a conventional bulk-fill composite for restoration of class II cavities - results after three years. J Dent 125:104275. https://doi.org/10.1016/j.jdent.2022.104275
14. Cieplik F, Scholz KJ, Anthony JC, Tabenski I, Ettenberger S, Hiller KA, Buchalla W, Federlin M (2022) One-year results of a novel self-adhesive bulk-fill restorative and a conventional bulk-fill composite in class II cavities—a randomized clinical split-mouth study. Clin Oral Investig 26:449–461. https://doi.org/10.1007/s00784-021-04019-y
15. Brueckner C, Schneider H, Haak R (2017) Shear bond strength and tooth-composite interaction with self-adhering flowable composites. Oper Dent 42:90–100. https://doi.org/10.2341/15-365-L
16. Liu X, Zhang R, Yu X, Hua F, Zhang L, Chen Z (2023) Self-adhesive flowable composite resins and flowable composite resins in permanent teeth with occlusal cavities: A systematic review and meta-analysis. J Dent 138:104691. https://doi.org/10.1016/j.jdent.2023.104691
17. Paolone G, Mandurino M, Scotti N, Cantatore G, Blatz MB (2023) Color stability of bulk-fill compared to conventional resin-based composites: A scoping review. J Esthet Restor Dent 35:657–676. https://doi.org/10.1111/jerd.13017
18. de Oliveira NG, da Silveira MT, da Silva LP, Barros KM, Costa LM, Soares ML, Carvalho MV (2021) Morphological analysis and immunohistochemical expression in restorations with self-adhesive resin: A randomized split-mouth design-controlled study. J Clin Exp Dent 13:e927–e934. https://doi.org/10.4317/jced.58413
19. Oz FD, Ergin E, Cakir FY, Gurgan S (2020) Clinical evaluation of a self-adhering flowable resin composite in minimally invasive class I cavities: 5-year results of a double blind randomized, controlled clinical trial. Acta Stomatol Croat 54:10–21. https://doi.org/10.15644/asc54/1/2
20. Çelik EU, Aka B, Yilmaz F (2015) Six-month clinical evaluation of a self-adhesive flowable composite in noncarious cervical lesions. J Adhes Dent 17:361–368. https://doi.org/10.3290/j.jad.a34556
21. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M et al (2015) PRISMA-P group, preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 4:1. https://doi.org/10.1186/2046-4053-4-1
22. Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J et al (2011) GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol 64:401–406. https://doi.org/10.1016/j.jclinepi.2010.07.015
23. Guyatt GH, Oxman AD, Kunz R, Brozek J, Alonso-Coello P, Rind D et al (2011) GRADE guidelines 6. Rating the quality of evidence—imprecision. J Clin Epidemiol 64:1283–1293. https://doi.org/10.1016/j.jclinepi.2011.01.012
24. Kalola AV, Sreejith SU, Kanodia S, Parmar A, Iyer JV, Parmar GJ (2022) Comparative clinical evaluation of a self-adhering flowable composite with conventional flowable composite in class I cavity: an in vivo study. J Conserv Dent 25:156–160. https://doi.org/10.4103/jcd.jcd_456_21
25. Peskersoy C, Oguzhan A, Gurlek O (2022) The effect of flowable composite resins on periodontal health, cytokine levels, and Immunoglobulins. Biomed Res Int 2022:6476597. https://doi.org/10.1155/2022/6476597
26. Maj A, Trzcionka A, Twardawa H, Tanasiewicz M (2020) A comparative clinical study of the self-adhering flowable composite resin vertise flow and the traditional flowable composite resin premise flowable. Coatings 10:800. https://doi.org/10.3390/coatings10080800
27. Ibrahim HA, Abdalla AI, El-Sayed HY (2023) Clinical evaluation of different composite resin systems in class I cavities (18-month randomized clinical trial). Braz Dent Sci 26:e3737. https://doi.org/10.4322/bds.2023.e3737
28. Oz FD, Meral E, Gurgan S (2021) Does a Self-adhesive flowable resin composite perform similarly to highly filled and conventional flowable resin composites in occlusal cavities? A 2-year follow-up study. J Adhes Dent 23:497–503. https://doi.org/10.3290/j.jad.b2288205
29. Sabbagh J, Dagher S, El Osta N, Souhaid P (2017) Randomized clinical trial of a self-adhering flowable composite for class I restorations: 2-year results. Int J Dent 2017:5041529. https://doi.org/10.1155/2017/5041529
30. Shaalan OO, Abou-Auf E, El Zoghby AF (2018) Clinical evaluation of self-adhering flowable composite versus conventional flowable composite in Conservative class I cavities: randomized controlled trial. J Conserv Dent 21:485–490. https://doi.org/10.4103/JCD.JCD_210_18
31. Ellithy MS, Abdelrahman MH, Afifi RR (2024) Comparative clinical evaluation between self-adhesive and conventional bulk-fill composites in class II cavities: A 1-year randomized controlled clinical study. J Esthet Restor Dent 36:1311–1325. https://doi.org/10.1111/jerd.13242
32. Albelasy EH, Hamama HH, Chew HP, Montasser M, Mahmoud SH (2024) Clinical performance of two ion-releasing bulk-fill composites in class I and class II restorations: A two-year evaluation. J Esthet Restor Dent 36:723–736. https://doi.org/10.1111/jerd.13193
33. Maghaireh GA, Albashaireh ZS, Allouz HA (2023) Postoperative sensitivity in posterior restorations restored with self-adhesive

and conventional bulk-fill resin composites: A randomized clinical split-mouth trial. J Dent 137:104655. https://doi.org/10.1016/j.jdent.2023.104655
34. AlHumaid J, Al Harbi FA, ElEmbaby AE (2018) Performance of self-adhering flowable composite in class V restorations. J Contemp Dent Pract 19:785–791. https://doi.org/10.5005/jp-journals-10024-2337
35. El-Shazly RK, El-Zayat IMA, Mohsen MMA, Labib MEM (2025) Clinical evaluation of self-adhesive bulk-fill composite versus conventional nano-hybrid composite in cervical cavities-a randomized controlled clinical trial. J Esthet Restor Dent 37:1907–1919. https://doi.org/10.1111/jerd.13472
36. Schenke IM, Pfister JL, Hiller KA, Buchalla W, Cieplik F, Ettenberger S et al (2025) Randomized clinical split-mouth study on a self-adhesive vs. a conventional bulk-fill composite in class II cavities: results after five years. J Dent 156:105663. https://doi.org/10.1016/j.jdent.2025.105663
37. Marquillier T, Doméjean S, Le Clerc J, Chemla F, Gritsch K, Maurin JC et al (2018) The use of FDI criteria in clinical trials on direct dental restorations: A scoping review. J Dent 68:1–9. https://doi.org/10.1016/j.jdent.2017.10.007
38. Da Rosa Rodolpho PA, Donassollo TA, Cenci MS, Loguercio AD, Moraes RR, Bronkhorst EM et al (2011) 22-year clinical evaluation of the performance of two posterior composites with different filler characteristics. Dent Mater 27:955–963. https://doi.org/10.1016/j.dental.2011.06.001
39. Casagrande L, Seminario AT, Correa MB, Werle SB, Maltz M, Demarco FF, Araujo FB (2017) Longevity and associated risk factors in adhesive restorations of young permanent teeth after complete and selective caries removal: a retrospective study. Clin Oral Investig 21:847–855. https://doi.org/10.1007/s00784-016-1832-1
40. Ishikiriama SK, Mondelli RF, Kano SC, Ishikiriama A, Mondelli J (2007) Role of additional retention on marginal adaptation and sealing of large resin composite class II restorations. Oper Dent 32:564–570. https://doi.org/10.2341/06-158
41. Gorseta K, Borzabadi-Farahani A, Vrazic T, Glavina D (2019) An in-vitro analysis of microleakage of self-adhesive fissure sealant vs. conventional and Gic fissure sealants. Dent J (Basel) 7:32. https://doi.org/10.3390/dj7020032
42. Chatra A, Nair PMS, D'Costa VF, Kukkila J, Mayya A, Chatra L, Mayya SS (2024) Shear bond strength of self-adhesive versus conventional flowable composites: an in vitro study. J Int Soc Prev Community Dent 14:362–368. https://doi.org/10.4103/jispcd.jispcd_14_24
43. Peterson J, Rizk M, Hoch M, Wiegand A (2018) Bonding performance of self-adhesive flowable composites to enamel, dentin and a nano-hybrid composite. Odontology 106:171–180. https://doi.org/10.1007/s10266-017-0324-3
44. Josic U, Teti G, Ionescu A, Maravic T, Mazzitelli C, Cokic S et al (2024) Cytotoxicity and Microbiological behavior of universal resin composite cements. Dent Mater 40:1515–1523. https://doi.org/10.1016/j.dental.2024.07.004
45. Alghamdi AA, Athamh S, Alzhrani R, Filemban H (2024) Assessment of the Micro-Tensile bond strength of a novel bioactive dental restorative material (Surefil One). Polym (Basel) 16:1558. https://doi.org/10.3390/polym16111558
46. Fu J, Kakuda S, Pan F, Hoshika S, Ting S, Fukuoka A et al (2013) Bonding performance of a newly developed step-less all-in-one system on dentin. Dent Mater J 32:203–211. https://doi.org/10.4012/dmj.2012-204
47. Szesz A, Parreiras S, Reis A, Loguercio A (2016) Selective enamel etching in cervical lesions for self-etch adhesives: a systematic review and meta-analysis. J Dent 53:1–11. https://doi.org/10.1016/j.jdent.2016.05.009

Authors and Affiliations

João Felipe Besegato[1] · Andrea Freire[1] · Joissi Ferarri Zaniboni[2] · Guilherme Loubet Melo[1] · Aryvelto Miranda Silva[3]

✉ João Felipe Besegato
joao.besegato@ufms.br

Andrea Freire
andrea.freire@ufms.br

Joissi Ferarri Zaniboni
joissi.zaniboni@usp.br

Guilherme Loubet Melo
loubet.melo@ufms.br

Aryvelto Miranda Silva
aryvelto.miranda@ufpi.edu.br

[1] School of Dentistry, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil

[2] Department of Restorative Dentistry, School of Dentistry of Ribeirão Preto (FORP), University of São Paulo (USP), Ribeirão Preto, SP, Brazil

[3] Department of Restorative Dentistry, Federal University of Piauí (UFPI), Teresina, PI, Brazil

cadernos Saúde Coletiva
ISSN 2358-291X (Online)

ARTIGO ORIGINAL

Relação da saúde bucal com reações hansênicas em município hiperendêmico para hanseníase

Relation between oral health and hansen's disease reactions in a hyperendemic city for hansen's disease

Adriano de Aguiar Filgueira[1], Maria Socorro Carneiro Linhares[2], Mariana Ramalho Farias[3], Angelo Giuseppe Roncalli da Costa Oliveira[4], Ana Karine Macedo Teixeira[3]

[1]*Residência Multiprofissional em Saúde da Família, Universidade Estadual Vale do Acaraú (UVA) - Sobral (CE), Brasil.*
[2]*Departamento de Enfermagem, Universidade Estadual Vale do Acaraú (UVA) - Sobral (CE), Brasil.*
[3]*Universidade Federal do Ceará (UFC), Campus Sobral - Sobral (CE), Brasil.*
[4]*Universidade Federal do Rio Grande do Norte (UFRN) - Natal (RN), Brasil.*

Como citar: Filgueira AA, Linhares MSC, Farias MR, Oliveira AGRC, Teixeira AKM. Relação da saúde bucal com reações hansênicas em município hiperendêmico para hanseníase. Cad Saúde Colet, 2020;28(1):44-55. https://doi.org/10.1590/1414-462X202028010033

Resumo

Introdução: Péssimas condições de saúde bucal vêm sendo apontadas como prejudiciais às pessoas acometidas pela hanseníase, pois contribuem para a piora do quadro clínico desses indivíduos. **Objetivo:** Este trabalho objetiva avaliar as condições de saúde bucal dos indivíduos atendidos por um serviço especializado no município de Sobral, no Ceará, e comparar dois grupos (com e sem reação hansênica) quanto às condições orais. **Método:** Trata-se de um estudo transversal realizado no Ambulatório de Hanseníase do município de Sobral, no Ceará, com 56 pacientes em tratamento. Foram realizados um levantamento epidemiológico em saúde bucal e uma entrevista que coletaram informações sobre condições de saúde bucal, dados socioeconômicos, utilização dos serviços odontológicos e percepção entre doença e saúde bucal. Para a análise estatística entre os grupos, foi realizada uma análise bivariada, seguida por uma análise multivariada. **Resultados:** Houve predomínio de sexo masculino, baixa escolaridade e diagnóstico multibacilar. Apesar da relação estatisticamente significante entre reação hansênica e cárie dentária, necessidade de exodontia e/ou endodontia, sangramento gengival, cálculo dentário e bolsa periodontal, apenas esta última apresentou associação significativa (p = 0,019) na análise multivariada. **Conclusão:** Os participantes deste estudo apresentaram precárias condições de saúde bucal, sendo piores naqueles com reações hansênicas.

Palavras-chave: hanseníase; saúde bucal; odontologia em saúde pública.

Abstract

Background: Poor oral health conditions are being considered as harmful for people affected by Hansen's disease, since they contribute to worsening the clinical condition of these individuals. **Objective:** This study aims to evaluate the oral health conditions of individuals attended by a specialized service in the city of Sobral, Ceará, and to compare two groups (with and without Hansen's disease reaction) regarding oral conditions. **Method:** A cross-sectional study was carried out at the Ambulatório de Hanseníase in the city of Sobral, Ceará, Brazil, with 56 patients being treated. An epidemiological survey was conducted on oral health and an interview that collected information on oral health conditions, socioeconomic data, use of dental services and perception between the disease and oral health. For the statistical analysis between the groups, a bivariate analysis was performed, followed by a multivariate analysis. **Results:** Predominance was the male gender, low level of schooling and multibacillary diagnosis. Despite the statistically significant relationship between presence of Hansen's disease reaction and the presence of dental caries, need for exodontia and / or endodontics, gingival bleeding, dental calculus and

Trabalho realizado no Ambulatório de Hanseníase, Centro de Infectologia – Sobral (CE), Brasil.
Correspondência: Adriano de Aguiar Filgueira. E-mail: adriano.odonto@yahoo.com.br
Fonte de financiamento: nenhuma.
Conflito de interesses: nada a declarar.
Recebido em: Mar. 04, 2017. Aprovado em: Jun. 04, 2019

periodontal pocket, only the latter had a significant association (p = 0.019) in the multivariate analysis. **Conclusion:** The participants of this study presented poor oral health conditions, being worse in those who presented Hansen's disease reactions.

Keywords: leprosy; oral health; public health dentistry.

INTRODUÇÃO

Segundo o Ministério da Saúde[1], hanseníase é uma doença crônica granulomatosa proveniente de infecção causada pelo *Mycobacterium leprae*. Esse bacilo afeta a pele e as células dos nervos periféricos, resultando em lesões cutâneas e neuropatia[2].

O estigma sobre a doença[3-5] faz com que o preconceito contra os pacientes acometidos pela hanseníase aumente à medida que os sinais e sintomas se tornam mais evidentes tanto para o indivíduo infectado quanto para a sociedade. As formas multibacilares da doença são mais susceptíveis a desenvolver sequelas e/ou reações advindas da hanseníase, o que torna mais notória a infecção pelo bacilo de Hansen e, consequentemente, o aumento da discriminação[3,5].

Quando não tratada precocemente, a hanseníase pode levar a sequelas neurológicas, oftalmológicas e motoras[4]. As deficiências físicas ocasionadas pelo comprometimento neurológico periférico, que podem afetar os indivíduos antes, no decorrer ou depois do tratamento, são mais frequentes nas formas mais graves da doença[6].

Apesar de os episódios reacionais poderem aparecer em qualquer uma das formas clínicas, são mais raros nos casos mais brandos da doença[7]. Estima-se que 25-30% das pessoas com hanseníase desenvolvem reações ou dano neural em algum momento, sendo desencadeada, principalmente, por processos infecciosos, infestações, distúrbios hormonais e fatores emocionais[8].

Embora poucos estudos tenham encontrado relação das infecções odontológicas com a presença de reações hansênicas, processos infecciosos em cavidade oral, assim como em todo o corpo, podem estimular ações do sistema imunológico, o que favorece o aparecimento de reações em pacientes acometidos pelo bacilo de Hansen[9-11].

O diagnóstico precoce, o tratamento oportuno para todos os casos diagnosticados, a prevenção e o tratamento de incapacidades, bem como a vigilância dos contatos domiciliares, são propostas para o controle da hanseníase no Brasil de acordo com o manual técnico-operacional das Diretrizes para Vigilância, Atenção e Eliminação da Hanseníase como Problema de Saúde Pública, normatizado pela Portaria nº 149, de fevereiro de 2016[12,13]. Para isso, uma abordagem mais integral deve ser realizada, considerando como relevantes o momento do diagnóstico, o período de tratamento com a poliquimioterapia (PQT) e o período de pós-alta da PQT, de acordo com questões como deficiência, limitação de atividade e restrição de participação social[10].

Em 2016, o Brasil perdeu em números de casos novos apenas para a Índia, registrando 25.218 ocorrências, atingindo uma taxa de detecção de 12,2/100 mil habitantes e fazendo com que o país fosse classificado como de alta carga para a doença. O país vem registrando queda no número de casos novos registrados ao longo do tempo por causa, principalmente, das estratégias do Ministério da Saúde quanto à abordagem da doença no Programa Saúde na Escola dos municípios com taxa de detecção elevada, não só para identificar casos entre as crianças, mas para informar os estudantes e mobilizar o conjunto das famílias[14,15].

No Ceará, a taxa de detecção em 2017 foi de 18,7 novos casos a cada 100 mil habitantes, padrão considerado alto pelo Ministério da Saúde, segundo definido pelo manual técnico-operacional das Diretrizes para Vigilância, Atenção e Eliminação da Hanseníase como Problema de Saúde Pública[12,13]. O município de Sobral, situado na região noroeste do Estado do Ceará, apresenta elevadas taxas de detecção de hanseníase e foi considerado hiperendêmico por muitos anos, com taxas superiores a 40 casos novos a cada 100 mil habitantes. Em 2017, apresentou taxa de detecção de 38,7 / 100 mil habitantes, sendo classificado no parâmetro muito alto[14,16,17].

Este estudo tem o objetivo de avaliar as condições de saúde bucal de indivíduos acometidos por sequelas de hanseníase e reações hansênicas atendidos no Ambulatório de

cadernos Saúde Coletiva

Hanseníase de Sobral, no Ceará, comparando os dois grupos quanto às doenças bucais e ao uso dos serviços odontológicos.

A crescente associação entre saúde bucal e reação hansênica tem tornado relevantes os estudos com essa temática. Além disso, a região geográfica de realização desta pesquisa é considerada hiperendêmica para a hanseníase e não possui um protocolo definido para o atendimento odontológico desses pacientes, o que pode favorecer a implementação de ações de vigilância em saúde bucal na região.

MÉTODO

Trata-se de um estudo transversal analítico, conduzido no Ambulatório de Hanseníase localizado no município de Sobral, no Ceará. Os sujeitos da pesquisa corresponderam a 56 pacientes diagnosticados com hanseníase e que, no período de coleta de dados desta pesquisa (setembro a dezembro de 2014), apresentavam alguma sequela advinda da hanseníase ou algum tipo de reação hansênica e estavam em tratamento no serviço especializado, independentemente do tempo de contato com o serviço de referência.

A microrregião de Sobral contém 24 municípios da 11ª CRES (Coordenadoria Regional de Saúde) e um contingente populacional de quase 600 mil habitantes[18]. O Ambulatório de Hanseníase localizado no município de Sobral é o principal centro de referência aos pacientes com hanseníase da região, possuindo serviço médico e de fisioterapia especializados principalmente aos indivíduos que apresentam condições mais graves da doença, como reações e/ou sequelas de hanseníase.

Foram consideradas sequelas de hanseníase qualquer deforminadade/incapacidade que dificultasse a realização de atividades do dia a dia e que foram consequências da infecção pelo bacilo de Hansen, como mãos em garra, pé caído, entre outras. As reações hansênicas, por outro lado, poderiam ser do tipo 1 ou 2. Ambas as condições foram diagnosticadas por uma médica especialista e uma fisioterapeuta em conjunto. Com base nessas condições, os pacientes foram alocados em dois grupos para que fosse possível a comparação das condições de saúde bucal com o uso dos serviços odontológicos entre eles.

Foram excluídos da pesquisa aqueles que estavam realizando tratamento simultâneo para reações hansênicas e sequelas de hanseníase por causa da dificuldade de enquadramento em um dos grupos. Como critério de inclusão, visando reduzir viés de seleção, foi adotada a presença de pelo menos um dente em cavidade oral para que se pudesse avaliar a presença de infecção oral.

A coleta de dados ocorreu no Ambulatório de Hanseníase, após a consulta médica e/ou fisioterápica para tratamento do agravo da hanseníase. A coleta consistiu em duas etapas: levantamento epidemiológico e entrevista com base em um roteiro estruturado.

O levantamento epidemiológico foi fundamentado na metodologia do Projeto Saúde Bucal Brasil (SB Brasil). Foi investigada a presença de cárie dentária a partir do índice CPO-D (número de dentes cariados, perdidos e restaurados) e ceo-d (número de dentes cariados, indicados para exodontia e restaurados), doença periodontal a partir do índice IPC (índice periodontal comunitário, no qual são avaliados o sangramento gengival, a presença de cálculo dentário e a bolsa periodontal) e PIP (indicativo de perda de inserção periodontal) e índice de uso e necessidade de prótese dentária. Para o exame odontológico, foram utilizados o espelho plano bucal e a sonda periodontal, ambos esterilizados, e o procedimento foi realizado sob luz natural e com examinador e paciente sentados, conforme preconizado pela Organização Mundial de Saúde (OMS)[19].

As seguintes informações foram coletadas: dados socioeconômicos (idade, sexo, escolaridade, renda familiar e emprego), condições de saúde (desde outros agravos à saúde que poderiam ocasionar respostas do sistema imunológico até condições que alteravam níveis hormonais, como puberdade e gestação), uso dos serviços odontológicos (se já foi ao dentista alguma vez na vida, última vez que procurou o serviço odontológico, tipo de serviço odontológico utilizado, se realizou e concluiu o tratamento nos últimos 12 meses), percepção do paciente em relação à doença, presença de reações hansênicas e classificação operacional.

A coleta de dados foi realizada por um residente de Odontologia da Residência Multiprofissional em Saúde da Família do município de Sobral, devidamente capacitado e calibrado.

As variáveis foram expressas na forma de frequências absolutas. Para avaliar a existência de associação entre condições socioeconômicas, utilização do serviço odontológico e condições de saúde bucal (variáveis independentes) com a presença de reação hansênica (variável dependente), foram empregados o teste do qui-quadrado de Pearson (x^2) e o teste exato de Fisher. Nas análises, foi considerado o nível de significância de 5%. As informações obtidas foram analisadas pelo programa *Statistical Package for the Social Sciences (SPSS), versão 20*.

A Comissão Científica do município de Sobral autorizou a realização da pesquisa, sendo esta posteriormente aprovada pelo Comitê de Ética em Pesquisa da Universa Estadual Vale do Acaraú (CEP/UVA – processo nº 793.452, de 10 de setembro de 2014). Foram respeitados todos os princípios éticos da Resolução nº 466/2012, e todos os pacientes assinaram o Termo de Consentimento Livre e Esclarecido (TCLE).

RESULTADOS

Participaram da pesquisa 56 indivíduos que estavam em tratamento no Ambulatório de Hanseníase de Sobral no período de setembro a dezembro de 2014, em que 50% eram pacientes acometidos por sequelas de hanseníase e os outros 50% estavam em tratamento para reações hansênicas. Além disso, 66% haviam concluído o tratamento poliquimioterápico para a hanseníase. A classificação operacional multibacilar correspondeu a 85,7% dos participantes. Um percentual de 38,3% apontou que estava passando ou passou recentemente por alguma das condições de saúde investigadas, dos quais 81,8% afirmaram ter hipertensão e/ou diabetes. Puberdade, gravidez, puerpério/amamentação e cirurgia recente não foram relatadas pelos participantes da pesquisa.

Na Tabela 1, são representados os dados socioeconômicos, o uso dos serviços odontológicos e a percepção dos pacientes em relação à doença. Dos participantes da pesquisa, 67,9% eram do sexo masculino. A faixa etária de adulto jovem e adulto (de 20 a 59 anos) representou a maior parcela dos indivíduos, com 69,6%, bem como aqueles que declararam possuir até 7 anos de estudo, com 60,7%, e renda familiar de até 1 salário mínimo, com 35,7%, sendo o valor máximo desta de 2,5 salários mínimos.

Tabela 1. Distribuição dos pacientes com hanseníase segundo perfil socioeconômico, utilização dos serviços odontológicos e compreensão da doença em relação à saúde bucal, Sobral, 2014

VARIÁVEL	n	%
Sexo		
Masculino	38	67,9
Feminino	18	32,1
Idade		
Até 9 anos	1	1,8
10-19 anos	4	7,1
20-59 anos	39	69,6
60 anos ou mais	12	21,4
Escolaridade		
Até 7 anos	39	69,6
8 anos ou mais	17	30,4

n: número de pessoas

cadernos Saúde Coletiva

Tabela 1. Continuação...

VARIÁVEL	n	%
Trabalha		
Sim	34	60,7
Não	22	39,3
Renda familiar		
Até 1 salário mínimo	20	35,7
Acima de 1 salário mínimo	36	64,3
Classificação operacional		
Paucibacilar	8	14,3
Multibacilar	48	85,7
Ida ao dentista alguma vez na vida		
Sim	49	87,5
Não	7	12,5
Última vez que foi ao dentista		
Menos de 1 ano	23	46,9
1 ano ou mais	26	53,1
Onde foi ao dentista pela última vez		
Serviço público	30	61,2
Serviço privado	19	38,8
Hanseníase traz prejuízo para a saúde bucal		
Sim	16	28,6
Não	38	67,9
Não soube responder	2	3,6
Dificuldade para realizar higiene bucal		
Sim	15	26,8
Não	41	73,2
Orientação para procurar o serviço odontológico		
Sim	22	39,3
Não	34	60,7
Conclusão de tratamento odontológico nos últimos 12 meses		
Sim	11	47,8
Não	12	52,2
Última visita ao dentista		
Antes do tratamento da hanseníase	20	35,7
Durante ou após o tratamento da hanseníase	29	51,8
Nunca foi ao dentista	7	12,5

n: número de pessoas

Um percentual de 12,5% relatou nunca ter ido ao dentista em algum momento da vida. Dos que frequentaram o dentista pelo menos uma vez na vida, 61,2% procuraram o serviço público. Com relação à última vez que foram ao serviço odontológico, 46,9% disseram ter ido há menos de um ano.

Ademais, 67,9% relataram não perceber algum prejuízo à saúde bucal advindo da hanseníase. No entanto, 26,8% dos indivíduos apontaram ter dificuldades para realizar a higiene oral, normalmente em razão das sequelas deixadas pela doença nos membros superiores.

Ainda, 60,7% afirmaram que não houve orientação para a procura do serviço odontológico no início do tratamento para a hanseníase, e 51,8% procuraram o dentista pela última vez durante a realização ou após o término do tratamento farmacológico da hanseníase. Dos indivíduos que realizaram tratamento odontológico nos últimos 12 meses, 47,8% conseguiram fazer tratamento completo.

Na Tabela 2, constam a média do índice CPO-D e seus componentes segundo a faixa etária. Verificou-se o aumento do índice com a idade: enquanto o CPO-D foi de 4,00 na faixa etária de 10 a 19 anos, alcançou 18,08 para aqueles com idade maior ou igual a 60 anos. A exceção foi uma criança de 6 anos que relatou nunca ter ido ao dentista e apresentou índice de ceo-d igual a 13, correspondendo exclusivamente ao componente cariado. De 10 a 19 anos, o componente "dente restaurado" foi o que prevaleceu, enquanto, nas idades mais elevadas, o componente "dente perdido" foi o predominante.

Tabela 2. Média do índice CPO-D e proporção de seus componentes segundo faixa etária, Sobral, 2014

Faixa etária	n	Cariado (SD)	Restaurado com cárie (SD)	Restaurado sem cárie (SD)	Perdido (SD)	CPOD (SD)
10-19 anos	4	1,25 (1,5)	0	3,75 (2,8)	0,25 (0,5)	4,00 (3,2)
20-59 anos	39	2,95 (4,3)	0,10 (0,3)	1,31 (2,7)	8,05 (7,4)	9,46 (7,0)
60 anos ou mais	12	2,08 (2,9)	0	0,50 (1,7)	17,58 (7,1)	18.08 (6,8)

n: número de pessoas; SD: desvio padrão

Na Tabela 3, constam os resultados relacionados à condição do periodonto. Na faixa etária de 10 a 19 anos, metade apresentou sangramento gengival à sondagem, com presença de cálculo dentário. Nas demais faixas etárias, a existência de cálculo foi a condição periodontal mais prevalente, seguida de sangramento gengival e bolsa periodontal.

Tabela 3. Frequência de indivíduos segundo o sextante com pior condição periodontal e faixa etária, Sobral, 2014*

Faixa etária	n	Sangramento		Cálculo		Bolsa periodontal	
		n	%	n	%	n	%
10-19 anos	4	2	50	2	50	0	0
20-59 anos	39	29	74,3	36	92,3	21	53,8
60 anos ou mais	12	9	75	10	83,3	5	41,6

*Um mesmo indivíduo pode apresentar mais de uma condição periodontal; n: número de pessoas

Com relação ao uso e à necessidade de prótese dentária, verificou-se que 75% dos pacientes não faziam uso de prótese superior, apesar de 46,4% necessitarem de algum tipo de prótese na arcada superior, sendo 5,4% de prótese total (PT) e 41% de prótese parcial removível (PPR). Dos 25% que faziam uso de prótese superior, 7,1% eram PT, 16,1%, PPR, e 1,8%, ponte fixa. Quanto à prótese inferior, 92,9% não faziam uso, porém 73,2% possuíam a necessidade dela, sendo 3,6% de PT e 69,7% de PPR. Dos 7,2% em uso de prótese inferior, todos eram de prótese parcial removível.

cadernos Saúde Coletiva

Na Tabela 4, em análise não ajustada, não houve associação estatisticamente significativa entre presença de reação hansênica e condições de saúde, estresse, sexo, renda familiar, escolaridade e última vez que procurou um cirurgião-dentista. Já em relação às condições de saúde bucal, presença de pelo menos um dente com cárie (p = 0,007), necessidade de exodontia/endodontia em pelo menos um dente (p = 0,004), presença de pelo menos um sextante com sangramento gengival (p = 0,024), cálculo dentário (p = 0,002) e bolsa periodontal (p = 0,001) apresentaram associação estatística significativa com a presença de reação hansênica.

Tabela 4. Distribuição dos pacientes com reação hansênica segundo variáveis sociodemográficas, uso de serviço odontológico e condições de saúde bucal, Sobral, 2014

Variável	Reação hansênica				Não ajustada		Ajustada	
	Sim		Não		p	RP (IC)	p	RP (IC)
	n	%	n	%				
Sexo								
Masculino	18	47,4	20	52,6	0,55	1,17 (0,68-1,99)	-	-
Feminino	10	55,6	8	44,4				
Renda								
Até 1 salário mínimo	7	35,0	13	65,0	0,12	0,6 (0,31-1,15)	-	-
Mais que 1 salário mínimo	21	58,3	15	41,7				
Escolaridade								
Até 7 anos	18	46,2	21	53,8	0,36	1,27 (0,75-2,14)	-	-
8 anos ou mais	10	58,8	7	41,2				
Última vez que procurou um cirurgião-dentista								
Antes do tratamento da hanseníase	10	50,0	10	50,0	0,71	0,89 (0,49-1,62)	-	-
No decorrer ou depois do tratamento da hanseníase	13	44,8	16	55,2				
Estresse								
Sim	6	66,7	3	33,3	0,21*	1,42 (0,81-2,47)	-	-
Não	22	46,8	25	53,2				
Condições de saúde								
Sim	10	45,5	12	54,5	0,591	0,85 (0,49-1,49)	-	-
Não	18	52,9	16	47,1				
Cárie								
Sim	22	68,8	10	31,2	0,007	2,75 (1,32-5,71)	0,085	2,06 (0,90-4,70)
Não	6	25,0	18	75,0				

*Teste exato de Fisher; n: número de pessoas; p: valor de p; RP (IC): razão de prevalência (intervalo de confiança)

Tabela 4. Continuação...

Variável	Reação hansênica				Não ajustada		Ajustada	
	Sim		Não		p	RP (IC)	p	RP (IC)
	n	%	n	%				
Necessidade de exodontia e/ou endodontia								
Sim	15	75,0	5	25,0	0,004	2,07 (1,25-3,43)	0,626	0,87 (0,49-1,52)
Não	13	36,1	23	63,9				
Sangramento								
Sim	25	62.5	15	37,5	0,024*	3,33 (1,16-9,50)	-	-
Não	3	18,8	13	81,2				
Cálculo								
Sim	28	58,3	20	41,7	0,002*	2,40 (1,71-3,35)	-	-
Não	0	0,0	8	100,0				
Bolsa periodontal								
Sim	20	79,6	6	23,1	0,001	2,88 (1,53-5,41)	0,019	2,33 (1,14-4,75)
Não	8	26,7	22	73,3				

*Teste exato de Fisher; n: número de pessoas; p: valor de p; RP (IC): razão de prevalência (intervalo de confiança)

Na análise ajustada do mesmo modelo, todas as variáveis relacionadas às condições de saúde, com exceção de sangramento e cálculo dentário (utilizado o teste de Fisher por causa da pequena amostra), apresentaram relação estatisticamente significante, porém apenas a presença de bolsa periodontal permaneceu com associação significativa (p = 0,019).

DISCUSSÃO

Os pacientes que participaram do estudo estavam em tratamento no Ambulatório de Hanseníase de Sobral para reações ou sequelas advindas da hanseníase. A pequena amostra de 56 indivíduos pode ser justificada pela dificuldade de enquadramento deles em apenas um dos grupos: com ou sem reação hansênica, bem como pelo número total de pacientes desdentados que foram excluídos da pesquisa. Além disso, o curto período de coleta de dados e a pequena demanda de pacientes novos no serviço (os mesmos pacientes retornavam várias vezes para continuidade das sessões de fisioterapia e/ou reavaliações médicas) contribuíram para o baixo número de pacientes avaliados.

Estudos apontam que as reações e as sequelas podem ser detectadas antes, no decorrer ou depois do tratamento poliquimioterápico da doença[7,8]. Como a medicação para o tratamento da hanseníase tem início logo após o diagnóstico por um profissional habilitado, o que era condição essencial para a inclusão nesta pesquisa, então todos os pacientes deste estudo haviam iniciado e/ou concluído o tratamento poliquimioterápico.

Dos indivíduos participantes, a maioria foi acometida pelas formas mais graves da doença, o que condiz com a literatura quando aponta que os pacientes mais propícios a desenvolver reações e/ou apresentar alguma sequela da hanseníase são aqueles que apresentam a forma multibacilar, pois possuem alto teor bacilífero[2,20,21].

Alguns estudos também encontraram uma maior frequência do sexo masculino nos pacientes infectados pelo *Mycobacterium leprae*[9,21]. O percentual de homens aumenta quando se trata das formas mais graves de hanseníase[2]. A explicação de os homens serem os mais acometidos pelas formas mais graves da doença se dá pela dificuldade histórica de reconhecer

suas necessidades, cultivando o pensamento mágico que rejeita a possibilidade de adoecer. A Política Nacional de Atenção Integral à Saúde do Homem afirma que eles adentram no sistema de saúde por meio da atenção especializada, o que leva ao agravo da morbidade pelo retardamento da atenção, gerando maior custo ao SUS[22].

A faixa etária mais prevalente nesta pesquisa foi a de adulto jovem e adulto (20 a 59 anos). Outros estudos também encontraram a idade adulta como a mais prevalente[11,23]. Capelo e Pagliuca[24] verificaram que há uma maior tendência das formas multibacilares com o avançar da idade e que a maior parcela dos pacientes com hanseníase está na faixa etária economicamente ativa, o que pode dificultar o diagnóstico, tratamento e acompanhamento desses indivíduos a fim de reduzir o aparecimento de sequelas e as reações advindas da hanseníase[24].

A baixa renda familiar e a escolaridade deficiente são evidências constantes nas pesquisas que abordam pacientes com hanseníase. Estudos apontam uma prevalência de 65 a 69,6% de pacientes com escolaridade inferior a 8 anos. Quanto à renda familiar, a quantia de até 2 salários mínimos está presente entre 73,8 a 89% das famílias que possuem pelo menos um indivíduo acometido pelo *Mycobacterium leprae*[9,11,14].

No que se refere à percepção de possíveis prejuízos à cavidade oral advindos da hanseníase, quase 68% dos pacientes disseram não perceber relação, valor que se aproximou de outros estudos que tiveram percentual variando de 64 a 76%[11,25]. Um estudo realizado por Almeida *et. al.* observou uma associação entre a necessidade de tratamento odontológico e a escolaridade do paciente, em que um maior grau de escolaridade favoreceu a percepção da pessoa quanto à necessidade de tratamento[9]. A baixa escolaridade pode justificar, portanto, a não percepção de possíveis prejuízos à cavidade oral por não conferir a devida importância à saúde bucal.

Uma pesquisa realizada em Fortaleza abordando pacientes com hanseníase verificou que 98% realizaram tratamento odontológico pelo menos uma vez na vida[25]. Neste estudo, o percentual de pacientes que frequentaram um dentista em algum momento da vida ficou em torno de 87,5%, número que se aproxima de outro estudo também realizado no município de Sobral, cujo número ficou em aproximadamente 89%[11]. Dados do Suplemento de Saúde da Pesquisa Nacional por Amostra de Domicílios (PNAD) de 2003 mostram que 28 milhões de brasileiros (15,9% da população) nunca foram ao dentista e que o percentual era maior nas áreas rurais, no sexo masculino e na população com até 1 salário mínimo[18]. Apesar do número crescente de equipes de saúde bucal (ESB) no país, o acesso aos serviços odontológicos ainda é algo deficiente[19]. Além disso, o desconhecimento da relação entre hanseníase e saúde bucal pelos cirurgiões-dentistas é um fator relevante para a falta de inclusão desses pacientes nas ações e assistências odontológicas. Almeida e colaboradores[25]) observaram em seus estudos que mais de 64% dos odontólogos nunca suspeitaram de algum caso da doença e que mais de 70% deles afirmaram desconhecer a relação entre hanseníase e saúde bucal.

Estudos realizados com pacientes diagnosticados com hanseníase encontraram valores aproximados sobre dificuldade de higienizar a cavidade oral por causa de problemas relacionados à hanseníase (valores variando de 13 a 15,3%)[25]. Neste estudo, foi verificado percentual mais elevado (26,8%), que pode ser justificado em razão de os sujeitos da pesquisa serem pacientes acometidos por formas mais graves da doença e em tratamento de problemas advindos da hanseníase (reações e sequelas hansênicas). Para Costa et al.[26], entre os motivos que podem dificultar a higiene estão as reações que comprometem a saúde sistêmica do paciente, tornando mais difíceis os hábitos de higienização corporal, incluindo a bucal, e as sequelas da doença, por exemplo, as mãos em garra e amputações de dedos, que podem interferir diretamente na saúde da boca[26].

Com relação à última vez que procurou o serviço saúde bucal, pouco mais da metade afirmou ter procurado o serviço durante ou após o tratamento da hanseníase, o que leva a crer que o fato de buscarem tratamento odontológico se deva à presença de focos de infecção em cavidade oral que podem ter levado ao agravamento da doença. Estudo realizado no mesmo município encontrou resultado diferente, relatando um percentual de 61,5% para aqueles que procuraram o serviço odontológico pela última vez antes do tratamento da hanseníase, encontrando associação estatisticamente significativa entre essa variável e a presença de

reações hansênicas[11]. Na pesquisa realizada por Filgueira e colaboradores, a procura do serviço antes pode ter reduzido os focos de infecção oral, levando ao não aparecimento de reações.

Apesar de a hanseníase ser uma das prioridades do Ministério da Saúde no Brasil e estar entre as doenças negligenciadas e em eliminação que devem ser priorizadas pelos serviços de saúde, a Odontologia ainda deixa a desejar no acesso aos serviços de saúde bucal pelos pacientes com hanseníase. Além disso, é rara a continuidade do tratamento odontológico, fato que pode ser tanto pela falta de abertura da Odontologia na priorização desses pacientes quanto pela resistência destes a realizar o tratamento odontológico[27].

Com o objetivo de superar o modelo biomédico de atenção às doenças, a Política Nacional de Saúde Bucal propõe duas formas de inserção transversal da saúde bucal nos diferentes programas integrais de saúde: por linhas de cuidado e por condição de vida. Esta última compreende saúde da mulher, saúde do trabalhador, portadores de necessidades especiais, hipertensos, diabéticos, entre outros. A hanseníase, sendo uma doença negligenciada e em eliminação, é considerada como uma das prioridades pelo Ministério da Saúde e deve ser, portanto, um dos focos de atenção à saúde bucal por condição de vida, visto que precárias condições bucais vêm sendo apontadas como uma das causas para a piora da qualidade de vida dos pacientes acometidos pela hanseníase[28].

Pacientes com hanseníase apresentam condições precárias de saúde bucal[10]. Os dados de cárie dentária, uso e necessidade de prótese dental em portadores de hanseníase apresentaram semelhanças com os resultados encontrados em outros estudos tanto na população geral[19] quanto na população hansênica[11]. As pesquisas verificaram aumento do CPO-D com o avançar da idade e elevada perda dentária na população idosa.

Quanto à condição periodontal, foram encontradas semelhanças com a população geral indicada pelo estudo SB Brasil. Ambos apontaram o cálculo dental como o mais prevalente dentre as condições do periodonto: enquanto, no SB Brasil, o percentual variou de 24 a 64,1%[19], esta pesquisa encontrou percentagem que variou de 50 a 92,3%.

A condição de saúde bucal precária dos pacientes com hanseníase foi relatada em alguns estudos que defenderam a priorização dos indivíduos acometidos pelo bacilo de Hansen na atenção odontológica, de modo que o encaminhamento ao cirurgião-dentista (CD) deve ser realizado tão logo ocorra a confirmação diagnóstica. Todavia, as pesquisas também ressaltaram que a ampliação das ações do cirurgião-dentista depende da integração aos programas de capacitação[9,10]. Atualmente, existe uma tendência a se modificar o fazer da Odontologia dentro do SUS por meio de mudanças nas grades curriculares das universidades, inserção dos acadêmicos de Odontologia dentro de programas como o pró-pet-saúde, além dos programas de pós-graduação como as residências multiprofissionais em saúde da família, saúde coletiva e saúde pública, que visam à formação de cirurgiões-dentistas aptos a desenvolver trabalhos em equipes multiprofissionais, ampliando a integralidade e melhorando a qualidade dos serviços públicos de saúde.

Apesar da maior tendência da ocorrência de reações hansênicas quando há presença de infecções dentoalveolares, somente a presença de bolsa periodontal apresentou-se significantemente associada. Souza et al.[29] acompanharam pacientes com hanseníase por três anos e verificaram associação estatisticamente significativa entre doença periodontal e surto reacional hansênico[29].

A literatura aponta processos infecciosos, infecções, distúrbios hormonais e fatores emocionais como fatores desencadeantes de episódios reacionais[7,8], pois ocasionam uma queda no sistema imunológico. A maioria dos pacientes que citaram estar passando por um período de estresse também estava sendo acometida por reações hansênicas, porém não se conseguiu estabelecer associação significativa. Tal fato pode ser explicado por essa condição ser autocitada pelos próprios pacientes, e alguns sujeitos da pesquisa podem ter omitido a condição, o que pode ser uma limitação do estudo.

Não foi encontrada relação significante também com problemas de saúde e presença de episódios reacionais. Apesar de 39,3% relatarem algum problema de saúde como hipertensão e diabetes, todos apontaram fazer uso de medicação, o que pode compensar os desequilíbrios advindos dos problemas de saúde e, consequentemente, não influenciar o aparecimento das

reações hansênicas. Além disso, a condição de saúde foi autorrelatada pelos próprios sujeitos da pesquisa, pois não foi realizada análise de prontuário, fato que representa uma limitação do estudo. Futuras pesquisas podem realizar aferição da pressão arterial e glicemia no momento da pesquisa para avaliar se existe algum problema de saúde ainda desconhecido pelo paciente acometido pelo *Mycobacterium leprae*.

Os pacientes acometidos pelo bacilo de Hansen em tratamento no Ambulatório de Hanseníase de Sobral apresentaram precárias condições de saúde bucal. Aqueles que tiveram reações hansênicas possuíam condições orais piores do que aqueles que realizavam tratamento apenas para sequelas, o que aponta que problemas bucais, principalmente a presença de bolsa periodontal, podem estar associados com o aparecimento de reações. Outros fatores, no entanto, como condições de saúde, estresse, renda e escolaridade não apresentaram associação com reação, mas podem ter efeito cumulativo com os problemas bucais para o aparecimento de episódios reacionais.

Os resultados apontam para uma real necessidade desses pacientes de realizar tratamento odontológico a fim de reduzir os focos de infecção em cavidade oral, diminuindo o aparecimento dos episódios reacionais e, consequentemente, melhorando as condições de vida desses indivíduos, além de evitar uma maior demanda nos serviços de atenção secundária especializados em hanseníase. O cuidado deve ser longitudinal e de forma integral, visto que mesmo aqueles que receberam alta da terapia medicamentosa podem ser acometidos tanto por sequelas quanto por reações hansênicas, sendo de extrema importância o acompanhamento de todos os profissionais da Estratégia Saúde da Família.

REFERÊNCIAS

1. Brasil. Ministério da Saúde. Doenças infecciosas e parasitárias: guia de bolso. 8. ed. Brasília; 2010.
2. Oliveira DT, Sherlock J, Melo EV, Rollemberg KCV, Paixão TRC, Abuawad YG, et al. Clinical variables associated with leprosy reactions e and persistence of physical impairment. Rev Soc Bras Med Trop. 2013;46(5):600-4. http://dx.doi.org/10.1590/0037-8682-0100-2013. PMid:24270251.
3. Monteiro SCL. Hanseníase: políticas públicas e qualidade de vida de pacientes e seus cuidadores [dissertação]. Belo Horizonte: Fundação Oswaldo Crusz; 2010.
4. Figueiredo APP. Hanseníase: do isolamento familiar ao social [dissertação]. Gurupi: Fundação UNIRG; 2012.
5. Dalillo D, Simões MJS. Estudo do comportamento social dos pacientes de hanseníase do município de São Carlos - SP. Revista Espaço para a Saúde. 2005;7(1):10-5.
6. Nardi SMT, Paschoal VDA, Chiaravalloti-Neto F, Zanetta DMT. Deficiências após alta medicamentosa da hanseníase: prevalência e distribuição especial. Rev Saude Publica. 2012;46(6):969-77. http://dx.doi.org/10.1590/S0034-89102013005000002. PMid:23358621.
7. Foss NT, Souza CS, Goulart IMB, Gonçalvez HS, Virmond M. Hanseníase: episódios reacionais. São Paulo: Associação Médica Brasileira e Conselho Federal de Medicina; 2003. http://dx.doi.org/10.11606/issn.2176-7262.v36i2/4p453-459.
8. Belo Horizonte. Secretaria do Estado de Saúde de Minas Gerais. Como tratar e reconhecer reações hansênicas. Belo Horizonte; 2005. 85 p.
9. Almeida JRS. Atenção à saúde bucal dos portadores de hanseníase no município de Fortaleza-CE, Brasil [dissertação]. Fortaleza: Universidade Federal do Ceará; 2010.
10. Cortela DCB, Ignotti E. Lesões visíveis na hanseníase: o papel do cirurgião-dentista na suspeita de casos novos. Rev Bras Epidemiol. 2008;11(4):619-32. http://dx.doi.org/10.1590/S1415-790X2008000400010.
11. Filgueira AA, Paresque MAC, Carneiro SMF, Teixeira AKM. Saúde bucal em indivíduos com hanseníase no município de Sobral, Ceará. Epidemiol Serv Saúde. 2014;23(1):155-164.
12. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Diretrizes para vigilância, atenção e eliminação da Hanseníase como problema de saúde pública: Manual técnico-operacional. Brasília; 2016.
13. Brasil. Ministério da Saúde. Portaria nº 149 de 3 de fevereiro de 2016. Legislação em Saúde. Diário Oficial da União [Internet], Brasília, DF, 3 de fevereiro de 2016 [citado em 2019 Fev 5]. Disponível em: http://bvsms.saude.gov.br/bvs/saudelegis/gm/2016/prt0149_04_02_2016.html

14. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Hanseníase. Boletim Epidemiológico. 2018;49(4):1-10.

15. Brasil. Ministério da Saúde. Brasil quer eliminar a hanseníase até 2015 [Internet]. 2012 [citado 2014 Dez 5]. Disponível em: http://www.brasil.gov.br/saude/2012/06/brasil-quer-eliminar-a-hanseniase-ate-2015

16. Taheri JB, Mortazavi H, Moshfeghi M, Bakhshi M, Bakhtiari S, Azari-Marhabi S, et al. Oral-facial manifestations of 100 leprosy patients. Med Oral Patol Oral Cir Bucal. 2012;15(5):e728-32. http://dx.doi.org/10.4317/medoral.17718. PMid:22322502.

17. Ceará. Secretaria de Saúde do Governo do Estado do Ceará. Informe epidemiológico hanseníase [Internet]. Fortaleza: Secretaria de Saúde; 2014 [citado em 2015 Mar 19]. Disponível em: http://www.saude.ce.gov.br/index.php/boletins?download=813%3Ahanseniase-janeiro-de-2012.

18. Instituto Brasileiro de Geografia e Estatística. Pesquisa nacional por amostra de domicílios [Internet]. Rio de Janeiro: IBGE; 2013 [citado em 2014 Dez 5]. Disponível em: http://www.ibge.gov.br/home/estatistica/pesquisas/pesquisa_resultados.php?id_pesquisa=40

19. Brasil. Ministério da Saúde. Departamento de Atenção Básica. Área Técnica de Saúde Bucal. Projeto SB2010: condições de saúde bucal da população brasileira no ano 2010: manual do examinador. Brasília: Ministério da Saúde; 2010.

20. Souza LWF. Reações hansênicas em pacientes em alta por cura pela poliquimioterapia. Rev Soc Bras Med Trop. 2010 nov-dez;43(6):737-9. http://dx.doi.org/10.1590/S0037-86822010000600029. PMid:21181036.

21. Pereira RMS, Silva TSO, Silva LS, Santos TC, Falcão CAM, Pinto LSS. Oral and dental condition in leprosy. Braz J Oral Sci. 2013 dez;12(4):330-4. http://dx.doi.org/10.1590/S1677-32252013000400010.

22. Brasil. Ministério da Saúde. Política Nacional de Atenção Integral à Saúde do Homem: princípios e diretrizes. Brasília: Ministério da Saúde; 2008.

23. Teixeira MAG, Silveira VM, França ER. Características epidemiológicas e clínicas em indivíduos paucibacilares e multibacilares, atendidos em dois centros de referência para hanseníase, na cidade de Recife, Estado de Pernambuco. Rev Soc Bras Med Trop. 2010 maio-jun;43(3):287-92. http://dx.doi.org/10.1590/S0037-86822010000300015. PMid:20563498.

24. Capelo SC, Pagliuca LMF. Correlação das formas clínicas de hanseníase com sexo e idade. Rev Rene. 2000;1(2):16-20.

25. Almeida JRS, Alencar CHM, Barbosa JC, Dias AA, Almeida MEL. Autopercepção de pessoas acometidas pela hanseníase sobre sua saúde bucal e necessidade de tratamento. Cien Saude Colet. 2013;18(3):817-26. http://dx.doi.org/10.1590/S1413-81232013000300027. PMid:23546208.

26. Costa APF, Nery JAC, Oliveira MLW, Cuzzi T, Silva MR. Oral lesions in leprosy. Indian J Dermatol Venereol Leprol. 2003;69(6):381-5. PMid:17642946.

27. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Política nacional de atenção básica. Brasília: Ministério da Saúde; 2017.

28. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Política nacional de saúde bucal. Brasília: Ministério da Saúde; 2004.

29. Souza VA, Emmerich A, Coutinho EM, Freitas MG, Silva EH, Merçon FG, et al. Dental and oral condition in leprosy patients from Serra, Brazil. Lepr Rev. 2009;80(2):156-63. PMid:19743619.

JAOS
JOURNAL OF APPLIED ORAL SCIENCE

Original Article
http://dx.doi.org/10.1590/1678-7757-2024-0455

Lipid nanocarrier containing eugenol for denture hygiene: evaluation of efficacy against *Candida* biofilms

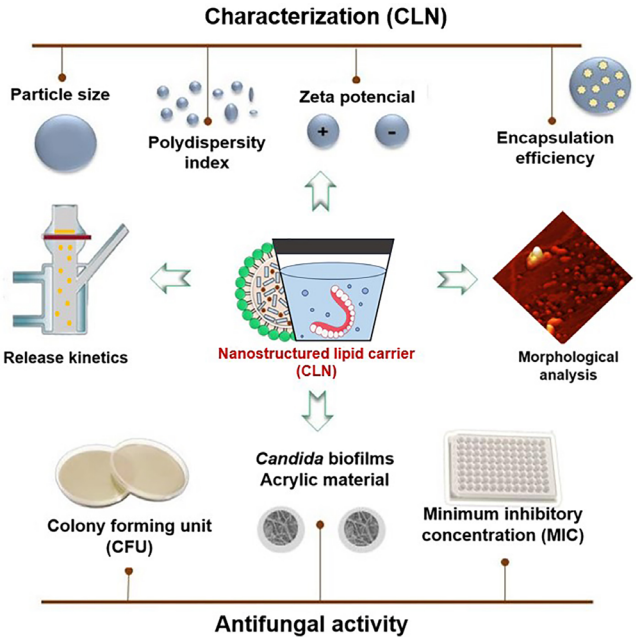
Irisvaldo Lima GUEDES[1] | Matheus Oliveira do NASCIMENTO[4] | Leandro de Sousa DIAS[3] | Alyne Rodrigues de ARAUJO-NOBRE[2] | Humberto Medeiros BARRETO[3] | Érika de Araújo ABI-CHACRA[3] | Ana Cristina Vasconcelos FIALHO[1] | Gláuber Campos VALE[1] | André Luis Menezes CARVALHO[1,4]

[1] Universidade Federal do Piauí, Programa de Pós-Graduação em Odontologia (PPGO), Teresina, Piauí, Brasil.
[2] Universidade Federal do Delta do Parnaíba, Núcleo de Pesquisa de Biodiversidade e Biotecnologia (Biotec), Parnaíba, Piauí, Brasil.
[3] Universidade Federal do Piauí, Departamento de Parasitologia e Microbiologia, Teresina, Piauí, Brasil.
[4] Universidade Federal do Piauí, Programa de Pós-Graduação em Ciências Farmacêuticas (PPGCF), Teresina, Piauí, Brasil.

Abstract

Eugenol has demonstrated efficacy against *Candida* spp., which is highly prevalent in denture wearers. However, the low water solubility and high volatility limit its application. The encapsulation in nanostructured lipid carriers (NLCs) may be a viable approach for developing new sanitizing agents for denture hygiene. Objective: To develop a sanitizing dispersion for denture hygiene using nanostructured lipid carriers (NLCs) containing eugenol and to evaluate the efficacy against *Candida* spp. biofilms. Methodology: The formulation was prepared using the ultrasonication method and characterized in terms of particle size (PS), polydispersity index (PDI), zeta potential (ZP), and encapsulation efficiency (EE). The minimum inhibitory concentration (MIC) was determined by the broth microdilution method and the antifungal activity was evaluated by four treatment groups (nanostructured formulation containing eugenol (NFE), free eugenol (FE), saline solution (SS), and the drug-free formulation NFW after eight hours of immersion in biofilms of two Candida species (*Candida albicans* and *Candida glabrata*) adhered to polymethyl methacrylate resin specimens. Results: The nanoparticles of NFE showed a particle size of 199.5±2.55 nanometers (nm) as measured by DLS, high homogeneity (0.07±0.02), an EE of 83.07±0.23, and a negative ZP (-25.86±0.65). The MICs of FE for *Candida albicans* and *Candida glabrata* were up to 10 times (64 µg/mL) and eight times (128 µg/mL) higher, respectively, than the MICs of NFE (6 µg/mL and 16 µg/mL). The biofilms of these microorganisms showed a significant reduction after immersion in NFE compared to the other tested groups (FE, NBF, and SS) (P<0.0001). Conclusion: The NFE demonstrated fungicidal activity against the isolated strains and significantly reduced *Candida* biofilms, thus showing promising performance for the sanitization of dentures over eight hours.

Keywords: Eugenol. Nanotechnology. Biofilms. *Candida*. Acrylic resins.



This article is derived from Irisvaldo Lima Guedes's Master's dissertation and is available at the address: https://sigaa.ufpi.br/sigaa/public/programa/noticias_desc.jsf?lc=pt_BR&id=370&noticia=519307121

Correspondence:
André Luis Menezes Carvalho - Universidade Federal do Piauí - Programa de Pós-graduação em Odontologia, PPGO - Laboratório de Dermofarmácia - Campus Universitário Ministro Petrônio Portella, Ininga, Teresina, PI, Brasil - Phone: (86) 99999-2309.
e-mail: aluismenezes@yahoo.com.br

Received: October 17, 2024
Revised: January 7, 2025
Accepted: January 16, 2025

Editor: Linda Wang
Associate Editor: Karin Hermana Neppelenbroek

Introduction

Candida spp. species are responsible for a significant portion of fungal infections in humans.[1] The most prevalent and pathogenic are *Candida albicans* (*C. albicans*) and *Candida glabrata* (*C. glabrata*), which can trigger infections that compromise individuals' health.[2,3] The pathogen negatively impacts users of removable dentures (RD), as it readily adheres to acrylic surfaces. Additionally, the fungus shows polymorphic characteristics, which contribute to the emergence of inflammatory processes in the oral cavity, such as oral candidiasis and prosthetic stomatitis.[4-6]

The hygiene of components of dentures is important for the prevention of oral fungal diseases. The chemical-mechanical method is the most recommended for cleaning and disinfecting dentures.[7] The most used chemical substances include sodium hypochlorite (SH), chlorhexidine digluconate (CHX), and alkaline peroxides (AP). However, the continued use of these substances can damage the structures of the dentures, alter their chemical and physical properties, and result in high costs.[8 -11]

In this context, the use of new and effective natural products with antibiofilm activity against *Candida* is becoming increasingly promising.[12] There is proven efficacy of eugenol, the main phenolic component of clove essential oil (70-90%), against *Candida* strains. However, it is volatile and has limited solubility and dose-dependent toxicity.[13,14]

Nanostructured lipid carriers (NLCs) are part of a binary pharmaceutical system composed of solid and liquid lipids that enable the retention of lipophilic actives, protecting them from degradation and improving their bioavailability due to their ability to modulate release.[15,16] The use of such systems makes isolated compounds such as eugenol even more promising for the development of antifungal sanitizing products.[17]

To date, no lipid nanocarrier containing encapsulated eugenol has been developed as a viable alternative for the hygiene of removable dentures (RDs). Therefore, this study aimed to develop a nanostructured lipid carrier dispersion containing eugenol, characterize it, and evaluate the activity against *Candida* biofilms adhered to an acrylic material used in RDs.

Methodology

Experimental design

This is an *in vitro* laboratory study. The formulation was developed, characterized, and tested to assess its potential antifungal effect on isolates and biofilms of two species of *Candida* (*C. albicans* and *C. glabrata*) that adhered to the surfaces of specimens of a heat-cured acrylic resin (RAT). These were polished and sterilized before the experiment. The number of specimens that were used in the study was determined based on a pilot study and a sample size of three or more was found to provide a good degree of reproducibility.

Preparation of the nanostructured formulation containing eugenol (NFE) and nanostructured formulation without eugenol (NFW)

The emulsification method followed by ultrasonication was used.[18] The formulation consisted of a solid lipid (carnauba wax (Lot: 0210701/2022)), liquid lipid (oleic acid (Synth, Diadema, Brazil)), aqueous surfactant solution (poloxamer 407® prepared at 5% (ChemSpecs, São Paulo, Brazil)) and eugenol (Biodinâmica, Ibiporã, Brazil)) at concentrations of 7%, 3%, 89.7% and 0.3%, respectively. All the components, except eugenol, were heated to 95 ºC (10 ºC above the melting point of the solid lipid (85 ºC)).[18] Homogenization was then performed using a macro ultrasonic probe sonicator (Eco-sonics, Indaiatuba, Brazil) set to a frequency of 20 kHz, an amplitude of 80 µm, and a power level of 70% for 10 minutes. Subsequently, the concentration of eugenol in the formulation was quantified via UV-VIS spectroscopy. The NFW was prepared following the same parameters as the NFE but without the incorporation of eugenol.

Characterization of the NFE

Particle size (PS), polydispersity index (PDI), and zeta potential (ZP)

The particle size (TP) and polydispersity index (PDI) were determined using the dynamic light scattering (DLS) technique, while the zeta potential (PZ) was measured via electrophoretic light scattering. The analyses were conducted using the Zetasizer NanoZS90 (Malvern Panalytical, Gondomar, Portugal) with a fixed detection angle of 90º, a resolution of 0.6 nanometers (nm), and sensitivity

across a wide range of sizes (0.6 nm to 6 µm). The measurements were performed at a temperature of 25 ºC, with a measurement time of 60 to 120 seconds for each analysis. Deionized water was used as the solvent for sample dilution. Measurements were performed in triplicate.

Encapsulation efficiency (EE) of eugenol

The method used by Vijayakumar, et al.[19] (2017) and Lopes, et al.[20] (2017) was employed to determine the drug encapsulation efficiency (EE). To verify the amount of free eugenol (EL), the NFE was centrifuged using an ultracentrifugation filter (Millipore, Darmstadt, Germany). Subsequently, the quantification of EL was performed using a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of wavelength of 291.4 nm (first-order derivative). The content (ET) was determined by reading the second dilution in the UV-VIS spectrophotometer. The analysis was conducted in triplicate. The amount of encapsulated active ingredient was determined using the formula: EE = (ET - EL) / ET × 100.

Evaluation of morphology by atomic force microscope (AFM)

The formulations containing eugenol (NFE) and without eugenol (NFW) were prepared by depositing a volume of 20 µL of the diluted nanoformulation at a ratio of 1:100 (in ultrapure water) onto a freshly cleaved mica surface at room temperature. After a drying period of 24 hours, analysis was performed using a TT-AFM model (Workshop, United States) in tapping mode, with silicon probes (TAP300-G, Ted Pella) and a resonance frequency of approximately 240 kHz. The images (512×512 pixels) were analyzed using Gwyddion 2.60 software, and the average size of the nanoparticles was expressed as the mean ± standard deviation (SD).[21]

In vitro release kinetics

The release drug substance was investigated using Franz diffusion cells, with a diffusion area of 1.15 cm². The receptor medium was prepared using a phosphate buffer solution and ethyl alcohol (Êxodo científica, Sumaré, Brazil) (8:2, pH 7.4),[22] and a dialysis membrane (Spectra/Por® Dialysis Membrane, MWCO 3500, Spectrum Laboratories Inc., USA) was used to separate the donor compartment from the receptor compartment and was prepared via an initial hydration using distilled water at 25 ºC for 30 minutes. Subsequently, the medium was rinsed to ensure the complete removal of impurities. The membrane was then immersed in a receptor medium for 24 hours prior to the start of the experiment to minimize variations during diffusion.

Two groups were prepared: test solutions (NFE (0.5 g)) and free eugenol solution (0.5 g). Six Franz cells were prepared, each containing 14 mL of the receptor medium for triplicate analysis. The temperature of the release medium was controlled at 37±0.5°C, and the magnetic stirring speed (SPLabor, São Paulo, Brazil) was set at 300 rpm. At time intervals of 0, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 24 h, 3 mL of the release medium was collected from each cell. The amount of released eugenol was quantified by reading the samples using a UV-VIS spectrophotometer.

Antifungal activity of NFE

Determination of the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

The microorganisms that were used are part of the microbiological collection of the Microbiology Research Laboratory at the Federal University of Piauí, where they are maintained on solid culture medium Sabouraud Dextrose Agar/Chloramphenicol Neogen (Kasvi, São José dos Pinhais, Brazil) at 8 ºC. The standard strains *C. albicans* ATCC 10231 and *C. glabrata* ATCC 2001 were inoculated and incubated in an oven at 37 °C in Brain Heart Infusion (BHI) medium (Kasvi, São José dos Pinhais, Brazil) at 3%. The optical density of the fungal suspensions was adjusted to be equivalent to 0.5 on the McFarland scale, corresponding to an approximate concentration of 1×10^6 to 5×10^6 colony-forming units (CFU)/mL. The adjustment was made using a spectrophotometer (Bel Photonics SP – 2000 UV, Piracicaba, Brazil), with absorbance measured at a wavelength of 530 nm.

The determination of the minimum inhibitory concentration (MIC) was evaluated using the broth microdilution method according to Leal, et al.[23] (2019). The determination of the minimum fungicidal concentration (MFC) was performed using the broth microdilution method, confirmed by the absence of growth on solid Sabouraud Dextrose Agar. To differentiate fungicidal from fungistatic activity, the MFC was equal to or up to four times greater than the minimum inhibitory concentration (MIC). The values

were compared between free eugenol and the test formulations, with and without eugenol, ensuring methodological rigor and reproducibility. Analyses were conducted in triplicate, and the results were expressed as the geometric mean.

Pre-formed *Candida* biofilms

Preparing and randomizing the specimens

The test specimens were fabricated with a thickness of 2 mm and a diameter of 12 mm in a circular shape using thermopolymerizable polymethyl methacrylate (PMMA) acrylic resin (Vipicril Plus clear, Florianópolis, Brazil). The finishing surface was performed with a polisher (Arotec, Cotia, Brazil) that was adapted with abrasive sanding discs (Sait, Guarulhos, Brazil) (grit sizes 600 and 1200). Polishing was conducted using acrylic polishers (brown, green, and yellow abrasive points (Exa-Technique, São Paulo, Brazil)). Simple randomization was used to allocate the test specimens into their respective pre-established groups. As a result, a randomly generated sequence was employed, using a table of random numbers corresponding to the groups.

Determining the treatment groups

Four comparison groups were used to evaluate the anti-biofilm activity of *C. albicans* and *C. glabrata* in 32 test specimens. Table 1 describes the sample division according to the treatment groups.

Biofilm formation methodology and treatment application

Standard strains of *C. albicans* ATCC 10231 and *C. glabrata* ATCC 2001 were used. Sabouraud Dextrose Agar with Chloramphenicol (Difco Laboratories) was used to reactivate and maintain the strains. To promote biofilm growth, 30 mL of Yeast Peptone Dextrose (YPD) broth was used, containing yeast extract (10 g/L; Isofar, Duque de Caxias, Brazil), dextrose (20 g/L; Dinâmica, Indaiatuba, Brazil), and peptone (20 g/L; Becton Dickinson, East Rutherford, United States). All media were prepared following the manufacturer's descriptions.

For biofilm formation, yeast-like cells were seeded in Sabouraud Dextrose Agar (SDA) (Difco Laboratories) and inoculated into 30 mL of Yeast Peptone Dextrose (YPD) broth. They were incubated at 37°C for 18 hours in a BOD incubator (7Lab, Rio de Janeiro, Brazil). Then, part of the suspension was transferred to a sterile YPD medium to readjust the cell concentration to 10^6 cells/mL, according to an optical density (OD) of 2.0 on the McFarland scale.

In the adhesion phase evaluation, 0.5 mL of the standardized cell suspension was transferred to a 24-well plate (Kasvi, São José dos Pinhais, Brazil) containing an acrylic resin specimen at the bottom of each well. Initial adhesion was conducted by incubating the plate in the incubator for six hours at a temperature of 37°C. After this period, the contaminated suspension was removed, and a new aliquot of 0.5 mL of YPD medium was added to each well, which remained for an additional 18 hours under the same conditions. After this period, the medium was again removed, and 0.5 mL of each treatment was added to each well. The plate was then incubated at 37°C for eight hours.

To assess the antifungal activity of the treatments, the specimens were transferred to a new plate with wells containing 0.5 mL of saline solution after 24 hours of biofilm development. Biofilm was removed from the solution by rigorous pipetting. Approximately 500 µL of the obtained suspension was transferred to sterile Eppendorf (Kasvi, São José dos Pinhais, Brazil). From this suspension, 100 µL were aliquoted to perform serial dilutions (10^{-1} to 10^{-7}) in Eppendorf tubes containing 900 µL of 0.9% saline solution. Each dilution was plated on ASD agar in quadruplicate and incubated for 48 hours at 37°C for subsequent

Table 1- Division of the specimens for the application of the treatment groups.

Groups	Treatments	Number of specimens	
		C. albicans	***C. glabrata***
Intervention	NFE	4	4
Intervention without active ingredient	NFW	4	4
Positive control	FE 0.3%	4	4
Negative control	SS 0,9%	4	4
Total	4	32	

NFE (Nanostructured formulation containing eugenol); NFW (Nanostructured formulation without eugenol); FE (Free eugenol); SS (saline solution).

counting of colony-forming units (CFUs).

Sterility and contamination control

All experimental procedures were conducted in a controlled environment, using laminar flow hoods and pre-disinfected surfaces. Materials and reagents were sterilized by autoclaving. Stringent aseptic techniques were employed, including the use of appropriate personal protective equipment.

Statistical analysis

The assumptions of variance equality and normal distribution of errors were checked for all tested response variables. The original CFU data were transformed into base 10 logarithms. The Graphpad Prism 9.02 software (Graphpad, La Jolla, CA, USA) was used for statistical analysis. The SHAPIRO-WILK's test was conducted to assess the normality of the data distribution. Since the data showed a normal distribution, an analysis of variance (ANOVA) was applied, followed by Tukey's test for multiple comparisons. These tests were chosen because they satisfy the statistical assumptions of the transformed data and provide robust and reliable analyses for comparing variables between groups. This approach is consistent with widely accepted statistical analysis used in experimental studies. The significance level was set at 5%.

Results

Characterization of the NFE

TP, IPD, PZ, and EE

The nanoparticles of NFE and NFW showed nanometric size, high homogeneity, and negative EE and ZP (Table 2). There were no significant changes in the parameters in the presence of the drug in the formulation (P>0.05).

Morphology determination by AFM

Figure 1 shows nanoparticles can be observed using Atomic Force Microscopy (AFM). The images reveal spherical nanoparticles in both analyzed samples, consistent with the extracted profile. The average size for NFW was 21.04±7.92 nm, whereas for NFE was 13.91±2.79 nm (Figure 2).

In vitro eugenol release kinetics

The cumulative amounts released (over 24 hours) from NFE and the free eugenol solution were 44.21% and 61.11%, respectively (Table 3). NFE showed a controlled release profile throughout the entire kinetic profile. The concentrations of the released free eugenol were significantly higher (P<0.05) at all collection points from the free eugenol solution compared to the concentrations that were released from NFE (Figure 3).

Microbiological analysis of CIM and CFM

The MICs of eugenol against the *C. albicans* and *C. glabrata* strains were 64 and 128 µg/mL, respectively. NFE reduced the values MIC by 10 and eight times (6 and 16 µg/mL, respectively). In addition, the MFCs were equal to the MIC values for both *Candida* strains in both solutions. The NFE showed fungicidal action against both test strains at the MIC values (Table 3).

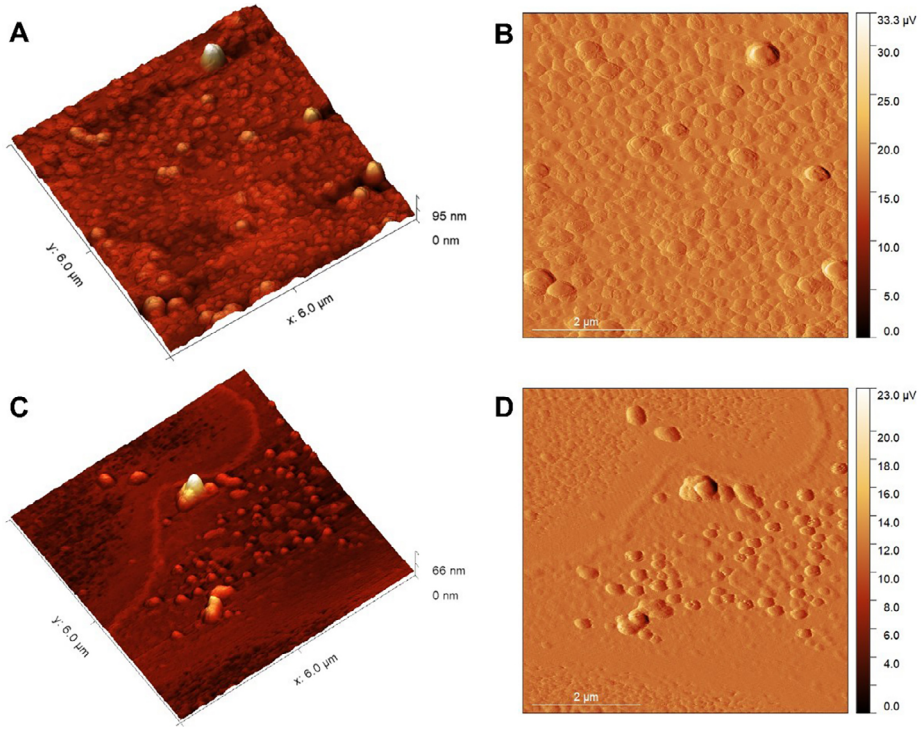
Antifungal activity of NFE on *Candid*a biofilms

Quantitative analysis of biofilm by viable cell count (expressed as colony forming units (CFU)) revealed that the NFE showed the highest antimicrobial activity, with a significant reduction of biofilms compared to the other groups (p<0.0001) (Figure 4 (1 and 2)). Free eugenol (FE), even at the same concentration as the NFE (0.3%), showed inferior efficacy against the biofilms of *C. albicans* and *C. glabrata* (p<0.0001). The NFW and the saline solution (SS) showed no significant difference in reducing biofilms (p>0.999)

Table 2- Physico-chemical characterization of the formulations (NFE and NFW).

Parameters	NFE	NFW
	Mean ± SD	Mean ± SD
PS (nm)	199.5±2.55	198.16±3.70
PDI	0.07±0.02	0.09±0.04
ZP (mV)	- 25.86±0.65	-24.33±0.23
EE (%)	83.07± 0.23	-

Legend: mV (millivolt); nm (nanometers); SD (standard deviation); NFE (Nanostructured formulation containing eugenol); NFW (Nanostructured formulation without eugenol); PS (Particle size); PDI (Polydispersity index); ZP (Zeta potential); EE (Encapsulation efficiency).

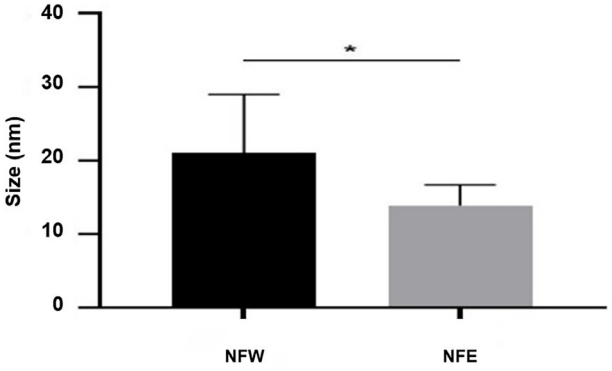


µV- microvolt ; 3D topography images (A and C) and 2D amplitude images (B and D). NFW (A and B); NFE (C and D). Scale = 2 µm (micrometer).

Figure 1- AFM images.

Table 3- Inhibitory effect of nanostructured formulations (NFE and NFW) and eugenol against Candida strains.

Formulations	*Candida* species	(µg/mL)			
		MIC	MFC	MFC/MIC	Inhibitory effect
NFW	*C. albicans* ATCC 10231	≥1024	≥1024	-	No activity
	C. glabrata ATCC 2001	≥1024	≥1024	-	No activity
Eugenol	*C. albicans* ATCC 10231	64	64	01:01	Fungicide
	C. glabrata ATCC 2001	128	128	01:01	Fungicide
NFE	*C. albicans* ATCC 10231	6	6	01:01	Fungicide
	C. glabrata ATCC 2001	16	16	01:01	Fungicide



*p<0.0001 after Mann-Whitney test. NFE (Nanostructured formulation containing eugenol); NFW (Nanostructured formulation without eugenol); nm (nanometer).

Figure 2- Nanoparticle size graph from AFM analysis.

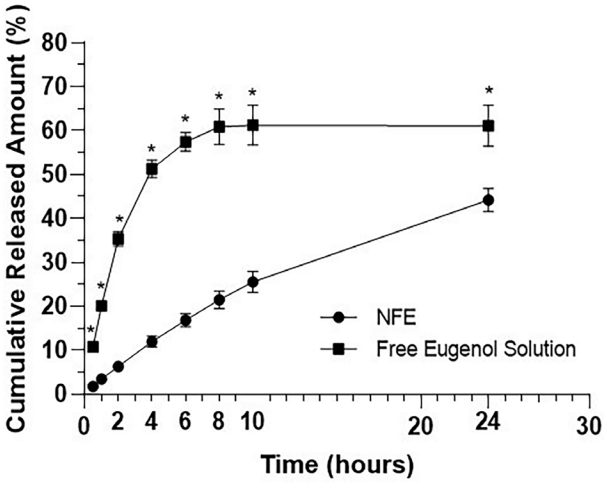
(Figure 4 (1 and 2)).

Discussion

A power of 70% and a stirring time of 10 minutes were selected to maximize emulsification efficiency and system stability, while preserving the integrity of the active component and ensuring compatibility, as reported by Fang and Bhandari[24] (2010), Silva, et al.[25] (2011), and Bolequi, et al.[26] (2016). The nanoparticles of the FNE showed nanometric sizes (199.5±2.55 nm as analyzed using the Zetasizer (Table 2) and 13.91±2.79 nm using atomic force microscopy (AFM) (Figure 1)). Particles are considered nanoparticles when their size falls within

the nanometric scale, ranging from 1 nm to 1000 nm. The main advantage is that physicochemical and functional properties improve as they transition to the nanoscale.[27] Additionally, the reduction in average diameter can enhance the stability of lipid nanoparticles, facilitating efficient target delivery and preventing rapid drug elimination.[28]

There was a reduction in particle size in the atomic force microscopy (AFM) readings compared to the Zetasizer measurements. This can be explained by the differences in the methods and precision levels of the analysis. The Zetasizer technique measures dynamic light scattering, which is used to calculate the average diameter rather than the size of the particles.[29,30] In contrast, AFM evaluates three-dimensional information in real-time about lipid systems, with a resolution close to one nanometer.[31,32] Therefore, it provides a more accurate nanometric analysis of the nanoparticles.



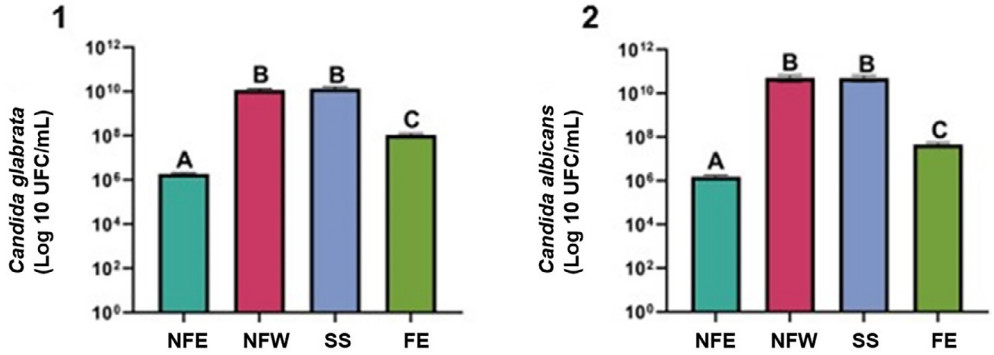
*P<0.05 after two-way ANOVA test. NFE (Nanostructured formulation containing eugenol).

Figure 3- *In vitro* release profile of eugenol encapsulated in nano-structured lipid carriers and free eugenol solution.

The polydispersity index (PDI) refers to the degree of non-uniformity in a particle size distribution.[33] The PDI value ranges from 0.0 (for a perfectly homogeneous sample regarding particle size) to 1.0 (for a highly heterogeneous sample with multiple particle size populations). Values that are less than or equal to 0.2 are generally more acceptable for optimizing nanoparticles containing polymers. According to Tamjidi, et al.[32] (2013), values above 0.5 indicate a very broad particle size distribution, characterizing high instability that leads to unpredictable behavior and reduces the efficacy of the bioactive system. In this study, monodisperse nanoparticles were obtained (0.07±0.02; Table 2), indicating acceptable stability.

ZP values above +30 mV or below -30 mV are considered ideal for colloidal dispersions to maintain good stability.[34,35] This study's results showed a negative ZP of -25.86±0.65 (Table 2). Although these values are below the average threshold, dispersion can remain stable. This stability can be attributed to the presence of the steric stabilizer Poloxamer 407. Surfactants such as Poloxamer, when adsorbed onto the particle surface, alter the particle's shear plane.[36] Its polymeric chain promotes particle repulsion (entropic forces), maintaining a considerable distance between them.

The high encapsulation efficiency obtained (83.07%±0.23, Table 2) can be attributed to the presence of the liquid lipid in the formulation, as it allows for the imperfect formation of the lipid matrix and, consequently, enhances the drug entrapment.[37] Eugenol shows high solubility in the oily phase, which can also be a contributing factor to the improved encapsulation efficiency.[38] Studies indicate that active substances with high lipid solubility tend to show



One-way ANOVA and Tukey test: NFE vs. NFW (P<0.0001); NFE vs. SS(P<0.0001); NFE vs. FE (P<0.0001); NFW vs. SS (P>0.999) NFW vs. FE (P<0.0001); FE vs. SS (P<0.0001). NFE (Nanostructured formulation containing eugenol); NFW (Nanostructured formulation without eugenol); FE (Free eugenol); SS (saline solution).

Figure 4- Activity of the test solutions on *C. albicans* and *C. glabrata* biofilms.

relatively high encapsulation efficiencies, typically above 80%.[39]

The NFE showed a controlled release profile with a cumulative amount of eugenol of 44.21% in 24 hours. This was expected due to the system's ability to encapsulate an active ingredient within a disordered lipid matrix, which hinders rapid release.[37,40] The type of stabilizer can also influence release control. Sulfactants such as poloxamer 407, which was used in this study, contribute to a slower degradation of the system due to their steric effect.[41]

NFE and FE demonstrated fungicidal activity against isolated *Candida* strains (Table 3) and in *Candida* biofilms (*C. albicans* and *C. glabrata*) (Figure 4). Several studies have confirmed the antifungal action of eugenol against this microorganism species.[12, 17,42,43] Regarding the action mechanism, this active compound can bind to the *Candida* membrane and decrease ergosterol biosynthesis due to its ability to interact with the fungal membrane and damage its cell wall.[44] Additionally, eugenol can increase levels of lipid peroxidation and reactive oxygen species, thereby inducing oxidative stress that leads to increased permeability of the fungal cell membrane.[45,46] This drug substance may also interact with adhesive proteins, such as ALS, resulting in a considerable decrease in the fungus's adhesion capacity and promoting the disruption of formed biofilms (*C.albicans*).[3]

The MIC (6 µg/mL) and MFC (6 µg/mL) of NFE against *C. albicans* strains were reduced by up to 10 times compared to the concentrations of FE (MIC (64 µg/mL) and MFC (64 µg/mL)) (Table 3). In addition, there was a significant reduction in pre-formed biofilms after application of NFE for eight hours compared to FE, even though both contained the same concentration (0.3%). These results can be justified by the presentation of FNE particles at the nanoscale (13.91 nm), which enables an increased surface area of contact and consequently enhances the chances of the nano-encapsulated active ingredient coming into contact with the fungal cell wall in the medium.[18] Furthermore, the system is capable of controlling eugenol release, thereby increasing its activity and enabling targeted action against the microorganism.[47,48]

Nanoencapsulation promotes specific molecular interactions that enhance the antifungal mechanisms of eugenol. For instance, encapsulation facilitates the incorporation of eugenol into the fungal cell membrane, strengthening its binding to ergosterol and compromising fungal membrane integrity.[45]

Additionally, the targeted delivery of encapsulated eugenol enhances its interaction with ALS adhesive proteins, significantly reducing adhesion and disrupting the biofilm structure.[49] These mechanisms explain the superior efficacy of the NFE compared to free eugenol, even at equivalent concentrations, in combating *Candida* biofilms.

Limitations of the study

Most tests were conducted in controlled laboratory settings (in vitro), which may not fully replicate the actual conditions of the oral cavity, such as the presence of saliva, pH variations, temperature fluctuations, and the complete oral microbiome. Although the tests demonstrated the efficacy of the NFE, it is crucial to assess its long-term effects. Prolonged use of the formulation over months or years may reveal factors such as microbial resistance or potential cumulative effects on acrylic materials.

The comparison was made using biofilms that were formed on specimens of heat-polymerized acrylic resin, which simulate dental prostheses. However, these conditions may not accurately reflect biofilm formation on real dentures used by patients, as they are influenced by individual factors such as oral hygiene, diet, and overall health. Furthermore, the formulation compatibility with different types of denture materials — not only acrylic resin — and metallic components, such as cobalt-chromium or titanium clasps in partial dentures, is yet to be investigated. Different materials may show varying reactions to formulation. These limitations highlight areas for future research and underscore that, despite the promising results, the practical application and generalizability of the findings require validation in a broader context.

Conclusions

The nano-structured formulation loaded with eugenol was successfully developed and showed characterization parameters within acceptable values. Furthermore, it showed fungicidal activity against isolated *Candida* strains and significantly reduced the biofilms of *Candida* (*C. albicans* and

C. glabrata). The NFE, containing 0.3% eugenol, demonstrated superior performance compared to free eugenol (FE) at the same concentration. Therefore, lipid nanocarriers (LNCs) demonstrated significant potential for administering eugenol in the hygiene of dental prostheses, offering promising prospects for future applications in dentistry

Acknowledgments

The authors would like to thank the financial support of the Coordination for the Improvement of Higher Education and Postgraduate Personnel (CAPES) - (88887. 675954/2022-00) and the Interdisciplinary Laboratory of Advanced Materials (LIMAV) of the Federal University of Piauí (UFPI)

Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets generated and/or analyzed during the current study are available in SciELO Data repository, https://doi.org/10.48331/scielodata.WWIV2M

Authors´contributions

Guedes, Irisvaldo Lima: Conceptualization (Equal); Formal analysis (Equal); Methodology (Equal); Visualization (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Nascimento, Matheus Oliveira**: Conceptualization (Equal); Data curation (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Validation (Equal). **Sousa Dias, Leandro:** Conceptualization (Equal); Investigation (Equal); Methodology (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Rodrigues de Araujo-Nobre, Alyne:** Methodology (Equal); Resources (Equal); Software (Equal); Validation (Equal); Visualization (Equal). **Medeiros Barreto, Humberto:** Conceptualization (Equal); Methodology (Equal); Validation (Equal). **Araújo Abi-chacra, Érika:** Investigation (Equal); Methodology (Equal); Validation (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Vasconcelos Fialho, Ana Cristina:** Conceptualization (Equal); Methodology (Equal); Supervision (Equal); Visualization (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Vale, Gláuber Campos:** Conceptualization (Equal); Funding acquisition (Equal); Software (Equal); Supervision (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Carvalho, André Luis Menezes:** Conceptualization (Supporting); Methodology (Equal); Project administration (Equal); Writing - original draft (Equal); Writing - review & editing (Equal)

References

1- Rocha R, Santos G, Duarte TN, Corrêa GO, Nampo FK, Ramos SP, et al. Chemical cleaning methods for prostheses colonized by *Candida spp*: a systematic review. J Prosthet Dent. 2020;124(6):653-58. doi: 10.1016/j.prosdent.2019.10.004

2- Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Microbiol Rev. 2010;23(2):253-73. doi: 10.1128/CMR.00076-09

3- El-Baz AM, Mosbah RA, Goda RM, Mansour B, Sultana T, Dahms TE, et al. Back to nature: Combating candida albicans biofilm, phospholipase and hemolysin using plant essential oils. Antibiotics (Basel). 2021;10(1):1-18. doi: 10.3390/antibiotics10010001

4- Kilic K, Koc AN, Tekinsen FF, Yildiz P, Kilic D, Zararsiz G, et al. Assessment of Candida species colonization and denture-related stomatitis in bar- and locator-retained overdentures. J Oral Implantol. 2014;40(5):549-56. doi: 10.1563/AAID-JOI-D-12-00048

5- Armstrong-James D, Brown GD, Netea MG, Zelante T, Gresnigt MS, van de Veerdonk FL, et al. Immunotherapeutic approaches to treatment of fungal diseases. Lancet Infect Dis. 2017;17(12):393-402. doi: 10.1016/S1473-3099(17)30442-5

6- Pereira R, Dos Santos Fontenelle RO, de Brito EH, de Morais SM. Biofilm of *Candida albicans*: formation, regulation and resistance. J Appl Microbiol. 2021;131(1):11-22. doi: 10.1111/jam.14949

7- Schmutzler A, Rauch A, Nitschke I, Lethaus B, Hahnel S. Cleaning of removable dental prostheses:a systematic review. J Evid Based Dent Pract. 2021;21(4):101644. doi: 10.1016/j.jebdp.2021.101644

8- Papadopoulos T, Polyzois G, Tapanli A, Frangou M. The effect of disinfecting solutions on bending properties and weight changes of Co-Cr and Ti-6Al-7Nb alloys for dentures. Odontology. 2011;99(1):77-82. doi: 10.1007/s10266-010-0135-2

9- Slaughter RJ, Watts M, Vale JA, Grieve JR, Schep LJ. The clinical toxicology of sodium hypochlorite. Clin Toxicol (Phila). 2019;57(5):303-311. doi: 10.1080/15563650.2018.1543889

10- Paranhos HF, Bezzon OL, Davi LR, Felipucci DN, Silva CH, Pagnano VO. Effect of cleanser solutions on the color of acrylic resins associated with titanium and nickel-chromium alloys. Braz Oral Res. 2014;28:0017. doi: 10.1590/1807-3107bor-2014.vol28.0017

11- Arruda CN, Salles MM, Oliveira VC, Macedo AP, da Silva CH, Paranhos HF. Using denture cleansers to control biofilm from dentures and brushes: a randomized crossover clinical trial. Int J Prosthodont. 2021;34(3):291-299. doi: 10.11607/ijp.6665

12- Saracino IM, Foschi C, Pavoni M, Spigarelli R, Valerii MC, Spisni E. Antifungal activity of natural compounds vs. candida spp.: a mixture of cinnamaldehyde and eugenol shows promising *in vitro* results. Antibiotics (Basel). 2022;11(1):73. doi: 10.3390/antibiotics11010073

13- Martins C, Doran C, Laires A, Rueff J, Rodrigues AS. Genotoxic and apoptotic activities of the food flavourings myristicin and eugenol in AA8 and XRCC1 deficient EM9 cells. Food Chem Toxicol. 2011;49(2):385-92. doi: 10.1016/j.fct.2010.11.013

14- Fuentes C, Fuentes A, Barat JM, Ruiz MJ. Relevant essential oil components: a minireview on increasing applications and potential toxicity. Toxicol Mech Methods. 2021;31(8):559-565. doi: 10.1080/15376516.2021.1940408

15- Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs: a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol. 2004;113(1-3):151-70. doi: 10.1016/j.jbiotec.2004.06.007

16- Souto EB, Müller RH, Gohla S. A novel approach based on lipid nanoparticles (SLN) for topical delivery of alpha-lipoic acid. J Microencapsul. 2005;22(6):581-92. doi: 10.1080/02652040500162378

17- Garg A, Singh S. Enhancement in antifungal activity of eugenol in immunosuppressed rats through lipid nanocarriers. Colloids Surf B Biointerfaces. 2011;87(2):280-8. doi: 10.1016/j.colsurfb.2011.05.030

18- Elmowafy M, Al-Sanea MM. Nanostructured lipid carriers (NLCs) as drug delivery platform: advances in formulation and delivery strategies. Saudi Pharm J. 2021;29(9):999-1012. doi: 10.1016/j.jsps.2021.07.015

19- Vijayakumar A, Baskaran R, Jang YS, Oh SH, Yoo BK. Quercetin-loaded solid lipid nanoparticle dispersion with improved physicochemical properties and cellular uptake. aaps pharmscitech. 2017;18(3):875-883. doi: 10.1208/s12249-016-0573-4

20- Lopes CE, Langoski G, Klein T, Ferrari PC, Farago PV. A simple HPLC method for the determination of halcinonide in lipid nanoparticles: development, validation, encapsulation efficiency, and *in vitro* drug permeation. Braz J Pharm Sci. 2017;53(2):e15250. doi: 10.1590/s2175-97902017000215250

21- Eaton P, Quaresma P, Soares C, Neves C, Almeida MP, Pereira E, et al. A direct comparison of experimental methods to measure dimensions of synthetic nanoparticles. Ultramicroscopy. 2017;182:179-90. doi: 10.1016/j.ultramic.2017.07.001

22- Monton C, Settharaksa S, Suksaereeb J, Chusuta T. The preparation, characterization, and stability evaluation of a microemulsion-based oral spray containing clove oil for the treatment of oral candidiasis. J Drug Deliv Sci Technol. 2020;57:101735. doi: 10.1016/j.jddst.2020.101735

23- Leal AL, Bezerra CF, Rocha JE, Santos AT, Cruz RP, Carneiro JN, et al. *Piper cernuum* Vell.: Chemical profile and antimicrobial potential evaluation. Ind Crops Prod. 2019;140:111577. doi: 10.1016/j.indcrop.2019.111577

24- Fang Z, Bhandari B. Encapsulation of polyphenols: a review. Trends Food Sci Technol. 2010;21:510-23. doi: 10.1016/j.tifs.2010.08.003

25. Silva AC, González-Mira E, García ML, Egea MA, Fonseca J, Silva R, et al. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. Colloids Surf B Biointerfaces. 2011;86(1):158-65. doi: 10.1016/j.colsurfb.2011.03.035

26- Beloqui A, Solinís MÁ, Rodríguez-Gascón A, Almeida AJ, Préat V. Nanostructured lipid carriers: promising drug delivery systems for future clinics. Nanomedicine. 2016;12(1):143-61. doi: 10.1016/j.nano.2015.09.004

27- Müller RH, Shegokar R, Keck CM. 20 years of lipid nanoparticles (SLN and NLC): present state of development and industrial applications. Curr Drug Discov Technol. 2011;8(3):207-27. doi: 10.2174/157016311796799062

28- Santos SV, Badan RA, Andrade SM. Solid lipid nanoparticles as carriers for lipophilic compounds for applications in foods. Food Res Int. 2019;122:610-626. doi: 10.1016/j.foodres.2019.01.032

29- Robles LV, García FB, Garzón SM, Hernández LA, Vázquez RM. Nanopartículas lipídicas sólidas [Solid lipid nanoparticles]. Rev Mex Cienc Farm. 2008;39(1):38-52. Spanish.

30- Yoon G, Park JW, Yonn I. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): recent advances in drug delivery. J Pharm Investig. 2013;43:353–362. doi: 10.1007/s40005-013-0087-y

31- Domingo C, Saurina J. An overview of the analytical characterization of nanostructured drug delivery systems: towards green and sustainable pharmaceuticals: a review. Anal Chim Acta. 2012;744:8-22. doi: 10.1016/j.aca.2012.07.010

32- Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. Innov Food Sci Emerg Technol. 2013;19:29-43. doi: 10.1016/j.ifset.2013.03.002

33- Bera B. Nanoporous silicon prepared by vapour phase strain etch and sacrificial technique. In: Proceedings of the International Conference on Microelectronic Circuit and System (Micro) [Internet]. 2015 [cited 2025 Jan 20];1:42-45. Available from: https://www.ijcaonline.org/proceedings/micro2015/number1/23705-1742/

34- Radtke M, Souto EB, Müller RH. Nanostructured lipid carriers: a novel generation of solid lipid drug carriers. Pharm Technol Eur. 2005;17(4):45-50.

35- Krambeck K, Silva V, Silva R, Fernandes C, Cagide F, Borges F, et al. Design and characterization of Nanostructured lipid carriers (NLC) and Nanostructured lipid carrier-based hydrogels containing *Passiflora edulis* seeds oil. Int J Pharm. 2021;600:120444. doi: 10.1016/j.ijpharm.2021.120444

36- Redhead HM, Davis SS, Illum L. Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: *in vitro* characterisation and in vivo evaluation. J Control Release. 2001;70(3):353-63. doi: 10.1016/s0168-3659(00)00367-9

37- Cheow WS, Hadinoto K. Factors affecting drug encapsulation and stability of lipid–polymer hybrid nanoparticles. Colloids Surf B Biointerfaces. 2011;85(2):214-220. doi: 10.1016/j.colsurfb.2011.02.033

38- Müller RH, Runge SA, Ravelli V, Thünemann AF, Mehnert W, Souto EB. Cyclosporine-loaded solid lipid nanoparticles (SLN®): Drug–lipid physicochemical interactions and characterization of drug incorporation. Eur J Pharm Biopharm. 2008;68(3):535-44. doi: 10.1016/j.ejpb.2007.07.006

39- Espinosa-Olivares MA, Delgado-Buenrostro NL, Chirino YI, Trejo-Márquez MA, Pascual-Bustamante S, Ganem-Rondero A. Nanostructured lipid carriers loaded with curcuminoids: Physicochemical characterization, *in vitro* release, *ex vivo* skin penetration, stability and antioxidant activity. Eur J Pharm Sci. 2020;155:105533. doi: 10.1016/j.ejps.2020.105533

40- Friedrich RB, Kann B, Coradini K, Offerhaus HL, Beck RC, Windbergs M. Skin penetration behavior of lipid-core nanocapsules for simultaneous delivery of resveratrol and curcumin. Eur J Pharm Sci. 2015;78:204-13. doi: 10.1016/j.ejps.2015.07.018

41- He M, Du M, Fan M, Bian Z. *In vitro* activity of eugenol against *Candida albicans* biofilms. Mycopathologia. 2007;163(3):137-43. doi: 10.1007/s11046-007-0097-2

42- Marcos-Arias C, Eraso E, Madariaga L, Quindós G. *In vitro* activities of natural products against oral Candida isolates from denture wearers. BMC Complement Altern Med. 2011;11:119. doi: 10.1186/1472-6882-11-119

43- Ahmad A, Wani MY, Khan A, Manzoor N, Molepo J. Synergistic Interactions of Eugenol-tosylate and Its Congeners with Fluconazole against *Candida albicans*. PLoS One. 2015;10(12):e0145053. doi: 10.1371/journal.pone.0145053

44- Lone SA, Ahmad A. Inhibitory effect of novel eugenol tosylate congeners on pathogenicity of *Candida albicans*. BMC Complement Med Ther. 2020;20:131. doi: 10.1186/s12906-020-02929-0

45- Didehdar M, Chegini Z, Shariati A. Eugenol: a novel therapeutic agent for the inhibition of *Candida* species infection. Front Pharmacol. 2022;13:872127. doi: 10.3389/fphar.2022.872127

46- Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288-303. doi: 10.4103/1735-5362.235156

47- Akel H, Ismail R, Katona G, Sabir F, Ambrus R, Csóka I. A comparison study of lipid and polymeric nanoparticles in the nasal delivery of meloxicam: formulation, characterization, and *in vitro* evaluation. Int J Pharm. 2021;604:120724. doi: 10.1016/j.ijpharm.2021.120724

48- Viegas C, Patrício AB, Prata JM, Nadhman A, Chintamaneni PK, Fonte P. Solid lipid nanoparticles vs. nanostructured lipid carriers: a comparative Review. Pharmaceutics. 2023;15(6):1593. doi: 10.3390/pharmaceutics15061593

49- Nett J, Andes D. *Candida albicans* biofilm development, modeling a host-pathogen interaction. Curr Opin Microbiol. 2006;9(4):340-5. doi: 10.1016/j.mib.2006.06.007

Received: 25 July 2024 | Revised: 6 December 2024 | Accepted: 18 December 2024

DOI: 10.1002/JPER.24-0462

1930-2025 95 YEARS JOURNAL OF Periodontology

ORIGINAL ARTICLE

Surveying coating strategies for peri-implantitis management: Clinical implications and classificatory approaches

Marta M. A. Pereira[1] | **Rafael Scaf de Molon**[2] | **Valentim A. R. Barão**[3] | **Jamil A. Shibli**[4] | **Anton Sculean**[5] | **Flavia Q. Pirih**[6] | **Erica D. de Avila**[1,7]

[1]Department of Dental Materials and Prosthodontics, São Paulo State University/UNESP, Araraquara, São Paulo, Brazil

[2]Department of Periodontology, São Paulo State University/UNESP, Araçatuba, São Paulo, Brazil

[3]Department of Prosthodontics and Periodontology, Universidade Estadual de Campinas/UNICAMP, Piracicaba, São Paulo, Brazil

[4]Department of Periodontology, Guarulhos University, Guarulhos, São Paulo, Brazil

[5]Department of Periodontology, University of Bern, Bern, Switzerland

[6]Section of Periodontics, University of California, Los Angeles, California, USA

[7]Department of Dental Materials and Prosthodontics, São Paulo State University/UNESP, Araçatuba, São Paulo, Brazil

Correspondence
Erica Dorigatti de Avila, Department of Dental Materials and Prosthodontics, São Paulo State University (UNESP) – School of Dentistry at Araçatuba, Rua José Bonifácio, 1193, Araçatuba, São Paulo, 16015-050, Brazil.
Email: erica.avila@unesp.br

Funding information
State of Sao Paulo Research Foundation; FAPESP, Grant/Award Numbers: 2021/10762-1, 2021/09434-0, 2018/20719-3,

Abstract

Background: Peri-implantitis, an inflammatory condition occurring in the supportive tissues, is triggered by a dysbiotic biofilm that grows on implant and/or abutment surfaces. Consequently, the entire surface becomes a notorious culprit, fostering bacterial adhesion that might lead to progressive loss of supporting bone. To combat peri-implantitis, research groups worldwide have diligently pursued the development of new antimicrobial coatings. However, for the successful development of coating materials, it is crucial to clarify their intended function. In this review, we propose a clear classification of coating strategies aimed at either preventing or treating peri-implantitis.

Methods: We first delve deep within the concepts of prevention and treatment, as well as the physicochemical properties and biological requirements of each dental implant component for interacting with host tissue cells, to unravel and guide materials and technique complexity according to each purpose.

Results: From a preventive standpoint, the goal is to impede disease initiation. This requires coating materials that can withstand the hostile oral environment indefinitely. In the treatment category, where the disease is already established, the coating material should act directly at the infected site. Furthermore, the physicochemical properties of the new antimicrobial coating must respect the properties required by each part of the implant to not compromise the interaction of the bone-biomaterial and soft tissue-biomaterial interfaces.

Conclusion: Despite considerable efforts in designing antimicrobial coatings, commercial success has remained elusive thus far. This underscores the need to consider essential components to facilitate the construction, validation, and eventual clinical potential of antimicrobial coatings for future marketing.

KEYWORDS
dental abutments, dental implants, peri-implantitis, therapeutics

2020/05231-4, 2023/15750-7; Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Grant/Award Numbers: 307471/2021-7, 314479/2023-6

Plain language summary

In this review, we have raised an essential point about the importance of considering both biological and chemical challenges in the development of antimicrobial coatings for preventing and treating peri-implantitis. From a preventive perspective, these coatings need to be designed to withstand the complex environment of the oral cavity while maintaining their integrity and functionality. This requires coatings that can resist changes in environmental factors. Conversely, in the treatment category, material coatings need to be responsive to either internal or external stimuli to activate the release of therapeutic agents. These coatings must be capable of switching on or off depending on the intensity of stimuli, allowing for targeted drug delivery to combat infection or inflammation.

1 | INTRODUCTION

Peri-implantitis is an inflammatory condition initiated by a dysbiotic biofilm that forms on implant and/or abutment surfaces.[1,2] During the inflammatory process, the chemical signaling cascade through the production of proinflammatory cytokines, promotes the activation of osteoclasts and, consequently, increased bone resorption.[1–3] The persistent inflammation disrupts the equilibrium between bone formation and resorption, leading to bone loss and jeopardizing the bone-implant interface.[1–3] However, peri-implantitis advances even more rapidly than periodontitis, probably, due to notable histological differences, such as the absence of a periodontal ligament, as well as the orientation of connective tissue fibers around implants, which makes them less effective in resisting infection.[4–7] Without timely intervention, this condition continues to erode bone structure, ultimately leading to implant instability and failure.[1,2]

Traditional treatment methods for peri-implantitis, such as mechanical debridement, antiseptics, antibiotics, and surgical procedures, often fail to fully address peri-implantitis.[8–10] Mechanical approaches may leave residual biofilm, systemic antibiotics raise concerns about resistance, and surgical options can be invasive with inconsistent outcomes.[8–10] These challenges have led to interest in alternative strategies, including local drug delivery systems and advanced surface modifications. As the inflammatory process in peri-implantitis is often triggered by biofilm adhesion on the implant/abutment surface, the entire surface becomes a notorious culprit, facilitating bacterial adhesion, given the ubiquitous presence of bacteria in the oral cavity.[6,7,11] This persistent biofilm creates a resistant microenvironment, compromising the surrounding bone and soft tissues.[6,7] In response, research efforts have focused on developing surfaces designed either to prevent bacterial adhesion or to assist in treating the disease once it has been established.[12,13]

Many studies have explored new coatings for dental implants, increasingly focused on bioactive coatings, antimicrobial surfaces, and stimuli-responsive materials that activate in response to inflammation.[12, 14–20] However, some of them often neglect critical questions regarding the specific area targeted (see Figure S1 in the online *Journal of Periodontology*) and the coating's intended purpose, whether for prevention or treatment of disease. For the successful development of coating material, it is crucial to clearly define the purpose for which it will be applied. In this context, we propose a new classification to elucidate biomaterial coating strategies used for preventing and treating peri-implantitis. From a prophylactic standpoint, the objective is always to prevent disease initiation. This necessitates that the material coating resists nonspecific interactions with cells, proteins, and other biomolecules present in the oral environment. Furthermore, in terms of prevention, strategies can be directed toward promoting biological sealing (an indirect approach) on abutments and/or deterring bacterial adhesion on overall surfaces, including both the implant itself and the abutment (a direct approach). In the treatment category, where the disease is already established, the material coating should possess direct action at the infected site.

2 | COATING STRATEGIES TO PREVENT PERI-IMPLANTITIS

According to a medical dictionary, the term "prevention" refers to the proactive measures taken to avert a disease or condition before it occurs. This means that overall coatings as a strategy to prevent peri-implantitis should resist degradation, potentially delaying or preventing disease onset,

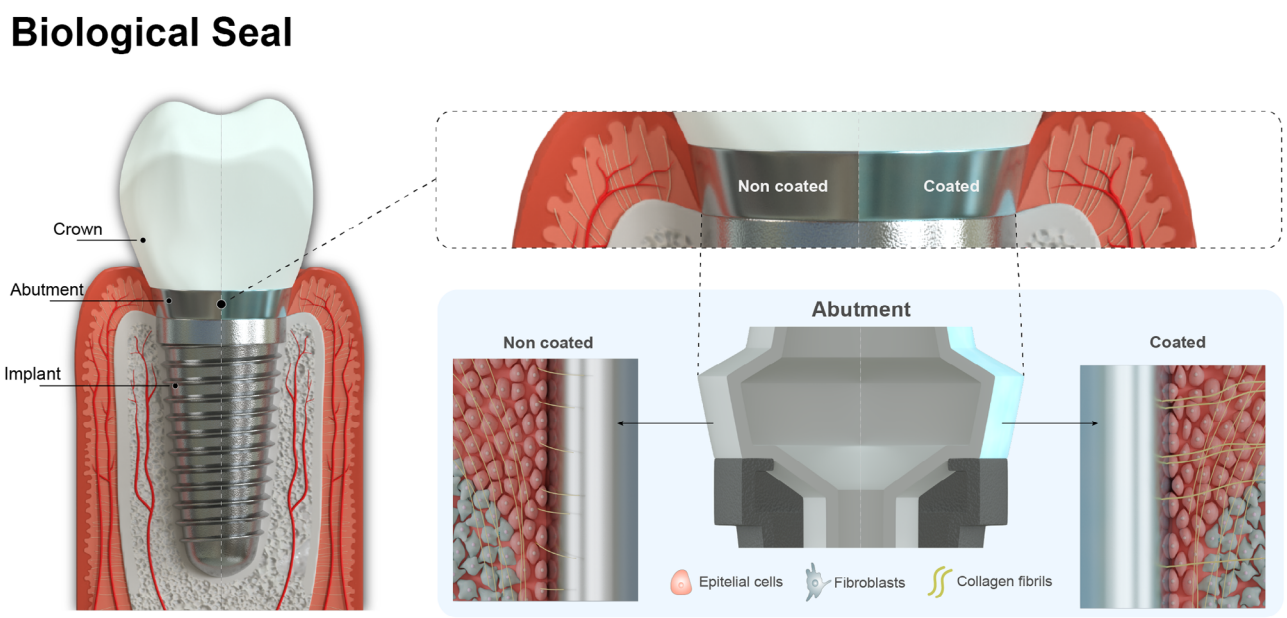


FIGURE 1 Representational illustration of each dental implant component, focusing on the abutment surface as the target substrate within coating development for soft tissue integration. Schematic differences of soft tissue and collagen fibers between non-coated-abutment surface (left) and coated-abutment surface (right). Peri-implanter connective tissue achieves tight adhesion to the coated via the biological interaction between collagen fibers running in a perpendicular and circular orientation in the connective tissue and the biological film on the abutment surface. Differently, peri-implant soft tissue fails to attach to the abutment implant surface due to the parallel instead of perpendicular orientation of collagen fibers running in the connective tissue.

even in patients with risk factors. For direct translation of the coating materials toward realistic applications, it is appropriate to highlight that patients considered risk factors for peri-implantitis would be potential candidates to receive a veneered dental implant to prevent the onset of the disease.

2.1 | Indirect strategies on abutment surfaces for soft tissue integration

Despite the biocompatibility largely explained by the stable oxide layer at the Ti surface, titanium remains bioinert and does not actively promote soft tissue adhesion.[21–23] Consequently, the weak interaction between soft tissue cells and titanium may facilitate the rapid progression of the inflammatory response, fibrous tissue formation, bone loss, and ultimately, implant failure.[23–25] Indirect strategies involve coating materials developed for soft tissue integration on abutment surfaces (Figure 1), given that abutments are in constant contact with soft tissue. While biological sealing does not entirely prevent peri-implantitis from appearing, it promotes a tighter seal between the implant and surrounding soft tissue to reduce the progression of inflammatory responses triggered by bacterial presence.[26,27] Although it does not eliminate the risk of peri-implantitis, it may enhance tissue health and resilience, contributing indirectly to the long-term success of dental implants.[26–30]

To achieve a biological seal on abutment surfaces, biological coatings can be applied to mimic dental properties without altering the roughness, since it is essential for abutment to have smoother and more regular surfaces.[31–35] The materials and techniques used for this proposal must be capable of providing coatings that are resistant to the hostility of the oral cavity and capable of stimulating hemidesmosome proteins to adhere to the film and/or redirect collagen fibers when fibroblast cells are exposed to the material. To this end, the biological coating can be constructed based on non-responsive polymers through the immobilization of substances such as proteins/peptides (such as collagen), glycoproteins in general (such as laminin-332), cationic polysaccharides (such as chitosan) and even synthetic/natural polymers. For instance, a recent study[36] developed a bioinspired peptide coating on titanium to prevent subgingival biofilm expansion by forming a long-lasting permucosal seal between soft tissue and abutment surfaces. This coating not only increased cell proliferation but also promoted the formation of hemidesmosomes by keratinocytes, showing promise for inducing a permucosal seal around dental implants. Another study[35] developed a lactoferrin-derived amyloid coating on titanium surfaces, demonstrating in vitro and in vivo evidence of soft tissue integration

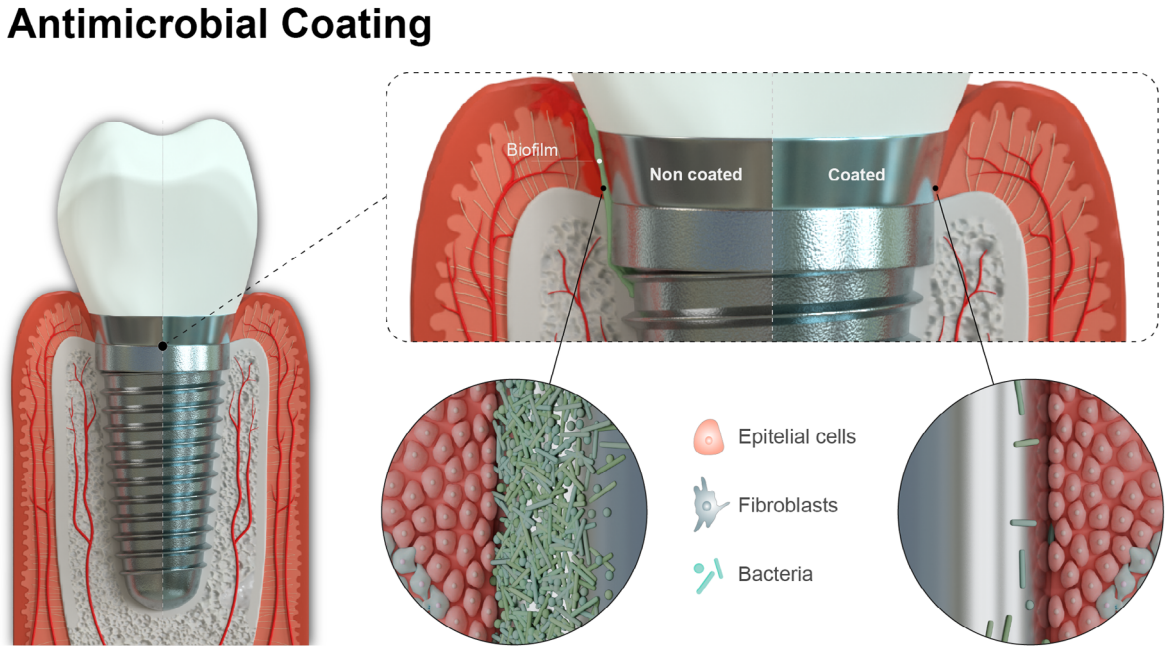


FIGURE 2 Representational illustration of each dental implant component, focusing on both abutment and implant surfaces as target substrates within the antimicrobial coating field. Schematic differences of biofilm formation between non-coated abutment/implant surface (left) and coated abutment/implant surface (right). LbL, layer-by-layer.

capacity and antibacterial activity. The coating, rich in amino and carboxyl groups, is bound to integrins on cell membranes, promoting cell proliferation and enhancing soft tissue sealing capability on titanium surfaces. This coating also stimulated junctional epithelium formation adjacent to metal surfaces and generated an integral soft tissue seal in rat models.

Strategies for enhancing soft tissue seals may also combine surface modification with biological coating. Two studies[37,38] evaluated soft tissue integration on abutment surfaces with topography modifications with and without bioactivation through argon plasma. These studies found favorable effects of plasma treatment on connective tissue, likely due to increased surface energy, enhanced wettability, and reduced biofilm accumulation and inflammation. However, the physical changes induced by plasma treatment are reversible, limiting its long-term effectiveness.

2.2 | Direct strategies on abutment/implant surfaces to prevent bacterial adhesion and biofilm formation

Unlike indirect strategies aimed at promoting biological sealing, direct strategies focus on the surface's ability to repel or kill bacteria upon contact, acting as antifouling surfaces with the potential to prevent bacterial attachment or inactivate bacteria via direct contact[39,40] (Figure 2). Therefore, within the prevention category and direct strategy, both implant and abutment surfaces could be coated.

2.2.1 | Direct strategies on abutment surfaces to prevent bacterial adhesion and biofilm formation

The method chosen for building antimicrobial coatings must preserve the original physical and topographic features required for abutment components without compromising their biomechanical properties. This means that aggressive techniques capable of modifying abutment structures should be avoided. Since bacterial adhesion is the reversible first stage of biofilm formation, antibiofouling coatings can be designed to create unfavorable topography and chemistry surfaces to prevent microbial settlement and attachment.[41,42]

Antifouling polymers, which resist nonspecific interactions between cells, proteins, and other biomolecules and surfaces, are considered promising materials.[43] Typically, these polymers have hydrophilic properties with polar or charged moieties that interact strongly with water molecules. Hydrophilic polymers form a hydrated layer on the surface through hydrogen bonding with water molecules, creating a physical barrier that reduces nonspecific interactions with proteins or bacteria, leading to steric repulsion.[44–49] The strength of surface hydration depends

on surface chemistry, molecular weight of polymers, packing density, film thickness, and chain conformation.[47–49] Major hydrophilic polymer classes include poly(ethylene glycol) (PEG), polyzwitterions, poly(oxazoline)s, and other nonionic hydrophilic polymers. To ensure long-term stability and fouling resistance, the antifouling polymer must be firmly anchored onto the abutment surface, preferably through covalent bonding.

Several studies have demonstrated the efficacy of PEG coatings in preventing bacterial colonization on medical devices.[47,50–52] For example, PEG-functionalized materials inhibited bacterial adhesion and biofilm formation due to hydration and steric hindrance effects. However, PEG degradation in the presence of oxygen[44,53] might limit its long-term utility.[44,54,55] Another study engineered a stable cell membrane coating based on CD47 receptors from red blood cell membranes, demonstrating natural antibiofouling and macrophage immunoregulatory properties for orthopedic and dental implant devices. While antifouling coatings based on hydration layers offer promising antibacterial properties, they may be affected by changes in temperature, pH, and salinity, requiring periodic component replacement.[56,57]

2.2.2 | Direct strategies on implant surfaces

Unlike abutments, surface roughness is one of the most crucial properties of implant surfaces as it promotes osseointegration.[58] Surface roughness enhances the surface area of the implant material, facilitating greater initial matrix deposition and earlier bone ingrowth.[59] However, roughness also poses a significant risk by potentially favoring bacteria attachment.[4,60,61] Regardless of the reasons favoring biofilm formation on rough surfaces,[62] the challenge lies in developing antimicrobial surfaces that maintain the natural physicochemical properties necessary for improving osseointegration and host response.[63]

From a clinical perspective, the significant impact of surface roughness on biofilm formation underscores the challenge of creating antimicrobial surfaces to prevent peri-implantitis while maintaining the necessary roughness values for implant surfaces. In this regard, surface modifications at the nanotopographical level have been investigated to interfere with bacterial attachment and prevent infection.[63] Nanopillar topographies, inspired by nature, have gained attention following studies demonstrating the surface bactericidal activity of cicadas' wings covered by nanopillars against *Pseudomonas aeruginosa* within minutes of contact.[64] Although the exact effect of topographical scale on bacterial attachment remains incompletely understood, several studies have indicated that topographical elements ranging from nanometers to micrometers can exert some control over bacterial attachment.[14,19,65–68] The mechanical bactericidal mechanism is associated with the physical capacity of nanopillars to kill bacteria by rupturing or deforming bacterial cell membranes, resulting in flattened cell morphology.[64,66] Similar bactericidal effects have been observed in natural topographical features such as dragonfly wings.[64] A proposed "ripping" model suggests that bacterial membranes are "ripped" by the shear forces caused by the movement of cells adhered to uneven nanopillars.[69]

Antimicrobial surfaces using charge-switchable coatings offer an innovative approach to surface modification for Ti implants. Covalent grafting is a particularly noteworthy technique in this context, as it enables the stable immobilization of positive charges on implant surfaces through various chemical reactions.[70] For example, oxidized Ti surfaces have been grafted with poly(sodium styrene sulfonate) groups via covalent bonding using radical polymerization, resulting in antimicrobial coating surfaces. Studies have shown that these coatings can inhibit the adhesion of bacteria such as *Staphylococcus aureus* by >90% and promote enhanced bone formation compared with non-grafted Ti surfaces.[71] However, one of the main challenges associated with this method is controlling the reaction conditions, including the concentration of the silane and reaction time.

In an interesting study, researchers successfully demonstrated the alkalinization of Ti using plasma electrolytic oxidation (PEO), followed by signaling with aminopropyltriethoxysilane (APTES) using plasma electrolytic oxidation (PEO) as a tattoo. PEO, created by high-voltage microdischarges, introduces -OH functional groups to Ti, resulting in high-quality coatings suitable for implant applications in terms of adhesion, topography, structure, and chemistry. In this specific case, PEO provided the -OH functional groups necessary for the bonding of aminosilane, which have the potential to positively charge the surface of materials. The outcomes of this study revealed improved electrochemical behavior, greater resistance to corrosion, and a bactericidal effect of APTES groups against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Additionally, the new coating surface exhibited favorable results in terms of protein adsorption and cytocompatibility with preosteoblastic cells.[72]

3 | COATING STRATEGIES TO TREAT PERI-IMPLANTITIS

In the context of peri-implantitis treatment, the focus shifts to direct action at the infected site. Treatment aims to either cure or slow down the progression of the

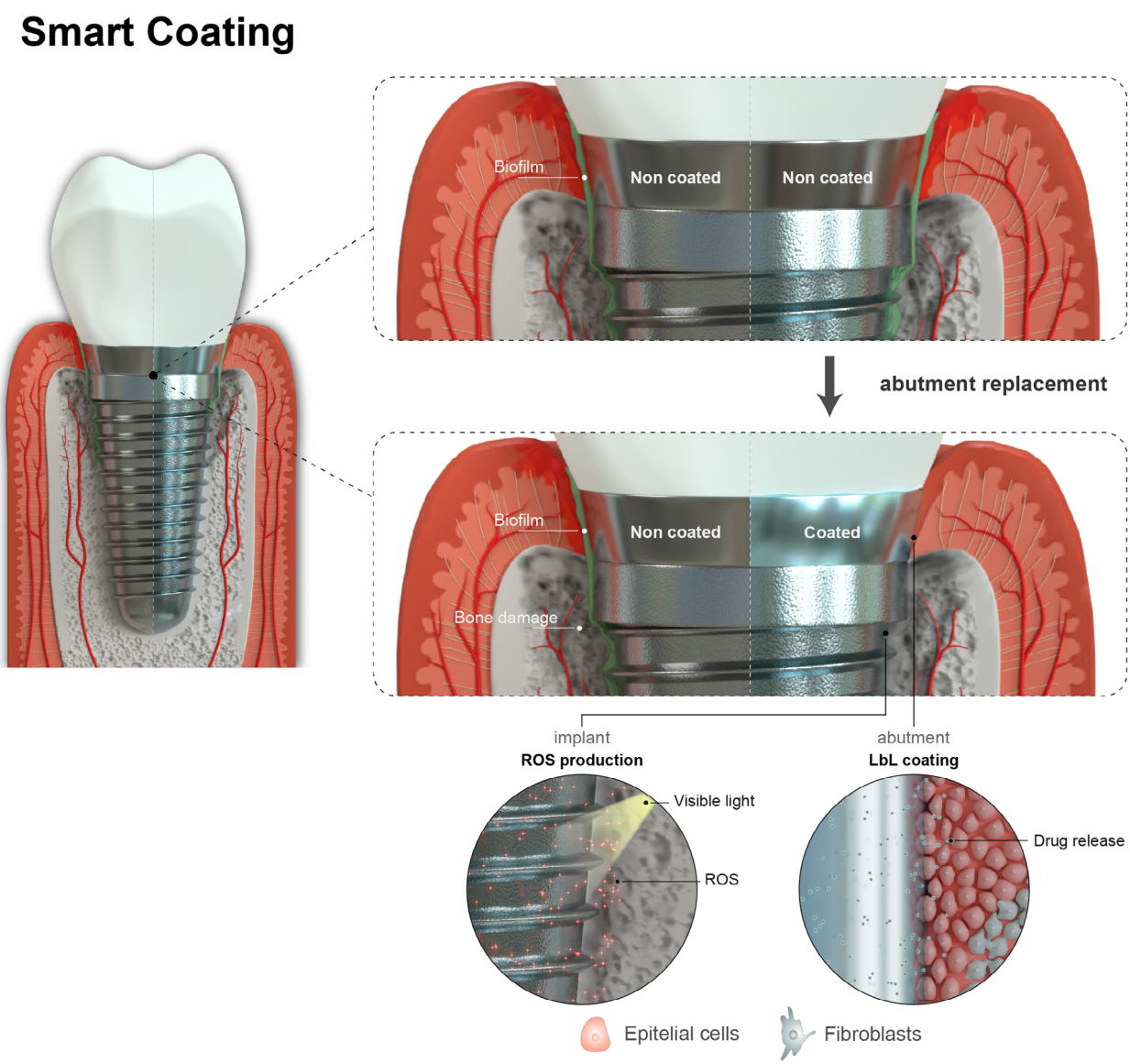


FIGURE 3 Representational illustration of each dental implant component, focusing on both abutment and implant surfaces as target substrate within smart coating field. Schematic differences of biofilm formation between non-coated abutment/implant surface (left) and coated abutment/implant surface (right), underlining two different approaches to drug release: from abutment (through smart/responsive coating) and implant (under visible light activation).

medical condition. However, curing peri-implantitis, defined as restoring health or normality to the affected area, remains questionable due to the complex and poorly understood nature of the disease. Coating strategies for treatment must be capable of immediate action against the infection and inflammatory process and can be applied to both abutment and implant surfaces. These coatings, known as smart coatings, are designed to be responsive to internal or external stimuli such as temperature, light, pressure, pH, or biological factors. By incorporating materials that can detect and respond to these stimuli, smart coatings can effectively combat peri-implantitis by adapting to changing conditions at the implant site (Figure 3).

3.1 | Smart systems strategies on abutment implant surfaces

Smart systems for treating peri-implantitis involve the controlled release of antimicrobial agents and/or substances that stimulate receptors responsible for releasing osteoclast-activating cytokines.[35,73–77] The term "Smart" was introduced for the first time in 2004 to describe overall biomaterials that responded to specific cellular signals.[78] The concept initially applied to biomaterials was extended to define the behavior of specific coatings capable of sensing their environment and making an appropriate response to that stimulus. These systems are designed to incorporate drugs and respond to external

stimuli to regulate their release over time. Typically constructed using natural and/or synthetic polymers,[79] smart coatings are primarily applied to abutment surfaces due to the flexibility of working with screw-in components that allow for replacement when needed. At this stage of the disease, where biological sealing is absent, coating abutment surfaces pose no clinical issues to be avoided.

Layer-by-layer (LbL) system are intelligent strategies for controlling the release of loaded drugs.[70,73,77,80] In essence, this technique is based on electrostatic attractions between positively charged polymers, as the cationic layer, and anionic polymers, as the anionic counterpart, during multilayer construction. The wide range of polymers capable of assembling the coating system makes it even more interesting: synthetic polymers (natural polymers, DNA, RNA, proteins, peptides, polysaccharides),[81,82] macromolecular assemblies (polymeric micelles),[83] (metal-) nanoparticles, and liposomes.[84] For the coating function as a drug delivery system, several drugs might be incorporated into nanometer scale coatings. Importantly, the intermolecular strength between drug and LbL will determine whether the drug release will occur in a controlled manner. A recent study by He et al. (2020)[85] demonstrated the creation of a smart coating through a simple LbL method without cross-linkers. The coated titanium substrate exhibited excellent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with sustained release of gentamicin for up to 11 days following an initial burst release in the first 24 h. However, challenges arise during drug incorporation due to the hydrophobic nature of polymers and the hydrophilic property of antibiotics. To confront this scenario, the formation of inclusion complexes through the synthesis between a drug and amphiphilic molecule emerges as a possible solution to retain drug within the LbL coating and control drug release overtime. In a recent study,[77] the authors improved the hydrophilicity of tetracycline (TC) and its capacity to entrap into the hydrophobic LbL coating, through a complexation between tetracycline and anionic beta cyclodextrin (BCD) molecules before antibiotic incorporation. This approach resulted in a constant release of TCBCD over the first 15 days, followed by a relatively steady and low release for up to 30 days, particularly pronounced in acidic pH conditions simulating an inflammatory process. Importantly, the sustained release of TCBCD exhibited strong antibacterial activity against *Staphylococcus aureus*, with more than a 5-log reduction in bacterial growth compared with commercial titanium substrates. Remarkably, the antibacterial activity of the system was maintained for up to 30 days regardless of the pH condition.[77]

Another approach to achieving antimicrobial coatings for treatment purposes is through chemical crosslinking. This process involves the use of natural and/or synthetic crosslinkers to form linkages within or between molecules in a polymer network, resulting in stronger covalent bonding or weaker interactions.[86] Crosslinking can enhance the biomechanical stability of materials under physiological conditions, leading to long-lasting antimicrobial effects.[87,88] In a recent study, quaternary ammonium carboxymethyl chitosan (QCMC) and collagen were chemically crosslinked with hydroxyapatite using poly dopamine as a binding agent to impart long-lasting, multi-antibacterial properties to implants.[89] Modified crosslinking LbL techniques were employed to form stable amide bonds, enabling the slow continuous release of QCMC for over 45 days. This approach holds significant promise for developing practical implant materials to treat implant-related infections.[89]

3.2 | Smart strategies on implant surfaces

Another treatment-focused system directed at the implant surface involves the construction of a responsive antimicrobial coating activated by external stimuli. This approach would enable the coating to acquire its desired function only when the implant surface is exposed due to bone resorption resulting from the inflammatory response.[90] However, it is crucial for titanium coatings to remain stable and non-degradable during the early phases after implantation. Therefore, both the materials and the methods applied must be thoroughly investigated to fabricate resilient and bioactive coatings.

An innovative solution involves creating a smart coating on implant surfaces using methods that incorporate doping elements to reduce the band gap of TiO_2 and enhance Ti photoactivity under visible light. In a recent study,[90] researchers doped TiO_2 coatings with nitrogen and bismuth via plasma electrolytic oxidation to develop an antimicrobial surface activated by visible light. While this research did not demonstrate significant biofilm reduction, the concept of creating dental implant surfaces capable of releasing ROS when activated by visible light, without harming tissue, holds promise for treating peri-implantitis. Clinically, these implants could be recommended for patients at risk of peri-implantitis. In such cases, antimicrobial activity would be targeted toward patients with risk factors once the implant is exposed due to peri-implantitis progression and bone resorption.

4 | CONCLUSION: ADVANCING CONCEPTUALIZATION TO EMERGE WITH ANTIMICROBIAL COATING CREATION

In this review, we have raised an essential point about the importance of considering both biological and chemical

challenges in the development of antimicrobial coatings for preventing and treating peri-implantitis. From a chemical perspective, these coatings need to be designed to withstand the complex environment of the oral cavity while maintaining their integrity and functionality. In the prevention category, coatings must maintain their properties consistently to preserve the biological seal and prevent the formation of biofilms. This requires coatings that can resist changes in external signals and environmental factors. Conversely, in the treatment category, smart coatings need to be responsive to specific signals to activate the release of therapeutic agents. These coatings must be capable of switching on or off depending on the presence or absence of external stimuli, allowing for targeted drug delivery to combat infection or inflammation.

While significant efforts have been devoted to developing antimicrobial coatings, their commercial success has been limited thus far. This underscores the complexity of the task at hand and the need for comprehensive approaches that consider various factors, including material properties, coating technology, validation methods, and clinical potential. By addressing these components systematically, researchers can enhance the likelihood of developing effective antimicrobial coatings for either preventing or treating peri-implantitis.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design of the study. Marta M. A. Pereira, Rafael Scaf de Molon, and Erica D. de Avila have been involved in data collection, data interpretation, drafting the manuscript and revising it critically. Valentim A. R. Barão, Jamil A. Shibli, Anton Sculean, and Flavia Q. Pirih have been involved in revising the manuscript critically and given final approval of the version to be published.

ACKNOWLEDGMENTS

This study was funded by the State of Sao Paulo Research Foundation (FAPESP, Sao Paulo, Brazil) (grant numbers 2021/10762-1 to M.M.A.P.; 2021/09434-0 to E.D.A; 2018/20719-3 to E.D.A; 2020/05231-4 to V.A.R.B.; 2023/15750-7 to R.S.M.), the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq, Brasília/DF, Brazil) (#307471/2021-7 to V.A.R.B.; #314479/2023-6 to J.A.S).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Rafael Scaf de Molon https://orcid.org/0000-0003-1110-6233

REFERENCES

1. Berglundh T, Armitage G, Araujo MG, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(Suppl 1):S313-S318.
2. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. *J Periodontol*. 2018;89(Suppl 1):S267-S290.
3. Bollen CM, Papaioanno W, Van Eldere J, Schepers E, Quirynen M, van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. *Clin Oral Implants Res*. 1996;7:201-211.
4. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater*. 1997;13:258-269.
5. Quirynen M, Vogels R, Peeters W, Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. *Clin Oral Implan Res*. 2006;17:25-37.
6. Belibasakis GN, Charalampakis G, Bostanci N, Stadlinger B. Peri-implant infections of oral biofilm etiology. *Adv Exp Med Biol*. 2015;830:69-84.
7. Villar CC, Huynh-Ba G, Mills MP, Cochran DL. Wound Healing Around Dental Implants. In: *Oral Wound Healing*: John Wiley and Sons, 2013:287-311.
8. Ephros H, Kim S, DeFalco R. Peri-implantitis: Evaluation and Management. *Dent Clin North Am*. 2020;64:305-313.
9. Herrera D, Berglundh T, Schwarz F, et al. Prevention and treatment of peri-implant diseases-The EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2023;50(Suppl 26):4-76.
10. Rokaya D, Srimaneepong V, Wisitrasameewon W, Humagain M, Thunyakitpisal P. Peri-implantitis Update: Risk Indicators, Diagnosis, and Treatment. *Eur J Dent*. 2020;14:672-682.
11. de Barros Lucena GA, de Molon RS, Moretti AJ, Shibli JA, Rego DM. Evaluation of Microbial Contamination in the Inner Surface of Titanium Implants Before Healing Abutment Connection: A Prospective Clinical Trial. *Int J Oral Maxillofac Implants*. 2018;33:853-862.
12. Alves Pereira MM, Piazza R, Santana AP, et al. Unraveling the Applicability of LbL Coatings for Drug Delivery in Dental Implant-Related Infection Treatment. *ACS Biomater Sci Eng*. 2025;11:13-32.
13. De Molon RS, Pereira MMA, De Avila ED. Implant surface features as key role on cell behavior. *Biocell*. 2022;46:1151-1156.
14. Feng G, Cheng Y, Wang SY, Borca-Tasciuc DA, Worobo RW, Moraru CI. Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough? *NPJ Biofilms Microbiomes*. 2015;1:15022.
15. Hasan J, Raj S, Yadav L, Chatterjee K. Engineering a nanostructured "super surface" with superhydrophobic and superkilling properties. *RSC Adv*. 2015;5:44953-44959.
16. Hsu LC, Fang J, Borca-Tasciuc DA, Worobo RW, Moraru CI. Effect of micro- and nanoscale topography on the adhesion of bacterial cells to solid surfaces. *Appl Environ Microbiol*. 2013;79:2703-2712.

17. Ivanova EP, Hasan J, Webb HK, et al. Bactericidal activity of black silicon. *Nat Commun*. 2013;4:2838.
18. MV G, NC C. Nano and Microscale Topographies for the Prevention of Bacterial Surface Fouling. . *Coatings*. 2014;4:37-59.
19. Serrano C, García-Fernández L, Fernández-Blázquez JP, et al. Nanostructured medical sutures with antibacterial properties. *Biomaterials*. 2015;52:291-300.
20. Whitehead KA, Colligon J, Verran J. Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. *Colloids Surf B Biointerfaces*. 2005;41:129-138.
21. Atsuta I, Ayukawa Y, Kondo R, et al. Soft tissue sealing around dental implants based on histological interpretation. *J Prosthodont Res*. 2016;60:3-11.
22. Abdallah MN, Badran Z, Ciobanu O, Hamdan N, Tamimi F. Strategies for Optimizing the Soft Tissue Seal around Osseointegrated Implants. *Adv Healthc Mater*. 2017;6.
23. Wang IC, Ou A, Johnston J, et al. Association between periimplantitis and cardiovascular diseases: A case-control study. *Journal of Periodontology*. 2022;93:633-643.
24. Ikeda H, Yamaza T, Yoshinari M, et al. Ultrastructural and immunoelectron microscopic studies of the peri-implant epithelium-implant (Ti-6Al-4V) interface of rat maxilla. *J Periodontol*. 2000;71:961-973.
25. Sani ES, Lara RP, Aldawood Z, et al. An Antimicrobial Dental Light Curable Bioadhesive Hydrogel for Treatment of Peri-Implant Diseases. *Matter-Us*. 2019;1:926-944.
26. Khang W, Feldman S, Hawley CE, Gunsolley J. A multi-center study comparing dual acid-etched and machined-surfaced implants in various bone qualities. *J Periodontol*. 2001;72:1384-1390.
27. Trisi P, Lazzara R, Rebaudi A, Rao W, Testori T, Porter SS. Bone-implant contact on machined and dual acid-etched surfaces after 2 months of healing in the human maxilla. *J Periodontol*. 2003;74:945-956.
28. Brito C, Tenenbaum HC, Wong BK, Schmitt C, Nogueira-Filho G. Is keratinized mucosa indispensable to maintain peri-implant health? A systematic review of the literature. *J Biomed Mater Res B Appl Biomater*. 2014;102:643-650.
29. Lin GH, Chan HL, Wang HL. The Significance of Keratinized Mucosa on Implant Health: A Systematic Review. *Journal of Periodontology*. 2013;84:1755-1767.
30. Roccuzzo A, Weigel L, Marruganti C, et al. Longitudinal assessment of peri-implant diseases in patients with and without history of periodontitis: A 20-year follow-up study. *Int J Oral Impl*. 2023;16:211-222.
31. Malheiros SS, Nagay BE, Bertolini MM, et al. Biomaterial engineering surface to control polymicrobial dental implant-related infections: focusing on disease modulating factors and coatings development. *Expert Rev Med Devices*. 2023;20:557-573.
32. van Oirschot B, Zhang Y, Alghamdi HS, et al. Surface Engineering for Dental Implantology: Favoring Tissue Responses Along the Implant. *Tissue Eng Part A*. 2022;28:555-572.
33. Dini C, Nagay BE, Cordeiro JM, et al. UV-photofunctionalization of a biomimetic coating for dental implants application. *Mater Sci Eng C Mater Biol Appl*. 2020;110:110657.
34. Emecen-Huja P, Eubank TD, Shapiro V, Yildiz V, Tatakis DN, Leblebicioglu B. Peri-implant periodontal wound healing. *Journal of Clinical Periodontology*. 2013;40:816-824.
35. Wang WR, Li J, Gu JT, et al. Optimization of Lactoferrin-Derived Amyloid Coating for Enhancing Soft Tissue Seal and Antibacterial Activity of Titanium Implants. *Advanced Healthcare Materials*. 2023;12(11):e2203086.
36. Koidou VP, Argyris PP, Skoe EP, et al. Peptide coatings enhance keratinocyte attachment towards improving the peri-implant mucosal seal. *Biomater Sci*. 2018;6:1936-1945.
37. Canullo L, Penarrocha Oltra D, Pesce P, et al. Soft tissue integration of different abutment surfaces: An experimental study with histological analysis. *Clin Oral Implants Res*. 2021;32:928-940.
38. Canullo L, Rakic M, Corvino E, et al. Effect of argon plasma pretreatment of healing abutments on peri-implant microbiome and soft tissue integration: a proof-of-concept randomized study. *Bmc Oral Health*. 2023;23(1):27.
39. Cloutier M, Mantovani D, Rosei F. Antibacterial Coatings: Challenges, Perspectives, and Opportunities. *Trends Biotechnol*. 2015;33:637-652.
40. Wang J, Vermerris W. Antimicrobial Nanomaterials Derived from Natural Products-A Review. *Materials (Basel)*. 2016;9(4):255.
41. Wei T, Yu Q, Chen H. Responsive and Synergistic Antibacterial Coatings: Fighting against Bacteria in a Smart and Effective Way. *Adv Healthc Mater*. 2019;8:e1801381.
42. Hasan J, Crawford RJ, Ivanova EP. Antibacterial surfaces: the quest for a new generation of biomaterials. *Trends Biotechnol*. 2013;31:295-304.
43. Faustino CMC, Lemos SMC, Monge N, Ribeiro IAC. A scope at antifouling strategies to prevent catheter-associated infections. *Adv Colloid Interface Sci*. 2020;284:102230.
44. Chen SF, Li LY, Zhao C, Zheng J. Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials. *Polymer*. 2010;51:5283-5293.
45. Francolini I, Vuotto C, Piozzi A, Donelli G. Antifouling and antimicrobial biomaterials: an overview. *Apmis*. 2017;125:392-417.
46. Lih E, Oh SH, Joung YK, Lee JH, Han DK. Polymers for cell/tissue anti-adhesion. *Prog Polym Sci*. 2015;44:28-61.
47. Razatos A, Ong YL, Boulay F, et al. Force measurements between bacteria and poly(ethylene glycol)-coated surfaces. *Langmuir*. 2000;16:9155-9158.
48. Zander ZK, Becker ML. Antimicrobial and Antifouling Strategies for Polymeric Medical Devices. *Acs Macro Lett*. 2018;7:16-25.
49. Zhang H, Chiao M. Anti-fouling Coatings of Poly(dimethylsiloxane) Devices for Biological and Biomedical Applications. *J Med Biol Eng*. 2015;35:143-155.
50. Gon S, Kumar KN, Nusslein K, Santore MM. How Bacteria Adhere to Brushy PEG Surfaces: Clinging to Flaws and Compressing the Brush. *Macromolecules*. 2012;45:8373-8381.
51. Roosjen A, van der Mei HC, Busscher HJ, Norde W. Microbial adhesion to poly(ethylene oxide) brushes: influence of polymer chain length and temperature. *Langmuir*. 2004;20:10949-10955.
52. Xing R, Lyngstadaas SP, Ellingsen JE, Taxt-Lamolle S, Haugen HJ. The influence of surface nanoroughness, texture and chemistry of TiZr implant abutment on oral biofilm accumulation. *Clin Oral Implants Res*. 2015;26:649-656.

53. Ulbricht J, Jordan R, Luxenhofer R. On the biodegradability of polyethylene glycol, polypeptoids and poly(2-oxazoline)s. *Biomaterials*. 2014;35:4848-4861.
54. Banerjee I, Pangule RC, Kane RS. Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Adv Mater*. 2011;23:690-718.
55. Liu YX, Chang CP, Sun T. Dopamine-Assisted Deposition of Dextran for Nonfouling Applications. *Langmuir*. 2014;30:3118-3126.
56. Zhang X, Liu L, Peng W, et al. Phosphonate/zwitterionic/cationic terpolymers as high-efficiency bactericidal and antifouling coatings for metallic substrates. *J Mater Chem B*. 2021;9:4169-4177.
57. DeFlorio W, Liu S, White AR, et al. Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Compr Rev Food Sci Food Saf*. 2021;20:3093-3134.
58. Elias CN, Oshida Y, Lima JH, Muller CA. Relationship between surface properties (roughness, wettability and morphology) of titanium and dental implant removal torque. *J Mech Behav Biomed Mater*. 2008;1:234-242.
59. Yeo A, Wong WJ, Khoo HH, Teoh SH. Surface modification of PCL-TCP scaffolds improve interfacial mechanical interlock and enhance early bone formation: An and characterization. *Journal of Biomedical Materials Research Part A*. 2010;92a:311-321.
60. Arnold JW, Bailey GW. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. *Poult Sci*. 2000;79:1839-1845.
61. Medilanski E, Wick LY, Wanner O, Harms H. Mutual influences of Pseudomonas aeruginosa and Desulfovibrio desulfuricans on their adhesion to stainless steel. *Biofouling*. 2003;19:125-132.
62. Scheuerman TR, Camper AK, Hamilton MA. Effects of Substratum Topography on Bacterial Adhesion. *J Colloid Interface Sci*. 1998;208:23-33.
63. Mas-Moruno C, Su B, Dalby MJ. Multifunctional Coatings and Nanotopographies: Toward Cell Instructive and Antibacterial Implants. *Adv Healthc Mater*. 2019;8:e1801103.
64. Ivanova EP, Hasan J, Webb HK, et al. Bactericidal activity of black silicon. *Nat Commun*. 2013;4:2838.
65. Graham MV, Cady NC. Nano and Microscale Topographies for the Prevention of Bacterial Surface Fouling. *Coatings*. 2014;4:37-59.
66. Hasan J, Raj S, Yadav L, Chatterjee K. Engineering a nanostructured "super surface" with superhydrophobic and superkilling properties. *Rsc Adv*. 2015;5:44953-44959.
67. Hsu LC, Fang J, Borca-Tasciuc DA, Worobo RW, Moraru CI. Effect of Micro- and Nanoscale Topography on the Adhesion of Bacterial Cells to Solid Surfaces. *Appl Environ Microb*. 2013;79:2703-2712.
68. Whitehead KA, Colligon J, Verran J. Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. *Colloid Surface B*. 2005;41:129-138.
69. Bandara CD, Singh S, Afara IO, et al. Bactericidal Effects of Natural Nanotopography of Dragonfly Wing on. *Acs Appl Mater Inter*. 2017;9:6746-6760.
70. Chouirfa H, Bouloussa H, Migonney V, Falentin-Daudre C. Review of titanium surface modification techniques and coatings for antibacterial applications. *Acta Biomater*. 2019;83:37-54.
71. Alcheikh A, Pavon-Djavid G, Helary G, Petite H, Migonney V, Anagnostou F. PolyNaSS grafting on titanium surfaces enhances osteoblast differentiation and inhibits Staphylococcus aureus adhesion. *J Mater Sci Mater Med*. 2013;24:1745-1754.
72. Silva J, Costa RC, Nagay BE, et al. Boosting Titanium Surfaces with Positive Charges: Newly Developed Cationic Coating Combines Anticorrosive and Bactericidal Properties for Implant Application. *ACS Biomater Sci Eng*. 2023;9:5389-5404.
73. de Avila ED, Castro AGB, Tagit O, et al. Anti-bacterial efficacy via drug-delivery system from layer-by-layer coating for percutaneous dental implant components. *Appl Surf Sci*. 2019;488:194-204.
74. Hammond PT. Building biomedical materials layer-by-layer. *Mater Today*. 2012;15:196-206.
75. Kulikouskaya V, Zhdanko T, Hileuskaya K, et al. Physicochemical aspects of design of ultrathin films based on chitosan, pectin, and their silver nanocomposites with antiadhesive and bactericidal potential. *Journal of Biomedical Materials Research Part A*. 2022;110:217-228.
76. Li X, Qi ML, Sun XL, et al. Surface treatments on titanium implants via nanostructured ceria for antibacterial and anti-inflammatory capabilities. *Acta Biomaterialia*. 2019;94:627-643.
77. Verza BS, van den Beucken JJJP, Brandt JV, et al. A long-term controlled drug-delivery with anionic beta cyclodextrin complex in layer-by-layer coating for percutaneous implants devices. *Carbohyd Polym*. 2021;257:117604.
78. Anderson DG, Burdick JA, Langer R. Materials science. Smart biomaterials. *Science*. 2004;305:1923-1924.
79. Wohl BM, Engbersen JFJ. Responsive layer-by-layer materials for drug delivery. *J Control Release*. 2012;158:2-14.
80. Thakur G, Rodrigues FC, Singh K. Crosslinking Biopolymers for Advanced Drug Delivery and Tissue Engineering Applications. *Cutting-Edge Enabling Technologies for Regenerative Medicine*. 2018;1078:213-231.
81. Stuart MA, Huck WT, Genzer J, et al. Emerging applications of stimuli-responsive polymer materials. *Nat Mater*. 2010;9:101-113.
82. Saurer EM, Jewell CM, Kuchenreuther JM, Lynn DM. Assembly of erodible, DNA-containing thin films on the surfaces of polymer microparticles: toward a layer-by-layer approach to the delivery of DNA to antigen-presenting cells. *Acta Biomater*. 2009;5:913-924.
83. Such GK, Johnston AP, Caruso F. Engineered hydrogen-bonded polymer multilayers: from assembly to biomedical applications. *Chem Soc Rev*. 2011;40:19-29.
84. Johnston AP, Read ES, Caruso F. DNA multilayer films on planar and colloidal supports: sequential assembly of like-charged polyelectrolytes. *Nano Lett*. 2005;5:953-956.
85. He LJ, Hao JC, Dai L, Zeng RC, Li SQ. Layer-by-layer assembly of gentamicin-based antibacterial multilayers on Ti alloy. *Mater Lett*. 2020;261:127001.
86. Oryan A, Kamali A, Moshiri A, Baharvand H, Daemi H. Chemical crosslinking of biopolymeric scaffolds: Current knowledge and future directions of crosslinked engineered bone scaffolds. *Int J Biol Macromol*. 2018;107:678-688.
87. Ballarre J, Aydemir T, Liverani L, Roether JA, Goldmann WH, Boccaccini AR. Versatile bioactive and antibacterial coating

system based on silica, gentamicin, and chitosan: Improving early stage performance of titanium implants. *Surf Coat Tech*. 2020;381.

88. Zhu M, Liu X, Tan L, et al. Photo-responsive chitosan/Ag/MoS(2) for rapid bacteria-killing. *J Hazard Mater*. 2020;383:121122.
89. Lin R, Wang Z, Li Z, Gu L. A two-phase and long-lasting multi-antibacterial coating enables titanium biomaterials to prevent implants-related infections. *Mater Today Bio*. 2022;15:100330.
90. Nagay BE, Dini C, Cordeiro JM, et al. Visible-Light-Induced Photocatalytic and Antibacterial Activity of TiO Codoped with Nitrogen and Bismuth: New Perspectives to Control Implant-Biofilm-Related Diseases. *Acs Appl Mater Inter*. 2019;11:18186-18202.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pereira MMA, de Molon RS, Barão VAR, et al. Surveying coating strategies for peri-implantitis management: Clinical implications and classificatory approaches. *J Periodontol*. 2024;1-11.
https://doi.org/10.1002/JPER.24-0462

Sleep Medicine 133 (2025) 106662

Contents lists available at ScienceDirect

Sleep Medicine

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

Association between children's sleep bruxism with that of their parents/guardians: A systematic review and meta-analysis

Francisco das Chagas Santos-Júnior [a], Ayah Qassem Ahmad Shqair [b,c], Marcoeli Silva de Moura [d,e], Marina de Deus Moura de Lima [e,f], Lúcia de Fátima Almeida de Deus Moura [e,g], Francisco Wilker Mustafa Gomes Muniz [h,j], Cacilda Castelo Branco Lima [e,i,*]

[a] Postgraduate Program in Dentistry, Federal University of Piauí (UFPI), Brazil
[b] Federal University of Pelotas (UFPel), Brazil
[c] Department of Dental Sciences, Faculty of Graduate Studies, Arab American University (AAUP), Ramallah, Palestine
[d] São Paulo State University Júlio de Mesquita Filho (Unesp), Brazil
[e] Department of Pathology and Dental Clinic, Postgraduate Program in Dentistry, Federal University of Piauí (UFPI), Brazil
[f] University of São Paulo (USP), Brazil
[g] University of Brasília (UnB), Brazil
[h] Periodontology from the Federal University of Pelotas (UFPel), Brazil
[i] Federal University of Minas Gerais (UFMG), Brazil
[j] Federal University of Rio Grande do Sul (UFRGS), Brazil

ARTICLE INFO

Keywords:
Sleep bruxism
Child
Heredity
Oral health

ABSTRACT

The aim of this was to analyze the association between sleep bruxism (SB) in children and their parents/guardians. A systematic review (registered in PROSPERO: CRD42023445486) was conducted using PubMed, Web of Science, Embase, and Scopus databases, as well as reference lists and gray literature, up to March 2025. Observational studies assessing SB in children and their parents/guardians were included, with no restrictions on date of publication or language. Risk of bias (RoB) was assessed using the Joanna Briggs Institute checklist and Newcastle-ottawa (NOS). Meta-analyses, subgroup analyses, sensitivity analyses, and meta-regression analyses were performed ($p<0.05$). Certainty of the evidence was also assessed. A total of 7,818 articles were retrieved, of which 15 met the eligibility criteria and were included in the qualitative analysis, while 14 were considered for meta-analysis. The majority exhibited a low RoB (66.7%). Children whose parents or guardians have SB are 3.23 times more likely to exhibit SB compared to those whose parents or guardians do not have SB (95% CI [confidence interval]: 2.41–4.32). In subgroup analyses, significant association was observed between SB in children and their fathers and/or mothers (odds ratio [OR]: 3.78; 95% CI: 2.87–4.97). Additionally, for possible (OR: 2.75; 95% CI: 1.79–4.24) and probable (OR: 3.90; 95% CI: 2.70–5.63) SB, as well as low (OR: 2.50; 95% CI: 1.71–3.65) and high/moderate (OR: 4.78; 95% CI: 3.76–6.08) RoB. The certainty of the evidence was assessed to be very low. There is an association between the occurrence of SB in children and their parents/guardians.

1. Introduction

Sleep bruxism (SB) is a repetitive activity of the masticatory muscles characterized by the involuntary grinding or clenching of teeth that occurs during sleep, which may be rhythmic (phasic) or non-rhythmic (tonic) [1,2]. The presence of SB in children, in addition to

Abbreviations: AB, awake bruxism; CI, confidence interval; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; JBI, Joanna Briggs Institute; NOS, Newcastle-Ottawa Scale; OR, Odds Ratio; PECO, population, exposure, comparison, outcomes; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement; PROSPERO, International Prospective Register of Systematic Reviews; SB, sleep bruxism; STAB, Standardized Tool for the Assessment of Bruxism.

* Corresponding author. Department of Pathology and Dental Clinic, Postgraduate Program in Dentistry, Federal University of Piauí, Campus Universitário Ministro Petrônio Portella, Bloco 5, Bairro Ininga, 64049-550, Teresina, Piauí, Brazil.
E-mail addresses: cacildacb@hotmail.com, cacildacbl@ufpi.edu.br (C.C.B. Lima).

https://doi.org/10.1016/j.sleep.2025.106662
Received 19 April 2025; Received in revised form 27 May 2025; Accepted 30 June 2025
Available online 1 July 2025

contributing to temporomandibular disorders, is associated with reports of headaches, tooth wear, and breathing difficulties, thereby interfering with the quality of life of both children and their families [3,4]. There is no consensus on which clinical dental management should be performed in children because the etiology of SB is not well understood [1,5–8].

The etiology of SB is multifactorial and may be systemic, psychological, hereditary, or related to other sleep disorders [9–11]. Sociodemographic factors associated with genetic factors may modulate the occurrence of SB in children, demonstrating the need for studies that investigate this relationship for better understanding [12]. Studies investigating the occurrence of SB in the family reinforce the genetic issue as a factor associated with SB in children [12–14].

The prevalence of SB in children and adolescents ranges from 3.5% to 49.6% and tends to decline with age [9]. In the absence of a family history of SB, the prevalence of SB in children is 22.5%. However, in cases where the family history of SB is present, this prevalence increases to 57.8% [15]. Furthermore, a similar pattern is observed when both parents/guardians exhibit SB, increasing its prevalence in children to 87.5% [16]. These numbers may be underestimated as many parents/guardians, due to lack of knowledge, may not recognize episodes of SB and most diagnoses are made based on self-reports [17].

Therefore, this systematic review aimed to examine the association between SB in children and their parents or guardians. The study hypothesis is that children whose parents/guardians have SB have higher chances to also have SB than children whose parents/guardians do not have the condition. In this way, aim to provide evidence for a better understanding of the role of genetic and hereditary factors in the etiology of SB.

2. Methods

2.1. Study design and protocol

A systematic review was developed to answer the question: "Do children whose parents/guardians have SB have a greater chance of having SB compared to children whose parents/guardians do not have the condition?". The protocol was registered in the *International Prospective Register* (PROSPERO – CRD42023445486). This review is reported in accordance with the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement* (PRISMA) [18].

2.2. Eligibility criteria

The eligibility criteria were chosen in accordance with the acronym PECOS.

- Population (P): Children aged 0–12 years;
- Exposure (E): parents/guardians with SB;
- Comparison (C): parents/guardians without SB;
- Outcome (O): occurrence of SB in children;
- Study type (S): Observational studies (cross-sectional, case-control, and cohort).

Observational studies that evaluated SB both in children and their parents/guardians whose diagnosis was based on possible SB, through self-report/positive report in a questionnaire/interview were included; in probable SB, through positive clinical examination associated or not with self-report/positive report; or definitive SB, through positive instrumental evaluation (audio and video devices, such as polysomnography, electromyography) with or without self-report/positive report and/or positive clinical [1]. Studies in which the diagnosis of SB did not follow the previously mentioned classification were grouped according to the definitions provided in the included studies, considering only the definitions of the current classification due to the methodological variations of the studies.

Studies were excluded that (a) they did not investigate SB in children and parents/guardians simultaneously; (b) did not have a comparison group; (c) did not specify the SB diagnostic method; (d) did not differentiate between SB and awake bruxism; (f) whose participants, children and parents/guardians, had disabilities/syndromes or used some systemic medication; (g) laboratory studies, editorials, case report, review. Studies with incomplete data or those generated doubts, the authors were contacted by email and if no answers were obtained, they were excluded.

2.3. Search strategy

The databases used were PubMed (National Library of Medicine), Scopus (Elsevier), Embase (Elsevier), and Web of Science (Clarivate Analytics). The search strategies used are available in Appendix A (supplementary data). No restrictions were imposed on the year of publication or language. The searches were carried out in March 2025.

A manual search was conducted in three journals that publish on the topic (Journal of Oral Rehabilitation, Journal of Sleep Research, and Journal of Dental Research), from February 2019 to March 2025. Furthermore, searches were conducted through the reference lists of the studies and reviews on the topic, initially selected after in full reading, obtained during the literature review stage [5,9,11–17,19–23].

Gray literature was searched, in Open Gray and Google Scholar, limited to the first 300 articles presented in order of relevance [24]. In these databases, adaptations of the previously reported search were conducted using the search terms "sleep bruxism" AND "children" AND 'father" AND "mother" both separately and in combination. Initially, the objective was to conduct a search in PROQUEST, however, this was not feasible due to restricted access from our institutions to this database at the time of the data collection.

2.4. Study selection

After carrying out the searches, the reference management software EndNote Web® (version X7; Clarivate Analytics) was used to organize references and exclude duplicates. Subsequently, the references were imported into the software Rayyan® (Rayyan Systems, Inc.).

The study selection process included two stages. In the first stage, two researchers (F.C.S.J. and A.Q.S.), independently selected the articles based on the titles and abstracts of all references located (inter-examiner *kappa*: 0.83). In the second stage, relevant articles with insufficient data in the title or abstract were read in full, independently (inter-examiner *kappa*: 0.95). When disagreements arose in any of these phases, a consensus meeting was convened, and if the discrepancy persisted, a third researcher was consulted (C.C.B.L.).

The flowchart of the search and article selection strategy is shown in Fig. 1.

2.5. Data extraction

Data collection was performed by two researchers (F.C.S.J. and A.Q.S.) independently, supervised by a third researcher in case of disagreements (C.C.B.L.).

The following data were extracted from eligible articles and recorded in a spreadsheet of Microsoft Excel® (version 2104, EUA): (1) study identification: name of the first author, location/country of corresponding authors, year of publication, journal title, language of publication, type of study, and country where the study was conducted; (2) sample: type of sample, number of included children, number of biological parents, number of non-biological parents, number of guardians, sex distribution (in each group), and age group; (3) results: prevalence of children with SB (in each group), prevalence of children without SB (in each group), prevalence of parents/guardians with SB (in each group), prevalence of parents/guardians without SB (in each group); parameters used for the diagnosis of SB; (4) main results of the study (including any adjustments such model adjustment variables, footnotes, as well as

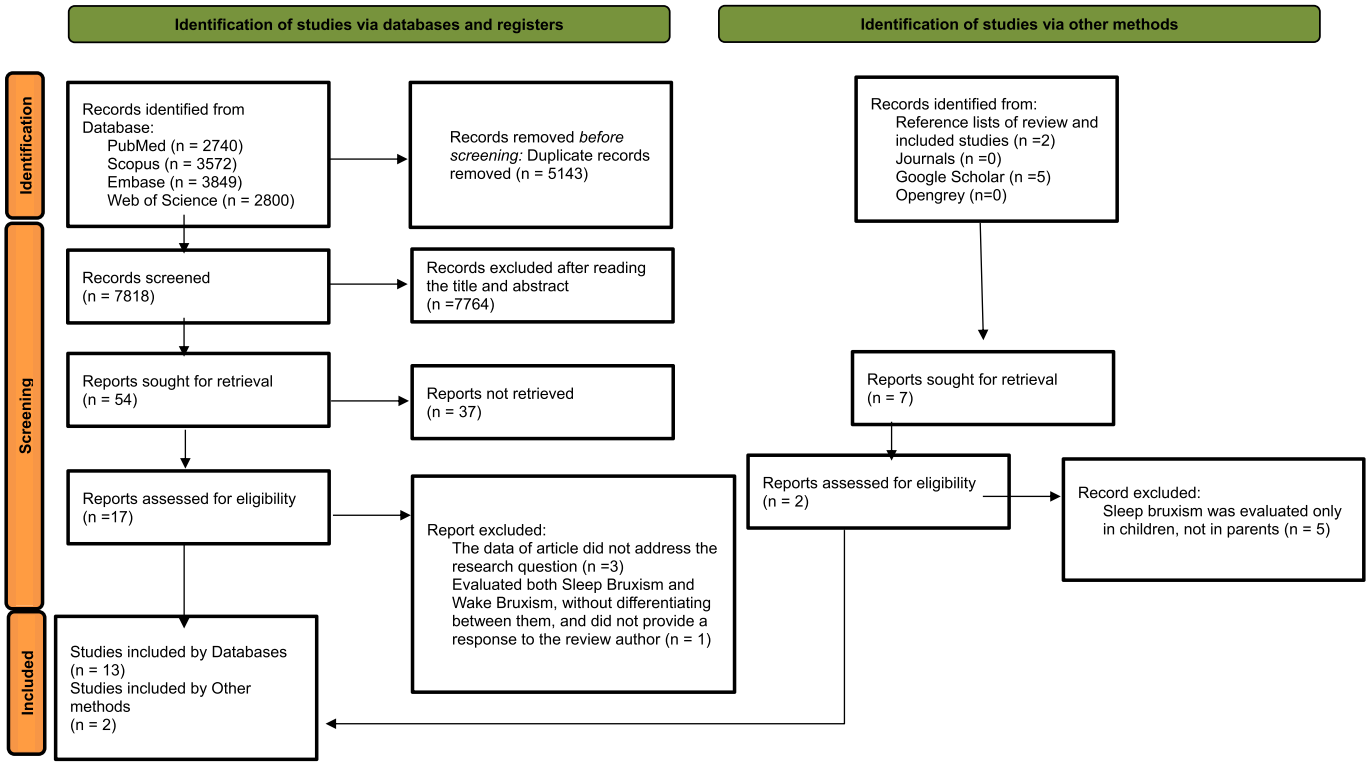


Fig. 1. Flowchart of the study selection process.

subgroup analyses); (5) declaration of conflicts of interest; and (6) reported financing.

In case of doubts or insufficient data, the authors were contacted via email to obtain further information.

2.6. Critical evaluation of included studies

Two researchers (F.C.S.J. and A.Q.S.) independently assessed the risk of bias in the selected studies. The quality and risk of bias assessment were conducted using the Joanna Briggs Institute (JBI) checklist for cross-sectional studies [25]. This tool consists of eight items, each with four response options: 'Yes' (indicating no risk of bias), 'No' (indicating a high risk of bias), 'Not clear' (indicating risk of bias not clear), and 'Not applicable' [25]. Studies were classified as low (more than 70% of items with "yes" answers), moderate (between 50% and 69% "yes") and high risk of bias (less than 49% "yes") [26]. The Newcastle-Ottawa Scale (NOS) was used to assessment the quality and risk of bias the case-control study. Using the NOS, eight items were assessed across three domains: (a) group selection, (b) comparability and (c) investigation of exposure. For each item in the domain a maximum of one star was assigned, with the exception of the comparability domain, which was assigned two stars [27], classifying studies above six star as low risk of bias [28].

2.7. Evidence synthesis and statistical analysis

Qualitative and quantitative analyses were performed. To analyze the presence of SB in children and parents/guardians, both studies that assessed the condition dichotomously (presence or absence) and those that assessed SB frequencies in both children and parents/guardians were considered, with the condition being considered present, as well as the diagnosis of SB at any frequency and/or period of time [29]. Data on associations between children having SB and parents/guardians having the same condition were included in the meta-analyses.

Three subgroup analyses were performed. In the first, the subgroups were: (1.) presence of SB only in fathers and/or mothers and (2.) presence of SB in parents and guardians. In the second, the SB diagnostic criteria reported in the studies included in the meta-analysis were considered: (1.) report of SB in children (possible SB) and (2.) clinical examination of the presence of dental wear associated or not with the report (probable SB). And third, analysis by risk of bias was carried out: (1.) studies with low risk of bias and (2.) studies with moderate and high risk of bias.

Odds ratio (OR) and their respective 95% confidence intervals (IC) were calculated for each study. The Q test evaluated heterogeneity and quantified by I^2. Due to the high heterogeneity, a random-effects model was applied to all analyses [30]. RevMan® software (Cochrane Collaboration, version 5.0, United Kingdom) was used to perform these analyses. The significance level was set at 5% for all analyses ($p < 0.05$).

Furthermore, to evaluate the effect of each study on the pooled estimate, sensitivity analysis was performed for all meta-analyses. To assess the risk of publication bias among the studies included in the meta-analysis, the Egger [31] and Begg [32] tests were performed. The assessment of the certainty of the evidence of each meta-analysis was performed using the scale *Grading of Recommendations Assessment, Development, and Evaluation* (GRADE) [33]. The criteria for lowering the certainty of the evidence were: analysis of risk of bias, inconsistency, indirectness, imprecision, and publication bias. To enhance the certainty of the evidence, the magnitude of the effect, potential confounding variables that might underestimate the intervention effect, and the dose-response gradient were analyzed [33].

3. Results

3.1. Study selection

After duplicate removal, a total of 7,818 articles were identified. Of these, only 17 met the eligibility criteria. Two articles were considered eligibility [34,35], identified through reference lists. Nineteen authors were contacted because of missing data, answered by only two authors.

Four studies were excluded, after contact by email, as they did not have sufficient data to answer the question of this review [36–38] or the study did not differentiate the diagnosis of SB or awake bruxism [19]. Therefore, 15 studies were included in this systematic review (Fig. 1).

3.2. Study characteristics

The characteristics of the included studies are presented in Table 1. Fourteen studies were classified as cross-sectional. The study of Sampaio et al. 2018 [12] applied a different classification from those performed by the authors of this review. The studies were carried out in six countries (Brazil, Turkey, Iran, Saudi Arabia, Spain, and China), with the majority in Brazil (53.3%), published from 2004 to 2024, with a total of 17,251 children and parents/guardians (Table 1).

Assessment of the association of SB in children and their parents/guardians was carried out in 14 of the 15 articles included [10,12,13,15,16,21,34,35,39–43]. One article did not report the direct association in the text but presents data on the association measure [17]. Significant associations between the child and the parent/guardian having SB was observed in 10 studies [10,12,13,15,16,21,35,40–42], however, in four studies there was no such association [34,39,43,44].

Eight studies reported the participation of only fathers and/or mother [12,15,16,40–44] and observed that there is an association between fathers and/or mothers and children having SB, except in two study [43,44] which was not significant (Table 1).

The diagnosis of SB in nine studies was made through report/self-report using questionnaires [10,13,16,17,34,35,39,40,43] and six through report/self-report using a questionnaire associated with the clinical examination [12,15,21,41,42,44] (Table 1).

3.3. Risk of bias in each study

The risk of bias for each study included in the qualitative analysis is presented in Figs. 2 and 3. The majority of articles (66.7%) presented a low risk of bias [12,15,17,21,34,35,39,40,43,44] (Figs. 2 and 3).

3.4. Summary of results

3.4.1. Global meta-analysis of the association between parents/guardians and their children having SB

To evaluate the association between parents/guardians having SB and their children, a meta-analysis was conducted with 14 studies [10,12,13,15,16,21,34,35,39–44]. It was not possible to perform a meta-analysis with one study due to insufficient data [17]. Children whose parents/guardians had SB are 3.23 times more chance to have SB compared to parents/guardians who do not have SB (OR: 3.23; 95% CI: 2.41–4.32; $p < 0.0001$; $I^2 = 72\%$; random-effect model) (Fig. 4).

3.4.2. Analysis of subgroups based on kinship, diagnostic criteria, and risk of bias

In the subgroup analysis of kinship, the significance of the findings was maintained only when the presence of SB was assessed exclusively in fathers and/or mothers and their children (OR: 3.78; 95% CI: 2.87–4.97; $p < 0.001$; $I^2 = 63\%$; random-effect model) (Table 2). For the subgroup analysis based on diagnostic criteria, the significance of the findings remained significant for both possible SB (OR: 2.75; 95% CI: 1.79–4.24; $p < 0.001$; $I^2 = 75\%$; random-effect model) and probable SB in children (OR: 3.90; 95% CI: 2.70–5.63; $p < 0.001$; $I^2 = 61\%$; random-effect model) (Table 2). However, no significant differences were observed in the heterogeneity of estimates of this subgroup ($I^2 = 31.4\%$; $p = 0.23$) (Table 2).

In the subgroup analysis according to the risk of bias, the significance of the findings remained significant in studies both low risk of bias (OR: 2.50; 95% CI: 1.71–3.65; $p < 0.001$; $I^2 = 68\%$; random-effect model) or with a high/moderate risk of bias (OR: 4.78; 95% CI: 3.76–6.08; $p < 0.001$; $I^2 = 14\%$; random-effect model) (Table 2). The difference test between the subgroups indicated a difference between the heterogeneity of the estimates of this analyzed subgroup ($I^2 = 87.5\%$; $p = 0.005$) (Table 2).

3.4.3. Sensitivity analysis

The sensitivity analysis demonstrated that the exclusion of a study did not change the significance of the results obtained in the meta-analysis. However, a lower OR was observed when removing the study of Chen et al. (2004) [41] (OR: 3.01; 95% CI: 2.27–3.98), while an increase was noted when removing the study by Clementino et al. (2017) [34] (OR: 3.62; 95% CI: 2.85–4.59) (Table 3).

3.5. Publication bias

When assessing the risk of publication bias among the 14 studies included in the meta-analysis, both Egger [31] ($t = -0.44$, $df = 12$, $p = 0.6656$) and Begg [32] ($z = -1.04$, $p = 0.2883$) tests indicated no publication bias among the studies. However, the distribution of studies in the funnel plot was quite heterogeneous (Fig. 5).

3.6. Meta-regression of studies

Meta-regression was performed due to the moderate heterogeneity ($I^2 = 72\%$) of the studies. In the meta-regression a high R^2 was detected (67.45%), which may partially explain the main results. In addition, the risk of bias may be attributed for such heterogeneity ($p = 0.023$) (Table 4).

3.7. Certainty of evidence

The certainty of evidence was very low in the analysis of the association between the occurrence of SB in children and their parents/guardians (Table 5).

4. Discussion

This is the first systematic review that aimed to determine the association between the occurrence of SB in children and SB in parents/guardians. Children whose parents/guardians had SB had three times more chance to have SB compared to parents/guardians who do not have SB, according to the global meta-analysis. However, in the subgroup analysis this association only remains when the parents also have SB, indicated a genetic/hereditary relationship with the condition. These results are consistent with previous studies involving family members, such as parents and twins, as well as genetic polymorphisms [45,46]. Primary studies evaluating the presence of SB in children and their family members indicate that the prevalence of SB in children increases when at least one parent exhibits SB [14–16].

In this context, these findings suggest the heritability of SB, or at least the heritability of the habits that lead to SB being partially genetically determined [45,47,48]. Since the etiology of SB is multifactorial, environmental and emotional factors also modulate the occurrence of SB [12,22], as family characteristics and social behaviors can affect the child's emotional state [1,17,44]. However, in this systematic review the influence of these factors was not studied. Furthermore, parents/guardians with SB are more attentive to their children's behavior during sleep, as they may know the signs and symptoms of this condition [17].

Studies that evaluate the genetic polymorphism associated with sleep bruxism indicate that some serotonin 5-HT2A receptor genes (rs6313, rs2770304 and rs4941573) and the single nucleotide C allele are related to SB [49]. Furthermore, genetic polymorphism in the ACTN3 gene (rs678397, rs1671064 and rs1815739) and masseter muscle fiber genotype may contribute to the etiology in children [50] and be a risk factor for SB [51]. However, there are still no studies that evaluate the association of these genetic polymorphisms between

Table 1
General characteristics and main results of the included studies.

Author (year), country	Study type	n, age (years)	Diagnosis of SB	Children with and without SB *n* and (%)	Guardians with and without SB *n* and (%)	Parents with and without SB *n* and (%)	Parents/ guardians with e without SB *n* and (%)	Main Results
Alves et al. (2019) Brazil	Cross-sectional	103 7.36 years (±2.34)	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* Questionnaire on self-reported SB according to AASM	- With SB: 26(25.2%) - Without SB: 77 (74.8%)	- With SB: Not reported - Without SB: Not reported	- With SB: Not reported - Without SB: Not reported	- With SB: 17 (16.5%) - Without SB: 86 (83.5%)	23.1% of children with SB had parents/guardians with SB. There was no association between the child having SB and the parents/guardians having the same condition (p = 0.361).
Chen et al. (2004) † China	Cross-sectional	774 3–6 years	*Children:* Questionnaire on reports of teeth grinding during sleep and clinical examination *Parents/guardians:* Questionnaire on self-reported teeth grinding during sleep	- With SB: 299 (38.6%) - Without SB: 475 (61.4%)	- With SB: 0 individuals† - Without SB: 0 individuals †	- With SB: 156 (20%) - Without SB: 623 (80%)	- With SB: 156 (20%) - Without SB: 623 (80%)	Of the 156 parents with SB, in 115 (73%) the condition was present in both parents and their children. There was an association between parents and children having SB (p < 0.001).
Clementino et al. (2017) Brazil	Cross-sectional	148 3–12 years	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 48 (32.4%) - Without SB: 47 (67.6%)	Not reported	Not reported	- With SB: 21 (15%) - Without SB: 119 (85%)	Of the 21 parents/ guardians who had SB, 10 (47.6%) of their children also had the condition. There was no association between SB from parents/ guardians and children (p = 0.109).
Hafiz et al. (2021) † Saudi Arabia	Cross-sectional	1,499 6–10 years	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* Questionnaire on SB reporting according to AASM	- With SB: 685 (45.7%) - Without SB: 814 (54.3%)	- With SB: 0 individuals† - Without SB: 0 individuals†	- With SB: 253 (8.5%) - Without SB: 2,745 (91.5%)	- With SB: 253(8.5%) - Without SB: 2,745 (91.5%)	Of the 253 fathers and mothers with SB, 166 (65%) had the condition and so did their children. SB in children was associated with the presence of SB in fathers and mothers (p < 0.001).
Jahanimoghadam et al. (2023) † Iran	Cross-sectional	600 6–12 years	*Children:* Questionnaire on SB reporting and clinical examination *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 84 (14.2%) - Without SB: 503 (85.7%)	- With SB: 0 individuals † - Without SB: 0 individuals†	- With SB: 65 (11%) - Without SB: 524 (89%)	- With SB: 65 (11%) - Without SB: 524 (89%)	Children with a family history of SB had a 2.93 higher prevalence of having the same condition (RP = 2.93; 95% CI = 1.57–5.48).
Martínez et al. (2024) † Spain	Case-control	104 8–9 years	*Children: Questionnaire on SB reporting and clinical examination Parents/guardians: SB Self-Report Questionnaire*	- With SB: 52 (50%) - Without SB: 52 (50%)	- With SB: 0 individuals † - Without SB: 0 individuals†	- With SB: 58 (55.7%) - Without SB: 46 (44.3%)	- With SB: 58 (55.7%) - Without SB: 46 (44.3%)	Sixty-two percent of parents with SB had children who also exhibited the condition. However, in the adjusted model, the association with family history was not significant.
Prado et al. (2019) Brazil	Cross-sectional	1,325 1–12 years	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* Self-report questionnaire according to AASM criteria	- With SB: 293 (24%) - Without SB: 926 (76%)	Not reported	Not reported	- With SB: 188 (15.4%) - Without SB: 1,032 (84.6%)	The prevalence of SB in children was 24%. Parents/guardians with possible SB have a greater chance of their child having SB (OR = 3.11; 95% CI = 2.09–4.74; p < 0.001)
Ramos et al. (2021) Brazil	Cross-sectional	862 5 years	*Children:* Clinical examination of tooth wear due to attrition associated or not with reports of teeth grinding during sleep (questionnaire) *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 307 (35.6%) - Without SB: 555 (64.3%)	Not reported	Not reported	- With SB: 81 (9.4%) - Without SB: 781 (90.6%)	Children with parents/ guardians who had SB had a 1.65 higher prevalence of having SB than children whose parents did not have this condition (RP: 1.65; 95% CI: 1.35–2.10; p < 0.001)
Sampaio et al. (2018) † Brazil	Cross-sectional	60 6–10 years	*Children:* Clinical examination to evaluate wear facets, associated or not with	- With SB: 24 (40%)	- With SB: 0 individuals† - Without SB: 0 individuals†	- With SB: 20 (33.3%)	- With SB: 20 (33.3%) - Without SB: 40 (66.7%)	Children whose mothers have SB are 3.5 (OR: 3.5; 95% CI: 1.14–10.74) times more likely to have SB.

(*continued on next page*)

Table 1 (*continued*)

Author (year), country	Study type	n, age (years)	Diagnosis of SB	Children with and without SB *n* and (%)	Guardians with and without SB *n* and (%)	Parents with and without SB *n* and (%)	Parents/ guardians with e without SB *n* and (%)	Main Results
			reports of SB *Mothers:* Clinical examination to evaluate wear facets, associated or not with self-report of SB	- Without SB: 36 (60%)		- Without SB: 40 (66.7%)		
Seraj et al. (2010) † Iran	Cross-sectional	600 4–12 years (mean 7.4 ± 2.4)	*Children:* SB Reporting Questionnaire *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 157 (26.2%) - Without SB: 443 (73.8%)	- With SB: 0 individuals† - Without SB: 0 individuals†	- With SB: 96 (16%) - Without SB: 504 (84%)	- With SB: 96 (16%) - Without SB: 504 (84%)	Association between family history of SB and occurrence of the condition in children ($p < 0.001$), with SB prevalence being 20.8% in children without a family history of SB; 51.1% paternal history of SB; 51.2% maternal history of SB, and 87.5% with both parents with SB.
Serra-Negra et al. (2016), Brazil	Cross-sectional	111 4–15 years (mean 8.28 ± 2.35)	*Children:* Questionnaire on reports of teeth grinding during sleep *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 41 (44.5%) - Without SB: 51 (55.5%)	Not reported	Not reported	- With SB: 31 (33.6%) - Without SB: 61 (66.4%)	71% of children with SB also had parents/ guardians with SB (OR: 4.83; 95% CI: 1.46–15.94; $p < 0.001$).
Serra-Negra et al. (2013), Brazil	Cross-sectional	221 5–11 years	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* SB Reporting Questionnaire	- With SB: 106 (48%) - Without SB: 115 (52%)	Not reported	- With SB: 252 (58%) - Without SB: 181 (42%)	Not reported	The prevalence of SB was 48% in children, 40.2% in fathers and 76.6% in mothers. SB in the child was associated with SB in the father ($p < 0.001$) and mother ($p = 0.042$).
Tavares-Silva et al. (2016), Brazil	Cross-sectional	134 (mean 7.4 ± 3.3)	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 31 (23.1%) - Without SB: 103 (76.9%)	Not reported	Not reported	- With SB: 16 (11.9%) - Without SB: 118 (88.1%)	There was an association between SB in the child and their parents/ guardians ($p = 0.002$).
US, US. (2021) †, Turkey	Cross-sectional	200 6–12 years	*Children:* Questionnaire on reporting SB according to ICDS associated with clinical examination of tooth wear *Parents/guardians:* Questionnaire on self-report of SB as per ICDS	- With SB: 160 (32%) - Without SB: 340 (68%)	- With SB: 0 individuals† - Without SB: 0 individuals†	- With SB: (27%) - Without SB: (73%)	Not reported	The prevalence of SB in children if there was no SB in the family was 22.5%, and 57.8% if they had someone in the family with SB. Children who had a family member with SB were four times more likely to have SB (OR: 4.07; 95% CI: 2.45–6.78).
Yazıcıoğlu, Ray (2021) † Turkey	Cross-sectional	96 7–11 years	*Children:* Questionnaire on SB reporting according to AASM *Parents/guardians:* SB Self-Report Questionnaire	- With SB: 48 (50%) - Without SB: 48 (50%)	- With SB: 0 individuals† - Without SB: 0 individuals†	- With SB: (21.8%) - Without SB: (78.6%)	- With SB: (21.8%) - Without SB: (78.6%)	28.1% of children with SB had fathers and/or mothers with SB. There was no association between the child having SB and the parents having the same condition ($p > 0.05$).

Legend – SB: Sleep Bruxism; p: p-value; CI: Confidence Interval; OR: Odds Ratio; †: study in which only parents participated. PR: prevalence ratio; ICDS: International Classification of Sleep Disorder. AASM: American Academy of Sleep Medicine.

parents/guardians and their children.

In the subgroup analysis according to the diagnostic criteria, the association of SB in parents/guardians and children was significant when SB was diagnosed either by clinical examination associated or not with the report, or only by the child's report of the condition. However, a difference was observed in the value of the estimation measures, in which the OR for probable SB was greater than that for possible SB. The assessment of SB through report/self-report, despite being a tool widely used in research and clinical practice, is subjected to memory bias and/ or lack of knowledge on the part of parents/guardians resulting in underestimation of results [17,34,39,40,52], in addition to being more suitable for evaluating only the presence and frequency of SB [53]. However, when associated with clinical examination, in addition to dental wear [12,21,41,42], criteria such as muscle pain and headache, palpation of the masseter muscles, temporal [15] to confirm the diagnosis of SB, which reduces the possibility of underestimating the results [1]. The lack of standardized and valid tools for assessing SB in children contributes to the underestimation of results, in addition to making comparisons between the studies analyzed difficult.

Standardized tools for the assessment of bruxism, such as the

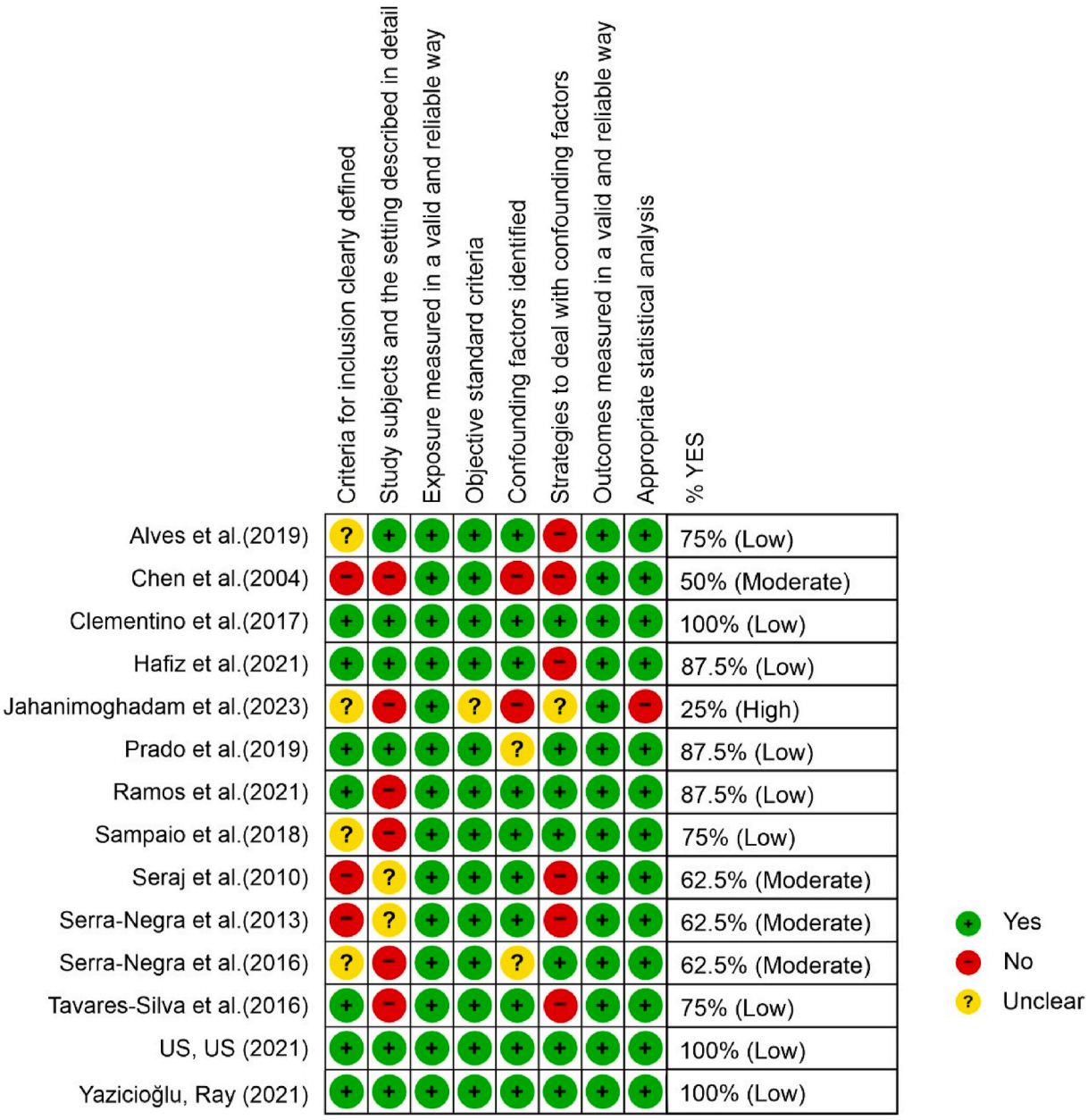


Fig. 2. Risk of bias analysis: Authors' judgment on each risk of bias item for the included studies using Joanna Briggs Institute criteria.

Study	Selection				Comparability	Exposure			Score	Interpretation
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8		
Martínez, Catalá-Pizzarro, Moreno	*	*	*	*	**	-	*	-	7/9	Low risk of bias

Fig. 3. Risk of bias analysis: Authors' judgment on each risk of bias item for the included study using Newcasting-Ottawa.

Standardized Tool for the Assessment of Bruxism (STAB), may be useful and accurate for diagnosing the condition. Still in the testing and validation phase, this tool, in addition to evaluating the state and consequences of bruxism, collects risk factors, etiological factors and comorbid conditions through the association of the patient's or guardian's report with clinical examination and instrumental approach, reducing the chances of underestimating the diagnosis of the condition in the evaluated patient [54]. Regarding the analysis according to the risk of bias of the studies, the association between the child having SB and the parents/guardians was observed in both studies with low and

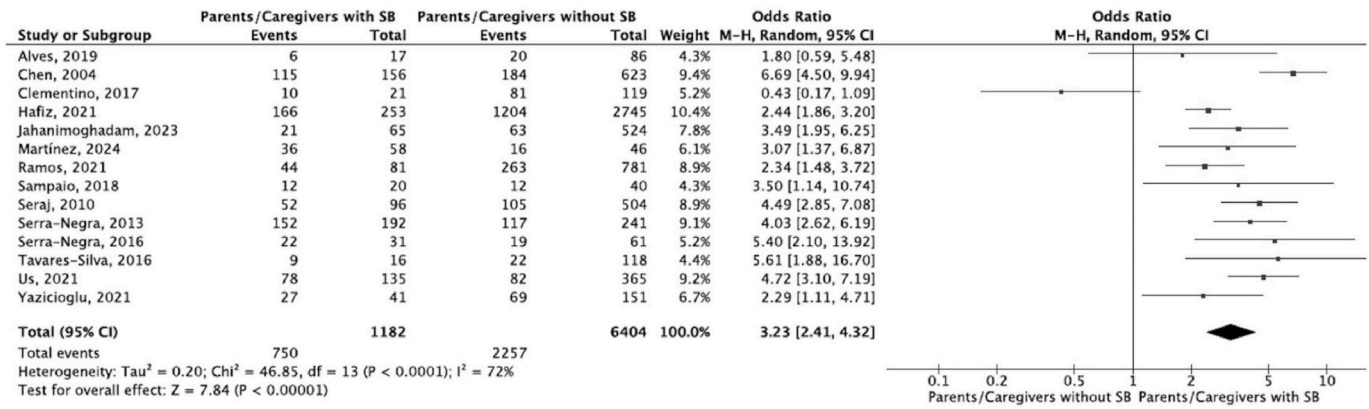


Fig. 4. Forest plot of the global meta-analysis of the association of sleep bruxism (SB) among parents/guardians and their children.

Table 2
Meta-analyses of subgroups, including kinship, diagnostic criteria, risk of bias and the association of sleep bruxism with their children.

Subgroups	N studies included/N of studies included in subgroup analysis	OR (95% CI) – Random-Effect Model	I^2 (p-value)	Test for Subgroup Difference: I^2/ p-value
Kinship				
Parents	14/9[a]	3.78 (2.87–4.97)	63% (0.005)	32.3%/0.22
Parents and guardians	14/5[b]	2.20 (0.97–5.02)	78% (0.001)	
Diagnostic Criteria				
Possible	14/8[c]	2.75 (1.79–4.24)	75% (0.0003)	31.4%/0.23
Probable	14/6[d]	3.90 (2.70–5.63)	61% (0.03)	
Risk of bias				
Low	14/9[e]	2.50 (1.71–3.65)	68% (0.001)	87.5%/0.005
High/ moderate	14/5[f]	4.78 (3.76–6.08)	14% (0.33)	

[a] [10,12,15,16,40–44].
[b] [13,21,34,35,39].
[c] [10,13,16,34,35,39,40,43].
[d] [12,15,21,41,42,44].
[e] [12,15,21,34,35,39,40,43,44].
[f] [10,13,16,41,42].

Table 3
Sensitivity analysis - Influence of each study on the association of sleep bruxism between parents/guardians and children.

Sleep Bruxism Association		
Study	OR (95% CI)	Heterogeneity I^2-p-value
Alves et al., 2019	3.31 (2.45–4.47)	74% – $p < 0.001$
Chen et al., 2004	3.01 (2.27–3.98)	64% – $p < 0.001$
Clementino et al., 2017	3.62 (2.85–4.59)	57% – $p = 0.006$
Hafiz et al., 2021	3.32 (2.42–4.56)	70% – $p < 0.001$
Jahanimoghadam et al., 2023	3.19 (2.33–4.38)	74% – $p < 0.001$
Martínez et al., 2024	3.23 (2.37–4.40)	74% – $p < 0.001$
Ramos et al., 2021	3.32 (2.43–4.54)	73% – $p < 0.001$
Sampaio et al., 2018	3.21 (2.37–4.35)	74% – $p < 0.001$
Seraj et al., 2010	3.11 (2.27–4.28)	73% – $p < 0.001$
Serra-Negra et al., 2013	3.14 (2.28–4.34)	74% – $p < 0.001$
Serra-Negra et al., 2016	3.13 (2.31–4.24)	74% – $p < 0.001$
Tavares-Silva et al., 2016	3.14 (2.32–4.25)	74% – $p < 0.001$
Us, Us 2021	3.10 (2.26–4.25)	73% – $p < 0.001$
Yazıcıoğlu, Ray 2021	3.30 (2.43–4.49)	74% – $p < 0.001$

OR: Odds Ratio; 95% CI: 95% Confidence Interval.

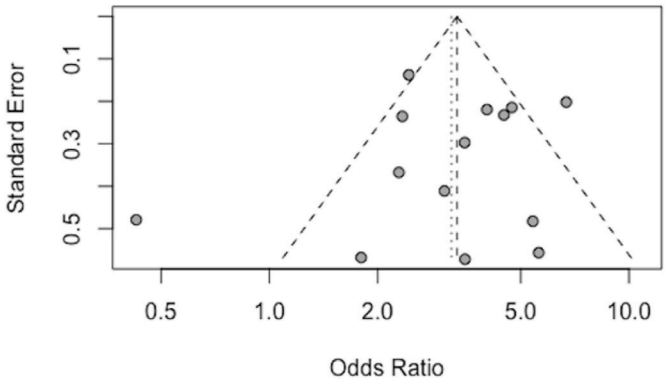


Fig. 5. Funnel plot of the 14 studies included in the meta-analysis.

Table 4
Meta-regression of the variables of the evaluated subgroups.

Variable	Estimation (EP)	R^2 adjusted	p-value	I^2
Kinship	0.34 (0.28)	67.45%	0.224	45.73%
Diagnosis	0.33 (0.23)		0.149	
Risk of bias	0.54 (0.24)		0.023	

EP: standard error

high/moderate risk of bias. The high estimate may have occurred due to the cross-sectional design of these studies, as well as due to the lower number of parent/guardian events without SB when compared to the low risk of bias. The inclusion of well-designed studies with a low risk of bias contributes to the evidence generated from them.

Sensitivity analysis demonstrated that the removal of two studies [34,41] has a different impact on the estimated association between parents/guardians and the child presenting SB. However, all of them were still significantly associated. The increase in the estimate, with the removal of the study from Clementino et al., 2017 [34], happened due to the observed association losing significance in the study's adjusted model. In the study by Chen et al., 2004 [41], the decrease in the estimate may have occurred due to the greater number of events (child having SB when the parent/guardian has the same condition), in addition to the authors considering both parents to have SB for the analysis. To explain the high heterogeneity of the studies, meta-regression was performed with the variables evaluated in each. However, only the risk of bias variable was significant, partly influencing the high heterogeneity. This corroborates the results mentioned previously, in which the inclusion of studies with high/moderate risk of bias overestimated the association. The certainty of evidence regarding the association between

Table 5
Assessment of certainty of evidence for all outcomes included in meta-analyses.

Assessment of certainty of evidence							Nº of patients		Effect		Certainty	Importance
Nº of Studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Parents and guardians with SB	Parents and guardians without SB	Relative (95% CI)	Absolute (95% CI)		
Sleep bruxism in children in relation to parents/guardians												
14	Observational studies	serious[a]	serious[b]	Not serious	Not serious	Strong association	750/1182 (63.5%)	2257/6404 (35.2%)	**OR 3.23** (2.41–4.32)	**285 more per 1000** (from 215 more to 349 more)	⨁◯◯◯ very low[a,b]	CRITICAL

CI: Confidence Interval; OR: *Odds ratio*.
[a] Five studies (n = 5) presented a high/moderate risk of bias, with a weight of 40.5% for the analysis. However, part of the heterogeneity among the studies is explained by this factor.
[b] A high heterogeneity was observed $I^2 = 72\%$. However, the meta-regression showed high R^2 ($R^2 = 67.45\%$).

SB in children and SB in parents or guardians was rated as very low. This was primarily due to the predominance of observational studies with moderate to high risk of bias and considerable heterogeneity, largely attributed to methodological differences among the included studies.

This systematic review has the limitations of the cross-sectional design of most of the primary studies included, which limits the establishment of a causal relationship between the outcome and the event studied. Furthermore, the high methodological variability and heterogeneity of the studies, as well as the lack of information, even after contact by email, about the biological relationship of parents/guardians, also limited some analyses of this review. Only two authors responded, one did not collect data on the biological relationship between parents and guardians [21], and the other provided data regarding the biological parents, which were used in the analyses included in this review [44].

Therefore, it is suggested that the development of well-designed longitudinal studies, use as diagnostic criteria for SB, clinical examination associated with the questionnaire or, when possible, an instrumental approach, such as polysomnography and electromyography, both in children and their parents/guardians, are warranted. It is necessary to use standardized and validated questionnaires, in addition to collecting information about the relationship between parents or guardians and whether they are biological or non-biological relatives.

The results of this systematic review with meta-analysis help to understand the etiology of SB. Understanding that the relationship between fathers and/or mothers having SB and how it can modulate the occurrence of SB in their children can awaken more interest in the scientific community on this topic. Since most studies included in this review primarily aimed to assess parents'/guardians' knowledge about sleep bruxism, the association between parental/guardian sleep bruxism and that of the child was observed as a secondary finding. Better understanding the etiology of sleep bruxism will help to incorporate questions into the anamnesis about the presence of SB in fathers and/or mothers, for a more cautious clinical look at the possible presence of the condition in the child, assisting pediatric dentists in early diagnosing this condition, in addition to establishing more effective treatment and/or management aimed, mainly, at modulating other etiological factors.

5. Conclusion

There is an association between parents/guardians having sleep bruxism and children also having the condition, indicating a possible genetic/hereditary contribution to the etiology of sleep bruxism. However, the results should be analyzed with caution due to the very low certainty of the evidence.

CRediT authorship contribution statement

Francisco das Chagas Santos-Júnior: Writing – review & editing, Visualization, Project administration, Investigation, Conceptualization, Writing – original draft, Validation, Methodology, Data curation. **Ayah Qassem Ahmad Shqair:** Methodology, Validation, Investigation. **Marcoeli Silva de Moura:** Writing – review & editing. **Marina de Deus Moura de Lima:** Writing – review & editing. **Lúcia de Fátima Almeida de Deus Moura:** Writing – review & editing. **Francisco Wilker Mustafa Gomes Muniz:** Writing – review & editing, Formal analysis, Supervision, Conceptualization. **Cacilda Castelo Branco Lima:** Project administration, Formal analysis, Writing – review & editing, Methodology, Conceptualization.

Data availability statement

Data relevant to the study are included in the article or uploaded as supplementary material. Other data that support the findings of this study are available from the corresponding author, upon reasonable request.

Funding

This work was supported by the Piauí State Research Support Foundation (Fapepi). This study was also supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Declaration of competing 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.

Acknowledgements

The authors acknowledge the Piauí State Research Support Foundation (FAPEPI) and the Postgraduate Program in Dentistry at the Federal University of Piauí (PPGO - UFPI) for their support in developing the research. Dr. Muniz holds research scholarships from the National Council for Scientific and Technological Development – CNPq.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2025.106662.

References

[1] Lobbezoo F, Ahlberg J, Raphael KG, et al. International consensus on the assessment of bruxism: report of a work in progress. J Reabilitação Oral 2018;45:837–44. https://doi.org/10.1111/joor.12663.
[2] Manfredini D, Ahlberg J, Lavigne GJ, Svensson P, Lobbezoo F. Five years after the 2018 consensus definitions of sleep and awake bruxism: an explanatory note. J Oral Rehabil 2024;51(3):623–4. https://doi.org/10.1111/joor.13626.
[3] Serra-Negra JM, Paiva SM, Auad SM, Ramos-Jorge ML, Pordeus IA. Signs, symptoms, parafunctions and associated factors of parent-reported sleep bruxism in children: a case-control study. Braz Dent J 2012;23(6):746–52. https://doi.org/10.1590/s0103-64402012000600020.
[4] Rodrigues JA, et al. Sleep bruxism and oral health-related quality of life in children: a systematic review. Int J Paediatr Dent 2020;30(2):136–43. https://doi.org/10.1111/ipd.12586.
[5] Castroflorio T, Bargellini A, Rossini G, Cugliari G, Deregibus A. Sleep bruxism and related risk factors in adults: a systematic literature review. Arch Oral Biol 2017;83(1):25–32. https://doi.org/10.1016/j.archoralbio.2017.07.002.
[6] Ierardo G, Mazur M, Luzzi V, Calcagnile F, Ottolenghi L, Polimeni A. Treatments of sleep bruxism in children: a systematic review and meta-analysis. Cranio 2021;39(1):58–64. https://doi.org/10.1080/08869634.2019.1581470.
[7] Bulanda S, Ilczuk-Rypuła D, Nitecka-Buchta A, Nowak Z, Baron S, Postek-Stefanska L. Sleep bruxism in children: etiology, diagnosis, and treatment—A literature review. Int J Environ Res Publ Health 2021;18(18):95–144. https://doi.org/10.3390/ijerph18189544.
[8] Bornhardt T, Iturriaga V. Sleep bruxism: an integrated clinical view. Sleep Med Clin 2021;6(2):373–80. https://doi.org/10.1016/j.jsmc.2021.02.010.
[9] Manfredini D, Restrepo C, Diaz-Serrano K, Winocur E, Lobbezoo F. Prevalence of sleep bruxism in children: a systematic review of the literature. J Oral Rehabil 2013;40:631–42. https://doi.org/10.1111/joor.12069.
[10] Serra-Negra JM, Paiva SM, Abreu MH, Flores-Mendoza CE. Pordeus IA relationship between tasks performed, personality traits, and sleep bruxism in Brazilian school children a population-based cross-sectional study. PLoS One 2013;8(11). https://doi.org/10.1371/journal.pone.0080075.
[11] Guo H, Wang T, Niu X, Wang H, Yang W, Qiu J, Yang L. The risk factors related to bruxism in children: a systematic review and meta-analysis. Arch Oral Biol 2018;86:18–34. https://doi.org/10.1016/j.archoralbio.2017.11.004.
[12] Sampaio NM, Oliveira MC, Andrade AC, Santos LB, Sampaio M, Ortega A. Relationship between stress and sleep bruxism in children and their mothers: a case control study. Sleep Sci 2018;11(4):239–44. https://doi.org/10.5935/1984-0063.20180038.
[13] Serra-Negra JM, Ribeiro MB, Prado IM, Paiva SM, Pordeus IA. Association between possible sleep bruxism and sleep characteristics in children. Cranio 2016 Sep;35(5):315–20. https://doi.org/10.1080/08869634.2016.1239894.
[14] Laganà G, Osmanagiq V, Malara A, Venza N, Cozza P. Sleep bruxism and SDB in Albanian growing subjects: a cross-sectional study. Dent J 2021;9(3):25–34. https://doi.org/10.3390/dj9030025.
[15] Us MC, Us YO. Evaluation of the relationship between sleep bruxism and sleeping habits in school-aged children. Cranio 2021;21:1–9. https://doi.org/10.1080/08869634.2021.1890454.
[16] Seraj B, Shahrabi M, Ghadimi S, Ahmadi R, Nikfarjam J, Zayeri F, et al. The prevalence of bruxism and correlated factors in children referred to dental schools of Tehran, based on parent's report. Iran J Pediatr 2010;20(2):174–80.
[17] Prado IM, Paiva SM, Fonseca-Gonçalves A, Maia LC, Tavares-Silva C, Fraiz FC, et al. Knowledge of parents/caregivers about the sleep bruxism of their children from all five Brazilian regions: a multicenter study. Int J Paediatr Dent 2019;29(4):507–23. https://doi.org/10.1111/ipd.12486.
[18] Page MJ, Mckenzie JE, Bossuyt P, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, Mcdonald S, Mcguinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021. https://doi.org/10.1136/bmj.n71.
[19] Cheifetz AT, Osganian SK, Allred EN, Needleman HL. Prevalence of bruxism and associated correlates in children as reported by parents. J Dent Child 2005;72(2):67–73.
[20] de Alencar NA, Leão CS, Leão ATT, Luiz RR, Fonseca-Gonçalves A, Maia LC. Sleep bruxism and anxiety impacts in quality of life related to oral health of Brazilian children and their families. J Clin Pediatr Dent 2017;41(3):179–85. https://doi.org/10.17796/1053-4628-41.3.179.
[21] Ramos PFC, de Lima MdM, de Moura MS, Bendo CB, Moura LFAD, Lima CCB. Breathing problems, being an only child and having parents with possible sleep bruxism are associated with probable sleep bruxism in preschoolers: a population-based study. Sleep Breath 2021;25:1677–84. https://doi.org/10.1007/s11325-020-02281-0.
[22] Guo H, Wang T, Li X, Ma Q, Niu X, Qiu J. What sleep behaviors are associated with bruxism in children? A systematic review and meta-analysis. Sleep Breath 2017;21(4):1013–23. https://doi.org/10.1007/s11325-017-1496-3.
[23] De Luca Canto G, Singh V, Gozal D, Major PW, Flores-Mir C. Sleep bruxism and sleep-disordered breathing: a systematic review. J Oral Facial Pain Headache 2014;28(4):299–305. https://doi.org/10.11607/ofph.1294.
[24] Haddaway NR, Collins AM, Coughlin D, Kirk S. O papel do Google Scholar em revisões de evidências e sua aplicabilidade à pesquisa de literatura cinzenta. PLoS One 2015;10(9). https://doi.org/10.1371/journal.pone.0138237.
[25] Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, Currie M, Qureshi R, Mattis P, Lisy K, Mu P-F. Chapter 7: systematic reviews of etiology and risk. In: Aromataris E, Munn Z, editors. Joanna Briggs Institute Reviewer's manual. The Joanna Briggs Institute; 2017. Available from: https://reviewersmanual.joannabriggs.org/.
[26] Melo G, Dutra KL, Rodrigues Filho R, Ortega AOL, Porporatti AL, Dick B, Flores-Mir C, De Luca Canto G. Association between psychotropic medications and presence of sleep bruxism: a systematic review. J Oral Rehabil 2018;45(7):545–54. https://doi.org/10.1111/joor.12633.
[27] Wells G, Shea B, O'Connell D, RobertsonJ Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Disponível em: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; 2012. Acesso em: 31 out/2022.
[28] Luchini C, Veronese N, Nottegar A, Shin JI, Gentile G, Granziol U, Soysal P, Alexinschi O, Smith L, Solmi M. Assessing the quality of studies in meta-research: Review/guidelines on the most important quality assessment tools. Pharm Stat 2021;20(1):185–95. https://doi.org/10.1002/pst.2068.
[29] Rostami EG, Touchette É, Huynh N, Montplaisir J, Tremblay RE, Battaglia M, Boivin M. High separation anxiety trajectory in early childhood is a risk factor for sleep bruxism at age 7. Sleep 2020;43(7). https://doi.org/10.1093/sleep/zsz317.
[30] Deeks JJ, Higgins JPT, Altman DG. Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editors. Cochrane handbook for systematic reviews of interventions. Cochrane; 2022 (updated February 2022), version 6.3. www.training.cochrane.org/handbook.
[31] Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Br Med J 1997;315:629–34. 1997.
[32] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088–101. 1994.
[33] Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, DeBeer H, Jaeschke R, Rind D, Meerpohl J, Dahm P, Schünemann HJ. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64(4):383–94. https://doi.org/10.1016/j.jclinepi.2010.04.026.
[34] Clementino MA, Siqueira MB, Serra-Negra JM, Paiva SM, Granville-Garcia AF. The prevalence of sleep bruxism and associated factors in children: a report by parents. Eur Arch Paediatr Dent 2017;18(6):399–404. https://doi.org/10.1007/s40368-017-0312-x.
[35] Tavares Silva C, Calabrio IR, Serra-Negra JM, Fonseca-Gonçalves A, Maia LC. Knowledge of parents/guardians about nocturnal bruxism in children and adolescents. Cranio 2017;35(4):223–7. https://doi.org/10.1080/08869634.2016.120163.
[36] Calıskan S, Delikan E, Ozcan-Kucuk A. Knowledge of parents about bruxism in their children. Odovtos 2020;22(1):123–32. https://doi.org/10.15517/ijds.2020.38712 [online].
[37] Talebian A, Sharif MR, Gilasi HR, Ghafeli Bidgoli M, Masoudi Alavi N. Risk factors of bruxism in children and adolescents: a case-control study. Iran J Child Neurol Summer 2023;17(3):81–7. https://doi.org/10.22037/ijcn.v17i2.34695.
[38] Gurunathan D, Varadarajan S, Balaji TM. Knowledge, attitude, and awareness of parents on bruxism in children: a questionnaire Study. World 2024;15(2):162.

[39] Alves CL, Fagundes DM, Soares PBF, Ferreira MC. Knowledge of parents/caregivers about bruxism in children treated at the pediatric dentistry clinic. Sleep Sci 2019; 12(3):185–9. https://doi.org/10.5935/1984-0063.20190083.
[40] Hafiz Z, Alhamid A, Alsaykhan M, Dakhil NB. Parental awareness of bruxism in saudi children: a public health concern. Biosc Biotech Res Comm 2021;14(3): 974–80.
[41] Chen YQ, Cheng HJ, Yu CH, Gao Y, Shen YQ. Epidemic investigation on 3 to 6 years children's bruxism in Shanghai. Shang Hai Kou Qiang Yi Xue 2004 Oct;13(5): 382–4.
[42] Jahanimoghadam F, Tohidimoghadam M, Poureslami H, Sharifi M. Prevalence and risk factors of bruxism in a selected population of Iranian children. Pesqui Bras Odontopediatria Clín Integr 2023;23:e210224. https://doi.org/10.1590/pboci.2023.020 [Internet].
[43] Yazıcıoğlu İ, Ray PÇ. Evaluation of anxiety levels in children and their mothers and appearance of sleep bruxism in Turkish children and associated risk factors: a cross-sectional study. J Oral Facial Pain Headache 2022;36(2):147–54. https://doi.org/10.11607/ofph.3011.
[44] Martínez IR, Catalá-Pizarro M, Moreno JR. Association between perfectionism, personality traits and probable sleep bruxism in Spanish schoolchildren: a case-control study. Int J Paediatr Dent 2024;34(5):516–23. https://doi.org/10.1111/ipd.13152.
[45] Lobbezoo F, Visscher CM, Ahlberg J, Manfredini D. Bruxism and genetics: a review of the literature. J Oral Rehabil 2014;41(9):709–14. https://doi.org/10.1111/joor.12177.
[46] Ahlberg J, Piirtola M, Lobbezoo F, Manfredini D, Korhonen T, Aarab G, Hublin C, Kaprio J. Correlates and genetics of self-reported sleep and awake bruxism in a nationwide twin cohort. J Oral Rehabil 2020;47(9):1110–9. https://doi.org/10.1111/joor.13042.
[47] Rintakoski K, Hublin C, Lobbezoo F, Rose RJ, Kaprio J. Genetic factors account for half of the phenotypic variance in liability to sleep-related bruxism in young adults: a nationwide Finnish twin cohort study. Twin Res Hum Genet 2012;15(6):714–9. https://doi.org/10.1017/thg.2012.54.
[48] Restrepo-Serna C, Winocur E. Sleep bruxism in children, from evidence to the clinic. A systematic review. Front Oral Health 2023;4:1166. https://doi.org/10.3389/froh.2023.1166091.
[49] Campello CP, Moraes SLD, Vasconcelos BCDE, Lima ELS, Pellizzer EP, Lemos CAA, Muniz MTC. Polymorphisms of the serotonin receptors genes in patients with bruxism: a systematic review. J Appl Oral Sci 2022;29:e20210262. https://doi.org/10.1590/1678-7757-2021-0262.
[50] Calvano Küchler E, Arid J, Palinkas M, Ayumi Omori M, de Lara RM, Napolitano Gonçalves LM, Hallak Regalo SC, Paes Torres Mantovani C, Rezende Vieira A, Diaz-Serrano K. Genetic polymorphisms in ACTN3 contribute to the etiology of bruxism in children. J Clin Pediatr Dent 2020;44(3):180–4.
[51] Nicot R, Raoul G, Vieira AR, Ferri J, Sciote JJ. ACTN3 genotype influences masseter muscle characteristics and self-reported bruxism. Oral Dis 2023;29(1): 232–44. https://doi.org/10.1111/odi.14075.
[52] Huynh N, Fabbro CD. Sleep bruxism in children and adolescents-A scoping review. J Oral Rehabil 2024;51(1):103–9. https://doi.org/10.1111/joor.13603.
[53] Yachida W, Arima T, Castrillon EE, Baad-Hansen L, Ohata N, Svensson P. Diagnostic validity of self-reported measures of sleep bruxism using an ambulatory single-channel EMG device. J Prosthodont Res 2016;60:250–7. https://doi.org/10.1016/j.jpor.2016.01.001.
[54] Manfredini D, Ahlberg J, Aarab G, Bender S, Bracci A, Cistulli PA, Conti PC, De Leeuw R, Durham J, Emodi-Perlman A, Ettlin D, Gallo LM, Häggman-Henrikson B, Hublin C, Kato T, Klasser G, Koutris M, Lavigne GJ, Paesani D, Peroz I, Svensson P, Wetselaar P, Lobbezoo F. Standardised tool for the assessment of bruxism. J Oral Rehabil 2024 Jan;51(1):29–58. https://doi.org/10.1111/joor.13411.

scientific reports

Check for updates

OPEN

Polysacharide of *Agaricus blazei* gel mitigates bone necrosis in model of the jaws related to bisphosphonate via Wnt signaling

Vanessa Costa de Sousa[1], Fátima Regina Nunes Sousa[2], Raquel Felipe Vasconcelos[3], Gisele Angelino Barreto[1], Conceição S. Martins[4], Nilson Romero Dias[4], Sislana Costa[4], Maria Jennifer Chaves Bernardino[4], George de Almeida Silva[5], Nadine Linhares[6], Delane Gondim[4], Mirna Marques[7], Helliada Chaves[8], Karuza Alves[4], Renata Leitão[4], Gerly A. C. Brito[4], Maria Elenir Nobre Pinho Ribeiro[5] & Paula Goes[10,9]✉

To investigate de effect of PAb gel on the bone tissue of rats submitted to Bisphosphonate-related osteonecrosis of the jaws (BRONJ). Initially, 54 animals were submitted to BRONJ model by Zoledronic Acid (ZA) (0.1 mg/kg 3x/wk for 9 wk, ip), followed by the 1st upper left molar extraction at the 8th wk. After tooth removal, the animals were divided into 3 groups, ZA that received placebo gel or PAb gel that received 1% PAb gel, inside the dental alveolus. The control Group (CONTROL) received 0.1 mg/kg of 0.9% saline and then placebo gel. Three weeks after tooth extraction, the animals were euthanized, and maxillae were colleted for macroscopic, radiographic, histological and Raman spectomery assays. Additionally, GSK3b, beta-catenin, and Runx2 mRNA expressions were determined. Blood samples were collected for the analysis of Bone-specific alkaline phosphatase (BALP) levels. PAb gel improved mucosal healing, increased the number of viable osteocytes, while it reduced the number of empty lacunae, as well as the amount of bone sequestration. Furthermore, PAb gel positively influenced the number and functionality of osteoblasts by stimulating Wnt signaling, thereby inducing bone remodeling. Additionally, PAb gel contributed to improved bone quality, as evidenced by an increase in bone mineral content, a decrease in bone solubility, and an enhancement in the quality of collagen, particularly type I collagen. PAb gel mitigated bone necrosis by stimulating of bone remodeling through Wnt signaling and concurrently improved bone quality. PAb gel emerges as a promising pharmacological tool for aiding in BRONJ therapy or potentially preventing the development of BRONJ.

Keywords BRONJ, Beta-glucan, Osteoblast, Wnt signaling, Bone remodeling, Bone quality

Medication-related osteonecrosis of the jaw (MRONJ) is a rare yet severe condition that can affect the upper or lower jaw[1]. The American Association of Oral and Maxillofacial Surgeons (AAOMS) introduced this term to encompass the increasing number of osteonecrosis cases involving the maxilla and mandible that are associated

[1]Post Graduation of Morphological Science, Department of Morphology, Medical School, Federal University of Ceará, Fortaleza, CE, Brazil. [2]Medical School, Federal University of Piauí, Picos, CE, Brazil. [3]Federal Institute of Ceará, Fortaleza, CE, Brazil. [4]Department of Morphology, Medical School, Federal University of Ceará, Fortaleza, CE, Brazil. [5]Department of Chemistry, Federal University of Ceará, Fortaleza, CE, Brazil. [6]Post Graduation of Dentistry, School of Dentistry, Federal University of Ceará, Fortaleza, CE, Brazil. [7]Faculty of Medical School, Federal University of Ceará, Sobral, CE, Brazil. [8]School of Dentistry, Federal University of Ceará, Sobral, CE, Brazil. [9]Department of Pathology and Legal Medicine, Medical School, Federal University of Ceará, Fortaleza, CE, Brazil. [10]Lab of Medical Immunology, Department of Pathology and Legal Medicine, Faculty of Medicine, Federal University of Ceará, Rua Coronel Nunes de Melo, 1315, Rodolfo Teófilo, Fortaleza, CE 60430-270, Brazil. ✉email: paulagpinheiro@yahoo.com.br

with other antiresorptive and antiangiogenic therapies[2]. Among the different types of MRONJ, bisphosphonate-related osteonecrosis of the jaw (BRONJ) has demonstrated a higher prevalence rate[3], ranging from 1.6 to 14.8% when intravenous bisphosphonates were used followed by tooth extraction[4].

The pathophysiology of BRONJ is complex and multifactorial. Although clinicians and researchers have engaged in extensive discussions about the etiological factors of BRONJ, several key factors contribute to the development of this condition, including bone remodeling inhibition, inflammation or infection, angiogenesis inhibition, innate or acquired immune dysfunction, and genetic predisposition[2]. When examining bone remodeling in more detail, it is well-established that osteoclast inhibition plays a central role in this process. However, the involvement of osteoblasts and osteocytes in BRONJ is not as thoroughly explored[5]. Our research group has recently demonstrated a reduction in osteoblast numbers in a rat BRONJ model, linked to the inhibition of Wnt signaling[6]. As a result, therapeutic strategies that target osteoblastogenesis could offer a promising approach to managing BRONJ.

The primary objective of BRONJ therapy is to prevent the onset of the disease by optimizing dental health and avoiding dentoalveolar surgical procedures. Additionally, international professional societies have recommended the use of drug holidays, although strong evidence supporting their effectiveness is lacking. Once the disease is established, the use of chlorhexidine and systemic antibiotics has been proposed, but it does not always lead to complete resolution and has limited long-term effectiveness. Furthermore, the removal of necrotic bone through surgical procedures is typically recommended, particularly in more advanced stages of the disease. Adjunct therapies, such as hyperbaric oxygen or ozone therapy, as well as the use of vitamin E and pentoxifylline, have been studied. However, highly effective treatment protocols are yet to be determined[2].

The urge of effective therapeutical approaches for BRONJ has stimulated the development of new biomaterials. *Agaricus blazei* (Ab) is a mushroom of the *Basidomycota* family that grows freely in Brazil. Ab has mainly been used by the local population as a food ingredient, but also as a medicine against a wide range of diseases, in particular infection and cancer[7]. The fruiting body of Ab is rich in β-glucans, characterized by chains of D-glucose linked by β-type glycosidic bonds, being β- (1–3) linked backbone with (1–6) linked side branches[8]. Biologically, this polysaccharide has shown immunomodulating effects[9,10]. On bone tissue, beta-glucan has shown antiresorptive effects in experimental periodontitis model[11,12] and positive effects on bone regeneration and metabolism[10,13]. However, no study has ever investigated the effect of the polysaccharide beta-glucan derived from *Agaricus blazei (PAb)*, neither topically nor systemically, on BRONJ.

In the context of addressing the need for effective therapeutic approaches for BRONJ, there has been a growing interest in the development of new biomaterials. Agaricus blazei (Ab) is a mushroom belonging to the Basidiomycota family and is commonly found in Brazil. While Ab has traditionally been used as a food ingredient by the local population, it has also been employed as a remedy for a wide range of diseases, particularly for infections and cancer[7]. The fruiting body of Ab is rich in β-glucans, which are characterized by chains of D-glucose linked by β-type glycosidic bonds, with a β-(1–3) linked backbone and (1–6) linked side branches[8]. Biologically, this polysaccharide has demonstrated immunomodulatory effects[9,10]. On bone tissue, beta-glucan has shown antiresorptive effects in experimental periodontitis models[11,12] and has had positive effects on bone regeneration and metabolism[10,13]. However, to date, no study has investigated the effects of the polysaccharide beta-glucan derived from Agaricus blazei (PAb), whether administered topically or systemically, on BRONJ.

Hence, given the promising and beneficial effects that PAb has demonstrated on bone metabolism, and considering that BRONJ is primarily linked to the inhibition of bone remodeling, we have hypothesized that PAb gel can stimulate bone formation and alleviate BRONJ in rats. This emphasizes the application of β-glucans as a biocompatible strategy and a potential candidate for the management of bone-related diseases.

Materials and methods

Study design and ethical aspects

This was a pre-clinical randomized and blinded study. The experiments were only initiated after approval by the Institutional Ethics Committee for Animal Research Federal University of Ceará (UFC) (number 4411060619).

All methods were performed in accordance with the relevant guidelines and regulations described in ARRIVE guidelines.

Animals and experimental groups

For this study, 54 female Wistar rats (12 weeks old, ± 200 g) (*Rattus novergicus)* were used. The sample size of 6 animals per group was determined in order to provide a power calculation of 80%, and significant level of $p < 0.05$, considering bone necrosis, defined by the percentage of empty lacunae of osteocytes (> 50% in 05 fields/slide) and presence of bone sequestration as the primary outcome variable[6,14].

Throughout the whole experiment the animals were kept in cages (n = 3 animals/cage) at temperature-controlled rooms, with free food and water. After two weeks of acclimation to the laboratory environment, the animals were divided in a blind and randomized manner. Randomization was performed by computer software, considering the weight of the animals. Three experimental groups were established as follows:

- Control group: where the animals received 0.1 mg/kg 0.9% saline solution 3x/wk intraperitoneally (ip) for 09 weeks and then placebo gel in the dental alveolus;
- Zoledronic acid (ZA) group: the animals were submitted to bisphosphonate-related osteonecrosis of the jaws (BRONJ) model, and then placebo gel in the dental alveolus;
- Polyssacharide of *Agaricus blazei* (PAb) gel group: the animals were submitted to BRONJ model, and then received 1.0% PAb gel in the dental alveolus;

The gels used in this study (placebo and 1.0% PAb) were inserted in the dental socket, after tooth extraction using a hypodermic needle (25 × 0.8 mm) previously prepared, pre-crooked and without bevel, in a single administration[15].

The study was divided into 3 sets of experiments: in the 1st set, the collected maxillae were used for macro and microscopic analyses. In the 2nd set the maxillae were used for radiographic and micro-Raman spectroscopy and the 3st for PCR in time real. All analyses were performed by an experienced examiner unaware of the groups.

BRONJ-like model

It was used a BRONJ-like rat model previous reported by de Sousa Ferreira et al.[6]. The animals received 0.1 mg/kg of Zoledronic Acid (Cristália, Itapira, SP, Brazil) intraperitoneally (ip) 3x/wk for 9 weeks[6,16]. On the 8th week (49th experimental day—D49/W8), all animals, previously anesthesized with ketamine and xylazine ip., were submitted to the extraction of 1st upper left molar [Refs.[6,16] with modifications]. Three weeks after tooth extraction the animals were euthanized by overdose of anesthetics (Fig. 1).

Preparation of *Agaricus blazei*-glucan polysaccharides (PAb) gel

Initially, 2.0 ml of a diluted graphene oxide (GOx) solution at a concentration of 0.75 mg/mL was prepared, then placed in an ultrasound bath for 5 min, undergoing a stirring fast and strong process. Following it was performed the incorporation of the active ingredient: it was added 20.0 mg of *Agaricus blazei* -glucan polysaccharides (PAb) register Sisgen number AC29F45 to GOx solution to the GOx solution. The gels were stirred overnight at room temperature, then 50.0 mg of Hydroxypropylmethylcellulose (HPMC) were added to the previously prepared suspension, obtaining the hydrogel (2.5% w/v of HPMC) according to the gel to be produced. Finally, a process of intense agitation was successfully carried out, followed by cooling at 5 °C for 3 days to obtain a homogeneous phase of polymer, solvent and drug. The gels were prepared after investigating the physical properties, specificity, viscosity, kinetics and stability of the drug, in the following concentrations: 1.0% *Agaricus blazei* -glucan polysaccharides Gel (1.0% w/v PAb)[17].

Macroscopic analysis

After euthanasia, detailed visual inspection of the maxillae was performed. Macroscopically, it was evaluated the presence or absence of bone exposure, as well as the oral mucosa continuity solution in the extraction region[6]. Data was presented in frequency.

Radiographic density analysis

For radiographic density analysis maxillae were radiographed by Digora® (Soredex, Finland), and then evaluated with Image J 1.31 software (ImageJ 1.32j, National Institute of Health, USA). A region of interest (ROI) consisting of 128 pixels was selected in the extraction site (considering as the upper limit the cervical of the second molar extending in the apical direction). The grayscale differences of both areas were considered radiographic density values. The analysis of the radiographic density of the ROI was performed using the histogram tool from Image J® software, using 256 shades of gray scale, where zero indicates black and 255 white. Data were expressed in arbitrary shades of gray[18].

Histopathological analysis

After macroscopic and radiographic analyses, maxillae were demineralized in 10% EDTA buffered solution. After 30 days of decalcification, the specimens were embedded in paraffin. Serial sections of 4 µm thickness, representing the area around the alveolar socket, obtained in a mesiodistal direction, were stained with

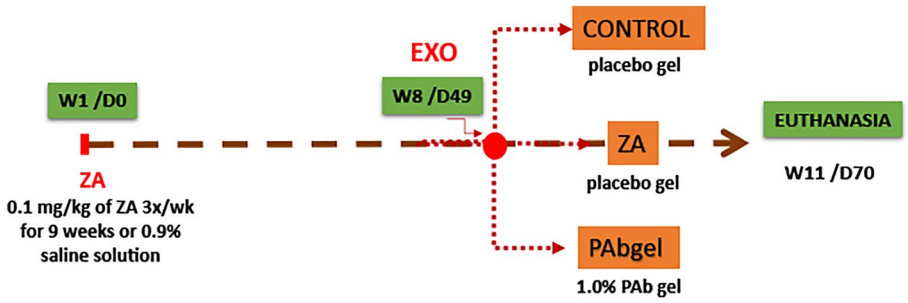


Figure 1. Experimental study design. Animals were divided into 3 groups, control, ZA and PAb gel groups. In the Control group, the animals were not submitted to BRONJ. They received a saline solution, and tooth extraction was carried out (D49/S8) and a placebo gel was placed on the dental socket. In the ZA group, the animals received AZ (0.1 mg/kg-i.p.), and the tooth extraction was carried out (D49/S8). In PAb group, the animals were submitted to BRONJ model, and immediately after tooth extraction, the animals received 1.0% of PAb gel the dental socket in. All gels were administered in a single application. The animals were followed until the day of euthanasia (D77/S11). *ZA* zoledronic acid, *PAb* polyssacharide of *Agaricus blazei*, *w* week, *d* day, *EXO* exodontia.

hematoxylin and eosin (HE) for histopathological and histomorphometric analysis. The histopathological analysis was performed, at 100 × magnification and the presence of bone sequestration was evaluated using scores where 0 indicates absence and 1 indicates[6,19]. These parameters were presented as median and extreme values.

Cells counts were also performed. Ten fields of HE stained slide, adjacent to the extraction site, were captured at 400 × magnification. In the same field, the number of osteocytes and empty lacuna/bone surface were counted as well as the number of osteoblast/bone perimeter (N.Ob./B.Pm.) using Image J® software (NIH, Bethesda, MD, USA) using the cell contain command[20]. The results were expressed as mean ± S.E.M.

Another section of the previously obtained paraffin block was collected for picrosirius red staining. The slides were analysed under a normal and polarized light filter. The quantitative estimation of collagen birefringence, as yellow–red for type I collagen and green for type III collagen[21], was determined from digital images of 6 fields of each section (from 6 specimens per group), at 200× magnification, using ImageJ® software, according to de Sousa Ferreira et al.[6]. Data was presented as the mean percentage ± S.E.M. of collagen content per group. The counts were performed using Image J 1.51 j8 software (NIH, Bethesda, MD, USA) and the data expressed as mean ± S.E.M.[6,19]. Data is presented as mean percentage ± S.E.M.[6,14].

Raman microspectroscopy

Micro Raman spectrometry was used to evaluate bone composition and remodeling. For that, samples were placed in a Micro-Raman spectrophotometer (XploRATM, Horiba JobinYvon, Paris, France) coupled to a Confocal microscope (model XploRATM, manufactured by Horiba JobinYvon). Three spectra of each sample were collected. For the standardization acquisitions were carried out in two distinct points, inside and outside the dental alveolus[6,14]. The data were obtained by a LabSpec 6 software data acquisition command system (Horiba, JobinYvon, Paris, France) and analyzed by the Origin 9 program (Originlab© Corporation, One Roundhouse Plaza, Northampton, MA 01060, USA). For a better understanding the change on bone tissue the ratio of the bands was calculated, as follows:

(1) Mineral-to-matrix ratio (MTMR) (~ 960 cm^{-1}/1454 cm^{-1}): indicates the amount of bone mineralization;
(2) Carbonated-to-phosphate ratio (CTPR) (~ 1070 cm^{-1}/ ~ 960 cm^{-1}): indicates "B-type" carbonate substitution and it is related to bone solubility.
(3) Mineral maturity ratio (~ 1030 cm^{-1}/ ~ 1020 cm^{-1}): reflects proportion of apatitic domain compared with non-apatitic surface domain and it is related to bone aging[22].
(4) Collagen crosslinks ratio (1660 cm^{-1}/1690 cm^{-1}): measures secondary structures of collagen indicating deterioration of collagen structural integrity[23].
(5) HA carbonate/amide I (~ 1070 cm^{-1}/ ~ 1667 cm^{-1}): used for remodeling evaluation[14,24].

RNA isolation and quantitative PCR

In another set of experiments, after euthanasia, the maxillae were collected, the gingival tissue removed, and the bone tissue was macerated in liquid nitrogen using Trizol (Thermo Fischer-Waltham, Massachusetts, USA). The extracted mRNA was quantified using Nanodrop (Thermo Fischer-Waltham, Massachusetts, USA) and then transcribed using Superscript II (Invitrogen). Subsequently, the RT-PCR assay was carried out using SYBR_green as a reference (ABI 7500 Fast; Applied Biosystems). The PCR condition was 50 °C for 2 min and 90 °C for 10 min, then 40 cycles at 95 °C for 15 s and 60 °C for 1 min, where the RT-PCR system at 7900HT from Applied Biosystems. To calculate the results obtained, the threshold cycle method[25] was used, where they were presented as relative fold increase related to beta-actin. Primer sequences were as following: ß-actina s: TGAGCTGAC CAGTTCCCTCT, ß-catenin as: AAGCTCGCTCCTGTGAGTTC; Runx2 s: CCTTCCCTCCGAGACCCTAA, Runx2 as: ATGGCTGCTCCCTTCTGAAC; GSK3b s: AGAAGAGCCATCATGTCGGG; GSK3b as: CCAAAA GCTGAAGGCTGCTG.

Determination of bone formation

Before euthanasia, 3 ml of blood samples were obtained from the abdominal aorta from all animals, previously anesthetized. The samples were distributed in tubes with clot accelerator for biochemical parameters and sent to Laboratory of Clinical and Toxicological Analysis (LACT) of the of the Federal University of Ceará (UFC). Serum levels of Bone Alkaline Phosphatase (BALP) for analysis of bone formation. Data was presented was expressed as mean ± S.E.M.

Statistical analysis

The normality of the data was verified through the Shapiro–Wilk test. Parametric data were presented as mean ± standart error of the mean (S.E.M) after ANOVA followed by Tukey test. Non-parametric data were presented as Median (extreme values) after Kruskal Wallis and Dunn's Tests. In all situations, the significance level of $p < 0.05$ was adopted. The software used for all analyzes was IBM® SPSS® statistics 20 and charts constructed using GraphPad Prism® version 6.0.

Ethics approval

Approval was obtained from the ethics committee of Animal Research Federal University of Ceará (UFC) (number 4411060619).

Results

PAb gel protected bone tissue of rats submitted to BRONJ

The model of BRONJ was effective, once the animals treated with ZA and submitted to tooth extraction presented a significant reduction of viable osteocytes (-52%) (Fig. 2A,C) with a 6 time increase in empty lacuna (Fig. 2B,C) compared to control. Bone sequestration was also seen in ZA group (Table 1). PAb gel, in the other hand, reversed these findings ($p < 0.05$), considered hallmarks of osteonecrosis.

PAb gel mitigated BRONJ-like lesion in rats

BRONJ-like lesions were evaluated by macroscopic and radiographic analyses. Macroscopically, BRONJ was marked by exposure of necrotic bone without mucosal healing (Fig. 3A). Radiographically, it was seen a reduction in the radiographic density in the site of extraction ($p < 0.05$) compared to control (Fig. 3B and C). Meanwhile, PAb gel promoted mucosal healing and reduced necrotic lesion in maxillary bone (Fig. 3A). The treatment also

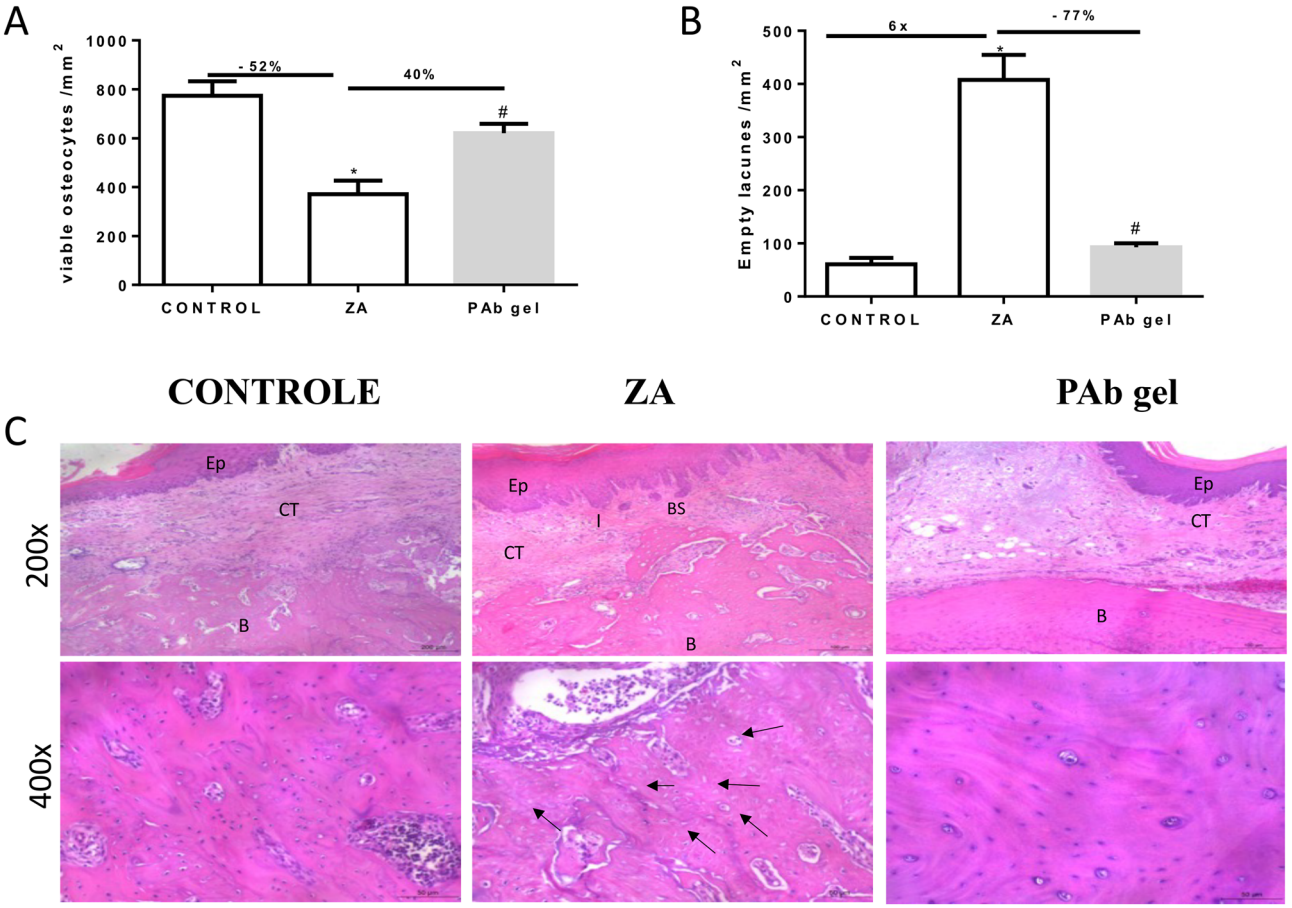


Figure 2. PAb gel protected bone tissue. (**A**) Number of viable osteocytes/mm^2 (N.Ocy./B.Ar.); (**B**) Number of empty lacunae/mm^2 (N.EL/B.Ar.); (**C**) Histological aspect of hemimaxillae. Bars represent the mean ± SEM of 6 animals per group. (*) indicates a significant difference when compared to the Control group. (#) indicates a significant difference when compared to the ZA group. Hematoxylin and Eosin (H&E). (200 × and 400 × magnification). Black arrows indicate empty gaps. *Ep* epithelium, *CT* connective tissue, *B* bone, *BS* bone sequestration. ANOVA and Tukey.

Bone sequestration/bone necrosis	CONTROL	ZA	PAb gel
(0) missing	05 (83%)	01(17%)	04 (67%)
(1) present	01 (17%)	05 (83%)	02 (33%)#
Median (extreme values)	0 (0–1)	2 (0–1)*	0 (0–1)#

Table 1. Histopathological analysis of PAb on the presence of bone sequestration/bone necrosis. Values are resented in Median (extreme values) of 6 animals per group. Kruskal–Wallis test followed by Dunn. *ZA* zoledronic acid, *PAb* polyssacharide of *Agaricus blazei*. *Indicates difference when compared to the control. #Indicates difference when compared to ZA group ($P < 0.05$). Data expressed as absolute frequency (percentage frequency).

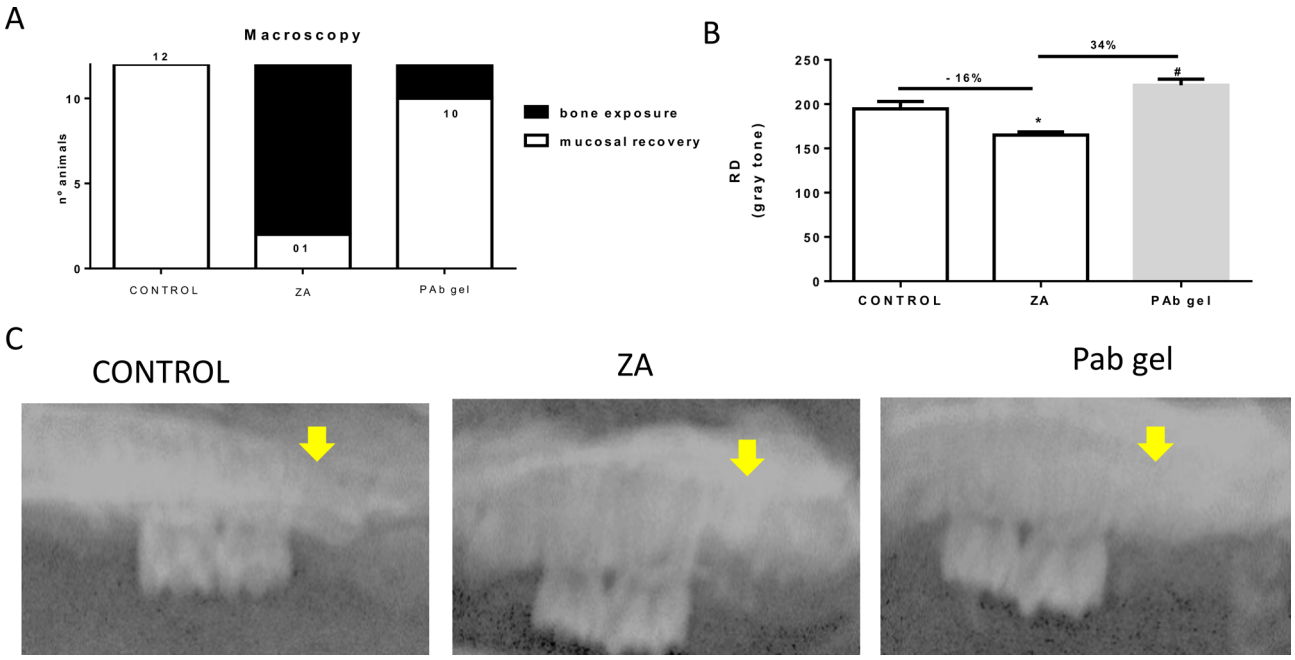


Figure 3. Pab gel mitigated BRONJ-like lesions. (**A**) Distribution of animals considering the macroscopic aspect of bone exposure and mucosal healing in hemimaxillae. Bars represent the mean ± SEM of 12 animals per group. (**B**) Radiographic density (RD) of the hemimaxillae; (**C**) Macroscopic and radiographic aspects of hemimaxilla. (*) indicates a significant difference when compared to the Control group. (#) indicates a significant difference when compared to the ZA group. Yellow arrows indicate radiographic density in the extraction site. ANOVA and Tukey.

increased, by 34%, the radiographic density in the area of dental alveolus when compared to ZA group ($p < 0.05$) (Fig. 3B and C).

PAb gels stimulated osteoblasts through Wnt signaling

Considering that delayed bone remodeling contributes do osteonecrosis and that osteoblasts plays a role on this process, we have decided to investigated if PAb gel would positively improve this cell activity somehow. Initiialy, we showed that ZA significantly reduced osteoblast count (Fig. 4A and C) and function (Fig. 4B) compared to control. However, when PAb gel was used it was seen a significant increase in both osteoblast number (Fig. 3A and C) and in BALP serum levels, a marker of osteoblast activity (Fig. 4B).

In order to understand the mechanism underlying the benefitial effect of Pab on osteoblast we performed the analysis on the expression of GSK3b and beta-catenin, main players of Wnt signaling, an important pathway related to osteoblastogenesis (Fig. 4D and E). As expected, ZA increased expression on GSK3b mRNA and decreased beta-catenin mRNA expression. Despite the negative impact on Wnt signaling, no significant reduction in the expression of Runx2, a marker of osteoblasts (Fig. 4F). On the contrary, PAb gel to stimulate Wnt signaling, due to the decrease in GSK3b mRNA expression coupled with a significant increased on beta-catenin mRNA expression. A marked increase in Runx2 mRNA expression was seen in the animals with BRONJ treated with PAb gel confirming the findings from histomorphometric analysis.

PAb gels improve bone quality in BRONJ model

Bone quality was assessed by collagen analysis and Raman spectrometry. The animals receiving ZA and subjected to tooth extraction presented a significant decrease (by 52%) in the amount of total collagen when compared to Control (Fig. 5A and B). This reduction was marked by the decrease on type I collagen on ZA group (37%) ($p > 0.05$) (Fig. 5C) ($p < 0.05$). No difference was observed regarding type III (Fig. 5D) ($p > 0.05$). In the order hand, the treatment with PAb gel increased the total amount of collagen, specially type I collagen, and significantly decreased type III collagen when compared to ZA group, corroborating our previous findings on osteoblast analysis.

On Raman spectrometry, it was seen that ZA reduced the amount of bone mineralization, seen by MTMR ratio. Maxillary bones, of animals from ZA group, showed higher CTPR, indicating increased solubility. These bones also presented increased mineral maturity ratio, compatible to older bones. Collagen crosslink ratio was significantly higher in ZA group, corroborating collagen analyses, previously described. BRONJ was reduced bone remodeling (HA carbonate/amide I ratio) suggested by the reduction on osteoblast cell count. PAb gel reversed all the findings caused by BRONJ on bone tissue (Table 2).

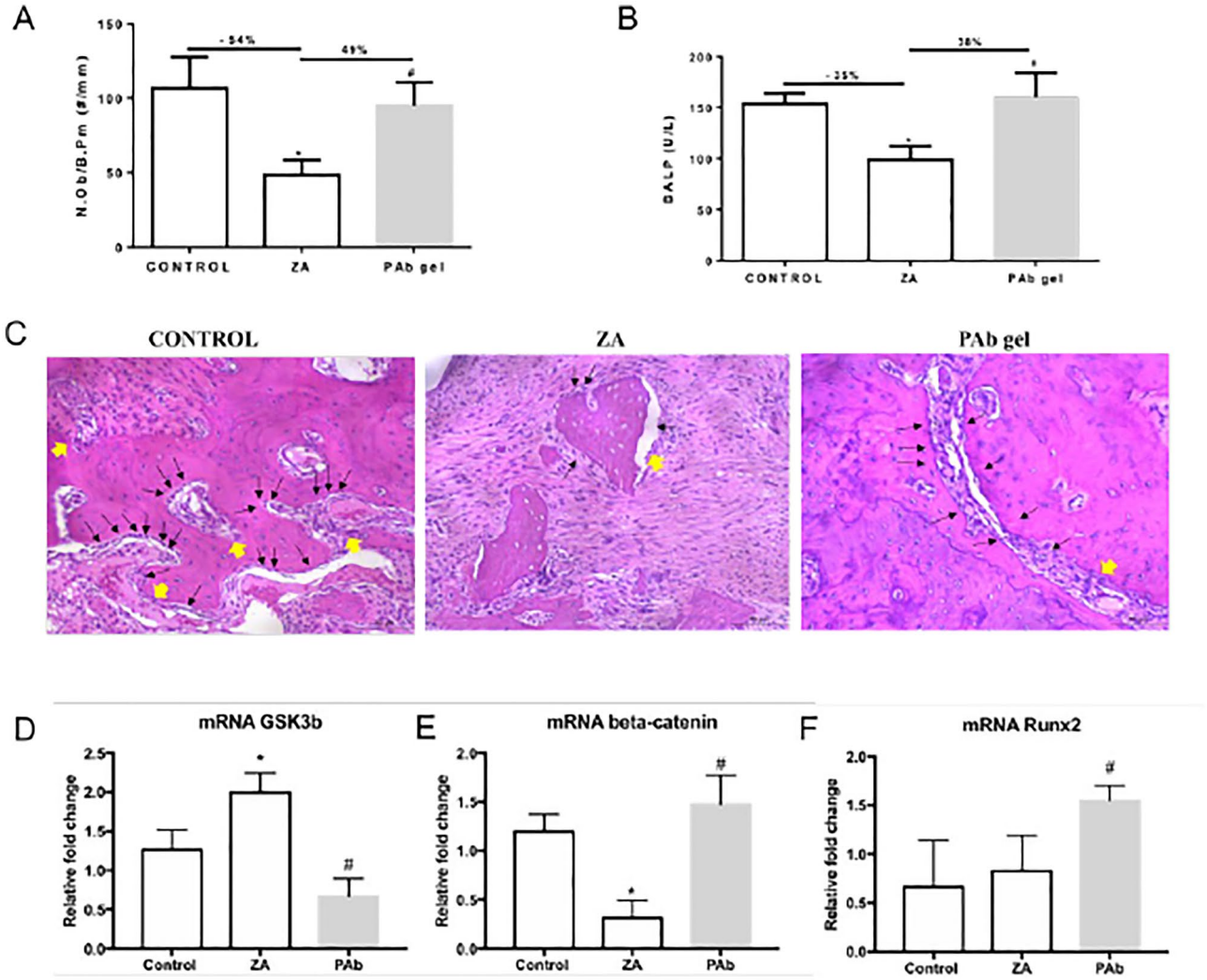


Figure 4. PAb gel stimulate osteoblastogenesis through Wnt signaling. (**A**) Number of osteoblast/bone perimeter (N.Ob./B.Pm.); (**B**) Serum levels of Bone-specific Alkaline Phosphatase (BALP); (**C**) Histological aspect of hemimaxillae; mRNA expression of (**D**) GSK3b; (**E**) Beta-catenin; (**F**) Runx2. Bars represent the mean ± SEM of 6 animals per group. (*) indicates a significant difference when compared to the Control group. (#) indicates a significant difference when compared to the ZA group. Hematoxylin and Eosin (H&E). (200 × magnification). ANOVA and Tukey.

Discussion

This study demonstrated that the BRONJ model in rats exhibited characteristics such as the exposure of necrotic bone without mucosal healing and, notably, a decrease in the number of viable osteocytes with an increase in empty lacunae[6,16,26,27], mirroring the features of BRONJ lesions in humans[28]. The BRONJ model also had a detrimental impact on osteoblast count and function by inhibiting Wnt signaling, thus confirming reduced bone remodeling. Moreover, ZA (zoledronic acid) led to a reduction in total collagen, especially type I collagen in bone tissue, as supported by collagen degradation observed in Raman spectrometry. The bone tissue subjected to the BRONJ model also exhibited reduced mineral content and increased solubility, resembling the characteristics of aged bone. Importantly, the use of PAb (polysaccharide from *Agaricus blazei*) counteracted all the findings observed in the ZA group. To the best of our knowledge, this is the first instance of reporting the local effect of a polysaccharide from Agaricus blazei in a BRONJ model in rats.

It was seen that, PAb gel protected bone subjected to the BRONJ model, reducing bone exposure and increasing the count of viable osteocyte cells. Bone necrosis is a hallmark of MRONJ. While osteocyte death occurs as a natural part of the skeletal life cycle, the suppression of bone resorption by bisphosphonates (BPs) has been suggested to lead to the accumulation of dead osteocytes. Prolonged exposure to BPs may also reach cytotoxic levels for osteocytes. These accumulated necrotic osteocytes release high levels of Damage-associated molecular patterns (DAMPs), further promoting inflammation that results in damage to oral soft and hard tissues[29]. Considering the effects of beta-glucan on bone tissue, a model of steroid-induced avascular necrosis of the femoral head in rabbits has shown that this polysaccharide reduced empty lacunae and decreased osteocyte apoptosis. This was marked by a decrease in the expression of pro-apoptotic regulators Bax and Caspase-3, as well

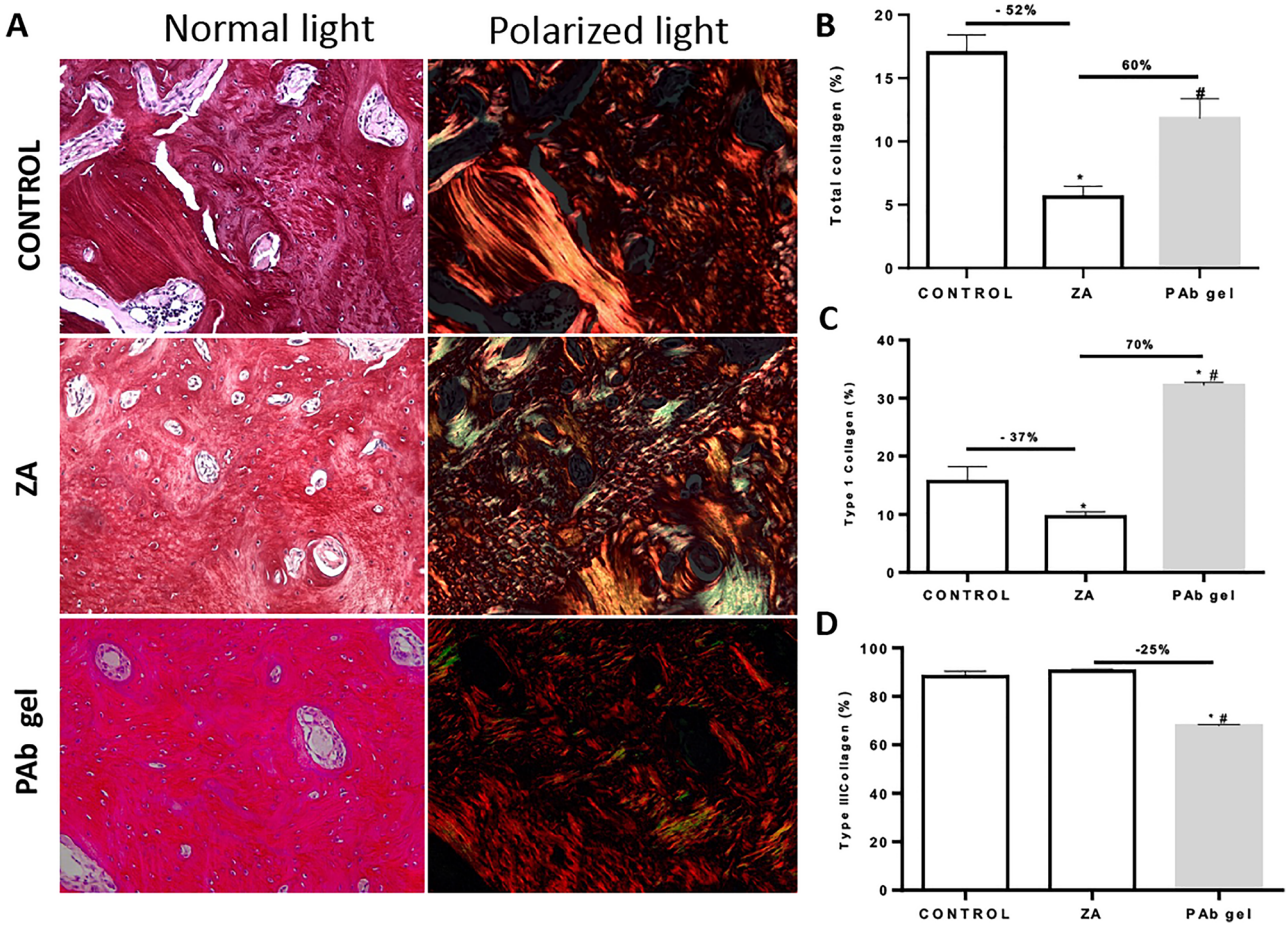


Figure 5. PAb gel improves bone quality. (**A**) Histological aspect of Picrosirius red stained hemimaxillae under normal light and polarized light; (**B**) Percentage of total collagen content on bone tissue; (**C**) Percentage of type I collagen on bone tissue; (**D**) Percentage of type III collagen on bone tissue. Bars represent mean ± SEM of 6 animals per group. Yellow–red indicates type I collagen and green indicates type III collagen. Yellow–red indicates type I collagen and green indicates type III collagen. (*) indicates difference when compared to the Control. (#) indicates difference when compared to ZA group. 200 × magnification. ANOVA and Tukey.

Ratio	CONTROL	ZA	PAb gel
MTMR	2.576 ± 0.453	0.502 ± 0.049*	2.073 ± 0.266#
CTPR	0.825 ± 0.136	1.694 ± 0.206*	0.464 ± 0.090#
Mineral maturity	1.697 ± 0.345	3.522 ± 0.703*	1.215 ± 0.055#
Collagen crosslinks	1.420 ± 0.940	3.640 ± 1.040*	0.70 ± 0.400#
HA carbonate/amide I	1.098 ± 0.080	0.420 ± 0.050*	1.060 ± 0.065#

Table 2. Effect of ATV on Raman spectroscopy parameters for bone substrates. Values are presented as mean ± SEM. *ZA* zoledronic acid, *PAb* polyssacharide from *Agaricus blazei*, *MTMR* mineral-to-matrix ratio, *CTPR* carbonate-to-phosphate ratio, *HA* hydroxyapatite. *Indicates difference when compared to the Control group ($P < 0.05$). #Indicates difference when compared to ZA group ($P < 0.05$). ANOVA and Tukey.

as an increase in the expression of the anti-apoptotic regulator Bcl-2. These findings suggest that Bax, Bcl-2, and Caspase-3 are involved in the anti-apoptotic effects of this polysaccharide[30], which aligns with our own findings.

Here, we have demonstrated that PAb increased the number of osteoblast cells and enhanced their function by stimulating the Wnt pathway, as evidenced by increased levels of β-catenin and Runx2. Previous research has indicated that extracts from Agaricus blazei can promote the expression of genes associated with osteoblast activity and bone formation[31]. While the impact of bisphosphonates (BPs) on bone remodeling delay is linked to the development of BRONJ, their effect on osteoblasts is less explored[5]. High doses of BPs have been reported to arrest the osteoblast cell cycle and induce apoptosis, consequently reducing osteoblast lineage proliferation[5]. Our previous research has also demonstrated the detrimental effect of BRONJ on osteoblasts and the Wnt pathway[6,32,33]. However, the intriguing effect of PAb gel on osteoblasts observed in this study can be attributed

to its ability to enhance osteoblast adhesion, growth, and proliferation[34]. Mushroom beta-glucan has also been found to stimulate Wnt/beta-catenin signaling in wound and healing models[35]. In bone tissue, beta-glucan has been shown to reduce osteoclast numbers and concentrations of inflammatory markers like IL-1β and TNF-α[4], as well as downregulate RANKL and upregulate osteoprotegerin (OPG)[36,37]. The reduction in the proliferation and activity of osteoclasts induced by beta-glucans undoubtedly supports osteoblast function, as evidenced by the increase in BALP levels[13], which aligns with our own results. Collectively, these findings demonstrate that PAb gel has the potential to restore bone remodeling, which is often reduced during the use of ZA.

In addition to the quantity of bone, the quality of this tissue is of paramount importance. PAb gel not only improved the quantity but also the quality of collagen. Collagen, as the primary component of the extracellular matrix[38], serves as a structural framework in tissues during the healing process, influencing cell proliferation and migration[39]. Type III collagen plays a crucial role in the initial stages of the healing process, where it is synthesized by fibroblasts in the granulation tissue[40]. As the wound matures and closes, type III collagen is broken down, and the synthesis of type I collagen increases[40]. Soundia et al.[41] demonstrated that necrotic bone exhibits disorganization in the collagen network with a predominance of type III collagen. The effects of beta-glucan on collagen have been previously demonstrated in wound and healing models, wherein it enhances collagen deposition and organization[42]. The improved quality of collagen after using PAb gel was further confirmed by assessing the collagen crosslink ratio through Raman spectrometry. Lower values of this ratio indicate a lesser degree of structural deterioration in collagen, making it a valuable tool for evaluating collagen quality and structural integrity in bone[23].

In this study, the improvement in bone quality after PAb use was further verified through Raman spectrometry, as it led to an increase in mineral content and a reduction in fragility, resulting in a more resilient and robust bone tissue. The increase in mineral-to-matrix ratio (MTMR) indicates a higher mineral content in the bone tissue, directly enhancing bone strength and making it more resistant to fractures[42]. A higher carbonate-to-phosphate ratio (CTPR) signifies that phosphate positions in the apatitic lattice, which are susceptible to ionic substitution, often referred to as "B-type" carbonate substitution, are associated with reduced bone solubility. Lastly, mineral maturity reflects the progressive transformation of non-apatitic domains into well-crystallized apatite, and it can be influenced by alterations in bone remodeling, such as the use of bisphosphonates (BPs), which tends to increase mineral maturity[22]. While Raman spectroscopy is widely used in various fields, including the detection of tumors in biology and medicine, its application in discriminating BRONJ has been reported only infrequently. Our findings are consistent with some authors[43] and contradicted by others[44], highlighting the need for additional studies to more comprehensively evaluate BRONJ lesions using Raman spectrometry.

Despite the beneficial effect of PAb gel in treating BRONJ, it is important to note that this study has certain limitations. The role of Wnt signaling warrants further in-depth investigation, and in vitro assays must be conducted to determine how PAb interacts with osteoblasts. Given the multifactorial etiology of BRONJ, additional aspects such as the potential anti-inflammatory, antimicrobial, and angiogenic effects of PAb should be examined in the near future.

Conclusion

In conclusion, the findings of this study suggest that the PAb gel derived from Agaricus blazei may have a mitigating effect on bone necrosis in the model of Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ). This study underscores the potential of PAb as a pharmacological tool to support or prevent BRONJ therapy. Nevertheless, further investigations, including clinical trials, are essential to validate its efficacy and safety in patients.

Data availability

The datasets generated and/or analysed during the current study are available in the Open Science Framework repository, at Identifier: https://doi.org/10.17605/OSF.IO/3MA5K.

Received: 9 November 2023; Accepted: 29 March 2024
Published online: 08 April 2024

References

1. Ferneini, E. M. Medication-related osteonecrosis of the jaw (MRONJ). *J. Oral Maxillofac. Surg. Off. J. Am. Assoc. Oral Maxillofac. Surg.* **79**(8), 1801–1802. https://doi.org/10.1016/j.joms.2021.05.014 (2021).
2. Ruggiero, S. L. *et al.* American Association of oral and maxillofacial surgeons'position paper on medication-related osteonecrosis of the jaws-2022 update. *J. Oral Maxillofac. Surg. Off. J. Am. Assoc. Oral Maxillofac. Surg.* **80**(5), 920–943. https://doi.org/10.1016/j.joms.2022.02.008 (2022).
3. Kuroshima, S., Sasaki, M. & Sawase, T. Medication-related osteonecrosis of the jaw: A literature review. *J. Oral Biosci.* **61**(2), 99–104. https://doi.org/10.1016/j.job.2019.03.005 (2019).
4. Kim, H. Y. Review and update of the risk factors and prevention of antiresorptive-related osteonecrosis of the jaw. *Endocrinol. Metab. (Seoul, Korea)* **36**(5), 917–927. https://doi.org/10.3803/EnM.2021.1170 (2021).
5. George, E. L., Lin, Y. L. & Saunders, M. M. Bisphosphonate-related osteonecrosis of the jaw: A mechanobiology perspective. *Bone Rep.* **8**, 104–109. https://doi.org/10.1016/j.bonr.2018.03.003 (2018).
6. de Sousa Ferreira, V. C. *et al.* Bisphosphonate-related osteonecrosis induced change in alveolar bone architecture in rats with participation of Wnt signaling. *Clin. Oral Investig.* **25**(2), 673–682. https://doi.org/10.1007/s00784-020-03551-7 (2021).
7. Wang, P., Li, X. T., Sun, L. & Shen, L. Anti-inflammatory activity of water-soluble polysaccharide of *Agaricus blazei* murill on ovariectomized osteopenic rats. *Evid.-Based Complement. Altern. Med. eCAM* **2013**, 164817. https://doi.org/10.1155/2013/164817 (2013).
8. Rop, O., Mlcek, J. & Jurikova, T. Beta-glucans in higher fungi and their health effects. *Nutr. Rev.* **67**(11), 624–631. https://doi.org/10.1111/j.1753-4887.2009.00230.x (2009).

9. Biedron, R., Tangen, J. M., Maresz, K. & Hetland, G. *Agaricus blazei* Murill—Immunomodulatory properties and health benefits. *Funct. Foods Health Dis.* **2**(11), 428–447. https://doi.org/10.3390/nu12051339 (2012).
10. Hetland, G. *et al.* Antitumor, anti-inflammatory and antiallergic effects of agaricus blazei mushroom extract and the related medicinal basidiomycetes mushrooms, hericium erinaceus and grifolafrondosa: A review of preclinical and clinical studies. *Nutrients* **12**(5), 1339. https://doi.org/10.3390/nu12051339 (2020).
11. Kim, Y. S. *et al.* Effects of polycan, a β-glucan, on experimental periodontitis and alveolar bone loss in Sprague-Dawley rats. *J. Periodontal Res.* **47**(6), 800–810. https://doi.org/10.1111/j.1600-0765.2012.01502.x (2012).
12. Park, H. S. *et al.* Functional polysaccharides from Grifola frondosa aqueous extract inhibit atopic dermatitis-like skin lesions in NC/Nga mice. *Biosci. Biotechnol. Biochem.* **79**(1), 147–154. https://doi.org/10.1080/09168451.2014.963500 (2015).
13. Choi, M. *et al.* Yeast beta-glucan mediates histone deacetylase 5-induced angiogenesis in vascular endothelial cells. *Int. J. Biol. Macromol.* **211**, 556–567. https://doi.org/10.1016/j.ijbiomac.2022.05.057 (2022).
14. de Sousa, V. C. *et al.* Atorvastatin reduces zoledronic acid-induced osteonecrosis of the jaws of rats. *Bone* **164**, 116523. https://doi.org/10.1016/j.bone.2022.116523 (2022).
15. Luthfi, M. *et al.* Expression of fibroblast cells after extraction of wistar rat teeth after topical application of okra fruit (*Abelmoschus esculentus*) gel. *Infect. Dis. Rep.* **12**(Suppl 1), 8726. https://doi.org/10.4081/idr.2020.8726 (2020).
16. Barba-Recreo, P. *et al.* Zoledronic acid - related osteonecrosis of the jaws. Experimental model with dental extractions in rats. *J. Cranio-Maxillo-Fac. Surg. Off. Publ. Eur. Assoc. Cranio-Maxillo-Fac. Surg.* **42**(6), 744–750. https://doi.org/10.1016/j.jcms.2013.11.005 (2014).
17. Campelo, M. D. S. *et al. Agaricus blazei* Murill extract-loaded in alginate/poly(vinyl alcohol) films prepared by Ca2+ cross-linking for wound healing applications. *J. Biomed. Mater. Res. B Appl. Biomater.* **111**(5), 1035–1047. https://doi.org/10.1002/jbm.b.35212 (2023).
18. Goes, P., Lima, A. P., Melo, I. M., Rêgo, R. O. & Lima, V. Effect of Atorvastatin in radiographic density on alveolar bone loss in wistar rats. *Braz. Dent. J.* **21**(3), 193–198. https://doi.org/10.1590/s0103-64402010000300003 (2010).
19. Yanık, S. *et al.* Histopathological features of bisphosphonates related osteonecrosis of the jaw in rats with and without vitamin d supplementation. *Arch. Oral Biol.* **65**, 59–65. https://doi.org/10.1016/j.archoralbio.2015.10.010 (2016).
20. Jilka, R. L., Noble, B. & Weinstein, R. S. Osteocyte apoptosis. *Bone* **54**(2), 264–271. https://doi.org/10.1016/j.bone.2012.11.038 (2013).
21. Junqueira, L. C., Bignolas, G. & Brentani, R. R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem. J.* **11**(4), 447–455. https://doi.org/10.1007/BF01002772 (1979).
22. Farlay, D. *et al.* The ratio 1660/1690 cm(− 1) measured by infrared microspectroscopy is not specific of enzymatic collagen cross-links in bone tissue. *PLoS One* **6**(12), e28736. https://doi.org/10.1371/journal.pone.0028736 (2011).
23. Unal, M., Jung, H. & Akkus, O. Novel Raman spectroscopic biomarkers indicate that postyield damage denatures bone's collagen. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* **31**(5), 1015–1025. https://doi.org/10.1002/jbmr.2768 (2016).
24. De Vasconcelos, R. F. *et al.* Milk kefir therapy improves the skeletal response to resistance exercise in rats submitted to glucocorticoid-induced osteoporosis. *Exp. Gerontol.* **167**, 111921. https://doi.org/10.1016/j.exger.2022.111921 (2022).
25. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25**(4), 402–408. https://doi.org/10.1006/meth.2001.1262 (2001).
26. Colditz, J. *et al.* Postnatal skeletal deletion of dickkopf-1 increases bone formation and bone volume in male and female mice, despite increased sclerostin expression. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* **33**(9), 1698–1707. https://doi.org/10.1002/jbmr.3463 (2018).
27. Gong, X., Yu, W., Zhao, H., Su, J. & Sheng, Q. Skeletal site-specific effects of zoledronate on in vivo bone remodeling and in vitro BMSCs osteogenic activity. *Sci. Rep.* **7**, 36129. https://doi.org/10.1038/srep36129 (2017).
28. Ruggiero, S. L. *et al.* American Association of Oral and Maxillofacial Surgeons. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw–2014 update. *J. Oral Maxillofac. Surg. Off. J. Am. Assoc. Oral Maxillofac. Surg.* **72**(10), 1938–1956. https://doi.org/10.1016/j.joms.2014.04.031 (2014).
29. Aguirre, J. I., Castillo, E. J. & Kimmel, D. B. Biologic and pathologic aspects of osteocytes in the setting of medication-related osteonecrosis of the jaw (MRONJ). *Bone* **153**, 116168. https://doi.org/10.1016/j.bone.2021.116168 (2021).
30. Cui, D., Zhao, D. & Huang, S. Structural characterization of a safflower polysaccharide and its promotion effect on steroid-induced osteonecrosis in vivo. *Carbohydr. Polym.* **233**, 115856. https://doi.org/10.1016/j.carbpol.2020.115856 (2020).
31. Nakamura, T. *et al. Agaricus blazei* extract supplementation promotes bone formation in ovariectomized rats. *Biosci. Biotechnol. Biochem.* **75**(4), 645–651. https://doi.org/10.1271/bbb.100685 (2011).
32. Huang, K., El-Seedi, H. R. & Xu, B. Critical review on chemical compositions and health-promoting effects of mushroom *Agaricus blazei* Murill. *Curr. Res. Food Sci.* **5**, 2190–2203. https://doi.org/10.1016/j.crfs.2022.10.029 (2022).
33. Patntirapong, S., Singhatanadgit, W., Chanruangvanit, C., Lavanrattanakul, K. & Satravaha, Y. Zoledronic acid suppresses mineralization through direct cytotoxicity and osteoblast differentiation inhibition. *J. Oral Pathol. Med. Off. Publ. Int. Assoc. Oral Pathol. Am. Acad. Oral Pathol.* **41**(9), 713–720. https://doi.org/10.1111/j.1600-0714.2012.01154.x (2012).
34. Castillo Diaz, L. A., Saiani, A., Gough, J. E. & Miller, A. F. Human osteoblasts within soft peptide hydrogels promote mineralisation in vitro. *J. Tissue Eng.* **5**, 2041731414539344. https://doi.org/10.1177/2041731414539344 (2014).
35. Hsiao, C. M. *et al.* Immunomodulator "mushroom beta glucan" induces Wnt/β catenin signalling and improves wound recovery in tilapia and rat skin: A histopathological study. *Int. Wound J.* **13**(6), 1116–1128. https://doi.org/10.1111/iwj.12427 (2016).
36. Chou, W. Y. *et al.* Association between atopic dermatitis and colorectal cancer risk: A nationwide cohort study. *Medicine* **99**, e18530. https://doi.org/10.1097/MD.0000000000018530 (2020).
37. Silva, P. G. *et al.* Effect of different doses of zoledronic acid in establishing of bisphosphonate-related osteonecrosis. *Arch. Oral Biol.* **60**(9), 1237–1245. https://doi.org/10.1016/j.archoralbio.2015.05.015 (2015).
38. Cuttle, L. *et al.* Collagen in the scarless fetal skin wound: Detection with picrosirius-polarization. *Wound Repair Regen. Off. Publ. Wound Heal. Soc. Eur. Tissue Repair Soc.* **13**(2), 198–204. https://doi.org/10.1111/j.1067-1927.2005.130211.x (2005).
39. Tracy, L. E., Minasian, R. A. & Caterson, E. J. Extracellular matrix and dermal fibroblast function in the healing wound. *Adv. Wound Care* **5**(3), 119–136. https://doi.org/10.1089/wound.2014.0561 (2016).
40. Olczyk, P., Mencner, Ł & Komosinska-Vassev, K. The role of the extracellular matrix components in cutaneous wound healing. *BioMed. Res. Int.* **2014**, 747584. https://doi.org/10.1155/2014/747584 (2014).
41. Soundia, A. *et al.* Zoledronate impairs socket healing after extraction of teeth with experimental periodontitis. *J. Dent. Res.* **97**(3), 312–320. https://doi.org/10.1177/0022034517732770 (2018).
42. Majtan, J. & Jesenak, M. β-glucans: Multi-functional modulator of wound healing. *Molecules (Basel, Switzerland)* **23**(4), 806. https://doi.org/10.3390/molecules23040806 (2018).
43. Kün-Darbois, J. D., Libouban, H., Mabilleau, G., Pascaretti-Grizon, F. & Chappard, D. Bone mineralization and vascularization in bisphosphonate-related osteonecrosis of the jaw: An experimental study in the rat. *Clin. Oral Investing.* **22**(9), 2997–3006. https://doi.org/10.1007/s00784-018-2385-2 (2018).
44. Xia, C., Pu, Y., Zhang, Q., Hu, Q. & Wang, Y. The feasibility of discriminating BRONJ lesion bone with Raman spectroscopy. *Front. Endocrinol.* **14**, 1099889. https://doi.org/10.3389/fendo.2023.1099889 (2023).

Acknowledgements
We would like to thank the Laboratory of the Study and Image Processing Center (NEMPI) of the Faculty of Medicine of the Federal University of Ceará (UFC) for all histology and digital imaging services, the Graduate Program in Dentistry (PPGO) of UFC for Raman analysis and Laboratory of Clinical and Toxicological Analysis (LACT) of the Federal University of Ceará (UFC) for biochemical analysis.

Author contributions
Vanessa Costa de Sousa performed the study design, data analysis and elaboration of manuscript. Nilson Romero Dias, Sislana Costa e Maria Jennifer Chaves Bernadino contributed to the execution of the experiment, drug administration and animal care; Gisele Angelino, Nadine Linhares, Fatima Regina Nunes Sousa and Raquel Felipe Vasconcelos: assistance in inducing BRONJ, monitoring of treatment and histological preparation. Conceição da Silva Martins was responsible for processing picrossirius red analysis. George de Almeida Silva and Maria Elenir Nobre Pinho Ribeiro produced the gel formulation; Karuza Maria Alves Pereira was responsible for histopathological analysis; Delane Viana Gondim, Mirna Marques and Hellíada Chaves conduced data analysis and manuscript review; Renata Ferreira Carvalho Leitão analyzed the radiographic density and performed manuscript editing; Gerly Anne Brito performed the critical review of the manuscript and Paula Goes supervised the whole study, performed data analysis, elaboration and revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Funding
This study was funded by the National Council for Scientific and Technological Development (CNPq) process number 402349/2021-0.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to P.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Is adenotonsillar hypertrophy associated with dentofacial morphology? A systematic review and meta-analyses

Tingting Zhao,[a,b,c,d] Min Wang,[e] Peter Ngan,[f] Zhendong Tao,[a] Xueqian Yu,[g] Fang Hua,[a,c,d,h,i,j] and Hong He[a,b,c,d]
Wuhan and Guangzhou, China, and Morgantown, WVa, and Manchester, United Kingdom

Introduction: As a common cause of upper airway obstruction in children, adenotonsillar hypertrophy (ATH) has been hypothesized to adversely affect dentofacial development and morphology. This systematic review aimed to summarize the existing evidence regarding the association between ATH and dentofacial characteristics of children. **Methods:** Four databases (PubMed, Embase, Web of Science, and VIP Chinese Journal Database) were searched from inception to November 1, 2024, for cross-sectional studies that compared the dental or craniofacial characteristics of children with and without adenoid hypertrophy (AH) and/or tonsillar hypertrophy (TH). The Newcastle-Ottawa Scale for Cross-Sectional Studies was used to assess the methodologic quality of included studies. Meta-analyses were performed with the random-effects model. **Results:** Thirty-six studies were included in this review. According to meta-analyses, the mandibular plane angle (SN-MP: mean difference [MD] = 2.20° [95% confidence interval {CI} 1.47-2.92]; P <0.00001), articular angle (ArGoMe: MD, 1.23° [95% CI, 0.68-1.79]; P <0.0001) were significantly greater in children with AH and/or TH. No significant differences were found between the ANB angle between the 2 groups (MD, 0.31° [95% CI, −0.35 to 0.61]; P = 0.59). However, the SNA (MD, −0.30° [95% CI, −0.53 to −0.06]; P = 0.01) and SNB angle (MD, −0.78° [95% CI, −1.33 to −0.24]; P = 0.005) were found to be significantly smaller in children with AH and/or TH. Regarding dental characteristics, the rate of Angle Class II and III malocclusions (relative risk = 1.29 [95% CI, 1. 14-1.45]; P <0.0001) and open bite (relative risk = 1.65 [95% CI, 1.21-2.25]; P = 0.001) were found to be higher in the AH and/or TH children. In addition, the width between the maxillary first molars (MD, −1.34 mm [95% CI, −2.12 to −0.56]; P = 0.0008) was found to be smaller both in AH and TH children. **Conclusions:** On the basis of evidence of low to very low certainty, children with ATH tend to exhibit craniofacial characteristics such as sagittal maxillary and mandibular retrognathia and an increased mandibular plane angle. In addition, children with ATH children appear to have a higher prevalence of Class II and III malocclusions, open bite, and a narrower maxillary arch width compared with their non-ATH counterparts. However, these findings must be interpreted with caution because of the limited quality and consistency of the available evidence. The statistically significant differences identified in this review are relatively small when compared with population deviations, raising questions about their clinical significance. Further high-quality studies with standardized methodologies are needed to confirm these associations and clarify their clinical relevance. (Am J Orthod Dentofacial Orthop 2025;■:■-■)

[a]State Key Laboratory of Oral and Maxillofacial Reconstruction and Regeneration, Key Laboratory of Oral Biomedicine Ministry of Education, Hubei Key Laboratory of Stomatology, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[b]Department of Orthodontics, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[c]Hubei Provincial Clinical Research Center for Dentofacial Deformities in Children, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[d]Center for Dentofacial Development and Sleep Medicine, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[e]Department of Stomatology, The Twelfth People's Hospital of Guangzhou, Guangzhou, China.
[f]Department of Orthodontics, School of Dentistry, West Virginia University, Morgantown, WVa.
[g]Library, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[h]Center for Orthodontics and Pediatric Dentistry at Optics Valley Branch, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[i]Center for Evidence-Based Stomatology, School and Hospital of Stomatology, Wuhan University, Wuhan, China.
[j]Division of Dentistry, School of Medical Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom.
Tingting Zhao and Min Wang are joint first authors and contributed equally to this work.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
The data may be obtained from the authors for academic purposes.
Address correspondence to: Fang Hua, Centre for Evidence-Based Stomatology, School and Hospital of Stomatology, Wuhan University, Luoyu Rd 237, Wuhan 430079, China; e-mail, huafang@whu.edu.cn or Hong He, Department of Orthodontics, School and Hospital of Stomatology, Wuhan University, Luoyu Rd 237, Wuhan 430079, China; e-mail, drhehong@whu.edu.cn.
Submitted, December 2024; revised and accepted, April 2025.
0889-5406/$36.00

https://doi.org/10.1016/j.ajodo.2025.04.024

INTRODUCTION

Tonsils and adenoids are integral components of the pharyngeal lymphatic loop, which plays a vital role in the development of the immune system and serve as defense against infections.[1] The palatine tonsils are situated in the tonsillar fossa between the palatoglossal arch and the pharyngopalatine arch, whereas the adenoid is located at the junction of the roof and the posterior wall of the nasopharynx. Generally, tonsils and adenoids undergo rapid postnatal development and subsequently atrophy aged between 12 and 14 years.[2-4] The prevalence of adenoid hypertrophy (AH) among children receiving specialized otolaryngology evaluations can reach as high as 42%-70%,[5] whereas the prevalence of tonsillar hypertrophy (TH) varied from 11% to 35% in children, depending on the population and diagnostic criteria used.[6,7] In addition, according to a previous study, the prevalence of TH in children receiving orthodontic treatment can be as high as 66.3%.[8]

The common diagnostic methods for AH and/or TH include nasopharyngoscopy, oral examination and cephalometric analysis. Nasopharyngoscopy is regarded as the gold standard for diagnosing AH and is widely employed in otolaryngology.[9] Oral examination offers a noninvasive approach to diagnosing TH by evaluating the percentage of oropharyngeal airway occupied by tonsils in coronal planes.[10] In orthodontic and orthognathic practices, the routine use of lateral cephalograms provides a simple and cost-effective means to gauge sagittal upper airway obstruction.[11]

Adenotonsillar hypertrophy (ATH) has conventionally been regarded as the predominant risk factor for pediatric obstructive sleep apnea, which can adversely affect children's growth and development, cognitive abilities, and cardiovascular health.[12] The existence of AH and/or TH can also lead to dysphonia and lower quality of life.[13-18] ATH is a common cause of upper airway obstruction in children. It has been shown to cause open mouth breathing resulting in morphologic and functional alterations in maxillofacial muscles, ultimately giving rise to abnormal maxillofacial development.[19] Sousa et al[20] found that the lower posterior facial height was higher in children with ATH than in those with isolated AH. Baroni et al[21] analyzed craniofacial features of children with ATH and observed a more anterior mandibular position in subjects with TH vs AH. Osiatuma et al.[22] reported reduced maxillary arch dimensions in the adenoid hypertrophy group compared with the control group. Furthermore, Hultcrantz et al[23] noticed a significant decrease in the number of children with open bite after tonsillectomy.

Although many studies have explored the relationship between ATH and maxillofacial morphology, the lack of evidence synthesis on the specific dentofacial characteristics of children with ATH remains. Therefore, the objective of this systematic review and meta-analysis is to synthesize existing evidence regarding the association between ATH and dentofacial characteristics in children.

MATERIAL AND METHODS

Protocol and registration

The protocol of this review was prospectively registered in the International Register of Systematic Review (PROSPERO; No. CRD42021291990). This review report was written in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[24]

Eligibility criteria

Types of studies. We included cross-sectional studies comparing the dental and/or craniofacial characteristics between children with and without AH and/or TH.

Types of participants. We included studies making comparison between children (aged <18 years) with AH and/or TH (the hypertrophy group [HG]) and those without ATH (the control group [CG]). Studies using cephalometric assessments, oral examination or nasopharyngeal endoscopy as the diagnostic tool for AH and/or TH were considered acceptable. Studies in which >30% of participants had craniofacial syndrome, metabolic disorders, orthodontic or orthognathic treatment history, adenotonsillectomy history, or were excluded.

Types of outcome measures. We included studies using cephalometric measurements or photographs to assess craniofacial morphology and clinical examinations or plaster casts to evaluate dental characteristics. The primary outcomes were SN-MP (for craniofacial morphology) and overjet (for occlusion), whereas the secondary outcomes included cephalometric variables (ANB, SNA, SNB, SN-GoGn, ArGoMe, FH-OP, ArGo, FMA, SUM, U1-SN, L1-MP, and U1-L1) and dental characteristics measured by clinical examinations (proportion of Class II and III malocclusions, arch width, overbite, open bite, crossbite, and arch length).

Language. No restrictions on language were applied for the electronic searches.

Information sources, search strategy, and study selection

Systematic searches were conducted in 4 databases including PubMed, Embase, Web of Science and VIP Chinese Journal Database from inception to November

1, 2024. The detailed strategy was initially developed for PubMed and subsequently adapted for the other 3 databases (Supplementary Table I). A supplementary manual search was also performed by examining the reference lists of eligible studies and relevant reviews.

The titles and abstracts of the retrieved records were screened and the full texts of potentially eligible studies were obtained for further evaluation by 2 reviewers (T.Z. and M.W.), independently and in duplicate. Collaborative discussions were organized with coauthors to address discrepancies. The reason for exclusion was meticulously documented for each study. In addition, we attempted to contact corresponding authors for supplementary information as required.

Data items and collection

Two authors (T.Z. and M.W.) extracted and recorded the following information independently and in duplicate: (1) general information (title, author, year, and country), (2) study characteristics (study design and sample size), (3) participant characteristics (age, sex, measurement tools, diagnostic criteria, and degree of AH and/or TH), and (4) data results (primary and secondary outcome indicators). For those graduated data that provided only the grading of AH and/or TH, we converted the graduated data into 2-category data.

Any disagreement was resolved by discussion with the other authors.

Quality assessment

The methodologic quality of the included studies was appraised using the Newcastle-Ottawa Scale of cross-sectional studies, which employs a "STAR system" concerning 3 perspectives: selection, comparability, and outcome.[25] The scale operates on a 0-10 star rating system, with higher values denoting superior quality. On the basis of the final score, the quality of studies were classified as unsatisfactory (1-4), satisfactory (5-6), good (7-8), or very good (9-10) in quality.[26] The quality assessment was independently conducted by 2 authors (T.Z. and M.W,), with any differences resolved through consultation with the other authors.

Assessment of publication bias

Funnel plots were used to check for possible publication bias in which at least 10 studies were included.[27] Publication bias was assessed by visual inspection of the funnel plot and by Egger's regression asymmetry test.

Data synthesis

RevMan (version 5.4, Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark) was used to perform the statistical analyses. A meta-analysis was performed when data of the same results from $\geq$2 studies were identified. The chi-square test and I^2 statistic were used to test the heterogeneity across studies. Considering the large variability commonly observed in relevant studies, it was determined a priori that the random-effects model be used for meta-analyses. For dichotomous outcomes, effect measures were computed as risk ratio (RR) with 95% confidence intervals (CI) values, whereas continuous outcomes were analyzed using mean difference (MD) with 95% CI values.

Subgroup and sensitivity analyses

In instances of substantial heterogeneity ($I^2 \geq 50\%$ or $P < 0.100$), we tried to explore the sources of heterogeneity with subgroup analysis or sensitivity analysis. We decided to undertake a subgroup analysis according to the type of AH and/or TH: TH, AH, and ATH.

To ensure the robustness of the results, we conducted sensitivity analyses on primary outcomes, evaluating the influence of AH and/or TH by excluding studies with a high risk of bias. Given the heterogeneity in diagnostic criteria for AH and TH, additional sensitivity analyses were conducted. For AH, only studies using endoscopy with a physician-confirmed diagnosis were included; for TH, only studies with clinical assessments by dentists or physicians were considered. The meta-analysis was performed again using these refined diagnostic criteria. If the sensitivity analysis identified specific factors significantly affecting the review's conclusions, we would explore plausible causes of uncertainty and interpret the results more carefully.

Certainty assessment

The certainty of the evidence was assessed using the grading of recommendations, assessment, development, and evaluations tool (GRADE), which considers factors that may either lower (risk of bias, inconsistency, indirectness, imprecision, and publication bias) or raise (large effect, dose response, and plausible confounding) the evidence quality.[28,29] The initial level of evidence for cross-sectional studies was classified as low. On the basis of the above assessments, certainty is ultimately designated as high, moderate, low, or very low for each main outcome. GRADEpro Guideline Development Tool was used to create summary of findings tables.

RESULTS

Study selection and characteristics

We identified 9042 records from electronic databases. After the removal of 2783 duplicates, 6259 records remained. During the screening of titles and abstracts, we removed 6177 irrelevant records. Then we retrieved and screened the full text of the remaining 80 articles based on the inclusion and exclusion criteria. Forty-two articles were excluded after screening the full text and the reasons for exclusion were described in Supplementary Table II. Finally, 36 studies (38 articles) that met our eligibility criteria were included in this review, of which 31 studies were included in the meta-analyses. The study selection process is depicted in Figure 1.

The characteristics of the included studies[7,20-22,30-63] are listed in Table I. The sample population of all the included studies was 8132, among which the AH

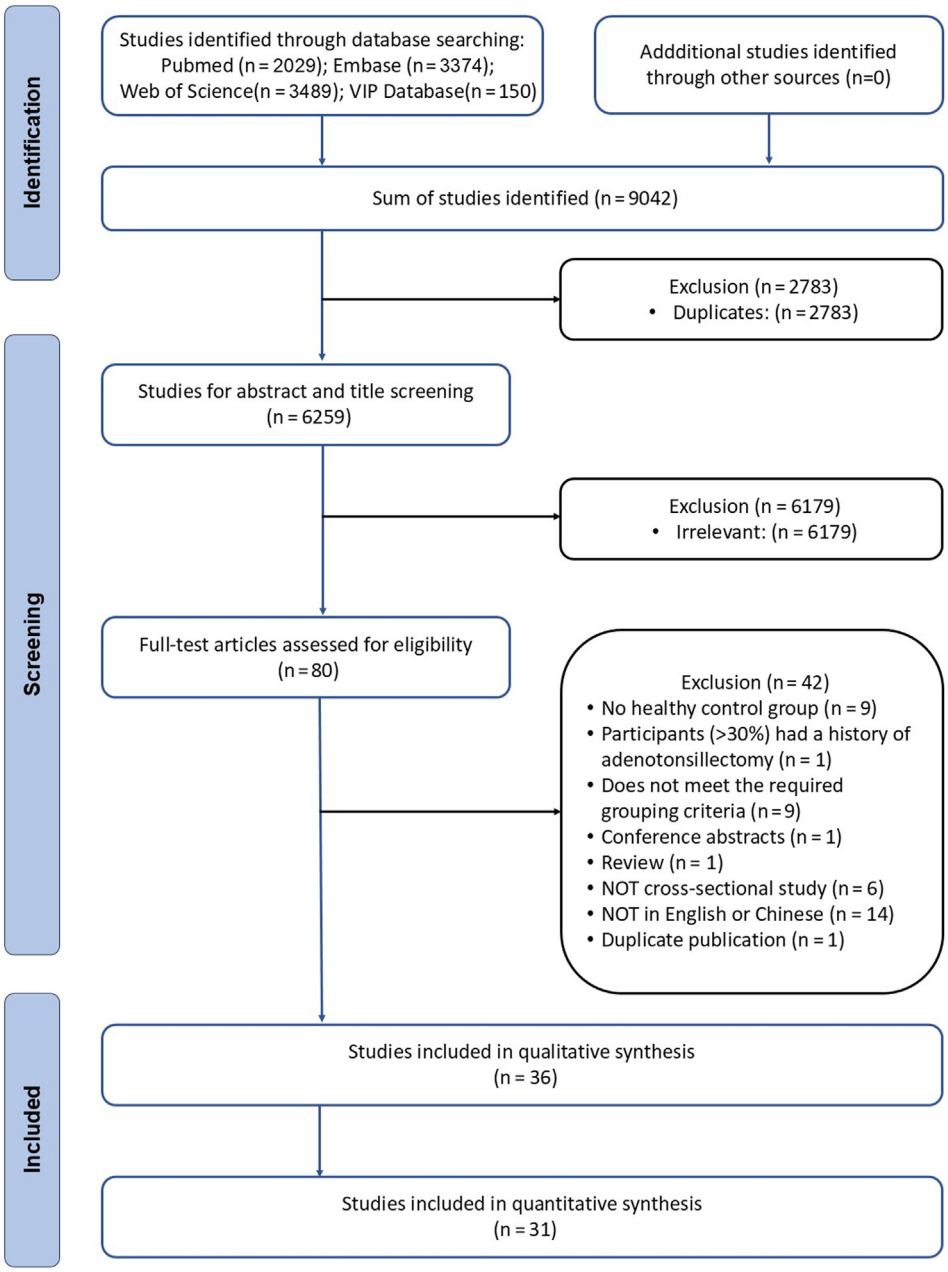


Fig 1. The flowchart illustrating the systematic search and studies selection.

Table I. Characteristics of included studies

No.	Study	Country	Sample size/gender/age (y)		Diagnostic criteria of AH	Diagnostic criteria of TH	Measurement of outcome indicators	Outcomes
			Study	Control				
1	Tarvonen and Koski[30]	FI	39/22M;17F/ 7.0-8.0	37/14M;23F/ 7.00-7.99	GII or GIII per Linder-Aronson method	–	Lateral cephalogram	U1-palatal, L-MP, U1-L1
2	Behlfelt et al[31,32]	DE	73/33M;40F/ 10.1 (mean)	73/33M;40F/ 10.1 (mean)	–	Judged by 2 otologists	Lateral skull radiographs and plaster models	Ils-NSL, Ili-ML, Ils-Ili, arch width, crossbite, ML-SL, ANB, SNA,SNB
3	Kawashima et al[33]	FI	15/10M;5F/4.67 ± 0.81	54/23M;31F/NA	–	The ratio of the tonsils to the oropharynx as measured transversally between the anterior pillars; >75% visible (GIII)	Lateral cephalogram	FH-ML, ArGoMe, U1-FH, L1-MP, U1-L1, overjet, overbite
4	Valera et al[34]	BR	44/NA/57.3 ± 11.6M	29/NA/62.4 ± 10.8M	Cohen and Konak's method	Classification of Brodsky and Koch; greater than GII	Orthodontic casts and lateral cephalometric	SN-GoGn, SNA, SNB, ANB, arch width, overjet
5	Sousa et al[20]	BR	ATH: 57/NA/NA	AH: 59/NA/NA	Cohen and Konak's method: the air column was smaller than the thickness of the soft palate; nasoendoscopy: adenoid was >50% of choana	Brodsky and Kock scale; GIII and GIV	Clinical examination and cephalometric analysis	SN-GoGn, SNB, ANB, ArGoMe, ArGo
6	Juliano et al[35]	BR	15/9M;6F/10.3 ± 1.4	12/9M;3F/9.5 ± 1.8	Nasofibroscopic evaluation according to Cassano's method: ≥75% obstruction	Nasofibroscopic evaluation: ≥75% obstruction	Lateral cephalometric	MP-HP, SNA, SNB, ANB
7	Baroni et al[21]	IT	AH: 20/NA/NA TG: 20/NA/NA ATH: 20/NA/NA	20/NA/NA	Lateral cephalogram; AS was >50% of TNAS	Lateral cephalogram; TS was >50% of TOAS	Lateral cephalogram	SN-GoGn, SNB, ANB,ArGoMe, FH-OP, ArGo,overbite, overjet
8	Zhang et al[36]	CN	30/18M;12F/ 3.0-12.0	28/17M;11F/ 3.0-12.0	Lateral cephalogram; A/N >0.71	–	Lateral cephalogram	SN-MP, SNA, SNB,ANB, ArGo
9	Diouf et al[37,38]	SN	12/NA/6.0-12.0	68/NA/6.0-12.0	Cohen and Kunak's method, the width of the air space is less than that of the velum	Brodsky and Koch	Plaster casts and lateral cephalogram	Open bite, Angle classification, SNA, SNB, ANB
10	Feres et al[39]	BR	58/NA/NA	42/NA/NA	Flexible nasofiberendoscopic examination; measured choanal obstruction ≥66.7%	–	Lateral cephalogram	SN-GoGn, SNA, SNB,ANB
11	Franco et al[40]	BR	AH: 42/NA/NA TH: 26/NA/NA ATH: 45/NA/NA	113/NA/6.4 ± 1.2	Clinical and endoscopic ENT examination; nasopharynx obstruction >80%	Clinical and endoscopic ENT examination; Brodsky and Kock scale GIII and GIV	Lateral cephalometry	SN-GoGn, SNB, ANB
12	Kim et al[41]	KR	846/524M;322F/ 5.9 ± 2.4	237/157M;80F/ 6.6 ± 2.8	Oropharyngeal endoscopy and lateral cephalometry; the ratio of the adenoid thickness to the nasopharyngeal airway width >50%	Oropharyngeal endoscopy and lateral cephalometry; Friedman staging system: GIII or GIV	Clinical examination	Angle classification

Table I. Continued

No.	Study	Country	Sample size/gender/age (y)		Diagnostic criteria of AH	Diagnostic criteria of TH	Measurement of outcome indicators	Outcomes
			Study	Control				
13	Šidlauskienė et al[43]	LI	74/NA/NA	20/NA/NA	Anterior and posterior rhinoscopy and pharyngoscopy; GII and GIII, when up to two-thirds of the choana was compromised	Anterior and posterior rhinoscopy and pharyngoscopy; GII-GIV, when there was <50% of normal space between tonsillar pillars	Study model and cephalometric radiograph	SN-MP, SNA, SNB, ANB, U1-ANS, L1-MP, overjet, overbite
14	Osiatuma et al[43]	NI	90/NA/3-12	90/NA/3-12	Diagnosed clinically and radiographically with hypertrophied adenoids	–	Dental casts	Arch width
15	Koca et al[44]	TR	12/NA/NA	175/NA/NA	Flexible fiberoptic nasopharyngolaryngoscopy	–	Photographs	g-sn, tra-anm
16	Ardehali et al[45]	IR	104/66M;38F/NA	71/NA/NA	Radiographic method of Cohen and Konak	–	Lateral cephalometric	SNA, SNB, ArGoMe
17	Osiatuma et al[22]	NG	90/55M;35F/3-12	90/38M;52F/3-12	Only reported clinically and radiographically judged	–	Dental casts	Arch width
18	Anderson et al[46]	KR	236/111M;125F/5-12	143/62M;81F/5-12	Lateral x-ray film of the neck or a flexible fiberoptic endoscopeGIII (75%-100% of hypertrophy)	Oral examination, GIII (75%-100% of hypertrophy)	Lateral cephalogram	SN-MP, SNA, SNB, ANB, ArGoMe
19	Diouf et al[47]	SN	42/NA/NA	44/NA/NA	Cohen and Konak's method and Holmberg and Linder-Aronson method	–	Dental plaster casts	Angle classification, arch width, overjet, overbite, open bite
20	Yap et al[48]	AU	10/8M;2F/11.85 ± 3.29 (range 7-14)	9/7M;2F/9.56 ± 2.14 (range 8-17)	Only reported hypertrophy	Oral examination TH ≥3 based on a standardized scale of 0-4	Extraoral photographs and dental model	Angle classification, overjet, overbite, crossbite
21	Poddębniak & Zielnik-Jurkiewicz[49]	PL	93/57M;36F/7-12	143/71M;72F/7-12	>75% in endoscopic examination	–	General dental and orthodontic examination	Open bite
22	Xu et al[50]	CN	40/NA/12-14	40/NA/12-14	A/N measured in the median sagittal plane of CBCT A/N >0.6	No obvious hypertrophy	CBCT	MP-HP, SNA, SNB, ANB, ArGoMe, ArGo, U1-SN, L1-MP
23	Perez et al[51]	CL	2/NA/6-11	33/NA/6-11	Cohen and Konak's method	Brodsky's tonsil grading scale (total of 5 grades)	Dental model	Angle classification
24	Festa et al[52]	IT	AH: 52/NA/NA TH: 22/NA/NA	CG1: 169/NA/NA CG2: 199/NA/NA	Nasal endoscopy, 4 grades, the ratio of adenoids occupying the choanal area	Oral examination, Brodsky and Koch	Dental examination	Angle classification
25	Wang et al[53]	CN	38/24M;14F/7.53 ± 2.14	35/20M;15F/7.54 ± 2.38	The electronic nasopharyngoscope, with the adenoid size occupying 50% of the posterior nostril	–	Lateral cephalogram	GoGn-SN, SNA, SNB, ANB, U1-NA, L1-NB
26	Zhang & Liu[54]	CN	52/29M;23F/8.15 ± 1.96 (range 5-12)	50/26M;24F/7.86 ± 2.04 (range 3-12)	A/N value of ≥0.71	–	Lateral cephalometric	Angle classification

Table I. Continued

No.	Study	Country	Sample size/gender/age (y)		Diagnostic criteria of AH	Diagnostic criteria of TH	Measurement of outcome indicators	Outcomes
			Study	Control				
27	Li et al[55]	CN	56/34M;22F/ 6.36 ± 1.41	42/23M;19F/ 6.76 ± 1.54	The A/N ratio of x-ray lateral cephalogram ≥0:71, and adenoid blockage of posterior nostril >51% by nasopharyngeal-fiberscope	–	Lateral cephalometric	MP-HP, SNA, SNB, ANB,ArGoMe, ArGo
28	Tong et al[56]	CN	29/NA/NA	684/NA/NA	–	Oral examinations,Friedman's method	Dental and orthodontic examination	Angle classification and lateral facial profile
29	Oku et al[57]	JP	20/7M;13M/9.38 ± 1.29	20/7M;13M/9.04 ± 1.08	–	Oral examination, tonsils extending more than three-quarters of the way to the midline	CBCT	SNA, SNB, ANB, maxillary and mandibular arch width
30	Huang et al[58]	CN	AH: 126/ 56M;70F/ 11.73 ± 2.51 TH: 59/ 21M;38F/ 12.47 ± 2.72 ATH: 69/ 33M;36F/ 11.07 ± 3.35	212/61M;151F/ 12.92 ± 2.34	Baroni's method, Ad/Np ratio of >0.5, and Tn/Op ratio of <0.5	Baroni's method, Tn/Op ratio of >0.5, and Ad/Np ratio of <0.5	Cephalometric examination	SN-MP, SNA, SNB, ANB
31	Kuskonmaz et al[59]	IT	1/NA/6-16	103/NA/6-16	–	Brodsky and Koch's classification	Dental casts	Angle classification
32	Tse et al[60]	CN	426/237M;189F/ 12	91/49M;42F/12	Fujioka's methods, At/Nd of >0.62	–	Lateral cephalometric radiograph	SN-MP, SNA, SNB, ANB
33	Lan et al[61]	CN	192/69F;123M/ 1-12	196/84F;112M/ 1-12	According to A/N >0.71 in MRI	–	Facial measurement	Facial convex angle, total facial convex angle, nasolabial angle
34	Huang et al[63]	CN	634/NA/6-15	308/NA/6-15	AS/TNAS ratio >0.5	TS/TOAS ratio >0.5	Lateral cephalogram	SN-MP, ANB, SNA, SNB, Angle classification
35	Zhao et al[7]	CN	486/250F;236M/ 6-12	112/72F;40M/6-12	Fujioka's and Baroni's method: >50%	Fujioka's and Baroni's method: >50%	Lateral cephalogram	SN-MP, ANB, SNA, SNB, ArGoMe, U1-SN, L1-MP, U1-L1
36	Eslami & Alipour[62]	IR	54/NA/5-7	66/NA/5-7	>75% of the nasopharynx in lateral neck radiography	–	Photographs	NFA, NMA, ULD, LLD

FI, Findland; *M*, male; *F*, female; *GII*, grade II; *GIII*, grade III; *DE*, Germany; *NA*, not available; *BR*, Brazil; *GIV*, grade IV; *ENT*, ear, nose, and throat; *IT*, Italy; *AS*, adenoid size; *TNAS*, total nasopharyngeal airway space; *TS*, tonsil size; *TOAS*, total oropharyngeal airway spance; *CN*, China; *A/N*, adenoids/nasapharynx; *SN*, Senegal; *KR*, South Korea; *LI*, Lithuania; *NI*, Nigeria; *TR*, Turkey; *IR*, Iran; *AU*, Australia; *PL*, Poland; *CBCT*, cone-beam computed tomography; *CL*, Chile; *JP*, Japan; *Ad*, adenoid; *Np*, nasopharynx; *Tn*, tonsil; *Op*, oropharynx; *At*, nasopharyngeal soft tissue thicknesses of adenoid; *Nd*, nasopharyngeal depth; *MRI*, magnetic resonance imaging.

and/or TH was 3854 and CG was 4278. These studies were from 16 countries: China (n = 11), Brazil (n = 5), Italy (n = 3), Senegal (n = 2), Finland (n = 2), Nigeria (n = 2), South Korea (n = 2), Iran (n = 2), Germany (n = 1), Australia (n = 1), Chile (n = 1), Japan (n = 1), Lithuania (n = 1), Poland (n = 1), and Turkey (n = 1). Lateral cephalometric analyses were performed in 21 studies, dental measurements were used in 14 studies, both photograph analysis and CBCT was used in 2 studies respectively. The samples for 2 articles written by Behlfelt et al[31,32] are the same, as well as 2 articles written by Diouf et al[37,38]; thus, these 2 articles are regarded as the same study.

Variation in the categorization of exposure factors was noted across the studies. In terms of obstruction sites, 16 studies[22,30,36,39,43-45,47,49,50,53-55,60-62] only mentioned AH but the tonsil status was unknown, whereas 5 studies[32,33,48,56,57,59] only mentioned TH but the adenoid status was unknown. In 5 studies,[34,35,41,42,46] obstruction was attributed to combined AH and TH. However, for the remaining 10 studies,[7,20,21,31,32,37,38,40,51,52,58,63] obstruction was attributed to isolated AH or isolated TH. Concerning the diagnostic criteria for ATH, a range of methods were applied. Six studies[20,34,37,38,45,47,51] used Cohen and Konak's method to determine AH, whereas 3 studies[30,31,47] employed Linder-Aronson's method for the same purpose. Meanwhile, 12 studies[20,35,39-42,44,46,49,52,53,55] used endoscopic otorhinolaryngology examination for assessing AH. In the instance of TH, 15 studies[20,33,34,37,40-42,46,48,51,52,56-59] relied on Brodsky and Koch's method for assessment, 5 studies[7,21,32,58,63] used lateral cephalogram, whereas 1 study[31] did not specify the particular method that used for assessment.

Quality assessment

The results of quality assessment of the included studies are shown in Table II. On the basis of the Newcastle-Ottawa Scale scores, the quality of the included studies ranged from unsatisfactory to very good. 9 studies were rated as unsatisfactory, 14 studies achieved a satisfactory rating, 11 studies were classified as good, 2 studies were classified as very good quality.

Results of syntheses

Craniofacial growth in the vertical dimension. Fifteen studies reported the outcomes of the SN-MP angle, among which 11 demonstrated satisfactory to very good quality ratings in the quality assessment. Meta-analysis showed the HG had a significantly increased angle of SN-MP in all subgroups (SN-MP: MD, 2.20° [95% CI, 1.47-2.92]; P <0.00001) (Fig 2; Table III). No publication bias was identified by visual assessment of the funnel plot (Supplementary Fig 1, *A*). Egger's test shows no significant publication biases among studies investigating the SN-MP (P = 0.271). After excluding 5 low-quality studies, the MD for the SN-MP angle was 1.98° (95% CI, 1.17-2.79; P <0.00001) (Supplementary Fig 2, *A*). Further exclusion of 7 studies with differing methodologic classification criteria yielded an MD of 2.86° (95% CI, 1.72-4.00; P = 0.006) (Supplementary Fig 3, *A*). The removal of these datasets did not result in statistically significant changes to the overall effect of the SN-MP, indicating the stability of the findings.

No significant difference in ramal height (ArGo) between the HG and CG based on 2 good quality and 3 unsatisfactory quality studies (P = 0.68) (Table III; Supplementary Fig 4, *A*). Eight studies reported the outcomes of ArGoMe, among which 6 demonstrated satisfactory to good quality ratings in the quality assessment. A significantly higher ArGoMe angle was found in the HG (ArGoMe: MD, 1.23° [95% CI, 0.68-1.79]; P <0.0001) (Table III; Supplementary Fig 4, *B*). The removal of these datasets from 5 low-quality studies did not result in statistically significant changes to the overall effect of the ArGo and ArGoMe (Supplementary Fig 2, *M* and *N*).

Craniofacial growth in the sagittal dimension. Fourteen studies reported on the outcomes of ANB angle, among which 10 demonstrated satisfactory to very good quality ratings in the quality assessment. The difference in ANB angle between the 2 groups was not statistically significant in any subgroup (ANB: MD, 0.31° [95% CI, −0.35 to 0.61]; P = 0.59) (Fig 3; Table III). After the exclusion of 4 low-quality studies, the overall effect remained nonsignificant. However, when further restricting the analysis to studies with consistent methodological criteria, a statistically significant higher ANB angle was found in the HG (MD, 0.91° [95% CI, 0.44-1.37]; P = 0.002) (Supplementary Figs 2, *B* and 3, *B*).

Ten studies with satisfactory or good quality reported on the SNA. Meta-analysis showed a significantly lower SNA angle in the HG (SNA: MD, −0.30° [95% CI, −0.53 to −0.06]; P = 0.01), as well as in the ATH subgroup (Table III; Supplementary Fig 4, *C*). Fifteen studies reported on the SNB angle, among which 10 demonstrated satisfactory to very good quality ratings in the quality assessment. The SNB angle showed a significant decrease in the HG compared with the CG (SNB: MD, −0.78° [95% CI, −1.33 to −0.24]; P = 0.005) as well as in the AH subgroup (Table III; Supplementary Fig 4, *D*). The funnel plots for ANB, SNA and SNB exhibited roughly symmetrical distribution (Supplementary Fig 1,

Table II. Risk of bias–Newcastle-Ottawa Scale of cross-sectional studies*

		Selection				*Comparability*	*Outcome*		
No.	*Study*	*Representativeness of the sample (1★)*	*Sample size (1★)*	*Nonrespondents (1★)*	*Ascertainment of the exposure (risk factor) (2★)*	*Based on design and analysis (2★)*	*Assessment of the outcome (2★)*	*Statistical test (1★)*	*Scores (10★)*
1	Tarvonen and Koski[30]	★	☆	★	★★	☆☆	★☆	★	6★
2	Behlfelt et al[31,32]	☆	☆	★	★★	★☆	★☆	★	6★
3	Kawashima et al[33]	☆	☆	★	★★	★☆	☆☆	★	5★
4	Valera et al[34]	☆	☆	★	★★	☆☆	☆☆	★	4★
5	Sousa et al[20]	☆	☆	★	★★	☆☆	☆☆	☆	3★
6	Juliano et al[35]	★	☆	★	★☆	★★	★★	★	8★
7	Baroni et al[21]	☆	☆	★	★★	★★	★☆	★	7★
8	Zhang et al[36]	☆	☆	★	☆☆	★★	☆☆	☆	3★
9	Diouf et al[37,38]	☆	☆	★	★★	☆☆	★☆	★	5★
10	Feres et al[39]	☆	☆	★	★★	☆☆	★★	★	6★
11	Franco et al[40]	★	★	★	★★	★★	★☆	★	9★
12	Kim et al[41]	★	☆	★	★★	★☆	☆☆	★	6★
13	Šidlauskienė et al[43]	☆	★	★	★★	☆☆	★☆	★	6★
14	Osiatuma et al[43]	☆	★	★	☆☆	☆☆	☆☆	★	3★
15	Koca et al[44]	☆	☆	★	★★	☆☆	☆☆	★	4★
16	Ardehali et al[45]	☆	☆	★	★★	☆☆	★☆	★	5★
17	Osiatuma et al[22]	☆	★	★	★★	★★	★☆	★	8★
18	Anderson et al[46]	☆	☆	★	★★	★★	★☆	★	7★
19	Diouf et al[47]	☆	☆	★	★★	☆☆	☆☆	★	4★
20	Yap et al[48]	★	☆	★	★★	★☆	★☆	★	7★
21	Poddębniak & Zielnik-Jurkiewicz[49]	★	☆	★	★★	☆☆	☆☆	★	5★
22	Xu et al[50]	☆	☆	★	★☆	☆☆	☆☆	★	3★
23	Perez et al[51]	☆	☆	★	★★	☆☆	★☆	★	5★
24	Festa et al[52]	★	☆	★	★★	☆☆	☆☆	★	5★
25	Wang et al[53]	☆	☆	★	★☆	★☆	☆☆	★	4★
26	Zhang & Liu[54]	☆	☆	★	★☆	★★	★☆	★	6★
27	Li et al[55]	☆	☆	★	★★	★★	☆☆	★	6★
28	Tong et al[56]	★	☆	★	★★	★☆	★☆	★	7★
29	Oku et al[57]	☆	★	★	★★	★☆	★☆	★	7★
30	Huang et al[58]	☆	☆	★	★★	★★	★☆	★	7★
31	Kuskonmaz et al[59]	☆	★	★	★★	★☆	★☆	★	7★
32	Tse et al[60]	★	☆	★	★★	★★	★★	★	9★
33	Lan et al[61]	★	★	☆	★★	★★	★☆	★	8★
34	Huang et al[63]	☆	☆	★	★★	★★	★☆	★	7★
35	Zhao et al[7]	☆	☆	★	★★	★☆	☆☆	★	5★
36	Eslami & Alipour[62]	☆	☆	☆	★☆	★☆	☆☆	★	3★

*The shaded stars indicate the score for that item, while the unshaded stars represent items that did not receive a score.

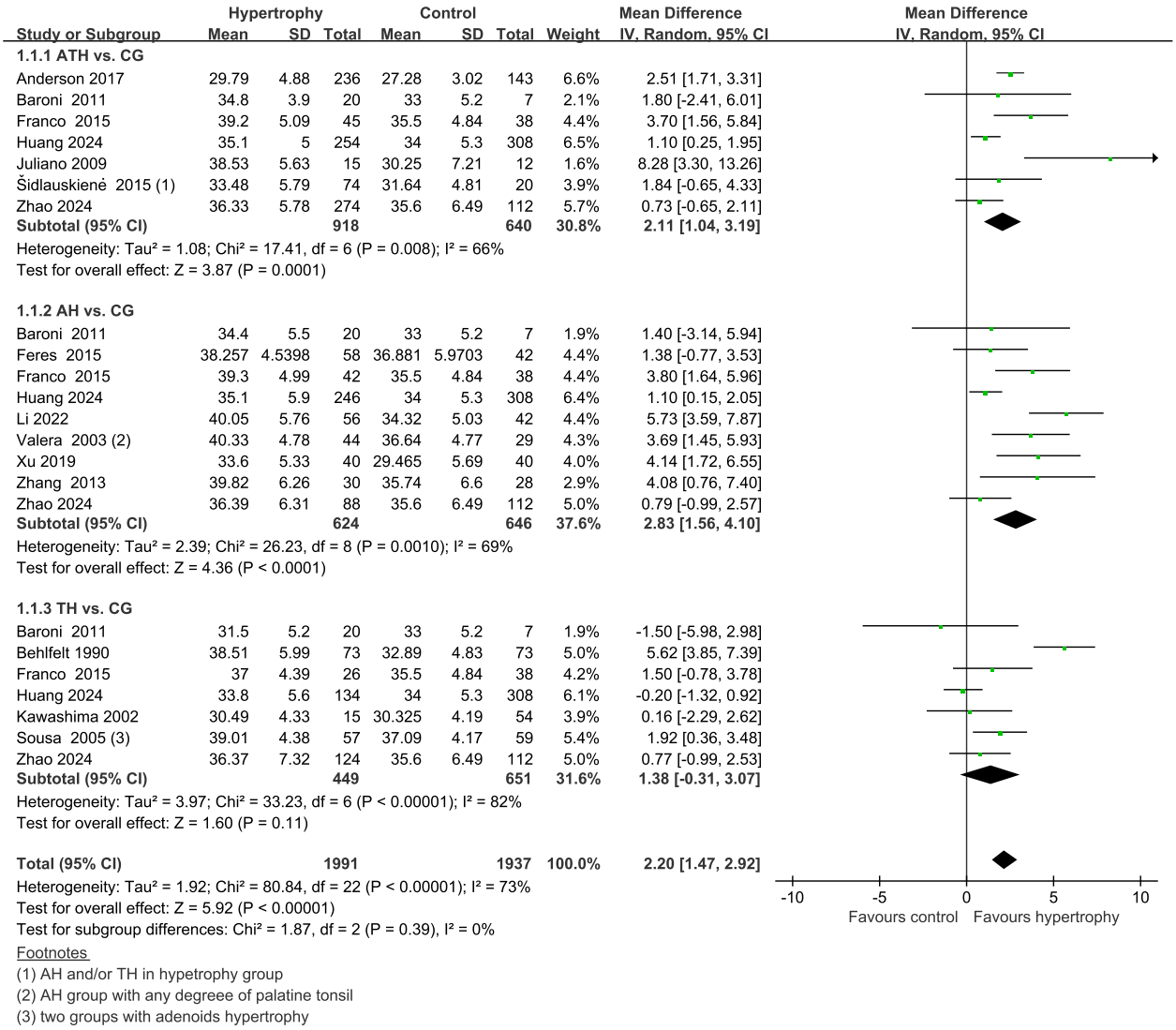


Fig 2. MDs in craniofacial vertical outcomes in children with and without ATH: SN-MP angle.

B-D) Egger's test shows no significant publication biases among studies investigating the ANB ($P = 0.529$). Sensitivity analysis confirmed the robustness of these results, as the exclusion of lower-quality studies did not substantially alter the effect estimates (Supplementary Fig 2, *K* and *L*).

Craniofacial growth in the transverse dimension. Five studies evaluated the maxillary arch width by measuring the intermolar distance between the maxillary left and right first molars. Among these, 2 studies were rated as good quality, 1 as satisfactory, and 2 as unsatisfactory. The result showed a significantly smaller maxillary arch width in the HG compared with the CG (intermaxillary first molar width: MD, −1.34 mm [95% CI, −2.12 to −0.56]; $P = 0.0008$) (Fig 4, *A*; Table III). The exclusion of lower-quality studies did not substantially alter the effect estimates (Supplementary Fig 2, *C*). After the exclusion of studies with inconsistent methodologic classification criteria, only 2 studies exploring the transverse difference remained eligible for analysis. The pooled results from the 2 studies demonstrated no statistically significant difference in transverse dimension development between children with ATH and non-ATH children (Supplementary Fig 3, *C*).

Four studies evaluated the mandibular arch width, measured as the intermolar distance between the mandibular left and right first molars. Of these studies, 2 were rated as good quality: 1 as satisfactory and 1 as

Table III. Pooled differences in continuous variables of craniofacial and dental outcomes in children with and without ATH

					Heterogeneity	
Group	*No. of studies*	*No. of children*	*MD (95% CI)*	P *value*	P *value*	*I (%)*
SN-MP						
ATH/CG	7	918/640	2.11 (1.04-3.19)	0.0001	0.008	66
AH/CG	9	624/646	2.83 (1.56-4.10)	<0.0001	0.001	69
TH/CG	7	449/651	1.38 (−0.31 to 3.07)	0.11	<0.00001	82
HG/CG	15	1991/1937	2.20 (1.47-2.92)	<0.00001	<0.00001	73
SNA						
ATH/CG	6	873/602	−0.48 (−0.86 to −0.11)	0.01	0.36	9
AH/CG	9	686/679	−0.31 (−0.71 to 0.10)	0.14	0.87	0
TH/CG	4	298/447	0.22 (−0.33 to 0.77)	0.43	0.45	0
HG/CG	13	1857/1728	−0.30 (−0.53 to −0.06)	0.01	0.54	0
SNB						
ATH/CG	7	918/640	−0.51 (−1.20 to 0.18)	0.15	0.02	61
AH/CG	10	728/717	−1.63 (−2.39 to −0.87)	<0.0001	0.006	61
TH/CG	6	381/544	0.42 (−0.75 to 1.59)	0.48	0.0009	76
HG/CG	15	2027/1901	−0.78 (−1.33 to −0.24)	0.005	<0.00001	76
ANB						
ATH/CG	7	918/640	0.27 (−0.58 to 1.12)	0.53	<0.00001	88
AH/CG	9	624/645	0.40 (−0.13,0.94)	0.14	0.009	61
TH/CG	6	397/543	−0.50 (−1.77,0.78)	0.45	<0.00001	90
HG/CG	14	1939/1828	0.13 (−0.35,0.61)	0.59	<0.00001	85
ArGoMe						
ATH/CG	3	530/262	1.43 (0.68-2.18)	0.0002	0.92	0
AH/CG	5	308/272	1.00 (−0.25 to 2.25)	0.12	0.87	0
TH/CG	4	216/232	0.99 (−0.12 to 2.09)	0.08	0.56	0
HG/CG	8	1054/766	1.23 (0.68-1.79)	<0.0001	0.97	0
ArGo						
ATH/CG	1	20/7	0.60 (−2.28 to 3.48)	0.68	–	–
AH/CG	4	146/117	−1.04 (−2.39 to 0.32)	0.13	0.40	0
TH/CG	2	243/190	1.52 (0.17-2.87)	0.03	0.63	0
HG/CG	5	243/190	0.27 (−1.01 to 1.55)	0.68	0.12	40
U1-SN						
ATH/CG	2	348/132	−0.06 (−1.87. 1.75)	0.95	0.50	0
AH/CG	3	167/189	−0.32 (−3.63 to 3.00)	0.85	0.02	76
TH/CG	3	201/226	−1.77 (−4.18 to 0.64)	0.15	0.08	60
HG/CG	6	716/547	−0.73 (−2.20 to 0.75)	0.33	0.02	57
L1-MP						
ATH/CG	2	348/132	−0.27 (−1.41 to 1.95)	0.75	0.67	0
AH/CG	3	167/189	−0.38 (−2.76 to 2.00)	0.76	0.11	55
TH/CG	3	207/231	−8.31 (−16.20 to −0.42)	0.04	<0.00001	97
HG/CG	6	722/552	−3.27 (−6.62 to 0.07)	0.06	<0.00001	93
U1-L1						
ATH/CG	1	274/112	−0.53 (−3.27 to 2.21)	0.70	–	–
AH/CG	2	127/149	1.20 (−9.16 to 11.56)	0.82	0.001	91
TH/CG	3	201/226	3.11 (−0.55 to 6.78)	0.10	0.08	61
HG/CG	4	602/487	1.72 (−1.33 to 4.78)	0.27	0.001	75
Overjet						
ATH/CG	2	94/27	−0.20 (−2.28,1.87)	0.85	0.18	44
AH/CG	3	106/80	−0.28 (−2.49,1.94)	0.80	0.008	79
TH/CG	3	45/70	−0.15 (−1.75,1.45)	0.85	0.02	74
HG/CG	6	245/177	−0.13 (−1.02 to 0.77)	0.78	0.008	63
Overbite						
ATH/CG	2	94/27	0.16 (−1.50 to 1.82)	0.85	0.17	46
AH/CG	2	62/51	1.43 (−1.48 to 4.34)	0.34	0.03	78
TH/CG	3	45/70	0.05 (−0.72 to 0.83)	0.89	0.69	0
HG/CG	4	201/148	0.44 (−0.42 to 1.30)	0.32	0.04	55

Table III. Continued

Group	No. of studies	No. of children	MD (95% CI)	P value	Heterogeneity P value	Heterogeneity I (%)
Intermaxillary first molar width						
AH/CG	3	176/163	−0.74 (−1.50 to 0.01)	0.05	0.50	0
TH/CG	2	87/85	−2.07 (−2.82 to −1.32)	<0.00001	0.32	0
HG/CG	5	263/248	−1.34 (−2.12 to −0.56)	0.0008	0.08	52
Intermandibular first molar width						
AH/CG	2	132/134	0.13 (−0.94 to 1.20)	0.81	0.27	18
TH/CG	2	87/86	0.58 (−0.17 to 1.34)	0.13	0.52	0
HG/CG	4	219/220	0.41 (−0.19 to 1.00)	0.18	0.54	0

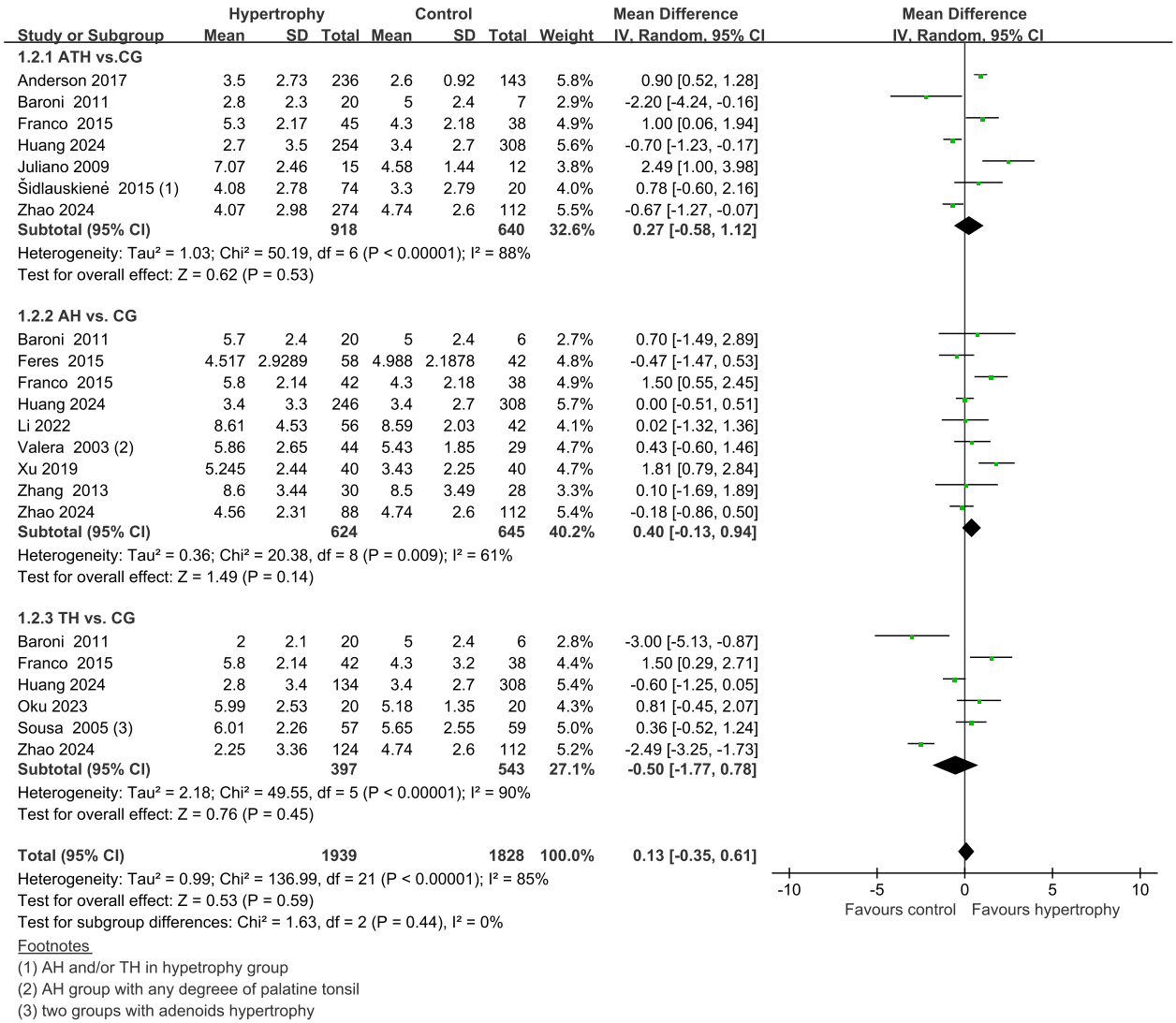


Fig 3. MDs in craniofacial sagittal outcomes in children with and without ATH: ANB angle.

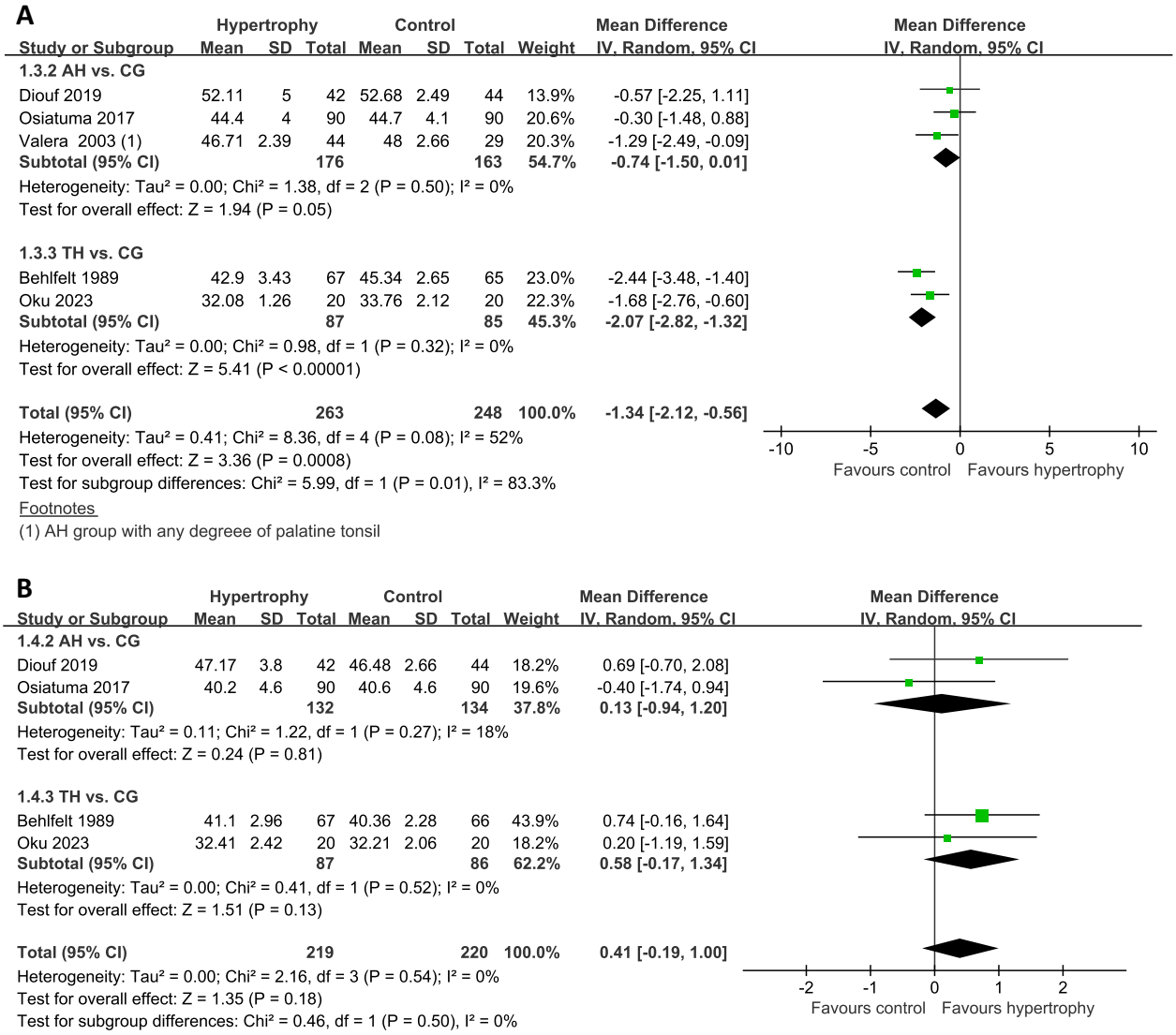


Fig 4. MDs in craniofacial transverse outcomes in children with and without ATH: **A,** Maxillary arch width; **B,** Mandibular arch width.

unsatisfactory. Meta-analysis revealed no significant differences between the HG and CG (MD, 0.41 mm [95% CI, −0.91 to 1.00]; $P = 0.18$) (Fig 4, *B*; Table III). Sensitivity analysis confirmed the robustness of these results (Supplementary Figs 2, *D* and 3, *D*).

Dental characteristics. Eleven studies reported on the Angle classification, among which 9 demonstrated satisfactory to good quality ratings in the quality assessment. Meta-analysis showed a significantly increased rate of Angle Class II and III malocclusions in the HG (RR, 1.29 [95% CI, 1.14-1.45]; $P < 0.0001$) (Table IV; Supplementary Fig 5, *A*). No publication bias was identified by visual assessment of the funnel plot (Supplementary Fig 1, *E*). Egger's test shows no significant publication biases among studies investigating the Angle classification ($P = 0.817$). The exclusion of lower-quality studies did not substantially alter the effect estimates (Supplementary Fig 2, *E*). However, when further restricting the analysis to studies with consistent methodologic criteria, no significant difference was found between the 2 groups in the rate of Angle Class II and III malocclusions ($P = 0.08$) (Supplementary Fig 3, *E*). Two studies evaluated crossbite and meta-analysis showed a significantly higher RR of crossbite in the TH

Table IV. Pooled differences in categorical variables of craniofacial and dental outcomes in children with and without ATH

					Heterogeneity	
Group	*No. of studies*	*No. of children*	*MD (95% CI)*	P *value*	P *value*	I^2 *(%)*
Angle Class						
ATH/CG	2	1100/545	1.54 (1.22-1.95)	0.0003	0.10	63
AH/CG	5	482/661	1.21 (0.98-1.48)	0.07	0.02	66
TH/CG	7	219/1408	1.22 (1.09-1.36)	0.0004	0.42	0
HG/CG	11	1801/2614	1.29 (1.14-1.45)	<0.0001	0.001	61
Crossbite						
TH/CG	2	82/82	6.31 (2.83-14.09)	<0.000001	0.72	0
HG/CG	2	82/82	6.31 (2.83-14.09)	<0.000001	0.72	0
Open bite						
AH/CG	3	227/277	1.55 (1.12-2.16)	0.009	0.008	80
TH/CG	1	12/68	3.15 (1.27-7.78)	0.01	–	–
HG/CG	4	239/345	1.65 (1.21-2.25)	0.001	0.008	74

group (RR, 6.31 [95% CI, [2.83-14.09]; P <0.00001) (Table IV; Supplementary Fig 5, *B*). However, it is important to note that the findings on posterior crossbite were derived from only 2 studies, both of which were graded as providing "very low certainty" evidence. After excluding studies with inconsistent methodologic classification criteria, only 1 study investigating crossbite remained, and it showed no statistically significant difference between groups (Supplementary Fig 3, *I*).

Five studies reported on the overbite and 6 studies reported on the overjet. Meta-analysis showed that anterior overbite and overjet showed no significant differences between the HG and CG (P >0.05) (Table III; Supplementary Fig 5, *C* and *D*). Four studies reported open bite and meta-analysis showed a significantly higher RR of open bite in the HG (RR, 1.65 [95% CI, 1.21-2.25]; P = 0.001) (Table IV; Supplementary Fig 5, *E*). After the exclusion of 4 low-quality studies and further restricting the analysis to studies with consistent methodologic criteria, the overall effect remained no change (Supplementary Figs 2, *F-H* and 3, *F-H*).

Six studies evaluated maxillary anterior teeth inclination (U1-SN angle), with 5 demonstrating satisfactory quality and 1 rated as unsatisfactory. Similarly, 4 studies examined mandibular anterior teeth inclination (L1-MP angle), of which 3 were satisfactory quality and 1 unsatisfactory. Meta-analysis showed no significant intergroup differences for either the U1-SN angle (P = 0.33) or L1-MP angle (P = 0.06). After the exclusion of low-quality studies, the overall effect remained nonsignificant (Supplementary Figs 2, *I* and *J* and 3, *J* and *K*). In addition, 4 satisfactory-quality studies assessing the U1-L1 angle found no significant differences between groups (P = 0.27). However, these studies employed varying diagnostic criteria for ATH, warranting cautious interpretation of the results (Supplementary Fig 3, *L*).

Certainty of evidence. The GRADE tool was used to assess the quality of each outcome indicator, revealing that 16 outcome indicators were graded as very low. A summary of these findings is shown in Supplementary Table III.

DISCUSSION

This systematic review substantiates that the children with AH and/or TH may exhibit different dental and craniofacial morphologic traits compared with children without hypertrophy. However, the statistically significant differences identified in this review are relatively small when compared with the corresponding population deviations, indicating a potential lack of clinical significance. Further research is needed to determine whether these subtle morphologic changes have meaningful implications for clinical decision-making.

ATH is a major cause of upper airway obstruction and the resultant mouth breathing or obstructive sleep apnea in children.[64] The association between ATH and dentofacial morphology has been focused by orthodontists for a period of time. The earliest article included in this systematic review was published in 1987. Of the 36 studies included, only 1 study reported no differences in the dentofacial features of the HG and CG. The other 35 studies showed some differences in skeletal or dental features between children with or without AH and/or TH.

For craniofacial growth in the vertical dimension, this systematic review found that the children with AH and/or TH have a higher mandibular plane angle and ArGoMe angle than the CG. In addition, subgroup analysis showed that for children with both AH and TH or isolated AH, they have higher mandibular plane angle, whereas for children with isolated TH, there is no significant difference with the CG. AH that obstructs the

nasopharynx forced patients to have chronic mouth-breathing habit, downward and backward rotation of the mandible, resulting in an increase vertical growth. This is also a typical characteristic of an "adenoid facies." Linder-Aronson et al[65,66] hypothesized that the reestablishment of nasal respiration in children is an important factor in determining the mandibular growth direction. In addition, several studies found that treatment of hypertrophic adenoids affects dentofacial deformity, showing the normalization toward a more horizontal mandibular growth pattern after adentonsillecotomy.[67]

For craniofacial growth in the sagittal dimension, the children with AH and/or TH have a decreased SNA angle and SNB angle. These findings suggest that ATH may constrain sagittal growth of maxilla and mandible in children. On the basis of Moss's theory of functional matrix, craniofacial development is the result of both genetic and functional factors, and the growth of the craniofacial occurs as a response to functional needs.[68] Our results may confirm Moss's theory that the major determinant of growth of the maxilla and mandible is the enlargement of the nasal and oral cavities, which grow in response to functional needs. During growth and development, nasal breathing pattern can promote normal dentofacial growth of children. Absence of normal function would have wide-ranging effects.

Regarding craniofacial growth in the transverse dimension, our analysis initially revealed that children with AH and/or TH exhibited a smaller maxillary arch width compared with controls. However, no significant difference was observed when specifically comparing the AH group with the CG. After the exclusion of studies with inconsistent methodologic classification criteria, only 2 studies remained eligible for analysis. The pooled results from these studies demonstrated no statistically significant difference in transverse dimension development between children with ATH and non-ATH children. This finding suggests that the current evidence regarding the relationship between ATH and transverse craniofacial development is insufficient and inconclusive. Furthermore, the available literature lacks orthodontic studies focusing on skeletal width measurements, highlighting a significant gap in our understanding of this aspect of craniofacial growth.

Regarding dental characteristics, our initial analysis suggested that the HG exhibited an increased prevalence of Angle Class II and III malocclusions, crossbite, and open bite. However, it is important to note that the findings on posterior crossbite were derived from only 2 studies, both of which were graded as providing "very low certainty" evidence. After excluding studies with inconsistent methodologic classification criteria, only 1 study investigating crossbite remained, and it showed no statistically significant difference between groups. Similarly, only 2 studies examining maxillary anterior teeth inclination met the inclusion criteria, and they also demonstrated no statistically significant difference. Given the limited number of studies and the very low certainty of the evidence, we have very little confidence in these effect estimates. It is highly likely that the true effect differs substantially from the estimated effect reported in these studies. These findings underscore the need for more rigorous, high-quality studies to draw definitive conclusions about the dental characteristics associated with ATH.

ATH is one of the most common reasons for upper airway obstruction and resultant mouth breathing in children.[69] When the ATH reach a certain severity, it obstructs normal nasal breathing, forcing children to adopt chronic mouth breathing. With a chronic mouth-breathing habit, the mandibular posture will change and the latter will lead to an imbalance of buccolingual muscle. Children with ATH related mouth breathing often develop a low tongue posture to facilitate airflow through the mouth. In this posture, the palatal vault will become deeper and narrower without enough supporting from the tongue.[37] Petraccone Caixeta et al[70] also reported that after adenotonsillectomy, children who have mouth breathing showed greater maxillary transverse development than the untreated control subjects. There are 5 studies[7,21,40,58,63] in this systematic review that clearly distinguish whether the upper airway obstruction was caused by patient's adenoids or tonsils and compare the differences in maxillofacial morphology caused by the two. AH may result in downward position of the tongue and the mandible, which leads to a retrognathic mandible and steep mandible plane angle. In contrast, tonsil obstruction appears to be associated with tongue protrusion, which may contribute to a more anterior position of the mandible. However, it is still worth noting that adenoid and TH are mostly occur simultaneously, and it is difficult to distinguish which dominates in most patients.

The evaluation of adenoid or tonsil was mostly based on lateral cephalogram, endoscopic examination or clinical examination. Most of the included studies used the methods of Brodsky and Koch[71] or Cohen and Konak,[72] whereas some did not specify diagnostic method for ATH. In addition, the diagnostic criteria are also different among studies even with the same method. Differences in the diagnostic methods or criteria reduce comparability between studies. Therefore, a unified standard for the diagnosis of AH and TH should be developed in the future, and research design should be carried out according to this standard. In addition, the evaluation of adenoid or tonsil are based on a given moment in all

the included studies, which could not reflect the airway condition of the whole dentofacial growth period. Upper airway assessment based on a past period time would be more meaning than immediate evaluation when exploring the association between upper airway obstruction and dentofacial development.

Strengths and limitations

One of the key strengths of this study is that it represents the first systematic review to specifically investigate the correlation between ATH and dentofacial characteristics in children. The findings of this systematic review and meta-analysis provide valuable insights into the influence of adenoids and tonsils on dentofacial development. Notably, the meta-analysis successfully differentiated between the effects of AH and TH on upper airway obstruction, offering a more nuanced understanding of their respective roles.

Despite these strengths, several limitations should be acknowledged. First, the number of studies available for each outcome was relatively small, primarily because of the heterogeneity in dentofacial measurement protocols across studies. Second, the lack of uniform diagnostic criteria for ATH limits the comparability of findings across studies. Third, a significant proportion of the included studies were found to have a substantial risk of bias, which may affect the overall reliability of the conclusion.

However, it is important to note that the observed heterogeneity reflects the real-world diversity in study populations and methodologies. Despite these challenges, this study provides a comprehensive synthesis of existing evidence, highlights knowledge gaps, and underscores the need for standardized approaches in future research. To address heterogeneity, we employed a random-effects model, complemented by rigorous subgroup and sensitivity analyses, which helped mitigate its impact and improve the interpretability of the pooled results.

CONCLUSIONS

This systematic review reveals that children with ATH are more likely to exhibit sagittal retrognathia in both the maxilla and mandible, as well as an increased mandibular plane angle. Compared with non-ATH children, those with ATH also demonstrated a higher incidence of Class II and III malocclusions, open bite, reduced maxillary incisor inclination, and a narrower maxillary dental arch width. However, these findings need to be viewed with caution because the body of evidence was of very low certainty and that the statistically significant differences identified in this review are relatively small when compared with the corresponding population deviations, indicating a potential lack of clinical significance. The dental and craniofacial features of children with ATH require more high-quality evidence to confirm, and the causal relationship between these features and children's ATH warrants further investigation.

AUTHOR CREDIT STATEMENT

Tingting Zhao contributed to conceptualization, data curation, methodology, and original draft preparation; Min Wang contributed to data curation, methodology, and original draft preparation; Peter Ngan contributed to conceptualization and manuscript review and editing; Zhendong Tao contributed to data curation, methodology, and original draft preparation; Xueqian Yu contributed to data curation, formal analysis, investigation, and methodology; Fang Hua contributed to conceptualization, data curation, formal analysis, funding acquisition, and manuscript review and editing; and Hong He contributed to conceptualization, data curation, formal analysis, funding acquisition, and manuscript review and editing.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Hospital Administration, National Health Commission of China (No. YLZLXZ24G036), the International Orthodontics Foundation (No. IOF2022C01), the Sanming Project of Medicine in Shenzhen Nanshan (No. SZSM202103005), and the Wuhan University School and Hospital of Stomatology Clinical Research Project (No. LYZX202101).

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ajodo.2025.04.024.

REFERENCES

1. Niedzielski A, Chmielik LP, Mielnik-Niedzielska G, Kasprzyk A, Bogusławska J. Adenoid hypertrophy in children: a narrative review of pathogenesis and clinical relevance. BMJ Paediatr Open 2023;7:e001710.
2. Manabe A, Ishida T, Yoon HS, Yang SS, Kanda E, Ono T. Differential changes in the adenoids and tonsils in Japanese children and teenagers: a cross-sectional study. Sci Rep 2017;7:9734.
3. Butler GE, McKie M, Ratcliffe SG. The cyclical nature of prepubertal growth. Ann Hum Biol 1990;17:177-98.
4. Ishida T, Manabe A, Yang SS, Yoon HS, Kanda E, Ono T. Patterns of adenoid and tonsil growth in Japanese children and adolescents: a longitudinal study. Sci Rep 2018;8:17088.

5. Pereira L, Monyror J, Almeida FT, Almeida FR, Guerra E, Flores-Mir C, et al. Prevalence of adenoid hypertrophy: a systematic review and meta-analysis. Sleep Med Rev 2018;38:101-12.
6. Kara CO, Ergin H, Koçak G, Kiliç I, Yurdakul M. Prevalence of tonsillar hypertrophy and associated oropharyngeal symptoms in primary school children in Denizli, Turkey. Int J Pediatr Otorhinolaryngol 2002;66:175-9.
7. Zhao T, Yang Z, Ngan P, Luo P, Zhang J, Hua F, et al. Association between adenotonsillar hypertrophy and dentofacial characteristics of children seeking for orthodontic treatment: a cross-sectional study. J Stomatol Oral Maxillofac Surg 2024;125:101751.
8. Zhao TT, Wang M, Yang Z, Zhang J, Hua F, He H. Percentage of tonsil hypertrophy in orthodontic patients with different sagittal skeletal relationship. Zhonghua Kou Qiang Yi Xue Za Zhi 2022;57:266-71.
9. Cho JH, Lee DH, Lee NS, Won YS, Yoon HR, Suh BD. Size assessment of adenoid and nasopharyngeal airway by acoustic rhinometry in children. J Laryngol Otol 1999;113:899-905.
10. Brodsky L. Modern assessment of tonsils and adenoids. Pediatr Clin North Am 1989;36:1551-69.
11. Major MP, Flores-Mir C, Major PW. Assessment of lateral cephalometric diagnosis of adenoid hypertrophy and posterior upper airway obstruction: a systematic review. Am J Orthod Dentofacial Orthop 2006;130:700-8.
12. Marcus CL, Brooks LJ, Ward SD, Draper KA, Gozal D, Halbower AC, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics 2012;130:e714-55.
13. Tatlıpınar A, Biteker M, Meriç K, Bayraktar Gİ, Tekkeşin Aİ, Gökçeer T. Adenotonsillar hypertrophy: correlation between obstruction types and cardiopulmonary complications. Laryngoscope 2012;122:676-80.
14. Stewart MG, Friedman EM, Sulek M, Hulka GF, Kuppersmith RB, Harrill WC, et al. Quality of life and health status in pediatric tonsil and adenoid disease. Arch Otolaryngol Head Neck Surg 2000;126:45-8.
15. Gomaa MA, Mohammed HM, Abdalla AA, Nasr DM. Effect of adenoid hypertrophy on the voice and laryngeal mucosa in children. Int J Pediatr Otorhinolaryngol 2013;77:1936-9.
16. İnönü-Sakallı N, Sakallı C, Tosun Ö, Akşit-Bıçak D. Comparative evaluation of the effects of adenotonsillar hypertrophy on oral health in children. BioMed Res Int 2021;2021:5550267.
17. Niedzielski A, Chmielik LP, Kasprzyk A, Stankiewicz T, Mielnik-Niedzielska G. Health-related quality of life assessed in children with adenoid hypertrophy. Int J Environ Res Public Health 2021;18:8935.
18. Galić MZ, Klančnik M. Adenoid size in children with otitis media with effusion. Acta Clin Croat 2022;60:532-9.
19. Macari AT, Haddad RV. The case for environmental etiology of malocclusion in modern civilizations—airway morphology and facial growth. Semin Orthod 2016;22:223-33.
20. Sousa JBR, Anselmo-Lima WT, Valera FCP, Gallego AJ, Matsumoto MAN. Cephalometric assessment of the mandibular growth pattern in mouth-breathing children. Int J Pediatr Otorhinolaryngol 2005;69:311-7.
21. Baroni M, Ballanti F, Franchi L, Cozza P. Craniofacial features of subjects with adenoid, tonsillar, or adenotonsillar hypertrophy. Prog Orthod 2011;12:38-44.
22. Osiatuma VI, Otuyemi OD, Kolawole KA, Amusa YB, Ogunbanjo BO. Dental arch dimensions of Nigerian children with hypertrophied adenoids. Turk J Orthod 2017;30:42-9.
23. Hultcrantz E, Larson M, Hellquist R, Ahlquist-Rastad J, Svanholm H, Jakobsson OP. The influence of tonsillar obstruction and tonsillectomy on facial growth and dental arch morphology. Int J Pediatr Otorhinolaryngol 1991;22:125-34.
24. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ 2021;372:n160.
25. Herzog R, Álvarez-Pasquin MJ, Díaz C, Del Barrio JL, Estrada JM, Gil Á. Are healthcare workers' intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. BMC Public Health 2013;13:154.
26. Aldhamin RA, Al Saif AZ. The mental health of healthcare workers in GCC countries during the COVID-19 pandemic: a systematic review and meta-analysis. J Taibah Univ Med Sci 2023;18:45-60.
27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
28. Foroutan F, Guyatt G, Zuk V, Vandvik PO, Alba AC, Mustafa R, et al. GRADE Guidelines 28: use of GRADE for the assessment of evidence about prognostic factors: rating certainty in identification of groups of patients with different absolute risks. J Clin Epidemiol 2020;121:62-70.
29. Iorio A, Spencer FA, Falavigna M, Alba C, Lang E, Burnand B, et al. Use of GRADE for assessment of evidence about prognosis: rating confidence in estimates of event rates in broad categories of patients. BMJ 2015;350:h870.
30. Tarvonen PL, Koski K. Craniofacial skeleton of 7-year-old children with enlarged adenoids. Am J Orthod Dentofacial Orthop 1987;91:300-4.
31. Behlfelt K, Linder-Aronson S, McWilliam J, Neander P, Laage-Hellman J. Dentition in children with enlarged tonsils compared to control children. Eur J Orthod 1989;11:416-29.
32. Behlfelt K, Linder-Aronson S, McWilliam J, Neander P, Laage-Hellman J. Cranio-facial morphology in children with and without enlarged tonsils. Eur J Orthod 1990;12:233-43.
33. Kawashima S, Peltomäki T, Sakata H, Mori K, Happonen RP, Rönning O. Craniofacial morphology in preschool children with sleep-related breathing disorder and hypertrophy of tonsils. Acta Paediatr 2002;91:71-7.
34. Valera FCP, Travitzki LVV, Mattar SEM, Matsumoto MAN, Elias AM, Anselmo-Lima WT. Muscular, functional and orthodontic changes in pre school children with enlarged adenoids and tonsils. Int J Pediatr Otorhinolaryngol 2003;67:761-70.
35. Juliano ML, Machado MAC, de Carvalho LBC, Zancanella E, Santos GMS, do Prado LBF, et al. Polysomnographic findings are associated with cephalometric measurements in mouth-breathing children. J Clin Sleep Med 2009;5:554-61.
36. Zhang H. Adenoidal hypertrophy and the mandibular growth pattern in children. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2013;27:955-8.
37. Diouf JS, Ngom PI, Sonko O, Diop-Bâ K, Badiane A, Diagne F. Influence of tonsillar grade on the dental arch measurements. Am J Orthod Dentofacial Orthop 2015;147:214-20.
38. Diouf JS, Ngom PI, Fadiga MS, Badiane A, Diop-Ba K, Sonko O, et al. Influence of tonsil size on sagittal cephalometric measurements. Int Orthod 2015;13:149-63.
39. Feres MFN, Muniz TS, de Andrade SH, Lemos M, Pignatari SSN. Craniofacial skeletal pattern: is it really correlated with the degree of adenoid obstruction? Dent Press J Orthod 2015;20:68-75.
40. Franco LP, Souki BQ, Cheib PL, Abrão M, Pereira TBJ, Becker HMG, et al. Are distinct etiologies of upper airway obstruction in mouth-

breathing children associated with different cephalometric patterns? Int J Pediatr Otorhinolaryngol 2015;79:223-8.
41. Kim DK, Rhee CS, Yun PY, Kim JW. Adenotonsillar hypertrophy as a risk factor of dentofacial abnormality in Korean children. Eur Arch Otorhinolaryngol 2015;272:3311-6.
42. Šidlauskienė M, Smailienė D, Lopatienė K, Čekanauskas E, Pribuišienė R, Šidlauskas M. Relationships between malocclusion, body posture, and nasopharyngeal pathology in pre-orthodontic children. Med Sci Monit 2015;21:1765-73.
43. Osiatuma VI, Otuyemi OD, Kolawole KA, Ogunbanjo BO, Amusa YB. Occlusal characteristics of children with hypertrophied adenoids in Nigeria. Int Orthod 2015;13:26-42.
44. Koca CF, Erdem T, Bayındır T. The effect of adenoid hypertrophy on maxillofacial development: an objective photographic analysis. J Otolaryngol Head Neck Surg 2016;45:48.
45. Ardehali MM, Zarch VV, Joibari M-E, Kouhi A. Cephalometric assessment of upper airway effects on craniofacial morphology. J Craniofac Surg 2016;27:361-4.
46. Anderson SM, Lim HJ, Kim KB, Kim SW, Kim SJ. Clustering of craniofacial patterns in Korean children with snoring. Korean J Orthod 2017;47:248-55.
47. Diouf JS, Ouédraogo Y, Souaré N, Badiane A, Diop-Bâ K, Ngom PI, et al. Comparison of dental arch measurements according to the grade and the obstructive character of adenoids. Int Orthod 2019;17:333-41.
48. Yap B, Kontos A, Pamula Y, Martin J, Kennedy D, Sampson W, et al. Differences in dentofacial morphology in children with sleep disordered breathing are detected with routine orthodontic records. Sleep Med 2019;55:109-14.
49. Poddębniak J, Zielnik-Jurkiewicz B. Impact of adenoid hypertrophy on the open bite in children. Otolaryngol Pol 2019;73:8-13.
50. Xu YF, Xu YL, Li ZH, Lei YH. Correlation between adenoid hypertrophy, maxillary sinus mucosal thickening and dentofacial development in children. Shanghai Kou Qiang Yi Xue 2019;28:529-35.
51. Perez I, Alves N, Lizana C, Deana NF. Influence of the palatine tonsil grade on the morphology of the maxillary and mandibular dental arches. Int J Morphol 2020;38:1201-7.
52. Festa P, Mansi N, Varricchio AM, Savoia F, Calì C, Marraudino C, et al. Association between upper airway obstruction and malocclusion in mouth-breathing children. Acta Otorhinolaryngol Ital 2021;41:436-42.
53. Wang H, Qiao X, Qi S, Zhang X, Li S. Effect of adenoid hypertrophy on the upper airway and craniomaxillofacial region. Transl Pediatr 2021;10:2563-72.
54. Zhang L, Liu H. Influence of adenoid hypertrophy on malocclusion and maxillofacial development in children. Evid Based Complement Alternat Med 2022;2022:2052359.
55. Li H, Wang H, Hao H, An H, Geng H. Influences of airway obstruction caused by adenoid hypertrophy on growth and development of craniomaxillofacial structure and respiratory function in children. Comput Math Methods Med 2022;2022:5096406.
56. Tong X, Li Y, Yang G, Zhang H, Jiang Y, Yu J, et al. The association of tonsil hypertrophy with pediatric dentofacial development: evidence from a cross-sectional study of young children in Shanghai, China. Nat Sci Sleep 2022;14:1867-75.
57. Oku Y, Iwasaki T, Tsujii T, Sakoda-Iwata R, Hisagai S, Ban Y, et al. Effect of palatine tonsil hypertrophy on tongue posture and maxillofacial dentition: a pharyngeal airway computational fluid dynamics study. Pediatr Dent J 2023;33:42-51.
58. Huang X, Gong X, Gao X. Age-related hypertrophy of adenoid and tonsil with its relationship with craniofacial morphology. BMC Pediatr 2023;23:163.
59. Kuskonmaz CS, Bruno G, Bartolucci ML, Basilicata M, Gracco A, De Stefani A. Correlation between malocclusions, tonsillar grading and Mallampati modified scale: a retrospective observational study. Children (Basel) 2023;10:1061.
60. Tse KL, Savoldi F, Li KY, McGrath CP, Yang Y, Gu M. Prevalence of adenoid hypertrophy among 12-year-old children and its association with craniofacial characteristics: a cross-sectional study. Prog Orthod 2023;24:31.
61. Lan Y, Chen J, Chen S, He Y, Huang F. Influences of adenoid hypertrophy on children's maxillofacial development. Healthcare (Basel) 2023;11:2812.
62. Eslami M, Alipour N. Evaluation of dentofacial angles in children with severe adenoid hypertrophy. Iran J Otorhinolaryngol 2024;36:587-93.
63. Huang L, Zheng L, Chen X, Bai Y. Age-group-specific associations between adenoid/tonsillar hypertrophy and craniofacial features. BMC Oral Health 2024;24:1212.
64. Marcus CL, Brooks LJ, Draper KA, Gozal D, Halbower AC, Jones J, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics 2012;130:576-84.
65. Linder-Aronson S, Woodside DG, Lundström A. Mandibular growth direction following adenoidectomy. Am J Orthod 1986;89:273-84.
66. Zettergren-Wijk L, Forsberg CM, Linder-Aronson S. Changes in dentofacial morphology after adeno-/tonsillectomy in young children with obstructive sleep apnoea—a 5-year follow-up study. Eur J Orthod 2006;28:319-26.
67. Becking BE, Verweij JP, Kalf-Scholte SM, Valkenburg C, Bakker EWP, van Merkesteyn JPR. Impact of adenotonsillectomy on the dentofacial development of obstructed children: a systematic review and meta-analysis. Eur J Orthod 2017;39:509-18.
68. Moss ML, Salentijn L. The primary role of functional matrices in facial growth. Am J Orthod 1969;55:566-77.
69. Ramos VM, Nader CM, Meira ZM, Capanema FD, Franco LP, Tinano MM, et al. Impact of adenotonsilectomy on nasal airflow and pulmonary blood pressure in mouth breathing children. Int J Pediatr Otorhinolaryngol 2019;125:82-6.
70. Petraccone Caixeta AC, Andrade I Jr, Bahia Junqueira Pereira T, Franco LP, Becker HMG, Souki BQ. Dental arch dimensional changes after adenotonsillectomy in prepubertal children. Am J Orthod Dentofacial Orthop 2014;145:461-8.
71. Brodsky L, Koch RJ. Anatomic correlates of normal and diseased adenoids in children. Laryngoscope 1992;102:1268-74.
72. Cohen D, Konak S. The evaluation of radiographs of the nasopharynx. Clin Otolaryngol Allied Sci 1985;10:73-8.

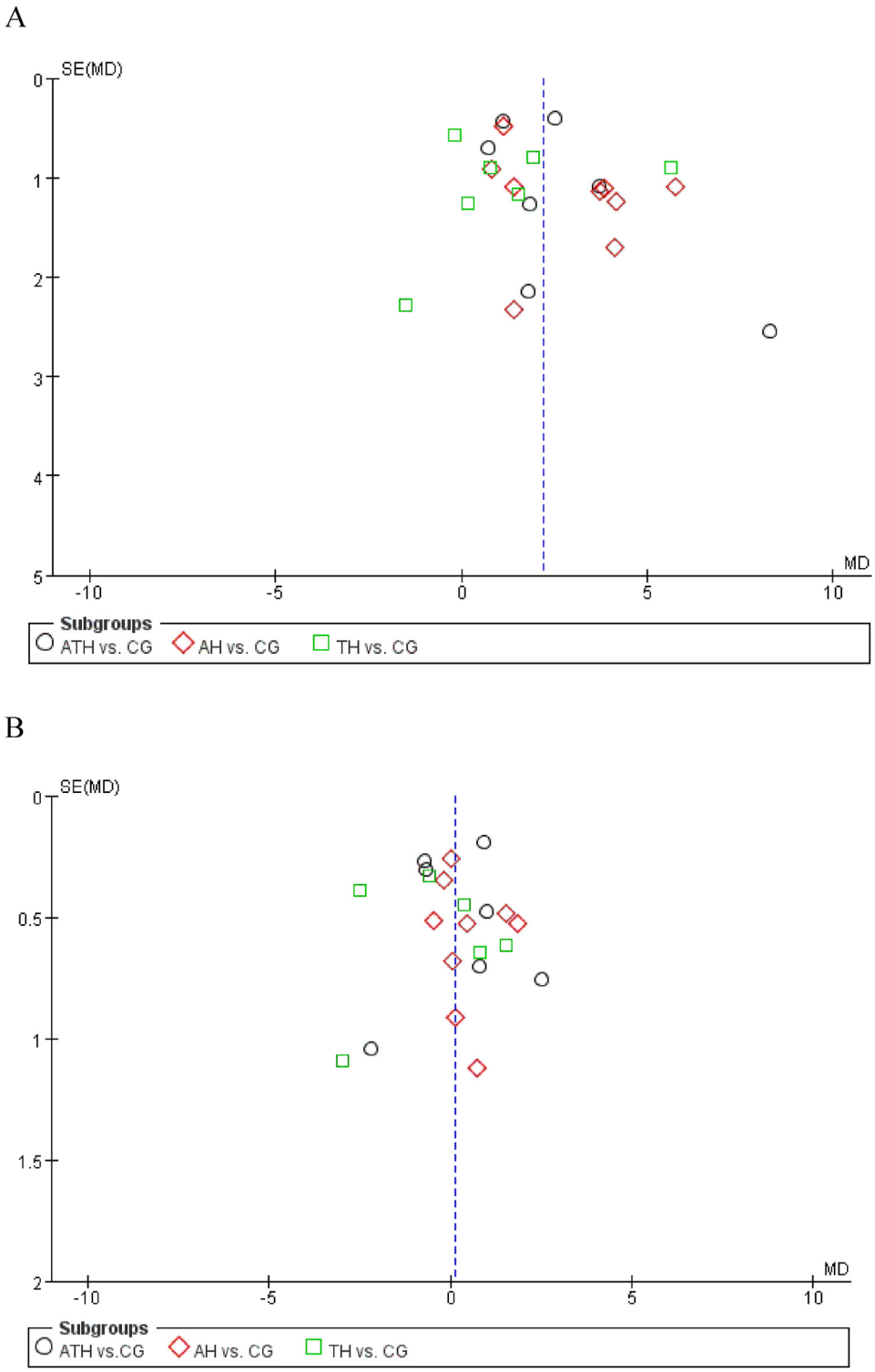
APPENDIX

Supplementary Table I. Search strategy for the electronic search

Source	*Search strategy*
Search strategy for PubMed	#1 (Pediatrics OR Adolescent OR Child) [mesh terms]
	#2 pediatric* OR teenager* OR juvenile* OR adolescen* OR child* OR kid OR kids[Title/Abstract]
	#3 #1 OR #2
	#4 (Tonsillitis OR Palatine Tonsil OR Adenoids OR Nasopharynx OR Oropharynx) [mesh terms]
	#5 (tonsil* OR adenotonsillar* OR adenoid* OR (adenoid face) OR nasopharyngeal* OR oropharyngeal*) [Title/Abstract]
	#6 #4 OR #5
	#7 ((Dental occlusion) OR Malocclusion) [mesh terms]
	#8 (craniofacial* OR maxillary* OR mandibular* OR facial* OR dentofacial* OR crossbite OR overbite OR overjet OR occlusion OR occlusal* OR "ANB" OR "SN-MP") [Title/Abstract]
	#9 ((arch morphology) OR (arch form) OR (arch width)) [Title/Abstract]
	#10 #7 OR #8 OR #9
	#11 Index of Orthodontic Treatment Need [mesh terms]
	#12 ((Index for Need of Orthodontic Treatment) OR (Index of Orthodontic Treatment Needs)) [Title/Abstract]
	#13 #11 OR #12
	#14 #10 OR #13
	#15 #3 AND #6 AND #14
Search strategy for Embase	#1 (pediatrics OR juvenile OR child) exp.
	#2 (pediatric* OR teenager* OR juvenile* OR adolescen* OR child* OR kid*):ti,ab,kw
	#3 #1 OR #2
	#4 ((tonsil disease) OR tonsil OR adenoid OR oropharynx OR nasopharynx) exp.
	#5 (tonsil* OR adenotonsillar* OR adenoid* OR (adenoid face) OR nasopharyngeal* OR oropharyngeal*):ti,ab,kw
	#6 #4 OR #5
	#7 ((tooth occlusion) OR malocclusion) exp.
	#8 (craniofacial* OR maxillary* OR mandibular* OR facial* OR dentofacial* OR crossbite OR overbite OR overjet OR occlusion OR occlusal* OR "ANB" OR "SN-MP"):ti,ab,kw
	#9 ((arch morphology) OR (arch form) OR (arch width)):ti,ab,kw
	#10 #7 OR #8 OR #9
	#11 (index of orthodontic treatment need) exp.
	#12 ((index for orthodontic treatment need) OR (index of orthodontic treatment needs) OR (IOTN index)):ti,ab,kw
	#13 #11 OR #12
	#14 #10 OR #13
	#15 #3 AND #6 AND #14
Search strategy for Web of Science	#1 TS=(pediatric* OR teenager* OR juvenile* OR adolescen* OR child* OR kid*)
	#2 TS=(tonsil* OR adenotonsillar* OR adenoid* OR (adenoid face) OR nasopharynx OR nasopharyngeal* OR oropharynx OR oropharyngeal*)
	#3 TS=(occlusion OR malocclusion OR craniofacial* OR maxillary* OR mandibular* OR facial* OR dentofacial* OR crossbite OR overbite OR overjet OR occlusion OR occlusal* OR "ANB" OR "SN-MP")
	#4 TS= ((arch morphology) OR (arch form) OR (arch width))
	#5 #3 OR #4
	#6 TS=((Index of Orthodontic Treatment Need) OR (Index of Orthodontic Treatment Need) OR (Index for Need of Orthodontic Treatment) OR (Index of Orthodontic Treatment Needs) OR (IOTN index))
	#7 #5 OR #6
	#8 #1 AND #2 AND #7
Search strategy for VIP Chinese Journal Database	#1 U=(儿童 OR 青少年 OR 未成年)
	#2 R=(扁桃体 OR 腺扁 OR 腺样体 OR 腺样体面容 OR 鼻咽 OR 口咽)
	#3 R=(颅面 OR 颌面 OR 上颌 OR 下颌 OR 面型 OR 面部 OR 牙𬌗 OR 覆𬌗 OR 反𬌗 OR 错𬌗 OR 咬合 OR 覆盖 OR 牙牙合 OR 覆牙合 OR 反牙合 OR 错牙合 OR 牙he OR 覆he OR 反he OR 错he OR 下颌平面角 OR ANB OR牙弓形态 OR 牙弓形状 OR 牙弓宽度 OR正畸治疗需求指数)
	#4 #1 AND #2 AND #3

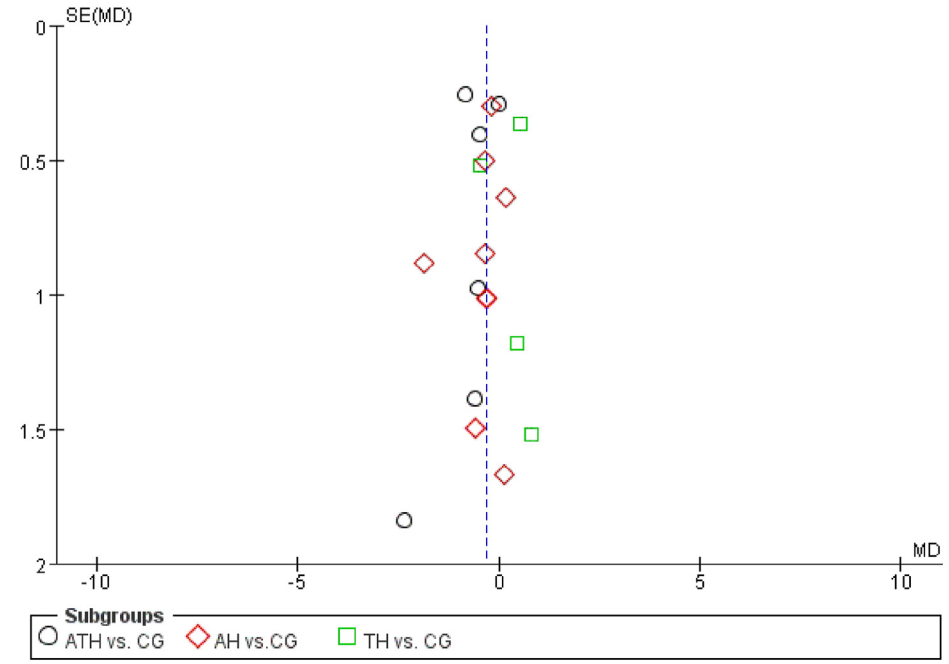
Supplementary Table II. Characteristics of excluded studies

Study	*Reasons for exclusion*
Petraccone Caixeta 2014	NOT cross-sectional study
Matsumoto 2012	NOT cross-sectional study
Mattar 2011	NOT cross-sectional study
Pereira 2011	NOT cross-sectional study
Vieira 2012	NOT cross-sectional study
倪 2022	Does not meet the required grouping criteria
张 2010	Duplicate publication
Basheer 2014	Does not meet the required grouping criteria
Subtelny 1975	Review
Diadchenko 1977 (1)	Not in English or Chinese
Diadchenko 1977 (2)	Not in English or Chinese
Mottl 1982	Not in English or Chinese
Adamidis 1983	Does not meet the required grouping criteria
Scara 1984	Not in English or Chinese
Klein 1986	No healthy CG
Hee-Kyung 1988	Not in English or Chinese
Hultcrantz et al 1988	No healthy CG
Woodside 1991	Does not meet the required grouping criteria
Rho 1993	Not in English or Chinese
Baumann 1996 (1)	Not in English or Chinese
Baumann 1996 (2)	Not in English or Chinese
Trotman 1997	No healthy CG
Agren 1998	No healthy CG
Zuccon 1999	Does not meet the required grouping criteria
Brasilei 2003	Conference abstracts
Ozdemir 2004	No healthy CG
Guilleminault 2007	Not in English or Chinese
Raffat 2009	No healthy CG
Kouassi 2009	Does not meet the required grouping criteria
Lofstrand 2010	Does not meet the required grouping criteria
Baroni 2011	No healthy CG
Huynh 2011	Does not meet the required grouping criteria
Esteller 2011	Not in English or Chinese
DiFrancesco 2012	No healthy CG
Diouf 2015	Not in English or Chinese
Diouf 2015 (2)	Not in English or Chinese
Indiarti 2017	No healthy CG
Diouf 2018	Not in English or Chinese
Pawlowska 2020	Does not meet the required grouping criteria
Inonu-Sakalli 2018	Participants (>30%) had a history of adenotonsillectomy
Pinna 1990	Not in English or Chinese
Zhang 2008	NOT cross-sectional study

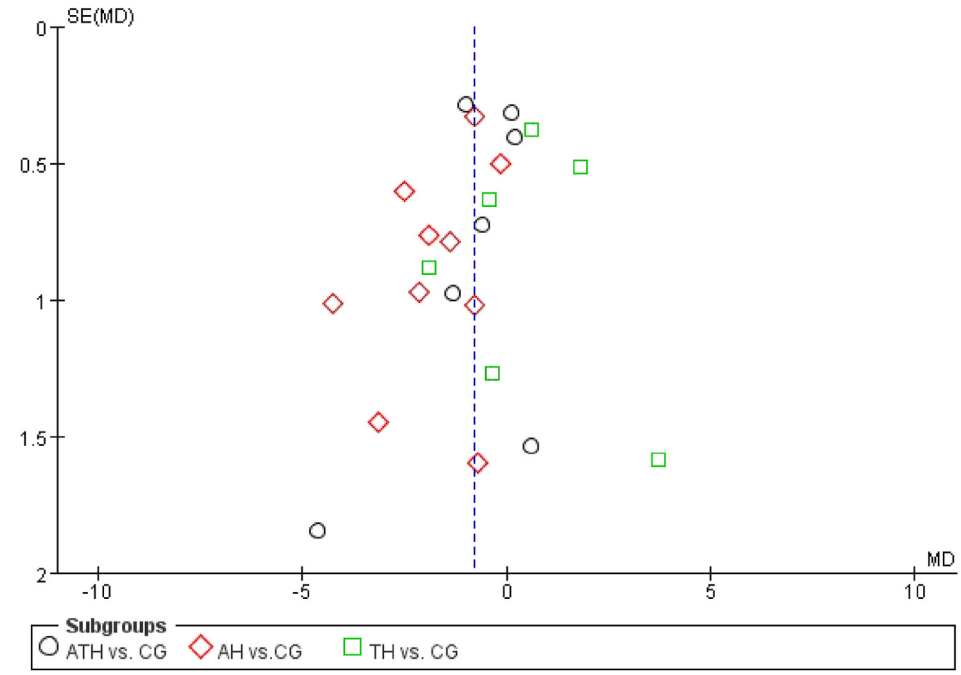


Supplementary Fig 1. Funnel plots: **A,** SN-MP; **B,** ANB; **C,** SNA; **D,** SNB; **E,** Angle's Classification.

C

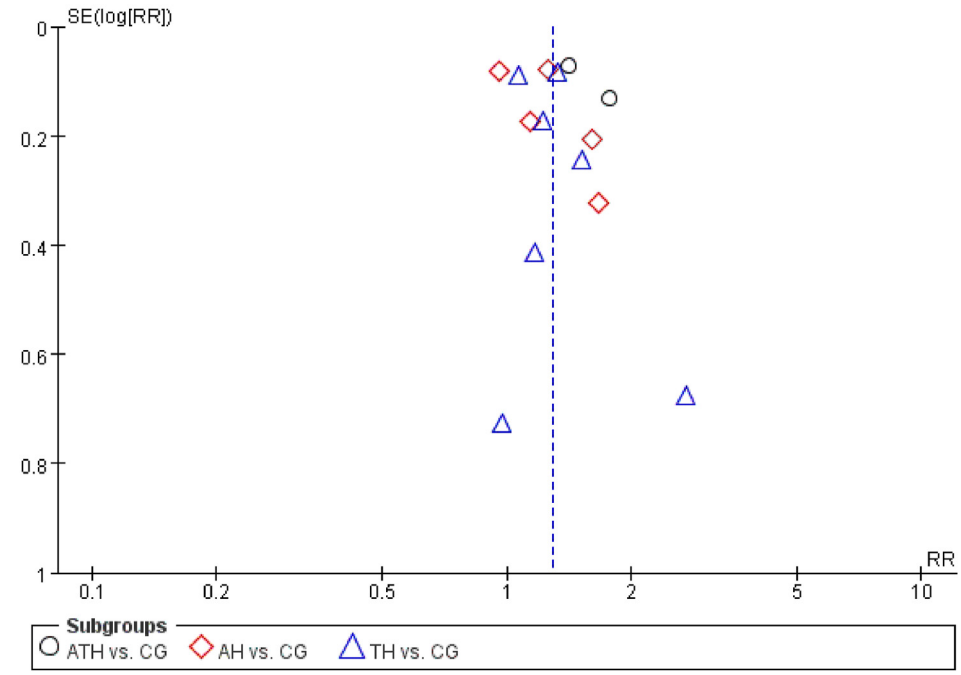


D



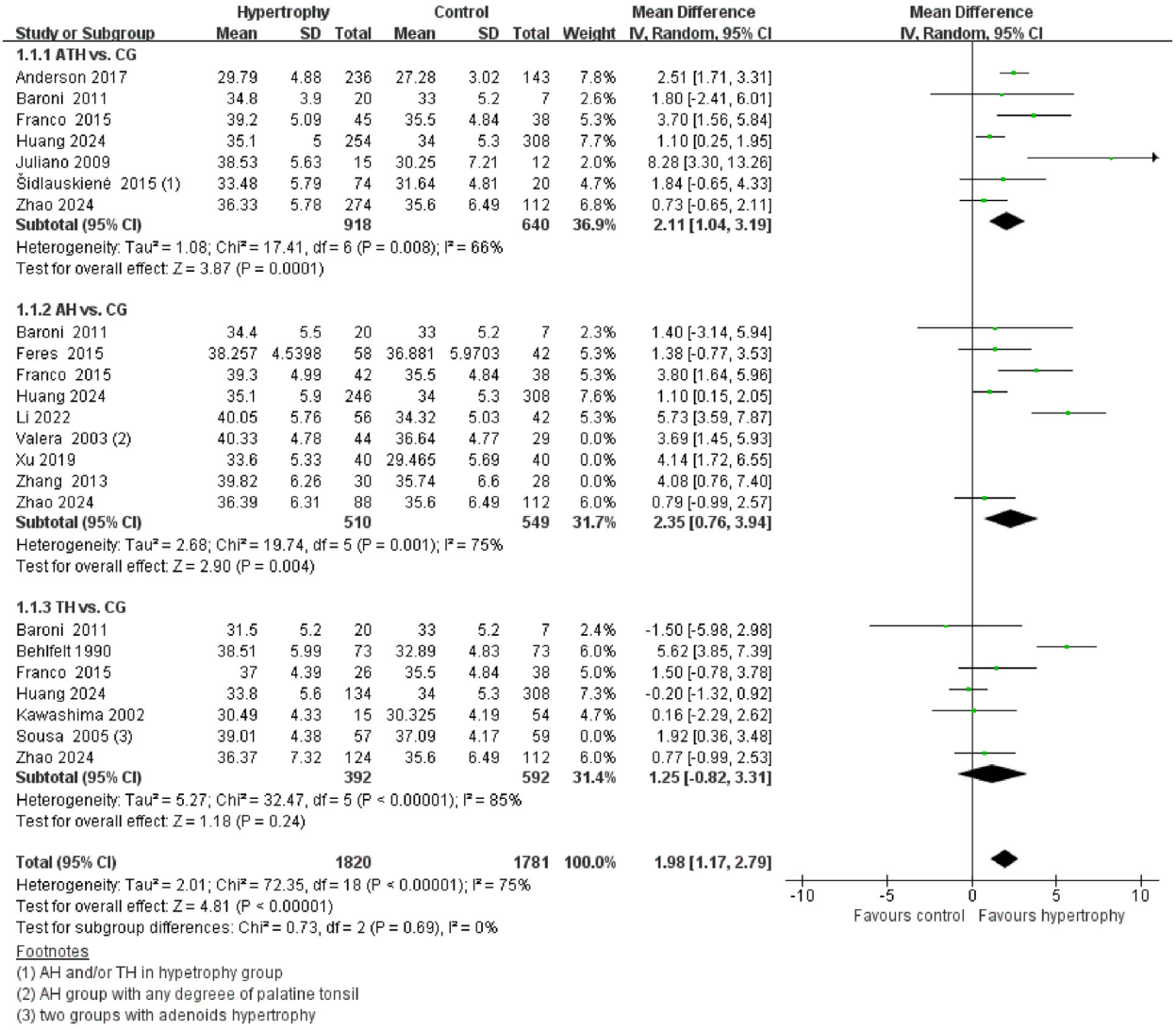
Supplementary Fig 1. (*continued*).

E



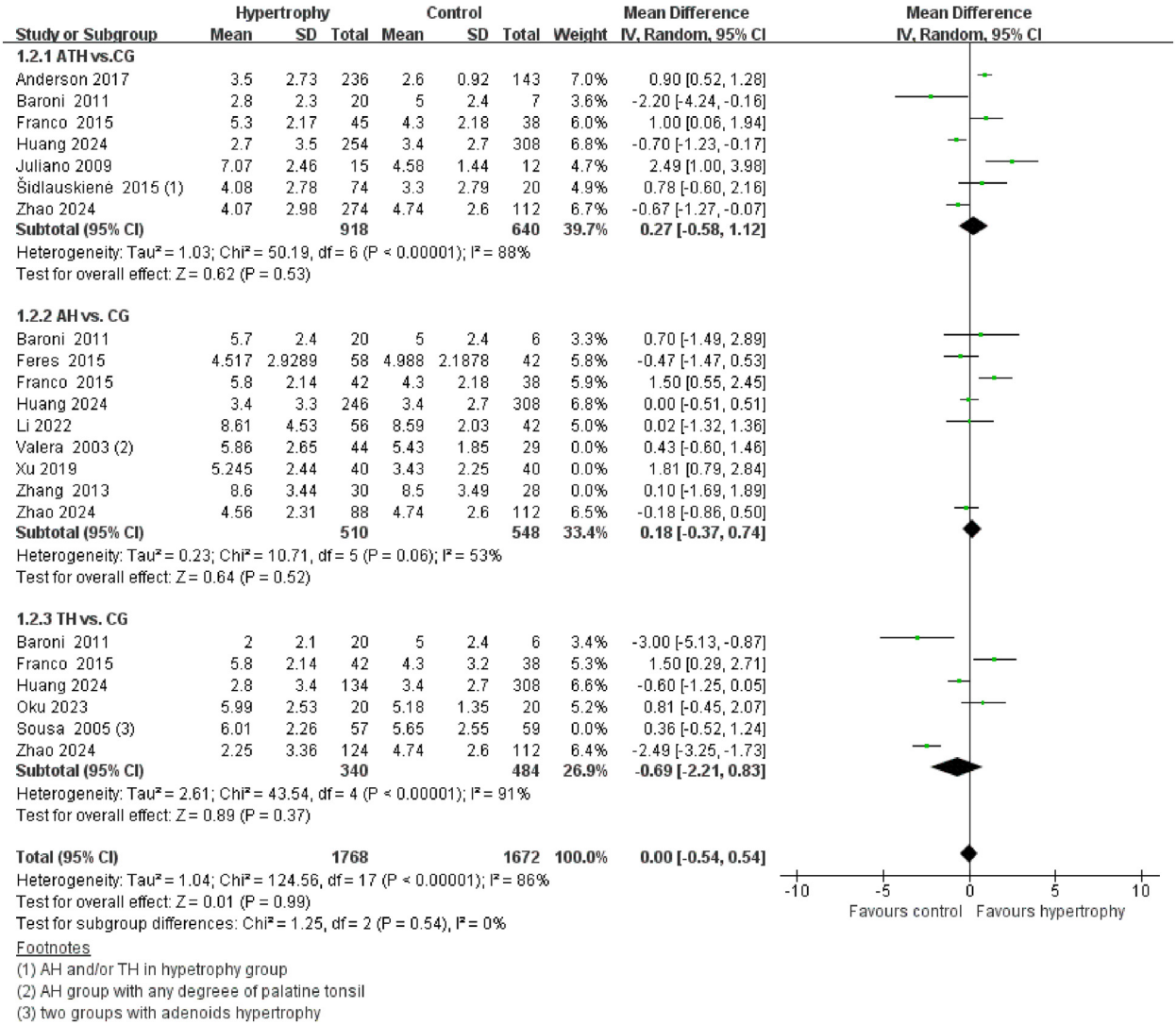
Supplementary Fig 1. (*continued*).

A



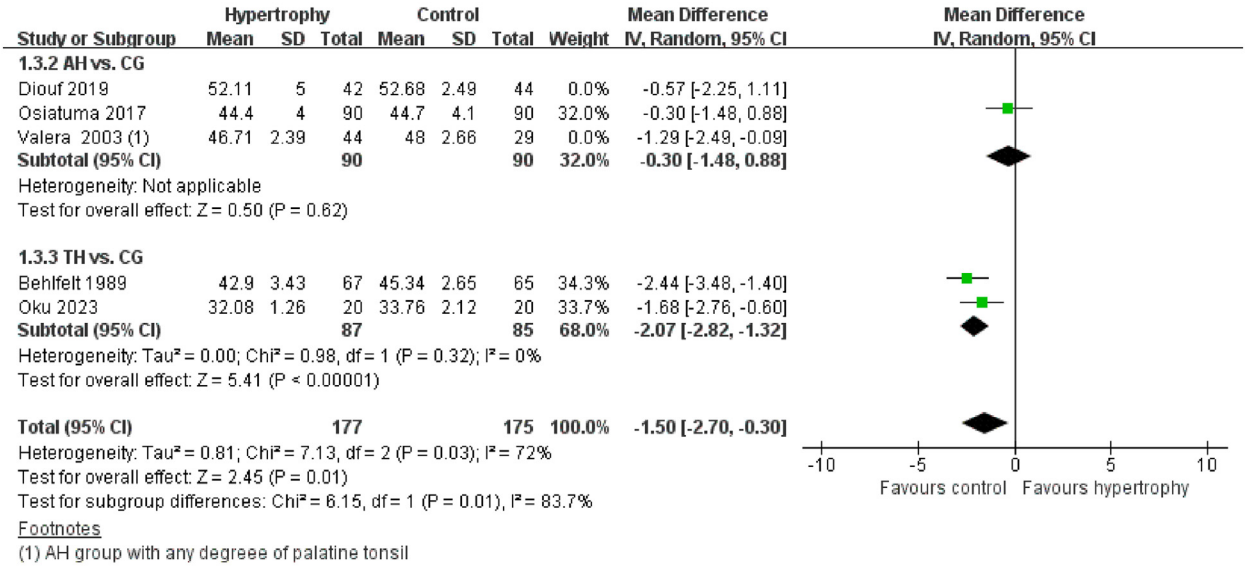
Supplementary Fig 2. Sensitivity analysis by excluding low-quality studies: **A,** SN-MP; **B,** ANB; **C,** Maxillary arch width; **D,** Mandibular arch width; **E,** Angle's Classification; **F,** Overjet; **G,** Overbite; **H,** Open bite; **I,** U1-SN; **J,** L1-MP; **K,** SNA; **L,** SNB; **M,** ArGo; **N,** ArGoMe.

B



Supplementary Fig 2. (*continued*).

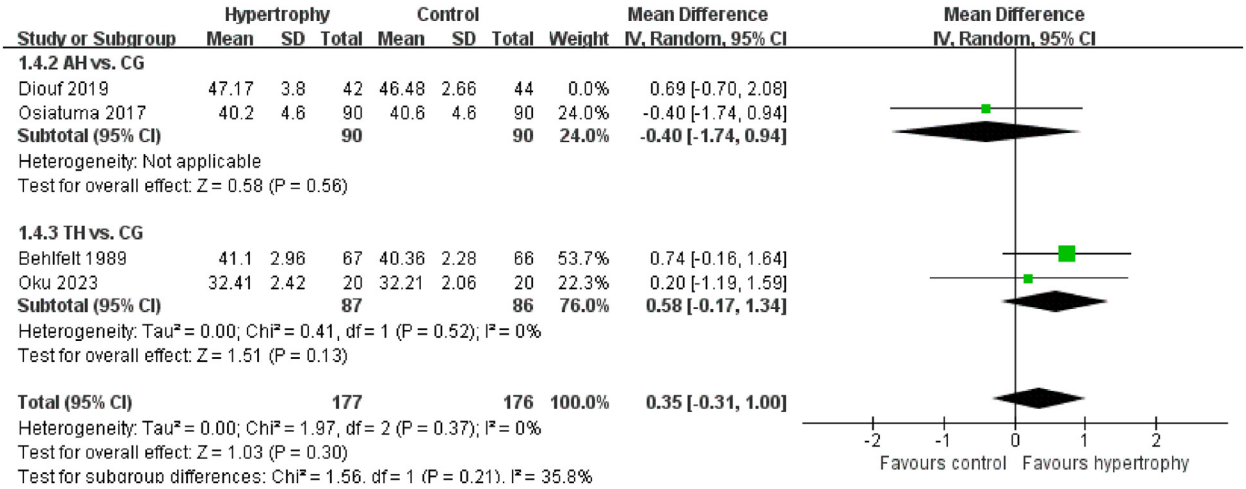
C



Footnotes

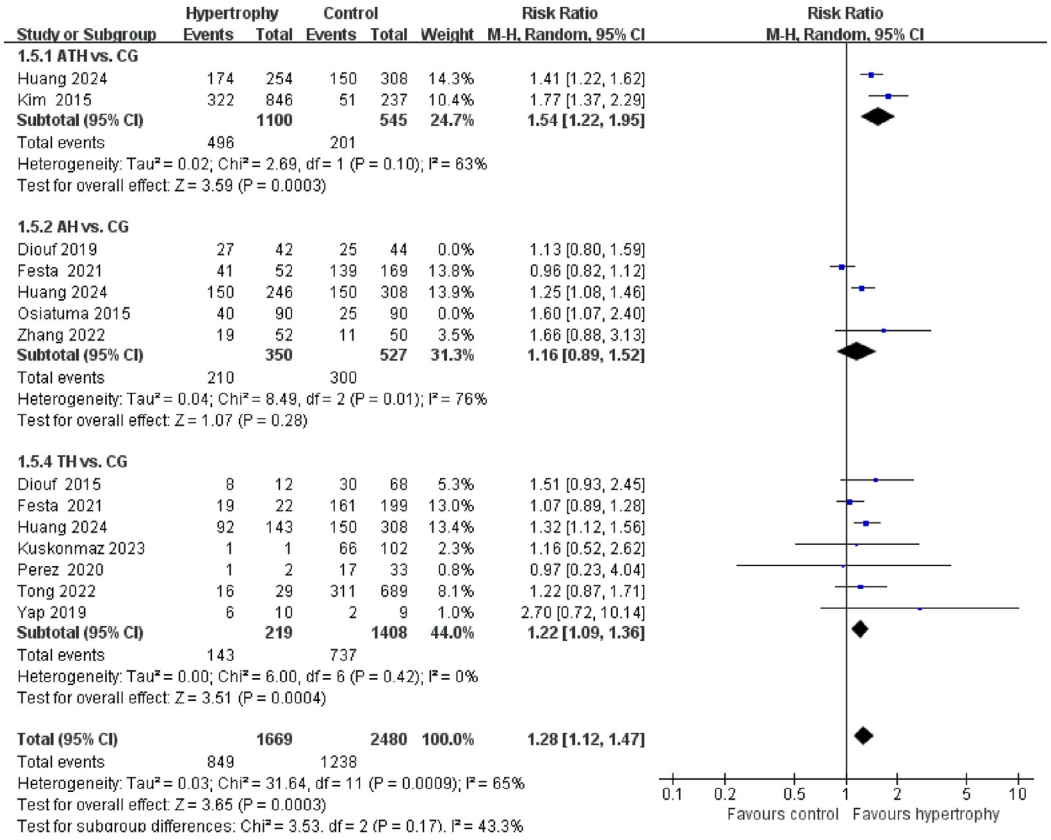
(1) AH group with any degreee of palatine tonsil

D

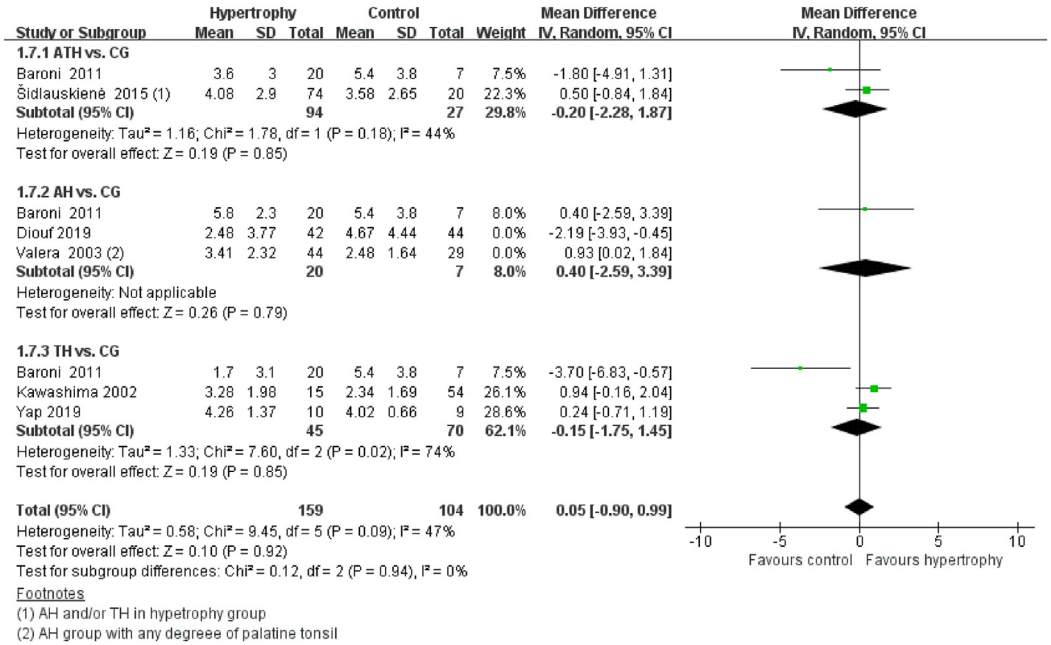


Supplementary Fig 2. (*continued*).

E

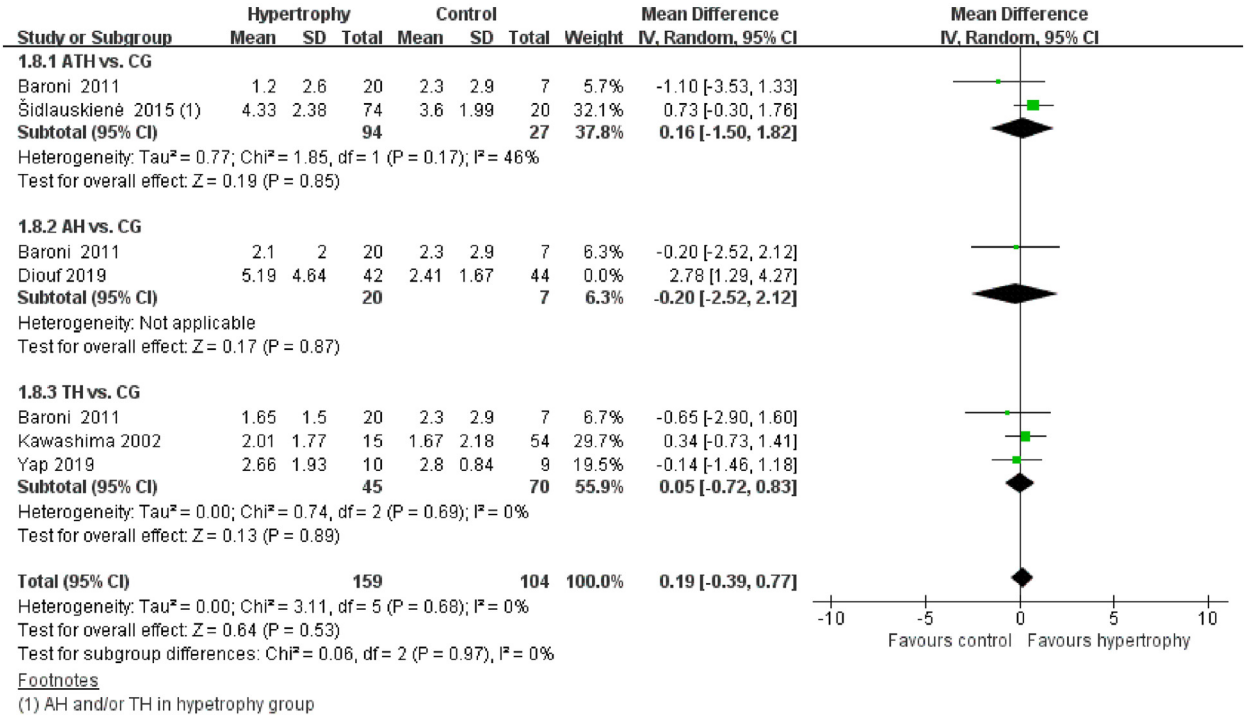


F



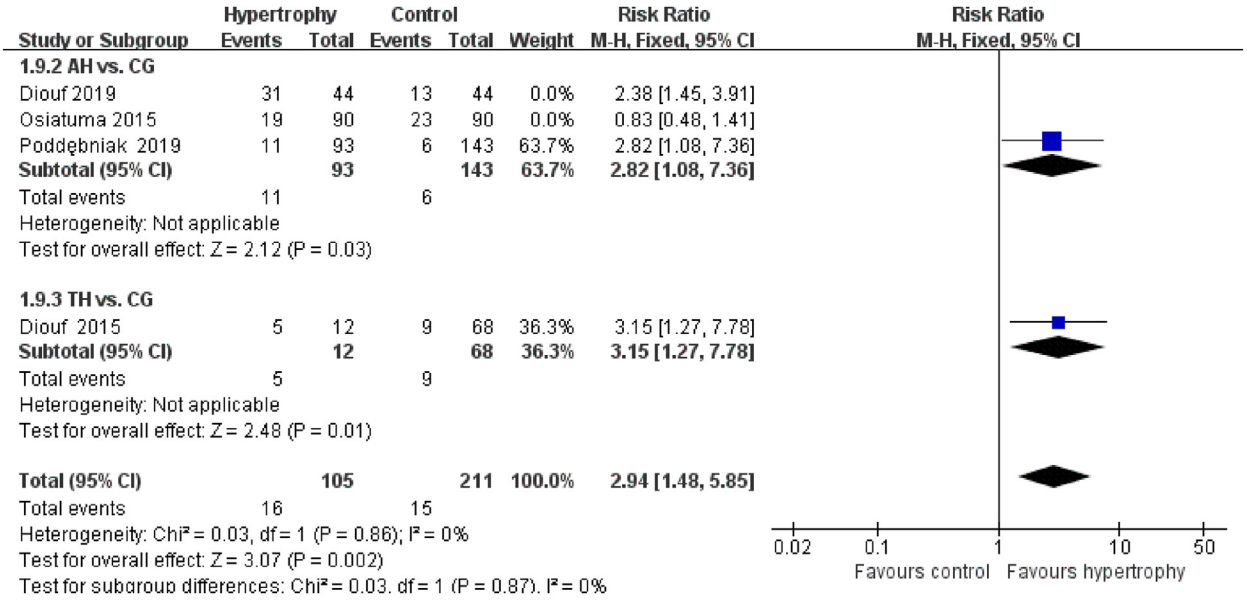
Supplementary Fig 2. (*continued*).

G



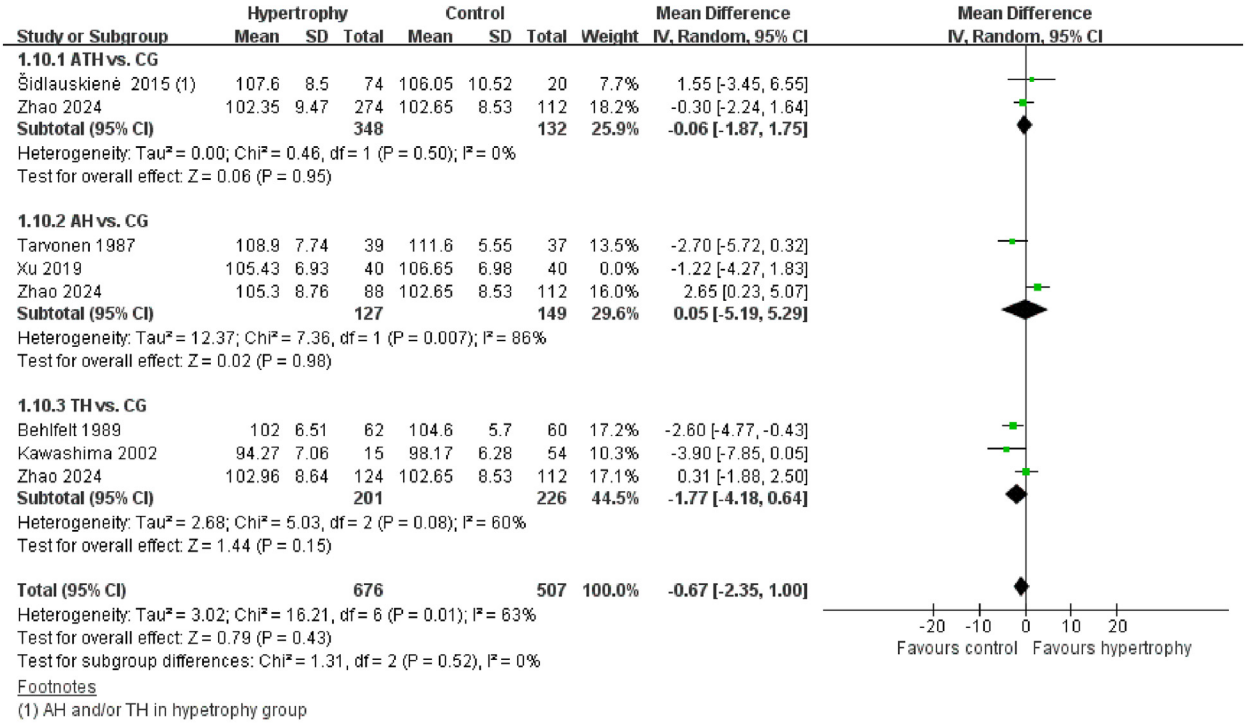
Footnotes
(1) AH and/or TH in hypetrophy group

H



Supplementary Fig 2. (*continued*).

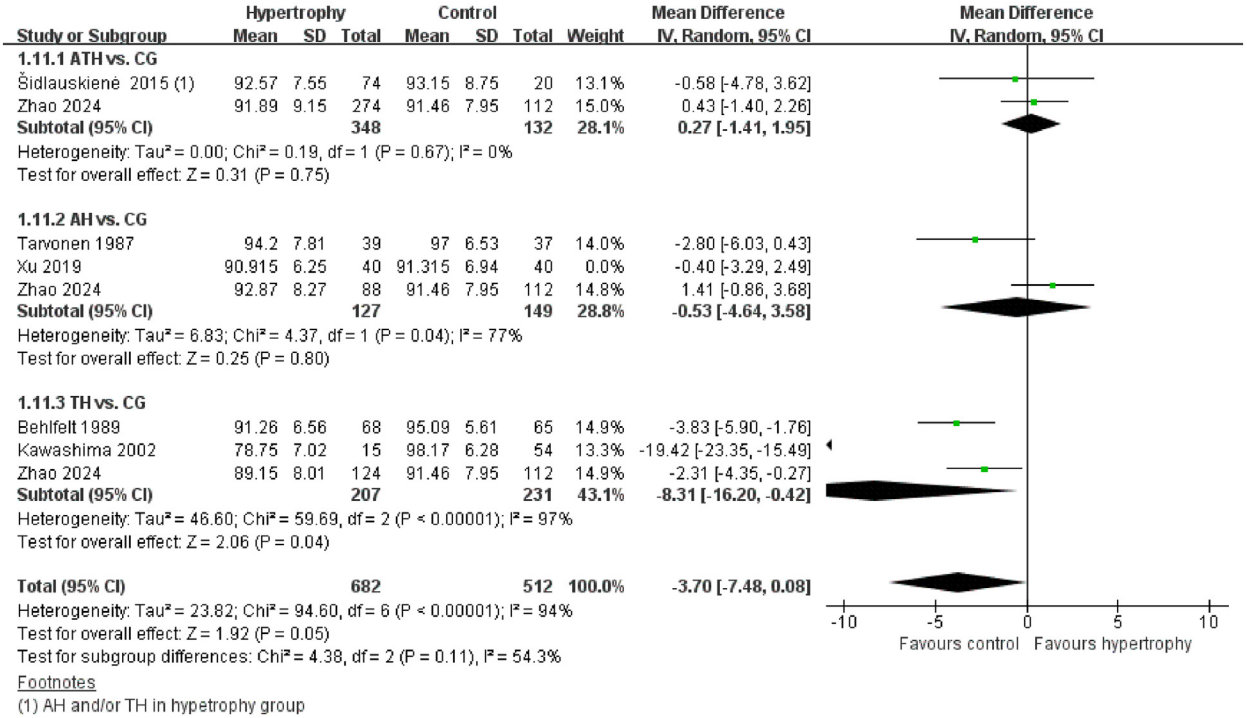
I



Footnotes

(1) AH and/or TH in hypetrophy group

J

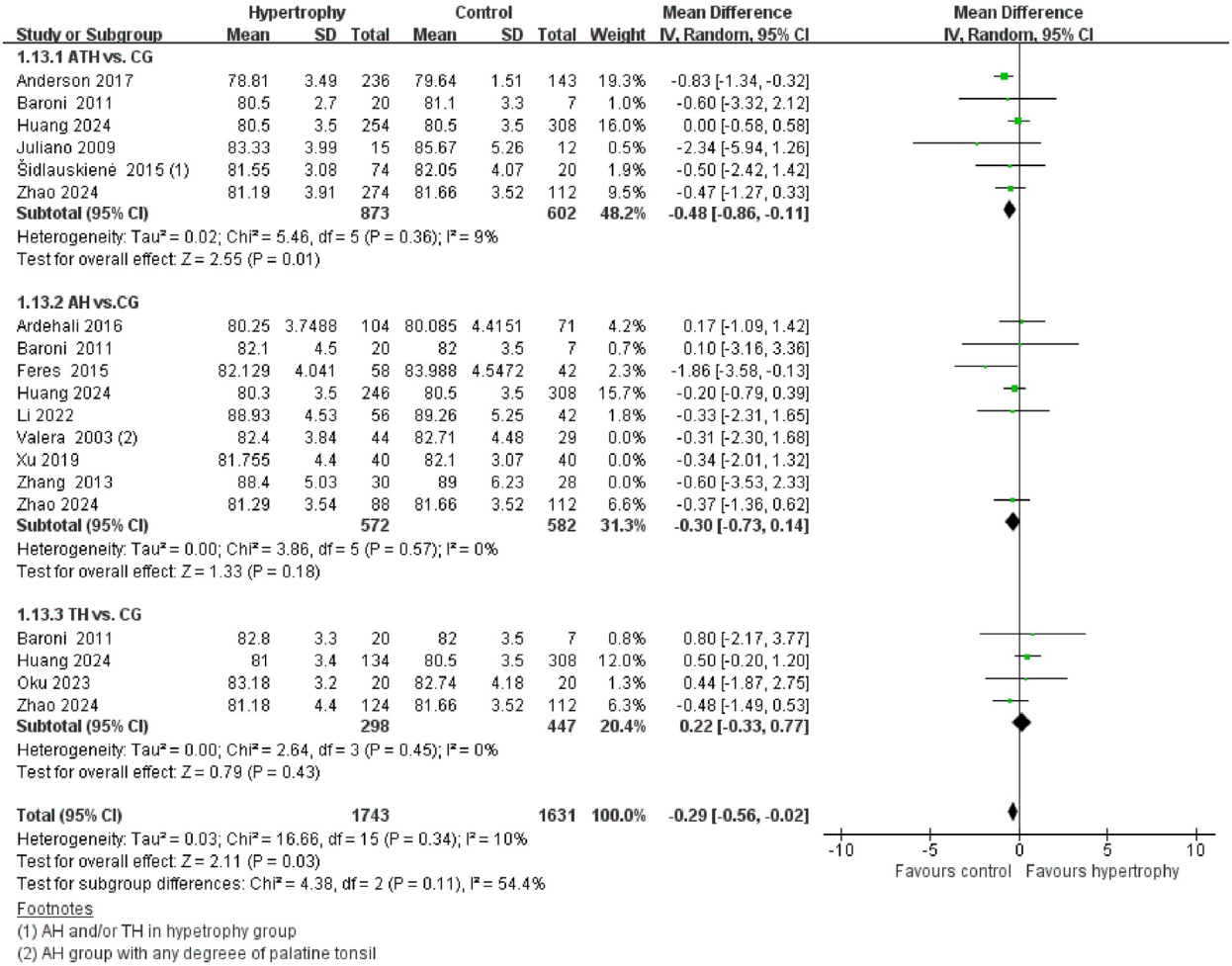


Footnotes

(1) AH and/or TH in hypetrophy group

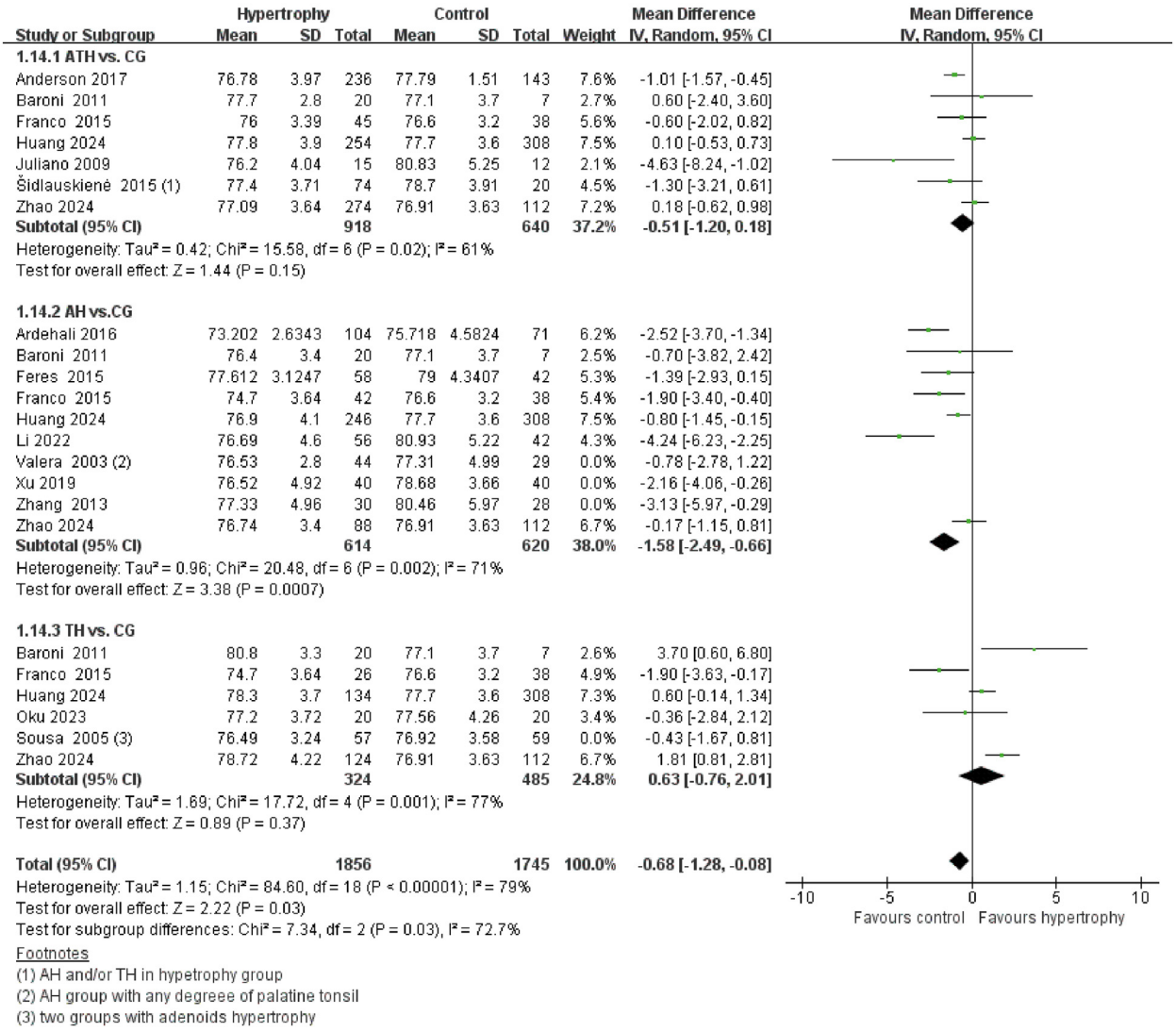
Supplementary Fig 2. (*continued*).

K



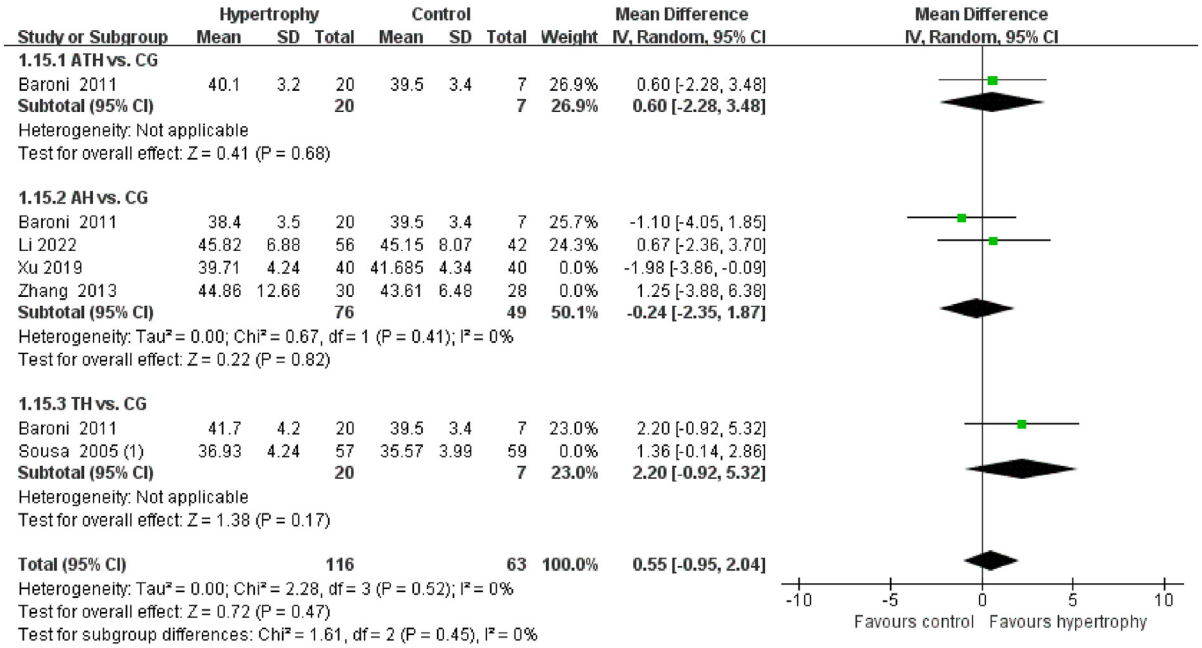
Supplementary Fig 2. (*continued*).

L



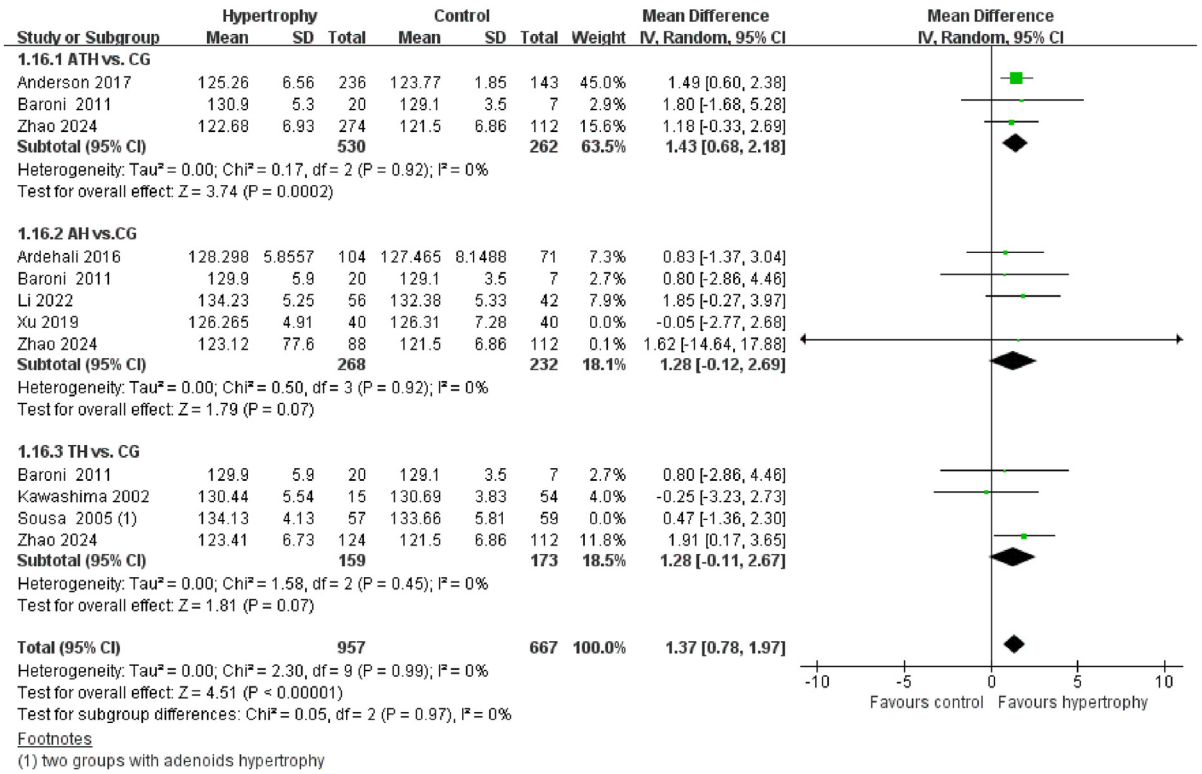
Supplementary Fig 2. (*continued*).

M



Footnotes
(1) two groups with adenoids hypertrophy

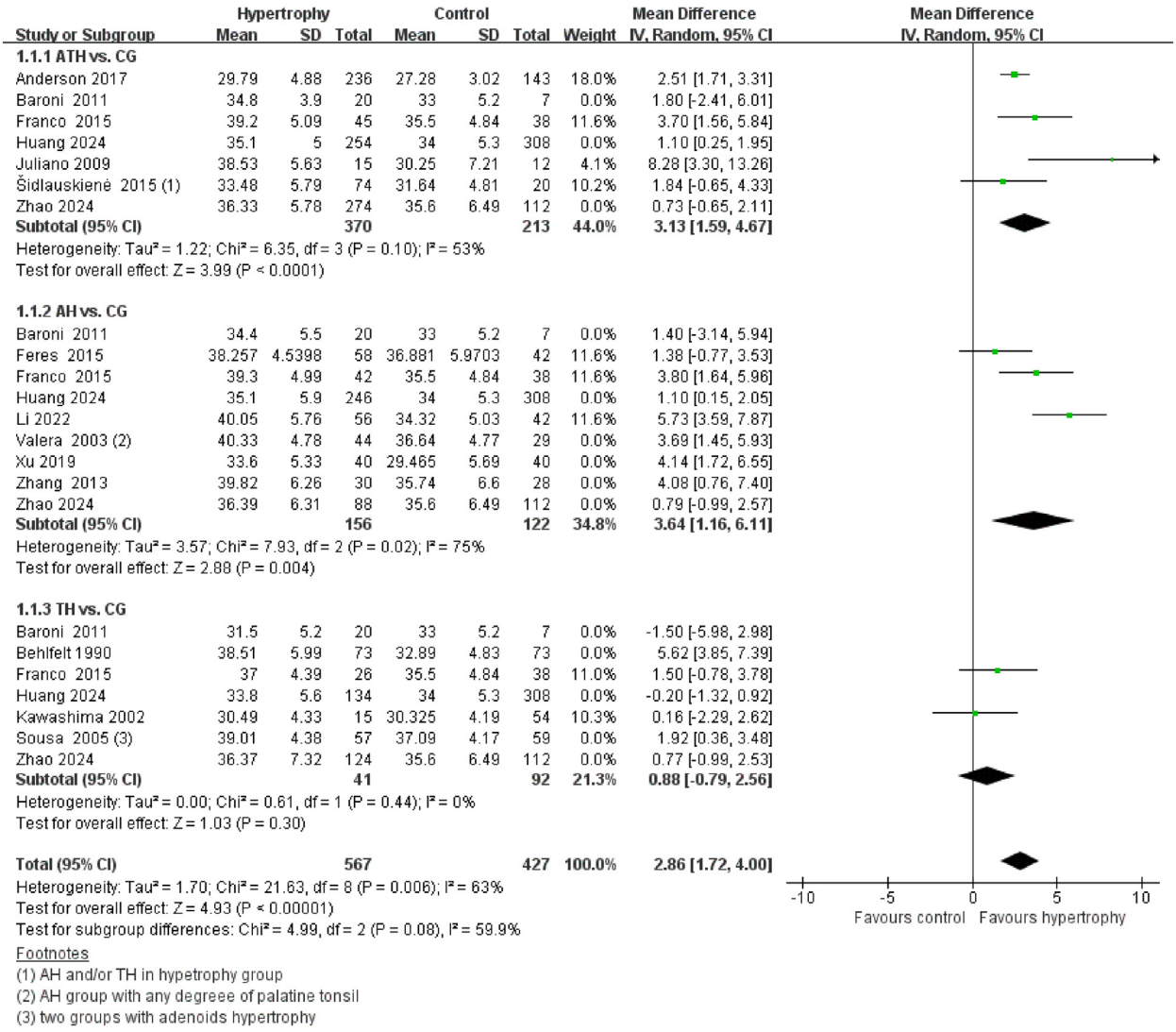
N



Footnotes
(1) two groups with adenoids hypertrophy

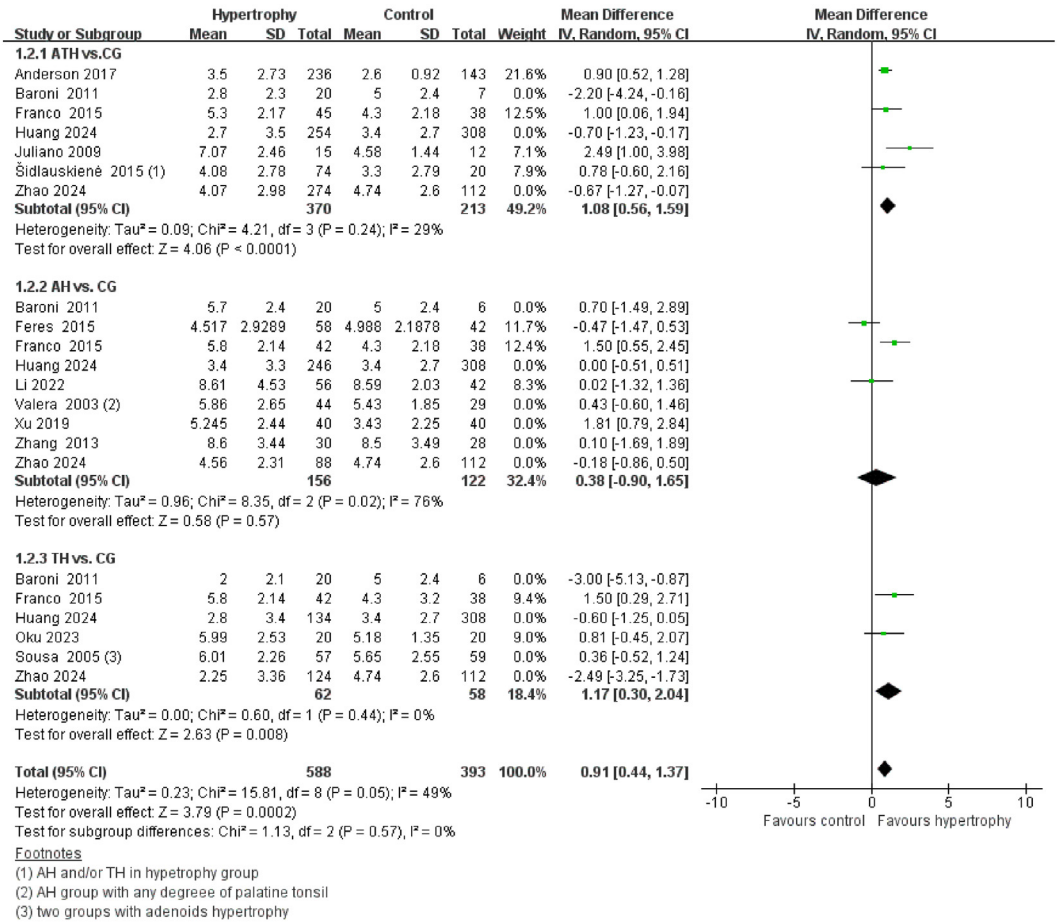
Supplementary Fig 2. (*continued*).

A



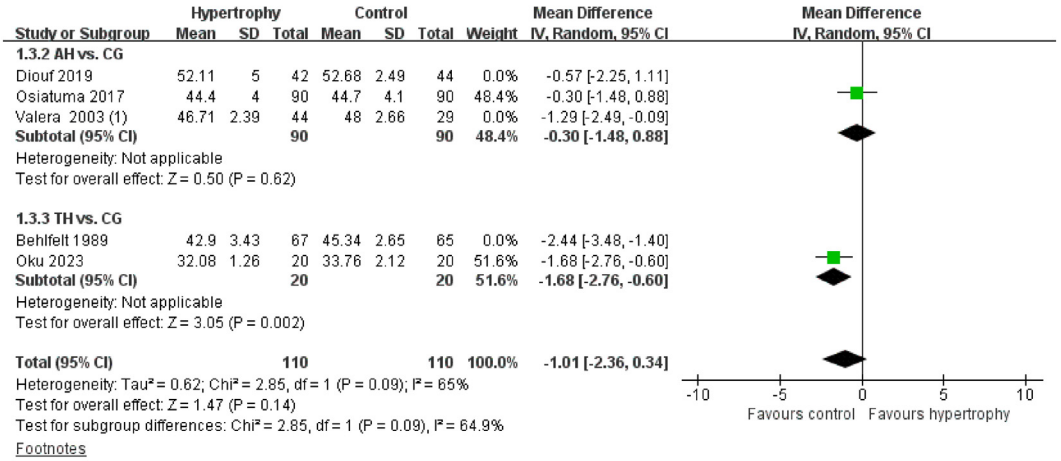
Supplementary Fig 3. Sensitivity analysis by excluding different methodologic classification criteria: **A,** SN-MP; **B,** ANB; **C,** Maxillary arch width; **D,** Mandibular arch width; **E,** Angle's Classification; **F,** Overjet; **G,** Overbite; **H,** Open bite; **I,** Crossbite; **J,** U1-SN; **K,** L1-MP; **L,** U1-L1.

B



Footnotes
(1) AH and/or TH in hypetrophy group
(2) AH group with any degreee of palatine tonsil
(3) two groups with adenoids hypertrophy

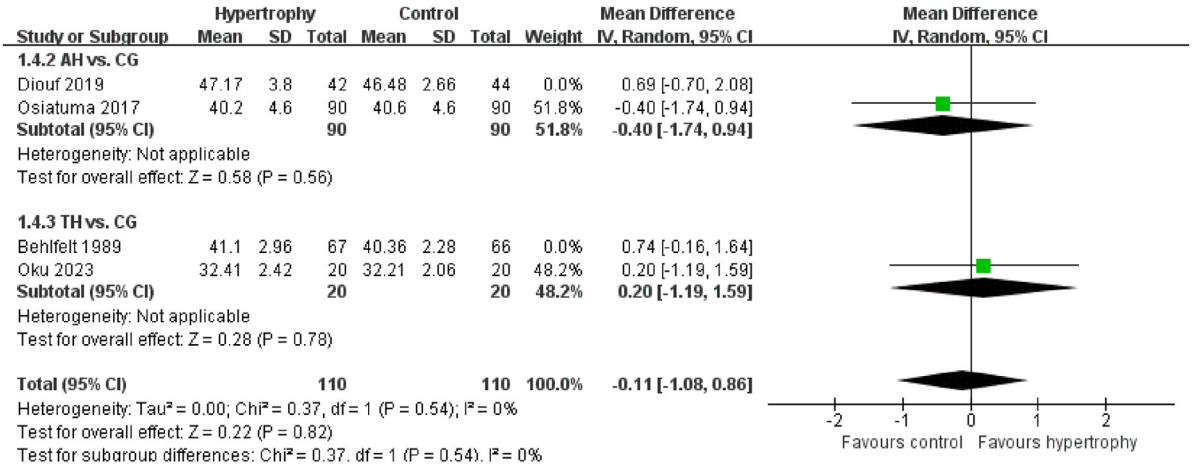
C



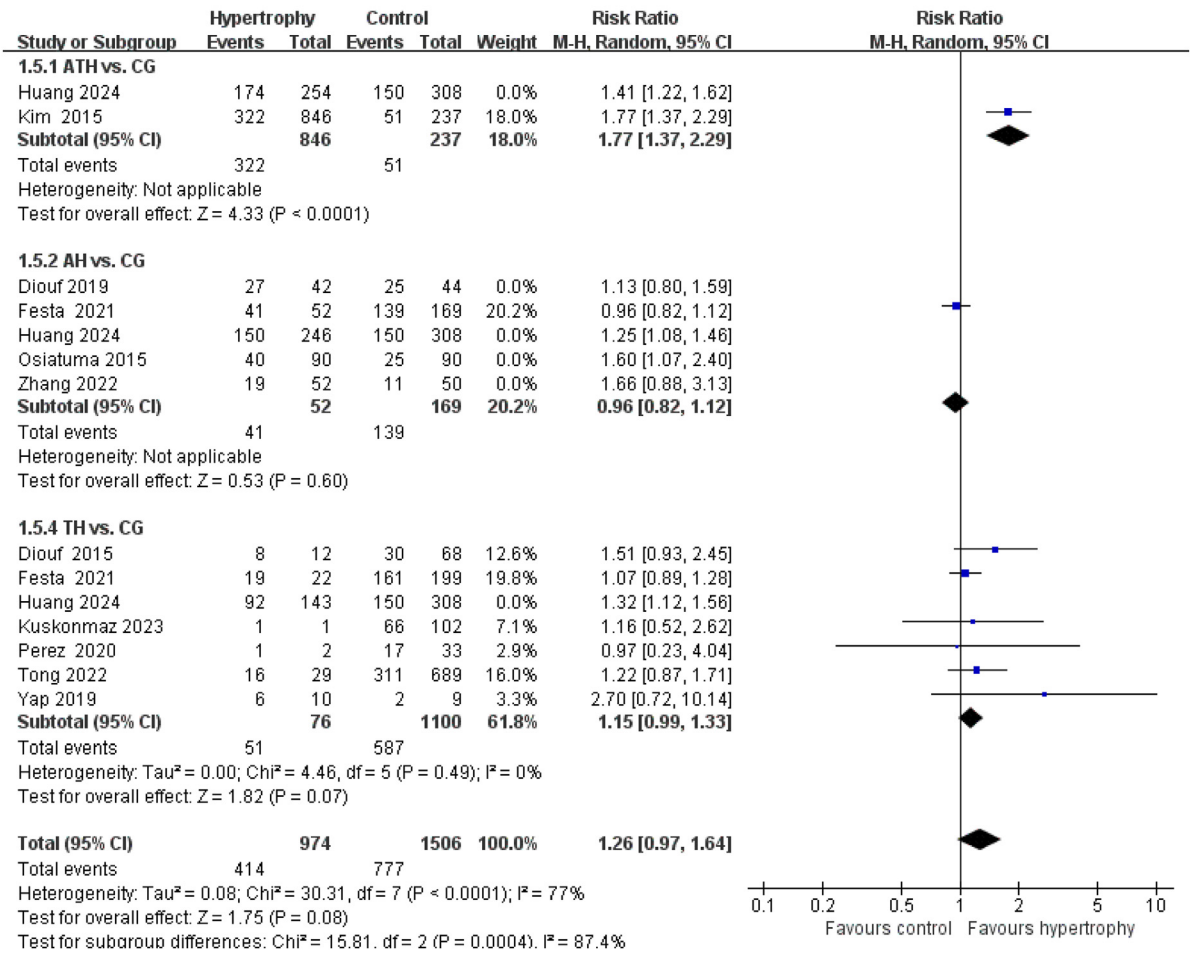
Footnotes
(1) AH group with any degreee of palatine tonsil

Supplementary Fig 3. (*continued*).

D

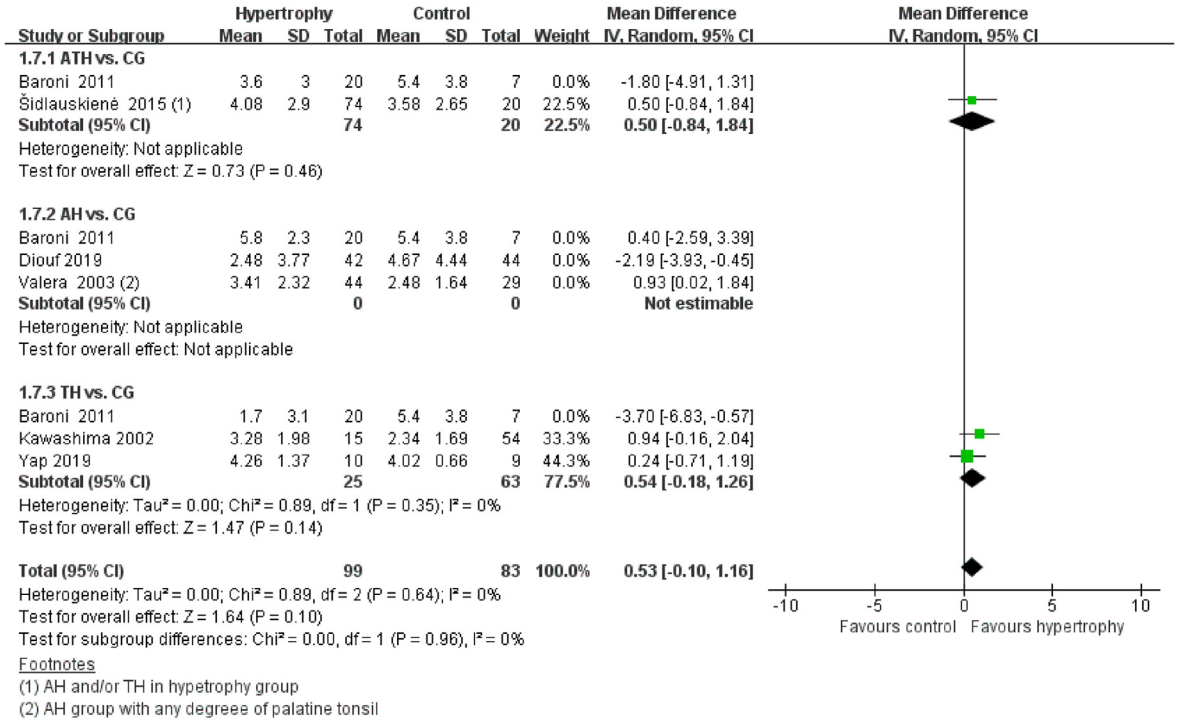


E



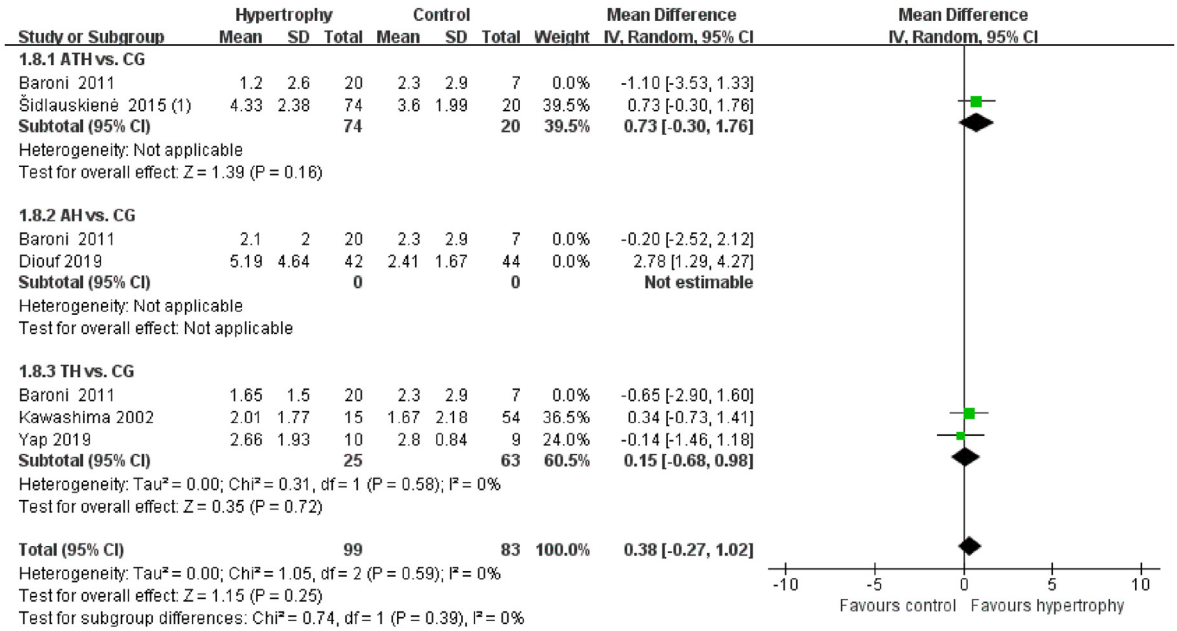
Supplementary Fig 3. (*continued*).

F



Footnotes
(1) AH and/or TH in hypetrophy group
(2) AH group with any degreee of palatine tonsil

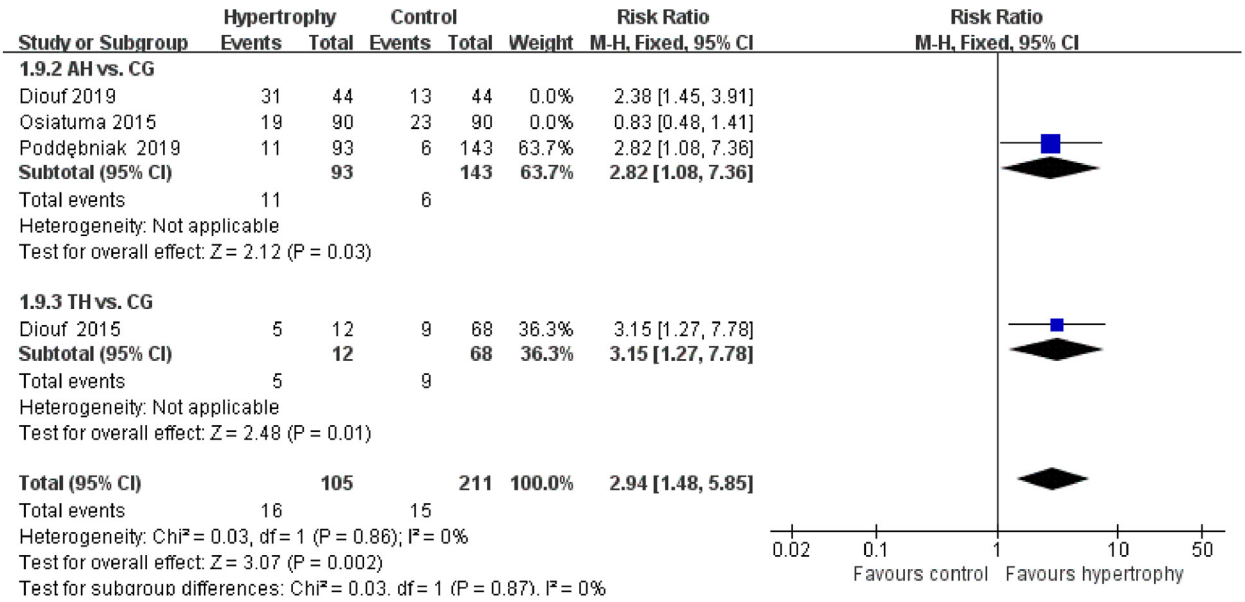
G



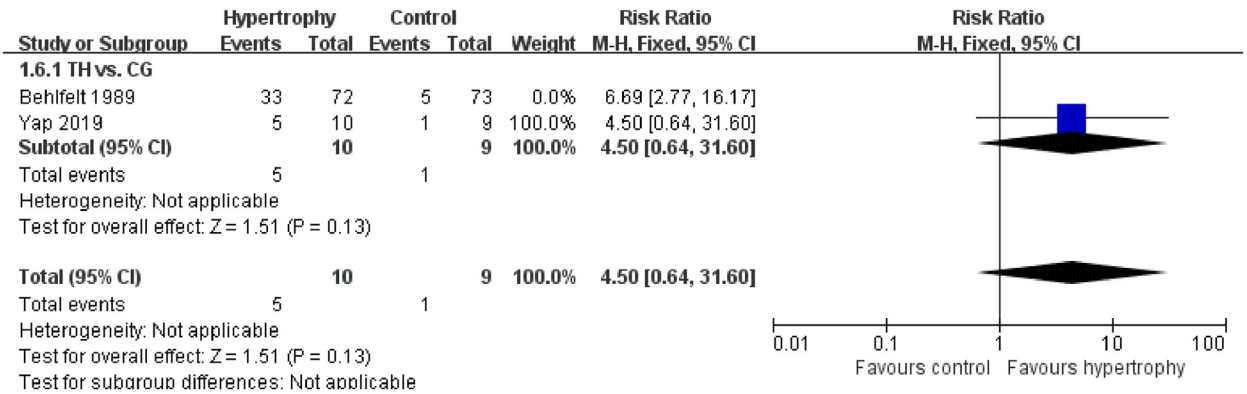
Footnotes
(1) AH and/or TH in hypetrophy group

Supplementary Fig 3. (*continued*).

H

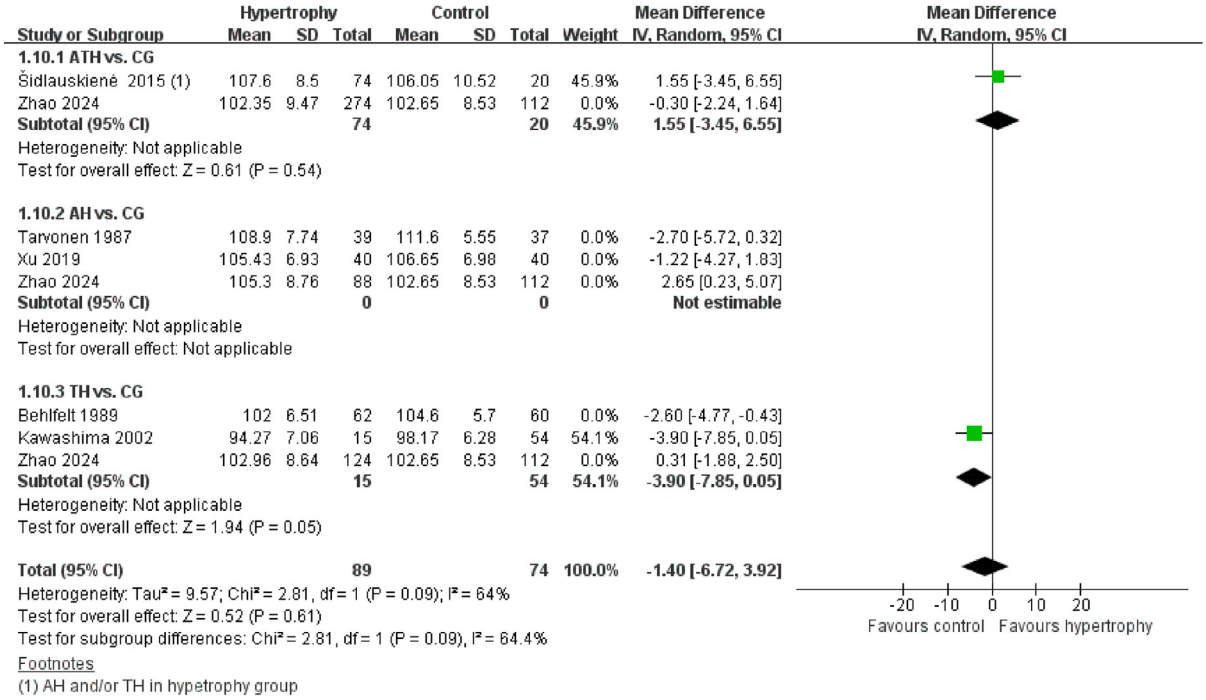


I



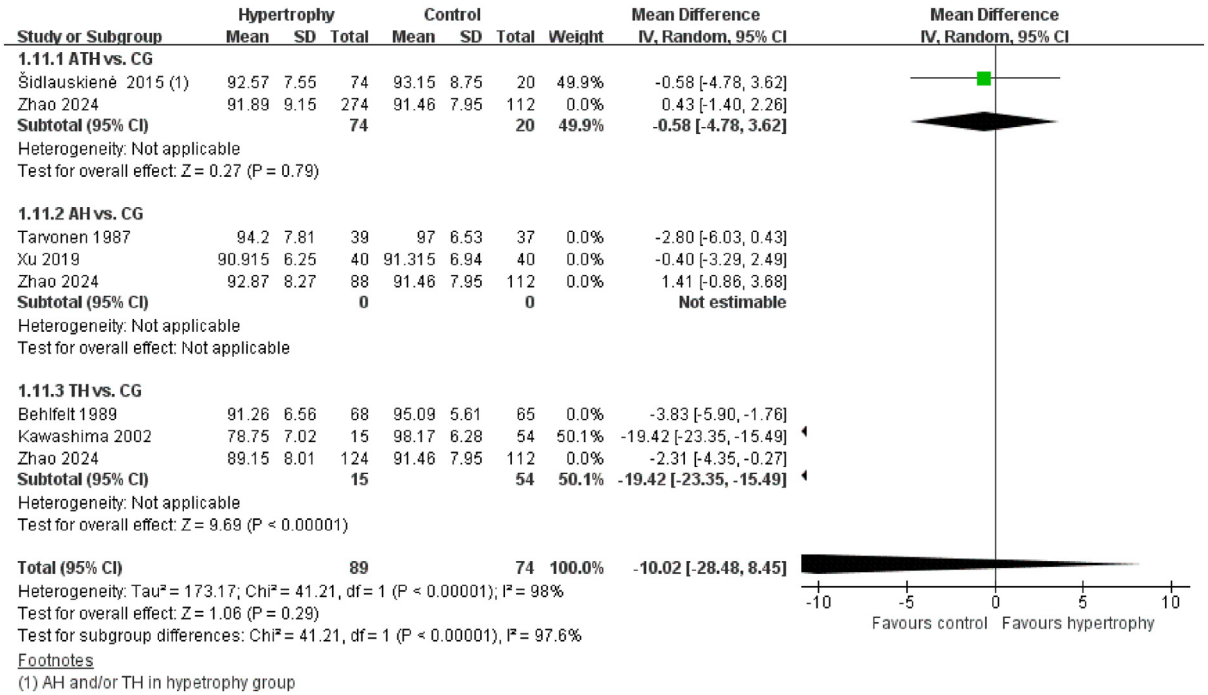
Supplementary Fig 3. (*continued*).

J



Footnotes
(1) AH and/or TH in hypetrophy group

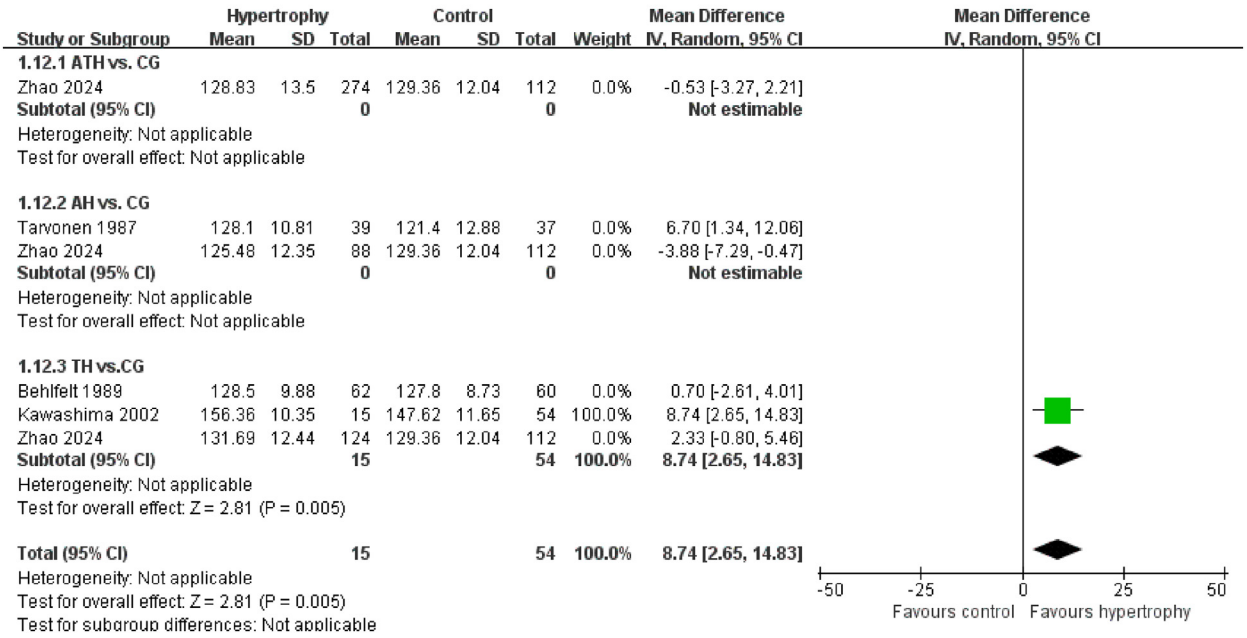
K



Footnotes
(1) AH and/or TH in hypetrophy group

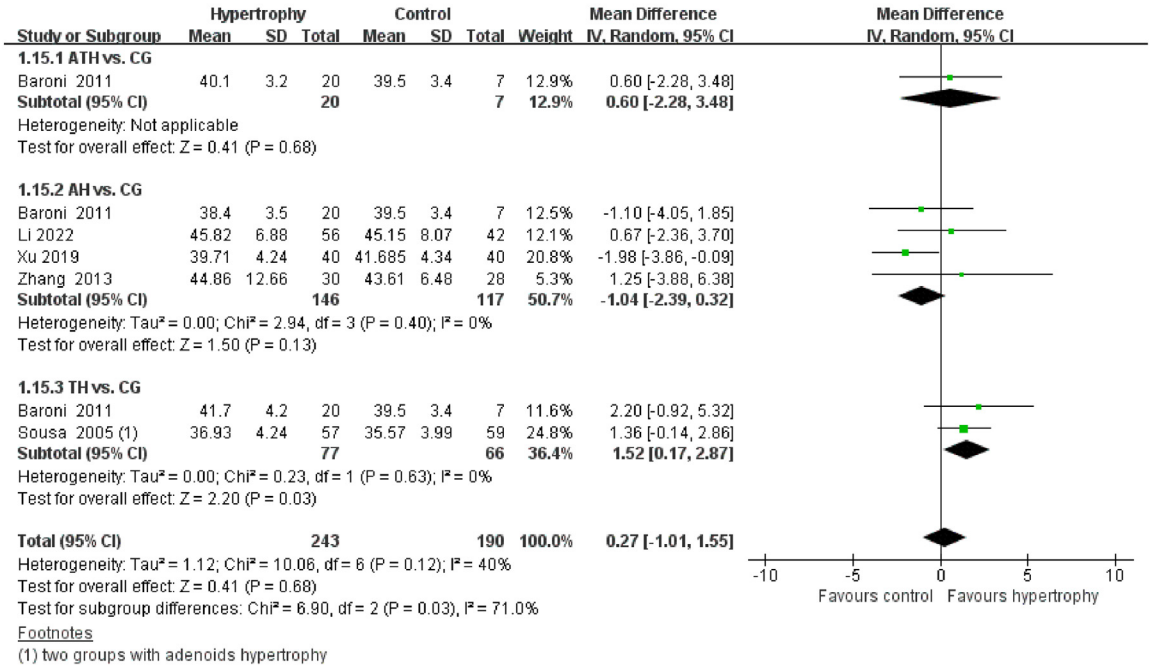
Supplementary Fig 3. (*continued*).

L

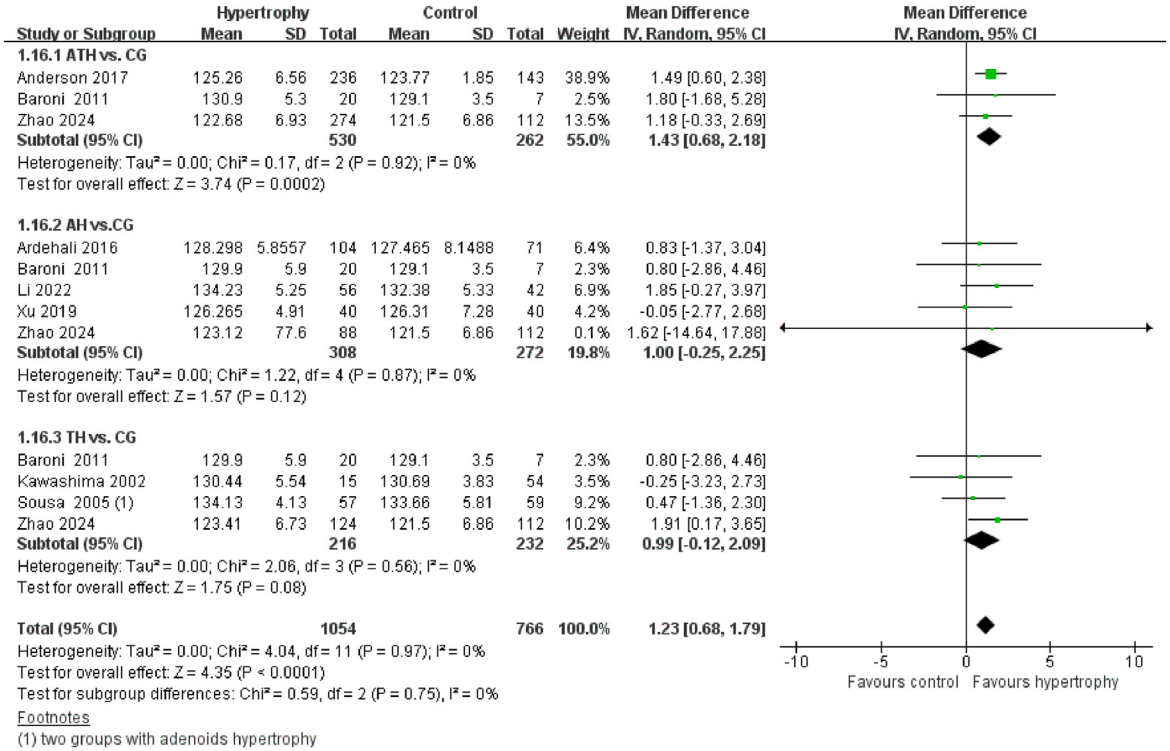


Supplementary Fig 3. (*continued*).

A

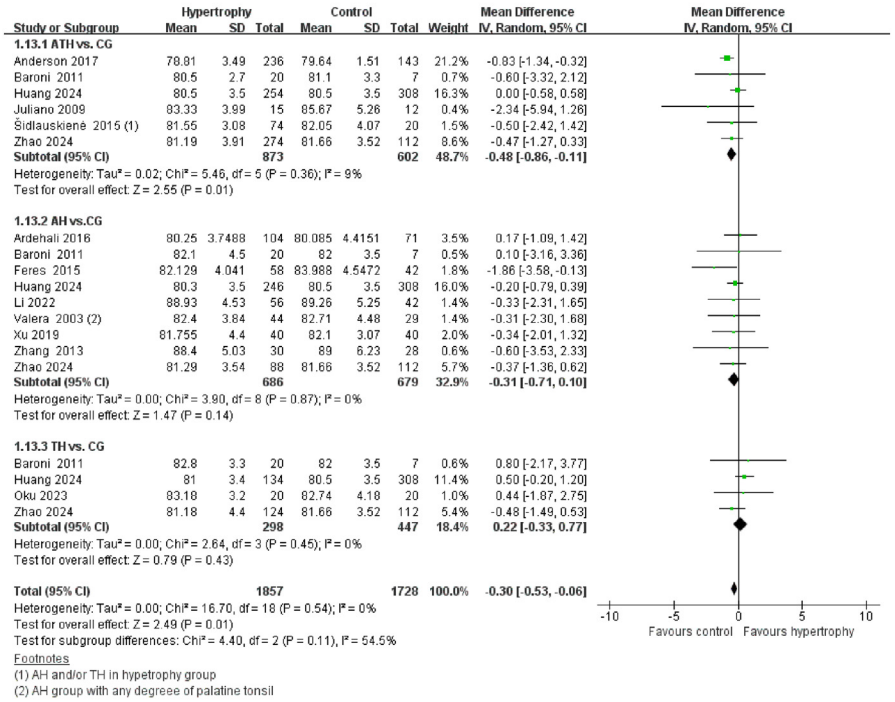


B

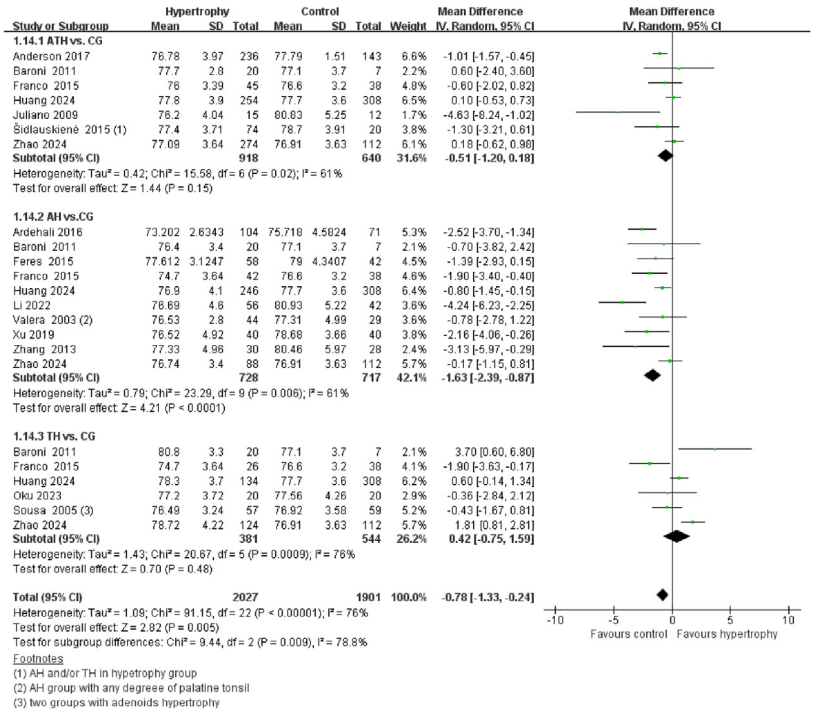


Supplementary Fig 4. MDs in craniofacial and dental outcomes in children with ATH and the controls: **A,** ArGo; **B,** ArGoMe angle; **C,** SNA; **D,** SNB.

C

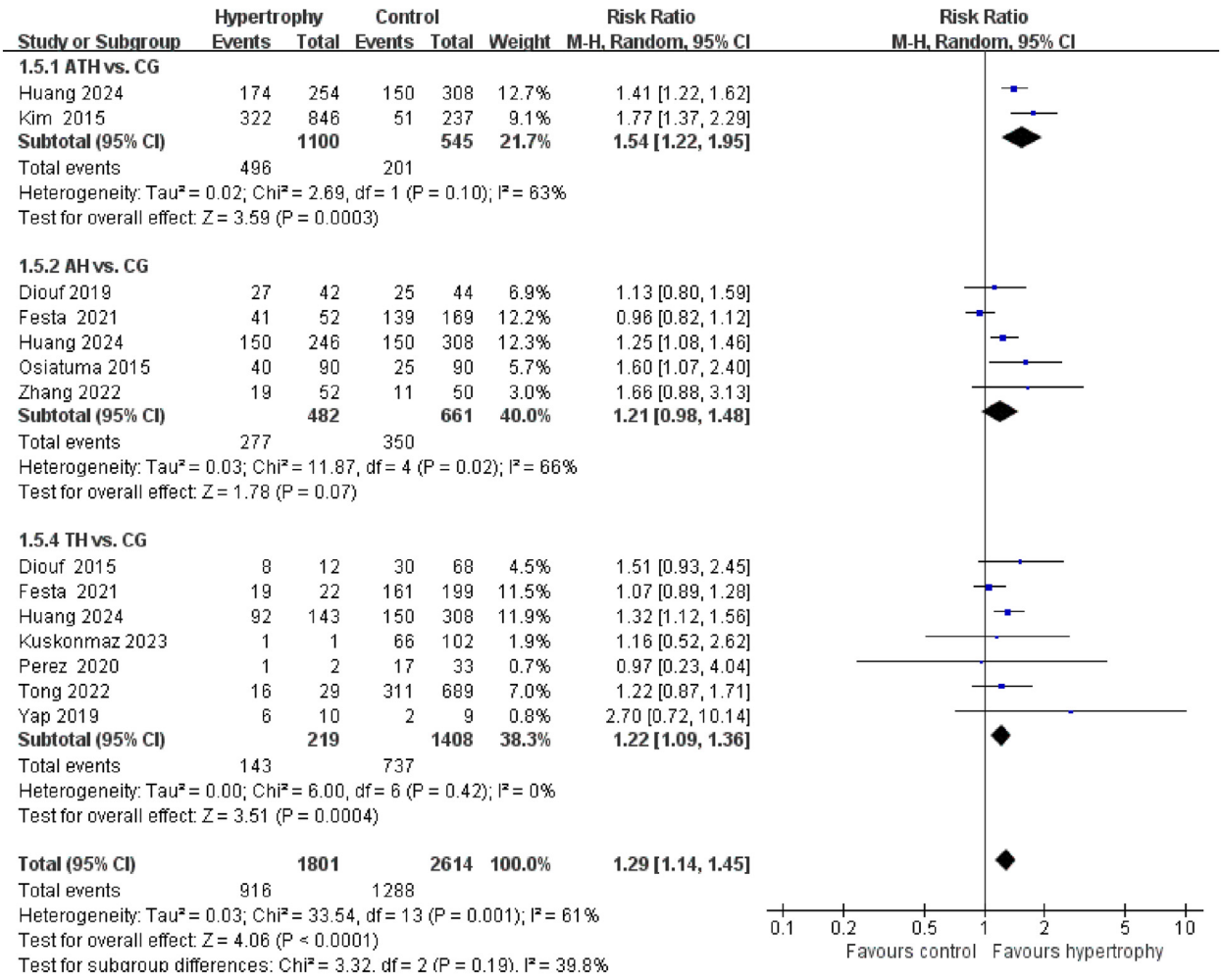


D

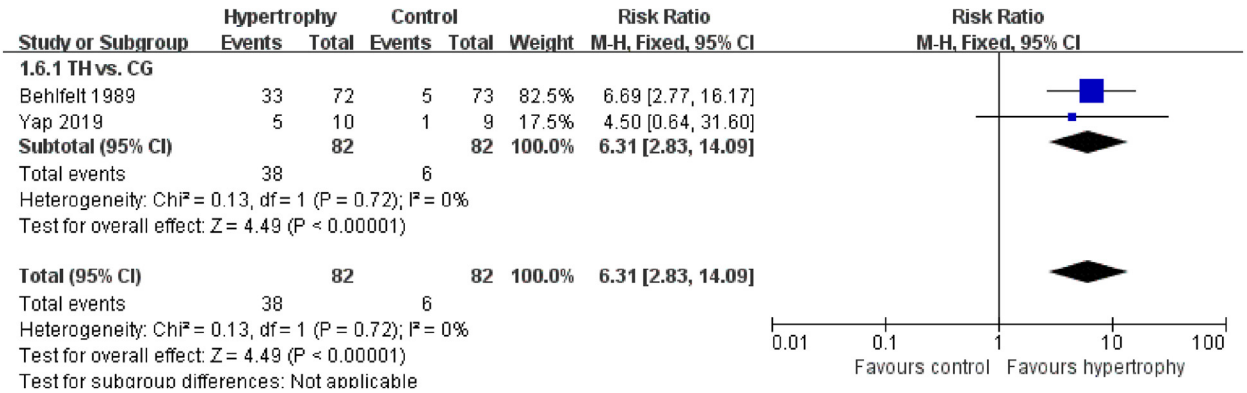


Supplementary Fig 4. (*continued*).

A

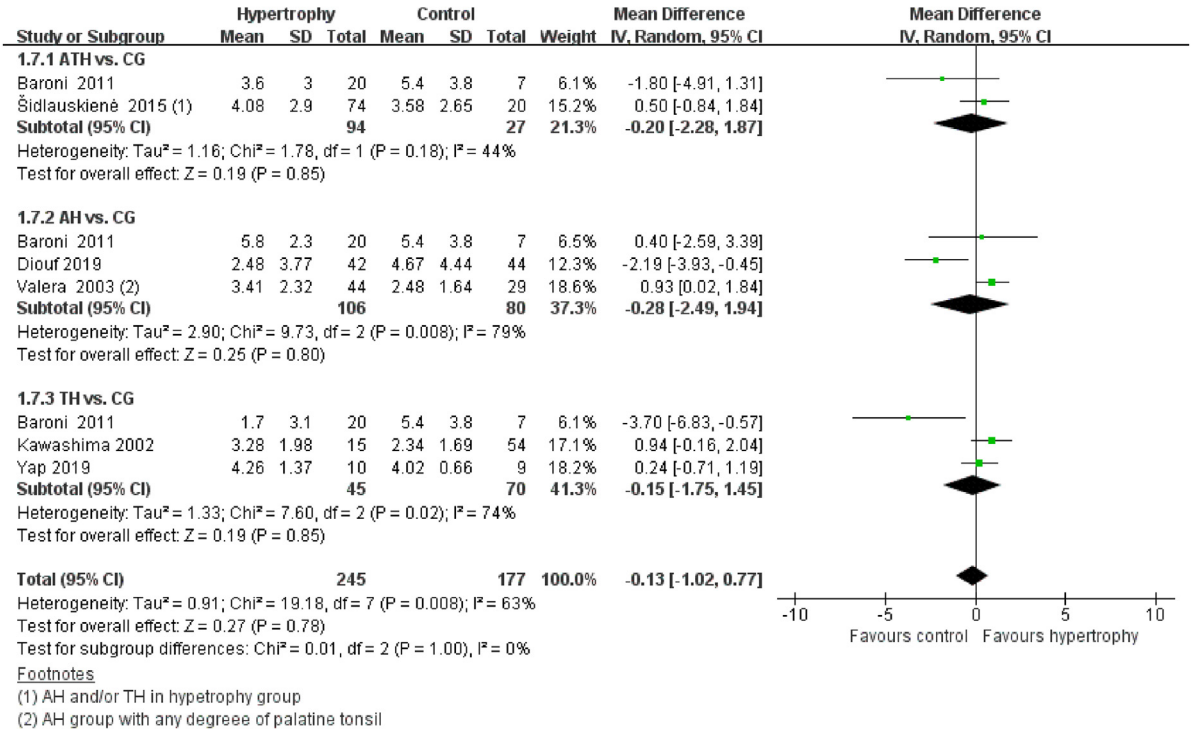


B



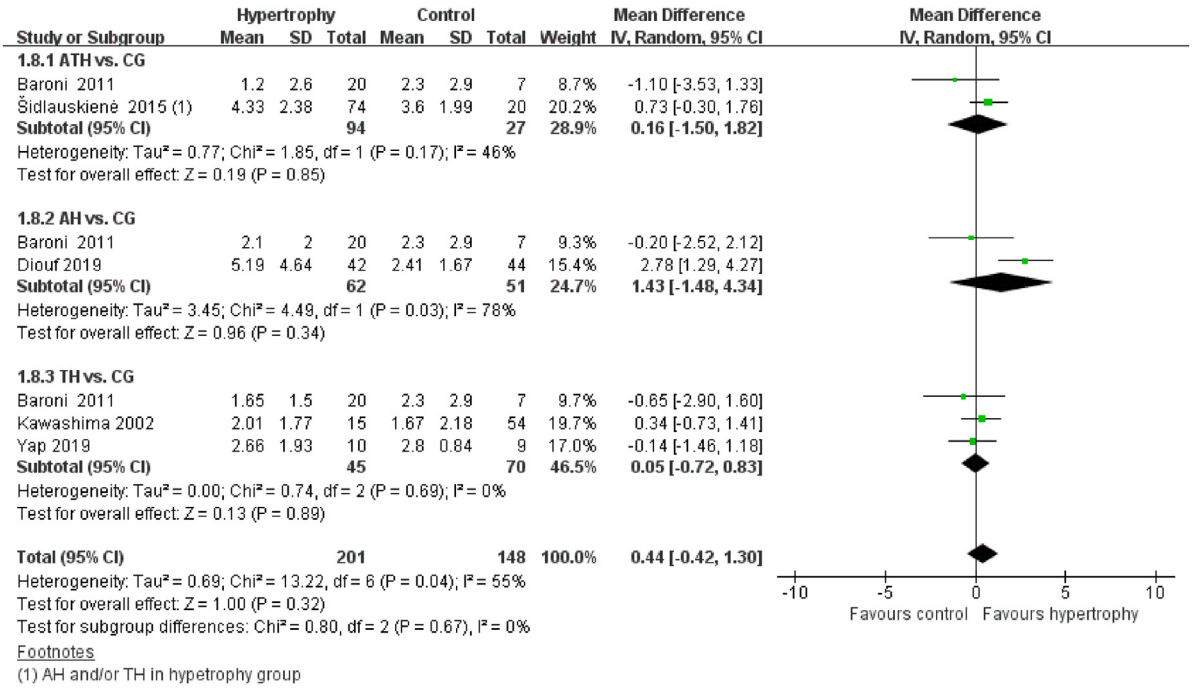
Supplementary Fig 5. MDs in anterior characteristics in children with and without ATH: **A,** Angle's classification; **B,** Crossbite; **C,** Overjet; **D,** Overbite; **E,** Open bite; **F,** U1-SN angle; **G,** L1-MP angle; **H,** U1-L1 angle.

C



Footnotes
(1) AH and/or TH in hypetrophy group
(2) AH group with any degreee of palatine tonsil

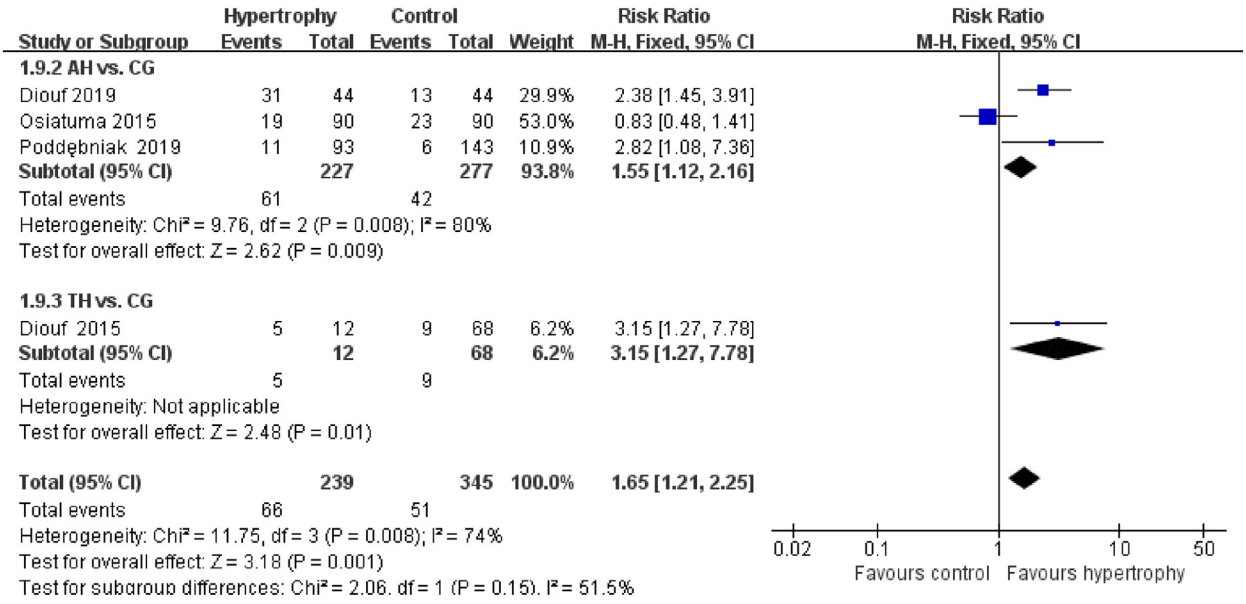
D



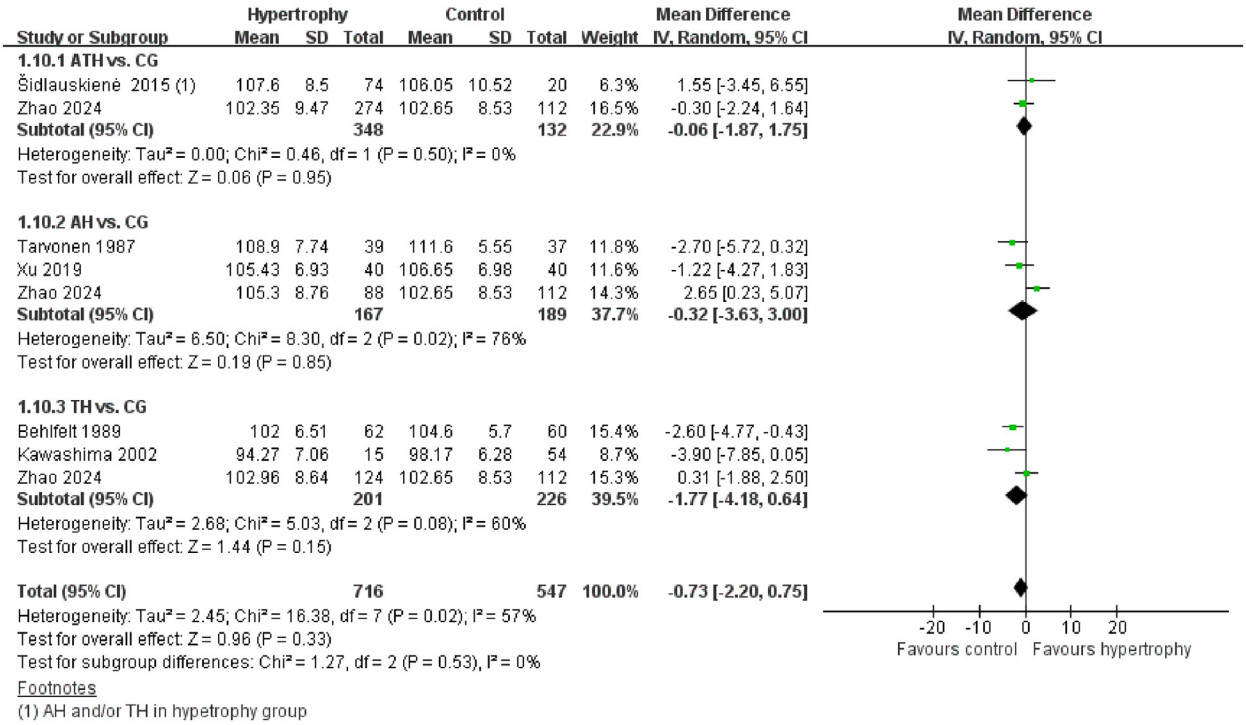
Footnotes
(1) AH and/or TH in hypetrophy group

Supplementary Fig 5. (*continued*).

E

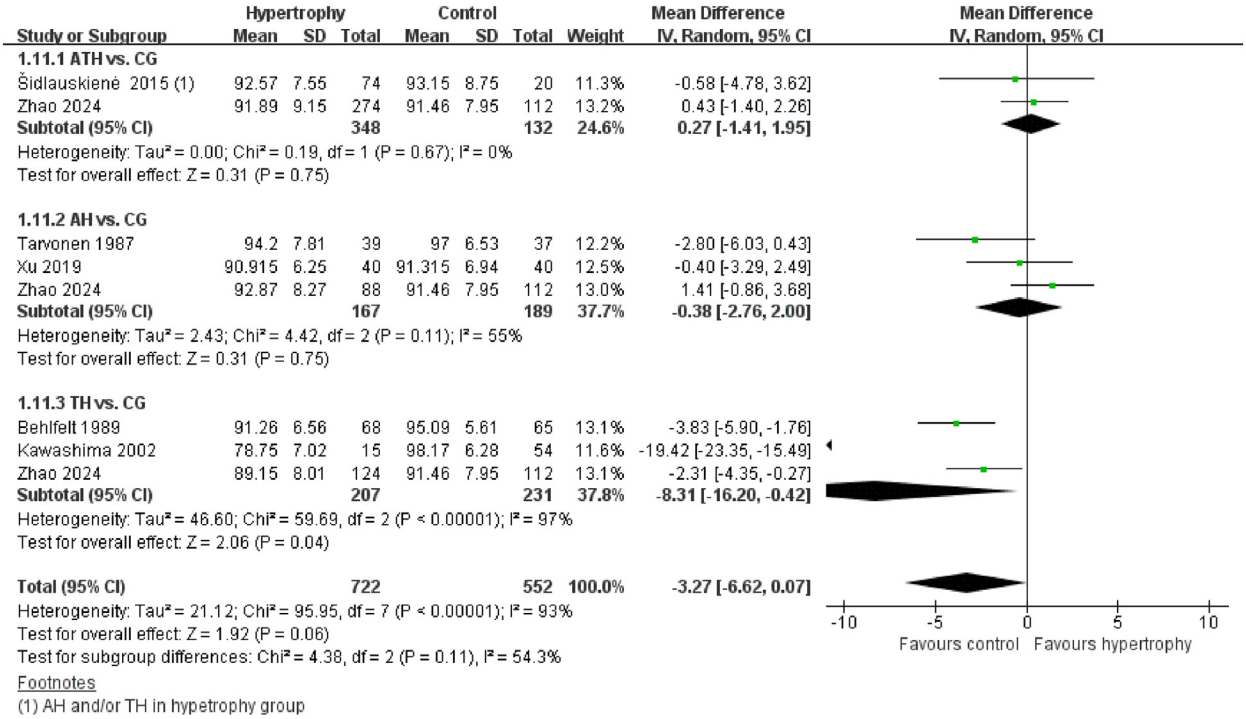


F



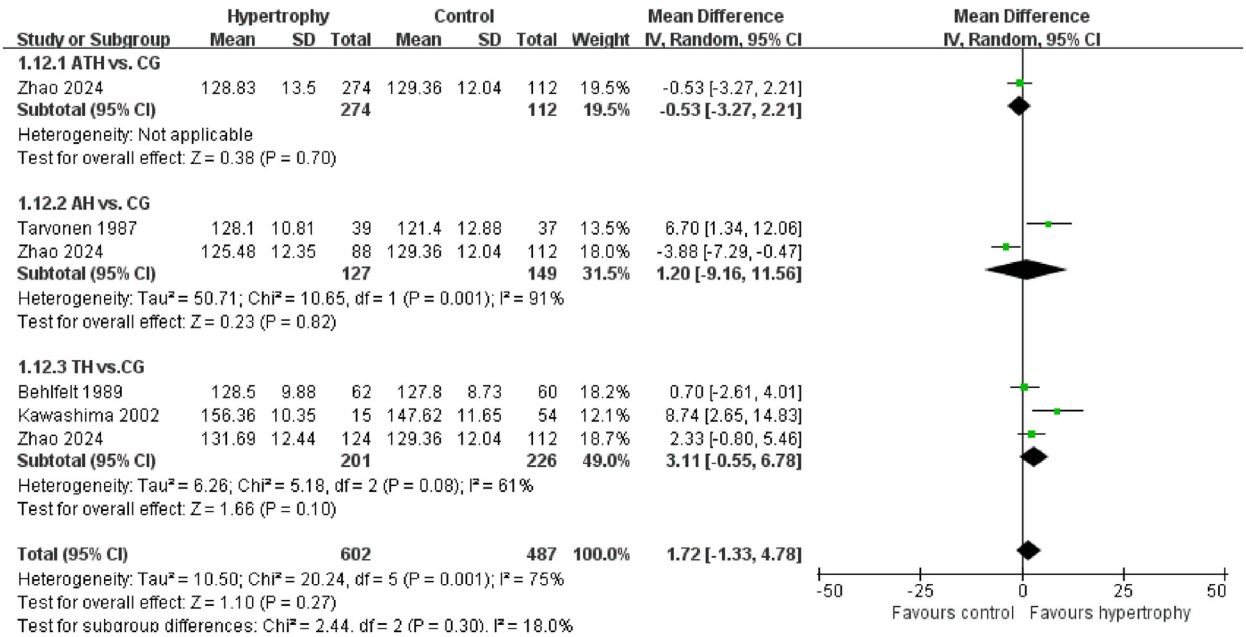
Supplementary Fig 5. (*continued*).

G



Footnotes
(1) AH and/or TH in hypetrophy group

H



Supplementary Fig 5. (*continued*).

Supplementary Table III. Summary of findings

Variables	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)†	Comments
	Assumed risk	Corresponding risk				
	Healthy children	Children with ATH				
SN-MP	The mean SN-MP angle in healthy children ranged 27.28-37.09	The mean SN-MP angle in ATH children was 2.20 higher (1.47-2.92)	–	3088 (15 studies)	⨁◯◯◯ Very low‡	There may be an increase in the SN-MP angle in ATH vs control
SNA	The mean SNA in the healthy children ranged 79.64-89.26	The mean SNA in the ATH children was 0.30 lower (0.53 lower to 0.06 lower)	–	2745 (13 studies)	⨁◯◯◯ Very low§	There may be a decrease in the SNA angle in ATH group vs control
SNB	The mean SNB angle in the healthy children ranged 75.72-80.93	The mean SNB angle in the ATH children was 0.78 lower (1.33 lower to 0.24 lower)	–	3079 (15 studies)	⨁◯◯◯ Very low¶	There may be a decrease in the SNB angle in ATH group vs control
ANB	The mean ANB angle in the healthy children ranged 2.60-8.59	The mean ANB angle in the ATH children was 0.13 lower (0.35 lower to 0.61 higher)	–	2918 (14 studies)	⨁◯◯◯ Very low#	The evidence does not show a difference in ANB
ArGoMe	The mean ArGoMe angle in the healthy children ranged 121.5-133.66	The mean ArGoMe angle in the ATH children was 1.23 higher (0.68 higher to 1.79 higher)	–	1596 (8 studies)	⨁◯◯◯ Very low‖	There may be an increase in the ArGoMe angle in ATH vs control
ArGo	The mean ArGo in the healthy children ranged 35.57-45.15	The mean ArGo in the ATH children was 0.27 higher (1.01 lower to 1.55 higher)	–	433 (5 studies)	⨁◯◯◯ Very low**	The evidence does not show a difference in ArGo
U1-ANS	The mean U1-ANS angle in the healthy children ranged 98.17-111.60	The mean U1-ANS angle in the ATH children was 0.73 lower (2.20 lower to 0.75 higher)	–	1039 (6 studies)	⨁◯◯◯ Very low††	The evidence does not show a difference in U1-SN
L1-MP	The mean L1-MP in the healthy children ranged 91.315-98.17	The mean L1-MP in the ATH children was 3.27 lower (6.62 lower to 0.07 higher)	–	1050 (6 studies)	⨁◯◯◯ Very low‡‡	The evidence does not show a difference in L1-MP
U1-L1	The mean U1-L1 in the healthy children ranged 121.40-147.62	The mean U1-L1 in the ATH children was 1.72 higher (1.33 lower to 4.78 higher)	–	765 (4 studies)	⨁◯◯◯ Very low§§	The evidence does not show a difference in U1-L1
Overjet	The mean overjet in the healthy children ranged 2.34-5.40	The mean overjet in the ATH children was 0.13 lower (1.02 lower to 0.77 higher)	–	422 (6 studies)	⨁◯◯◯ Very low¶¶	The evidence does not show a difference in overjet
Overbite	The mean overbite in the healthy children ranged 1.67-3.60	The mean overbite in the ATH children was 0.44 higher (0.42 lower to 1.30 higher)	–	349 (5 studies)	⨁◯◯◯ Very low##	The evidence does not show a difference in overbite
Angle classification	473 per 1000****	610 per 1000 (539-686)	RR, 1.29 (1.14-1.45)	3790 (11 studies)	⨁◯◯◯ Very low‖‖	Probably reduces the incidence of malocclusion

Supplementary Table III. Continued

Variables	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)†	Comments
	Assumed risk	Corresponding risk				
	Healthy children	Children with ATH				
Maxillary arch width	The mean maxillary arch width in the healthy children ranged 33.76-52.68	The mean maxillary arch width in the ATH children was 1.34 lower (2.12 lower to 0.56 lower)	–	511 (5 studies)	⨁◯◯◯ Very low[***]	There may be a decrease in the maxillary arch width in ATH vs control
Mandibular arch width	The mean mandibular arch width in the healthy children ranged 32.21-46.48	The mean mandibular arch width in the ATH children was 0.41 higher (0.19 lower to 1.00 higher)	–	439 (4 studies)	⨁◯◯◯ Very low[†††]	The evidence does not show a difference in mandibular arch width
Open bite	189 per 1000[****]	311 per 1000 (229-425)	RR, 1.65 (1.21-2.25)	584 (4 studies)	⨁◯◯◯ Very low[‡‡‡]	This probably reduces the incidence of open bite
Crossbite	90 per 1000[****]	568 per 1000 (255-1000)	RR, 6.31 (2.83-14.09)	164 (2 studies)	⨁◯◯◯ Very low[§§§]	This probably reduces the incidence of crossbite

Note. Study examined dental and craniofacial characteristics in children with or without ATH. Population included children with ATH. The setting was those aged <18 y. The intervention group was children with TH and/or AH, whereas the comparison group was children without TH and/or AH.

*The risk in the ATH group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the ATH (and its 95% CI); †GRADE Working Group grades of evidence: **High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect. **Moderate certainty:** we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. **Low certainty:** our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect. **Very low certainty:** we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect; ‡Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 73\%$) and serious risk of bias: 4 studies at high risk of bias; §Downgraded 2 levels due to study design and serious risk of bias: 3 studies at high risk of bias; ¶Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 76\%$) and serious risk of bias: 4 studies at high risk of bias; #Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 61\%$) and serious risk of bias: 4 studies at high risk of bias; ||Downgraded 2 levels due to study design and serious risk of bias: 2 studies at high risk of bias; **Downgraded 4 levels due to study design, serious imprecision and very serious risk of bias: 3 studies at high risk of bias; ††Downgraded 2 levels due to study design, substantial heterogeneity ($I^2 = 57\%$)and serious risk of bias: one study at high risk of bias; ‡‡Downgraded five levels due to study design, substantial heterogeneity ($I^2 = 93\%$), serious imprecision and serious risk of bias: one study at high risk of bias; §§Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 75\%$) and serious imprecision; ¶¶Downgraded 4 levels due to study design, substantial heterogeneity ($I^2 = 63\%$), serious imprecision and serious risk of bias: 2 studies at high risk of bias; ##Downgraded 4 levels due to study design, substantial heterogeneity ($I^2 = 55\%$), serious imprecision and serious risk of bias: one study at high risk of bias; ||||Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 61\%$), and serious risk of bias: 2 studies at high risk of bias; ***Downgraded 3 levels due to study design, substantial heterogeneity ($I^2 = 52\%$), and serious risk of bias: 2 studies at high risk of bias; †††Downgraded 3 levels due to study design, serious imprecision and serious risk of bias: one study at high risk of bias; ‡‡‡Downgraded 4 levels due to study design, substantial heterogeneity ($I^2 = 74\%$), and very serious risk of bias: 2 studies at high risk of bias; §§§Downgraded 2 levels due to study design and small sample size; ****Assumed risk was based on the positive events rate in the control groups of the included studies