Effects of chlorhexidine varnish on caries during orthodontic treatment: a systematic review and meta-analysis

Elaine Machado Pingueiro OKADA[a]
Larissa Nogueira Soares RIBEIRO[a]
Maria Bernadete Sasso STUANI[a]
Maria Cristina BORSATTO[a]
Tatiana Kelly da Silva FIDALGO[b]
Francisco Wanderley Garcia de PAULA-SILVA[a]
Erika Calvano KÜCHLER[a]

[a]Universidade de São Paulo - USP, School of Dentistry of Ribeirão Preto, Department of Pediatric Dentistry, Ribeirão Preto, SP, Brazil.

[b]Universidade do Estado do Rio de Janeiro-UERJ, School of Dentistry, Department of Community and Preventive Dentistry, Rio de Janeiro, RJ, Brazil.

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Corresponding Author:
Erika Calvano Küchler
E-mail: erikacalvano@gmail.com

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Abstract: This study aimed to perform a systematic review and meta-analysis to evaluate the effectiveness of chlorhexidine varnish on the reduction of caries incidence during fixed orthodontic treatment. The literature searches involved The Cochrane Library, Medline, Scopus, OpenSigle databases and manual searches. The search on OpenSigle did not produce any additional articles. Clinical studies conducted in patients with orthodontic fixed appliances that used professional application of chlorhexidine varnish were included. The effect-size was calculated and a meta-analysis was performed. From 182 abstracts, a total of six articles fulfilled the inclusion criteria. After reading the full articles, one was excluded because of lack of a control group. Three articles were used for continuous data analysis, and two articles were used for the dichotomous data analysis. The pooled meta-analysis with continuous data demonstrated chlorhexidine varnish effectiveness on caries reduction ($p = 0.003$), with a mean difference and confidence interval of −1.49 [−2.47, −0.51]. On the basis of the pooled meta-analysis of continuous data, we were able to conclude that professional application of chlorhexidine varnish is effective in caries incidence reduction during fixed orthodontic treatment.

Keywords: Chlorhexidine; Dental Caries; Orthodontics.

Introduction

Caries is a clinical and etiological heterogeneous condition[1] and is a major oral health problem in most industrialized countries.[2] Caries can present as a cavitated lesion, or as a non-cavitated lesion, also referred to as a white spot lesion (WSL). WSL is the incipient caries lesion and is limited to the enamel, in which its clinical presentation includes alterations in color of white, well-defined areas (According to The American Dental Association Caries)[3].

Different methods have been applied in caries prevention and control.[2] In orthodontic patients, caries prevention represents a significant challenge; in fact orthodontic treatment with fixed appliances is considered a risk factor for caries development.[4] A common complication in fixed orthodontic treatment is the development of WSL surrounding the orthodontic appliances[5,6]. Reportedly, 50% patients experienced an increase in WSL incidence

during orthodontic treatment.[6] In addition, caries progression occurs more rapidly in patients with orthodontic appliances.[7]

The extremely high cariogenic challenge that develops around brackets and beneath ilI-fitting bands explains the association between high caries experience and orthodontic fixed appliances. The biofilm formation on these regions acts as a highly organized microbial community[8] that is responsible for the WSL formation. This cariogenic environment of an orthodontic fixed appliance requires special preventive programs, such as the use of the chlorhexidine (CHX),[4] considered a safe, bacteriostatic and bactericidal agent to be indicated in dentistry.[9] Although several studies have reported an antimicrobial effect of CHX varnish in plaque formation, the clinical effectiveness of CHX varnish on caries prevention and control during fixed orthodontic treatment, (considered a high-risk factor for caries development), is still inconclusive. Therefore, the aim of this study was to evaluate the effectiveness of CHX varnish on the reduction of caries incidence during fixed orthodontic treatment.

Methodology

This systematic review and meta-analysis was performed according to Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement.[10] It was also registered with PROSPERO (CDR42015023395).

Search strategy

A search of the literature was performed manually during June 2015 using the following databases: Medline, Scopus, and The Cochrane Library. The gray literature was also consulted through Opensigle. Experts were contacted to identify unpublished and ongoing studies. The searches were complemented by the screening of references of selected articles to locate any study that did not appear in the database search.

The search strategy was based on the Medical Subject Heading terms (MeSH) or Text Word [tw] in different combination strategy. The following terms were used for each database:

a. Medline: chlorhexidine [MeSH Terms] AND orthodontic patients [tw] AND Demineralized White Lesion [tw] OR chlorhexidine [MeSH Terms] AND orthodontic patients [tw] AND White Spot Lesion [tw] OR chlorhexidine [MeSH Terms] AND Orthodontic Appliances [tw] AND Demineralized White Lesion [tw] OR chlorhexidine [MeSH Terms] AND Orthodontic Appliances [tw] AND White Spot Lesion [tw] OR chlorhexidine [MeSH Terms] AND orthodontics [MeSH Terms] AND Demineralized White Lesion [tw] OR chlorhexidine [MeSH Terms] AND orthodontics [MeSH Terms] AND White Spot Lesion [tw].

b. Scopus: chlorhexidine [Article Title/Abstract/Keyword] AND orthodontic patients [Article Title/Abstract/Keyword] AND Demineralized White Lesion [Article Title/Abstract/Keyword] OR chlorhexidine [Article Title/Abstract/Keyword] AND orthodontic patients [Article Title/Abstract/Keyword] AND White Spot Lesion [Article Title/Abstract/Keyword] OR chlorhexidine [Article Title/Abstract/Keyword] AND Orthodontic Appliances [Article Title/Abstract/Keyword] AND Demineralized White Lesion [Article Title/Abstract/Keyword] OR chlorhexidine [Article Title/Abstract/Keyword] AND Orthodontic Appliances [Article Title/Abstract/Keyword] AND White Spot Lesion [Article Title/Abstract/Keyword] OR chlorhexidine [Article Title/Abstract/Keyword] AND orthodontics [Article Title/Abstract/Keyword] AND Demineralized White Lesion [Article Title/Abstract/Keyword] OR chlorhexidine [Article Title/Abstract/Keyword] AND orthodontics [Article Title/Abstract/Keyword] AND White Spot Lesion [Article Title/Abstract/Keyword].

c. The Cochrane Library and Opensigle: chlorhexidine AND orthodontic patients AND Demineralized White Lesion OR chlorhexidine AND orthodontic patients AND White Spot Lesion OR chlorhexidine AND Orthodontic Appliances AND Demineralized White Lesion OR chlorhexidine AND Orthodontic Appliances AND White Spot Lesion OR chlorhexidine

AND orthodontics AND Demineralized White Lesion OR chlorhexidine AND orthodontics AND White Spot Lesion.

Eligibility criteria of the articles

The focused question was formulated according to the "*PICO* (Population, Intervention, Comparison, Outcome)" method. Specifically, clinical studies conducted in orthodontic young adult patients with orthodontic fixed appliances (P), with the use of professional CHX varnish (I), comparing the CHX varnish effectiveness with a control group (C) on the caries incidence (O).

Inclusion and exclusion criteria for the review were recognized as a priority, and applied independently by the authors. Split-mouth and parallel-group trials comparing CHX varnish to placebo or no treatment were included. The trials should have a minimum duration of 24 weeks. Articles that evaluated removable orthodontic appliances, fixed orthodontic appliances without brackets, or fixed orthodontic appliances with esthetic brackets (plastic or ceramic) were excluded.

The included articles were identified by the title and abstract, followed by the full text analyses. Two examiners (EMPO and LNSR) evaluated the articles. If there were differences in opinion, disagreement among examiners was reexamined in consensus meetings with a third examiner (ECK).

Data extraction and quality assessment

The examiners (EMPO, MBSS, and ECK) performed the independent data extraction and qualitative methodological quality assessment of the included articles. The data extraction included the articles' characteristics. We also attempted to extract any information regarding "effect modifiers" (factors possibly involved in the caries experience during orthodontic treatment) reported by the authors such as 1) water fluoridation; and 2) oral hygiene reinforcement program.

Qualitative scoring of 13 criteria was performed by two examiners (EMPO and MCB). The selected criteria were based on the guideline described by Fowkes and Fulton[11] and was used as a control for influence bias, to gain insight into potential comparisons and to guide interpretation of findings.

Meta-analysis

In order to standardize the continuous data for the quantitative analyses, when the results were presented as median, the mean value and the standard deviation was calculated using the formula proposed by Hozo et al.[12]

The calculation of the effect-size was performed in order to normalize the data, independent of the sample size and quantify the magnitude of the increase of caries incidence. The mean differences before–after debonding and their standard deviations were extracted. Means and standard deviations were converted into effect-size. Individual study effect-sizes were calculated and classified as suggested by Cohen,[13] as low effect ($d \leq 0.2$); medium effect ($0.21 \leq d \geq 0.79$); and high effect (≥ 0.8).

A meta-analysis was also performed to combine comparable results, using the Review Manager (version 5.3). The analyses were performed according to the data presented in each article; therefore, two Forest plots were constructed for the continuous data and for the dichotomous data.

For continuous data, the weighted mean differences of caries incidence between CHX and control groups were performed using the inverse-variance meta-analysis. For dichotomous data, the odds ratio was calculated in order to analyze the chance of an increase in caries incidence using the Cochran–Mantel–Haenszel test. We used a random-effects model because it takes into account the clinical heterogeneity of the included articles.[14,15]

We used the I^2 statistic to measure the extent of heterogeneity between articles, in which I^2 values of 25%, 50%, and 75% indicated low, medium, and high heterogeneity, respectively.

Results

The search in the databases resulted in 182 articles. Twenty-five duplicated articles were removed. Articles with titles and abstract not related to the subject were excluded, thus, seven articles remained to be read in entirety. One article was excluded because of the lack of a control group. Six articles[16,17,18,19,20,21] were included in the systematic

review. Figure 1 illustrates the search results. The contacted authors advised that there were no ongoing studies, and the search on OpenSigle yielded no additional articles.

The extracted data of the included articles are presented in the Table 1. Only one article[18] used 40% CHX varnish. The other five articles[16,17,19,20,21] used CHX varnish 1% with thymol 1%. Variances among the articles regarded the study design, CHX application, population, caries diagnosis and measurement, and duration of the trials.

Table 2 reported the qualitative scoring of the included articles. Only three articles[16,17,20] reported the use of randomization. None of the articles fully reported the sample size calculation.

Effect-size and meta-analysis

The effect-size calculation was performed for five[16,17,19,20,21] articles that presented the data as a continuous variable. The effect-size of each study is presented in Table 3.

The data from four[16,18,19,21] articles included studies that compared via meta-analysis. Three articles[16,18,19] were included in the continuous data meta-analysis, two articles[16,21] were included in the dichotomy data meta-analysis, and one article was

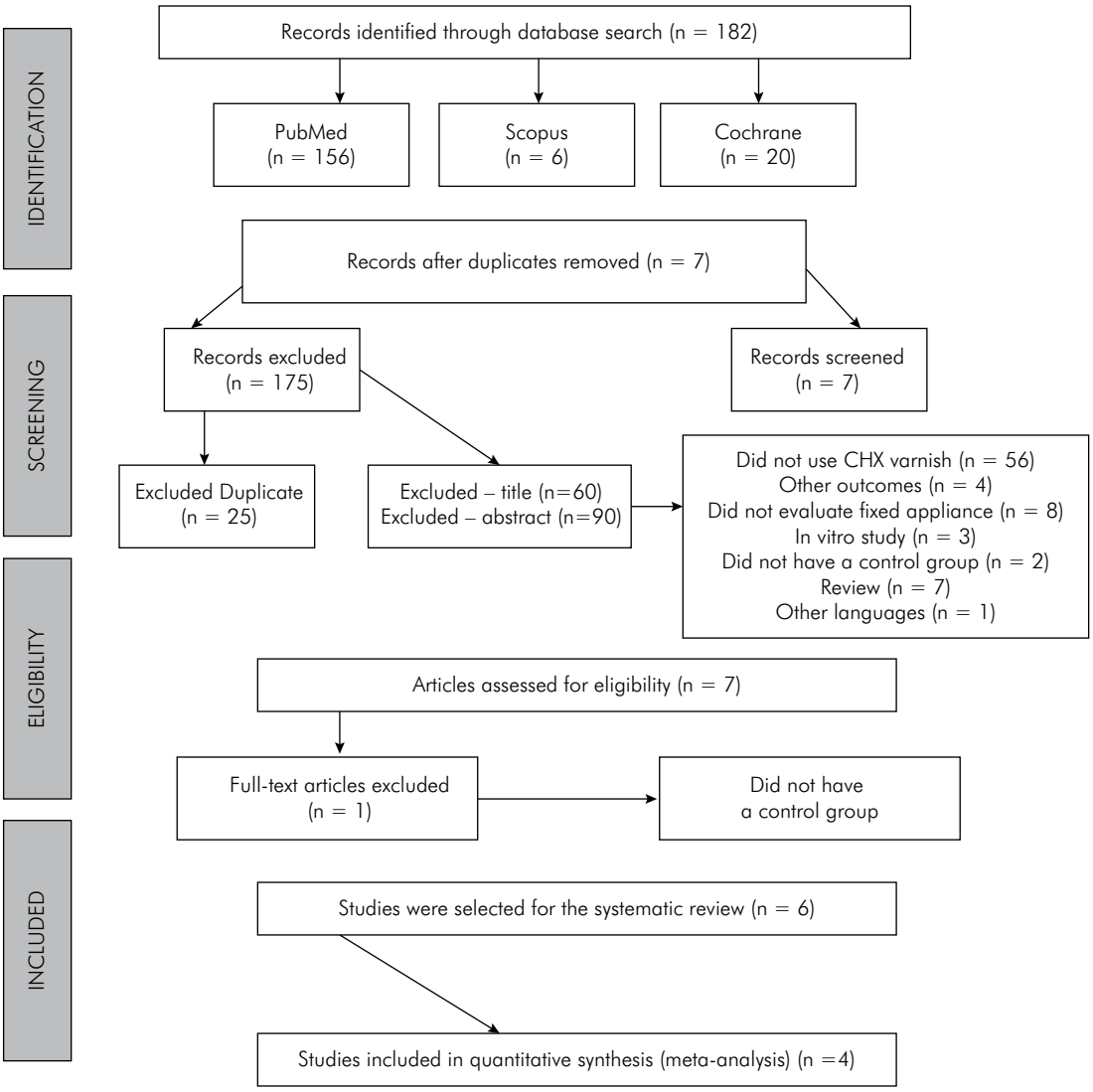


Figure 1. Literature search flow diagram.

Table 1. Characteristics of the included studies.

Author, year	Sample size (CHX/Control)	Comparison groups and type of intervention	Study design	Application frequency	Treated surfaces	Age rage	Caries experience measurement	Effect modifiers	Trial duration	Conclusion
Skold-Larsson et al., 2004[16]	24/21	Cervitec (1% CHX and 1% thymol) Placebo	Split-mouth	Every 6 weeks until debonding	Molars fissure	12–18	WSL using Diagnodent Laser fluorescence	Rinse daily 0,05% NaF + fluoride toothpaste and oral hygiene reinforcement	48 weeks	Caries were lower in CHX in the continuous variable analyses ($p < 0.05$)
Ogaard et al., 2001[17]	110/110	Cervitec (1% CHX/1% thymol) + fluoride varnish (0.7% F) Placebo + fluoride varnish	Case-control	Every 6 weeks until debonding	Around OFA and proximal	12–15	WSL index of Gorelick	Fluoride toothpaste and oral hygiene reinforcement	75 weeks	Not significant
Jenatschke et al., 2001[18]	18/15	EC-40 (40% CHX) Placebo	Case-control	Every 8 weeks until debonding	All	11–18	DMFS index system	Rinse daily 0,05% NaF + fluoride toothpaste and oral hygiene reinforcement	103 weeks	Not significant
Madléna and Vitalyos, 2000[19]	24/24	Cervitec (1% CHX/1% thymol) Placebo	Split-mouth	Every 13 weeks until debonding	Around OFA	12–23	DMFS index system	Oral hygiene reinforcement (fluoride exposure was unclear)	54 weeks	Caries were lower in CHX compared to placebo ($p < 0.05$)
Ogaard et al., 1997[20]	101/97	Cervitec (1% CHX/1% thymol) + fluoride varnish (0.7% F) Placebo + fluoride varnish (0.7% F)	Case-control	Every 6 weeks until debonding	Around OFA and proximal	12–15	WSL index of Gorelick	Fluoride toothpaste and oral hygiene reinforcement	24 weeks	Not significant
Twetman et al., 1995[21]	18/18	Cervitec (1% CHX/1% thymol) Placebo	Split-mouth	First week, 4th and 13th week	Around OFA	11–18	WSL index	Rinse daily (0,025%) NaF, fluoride toothpaste	27 weeks	Not significant

CHX: chlorhexidine; OFA: Orthodontic fixed appliances.

Table 2. Qualitative scoring of the included articles.

Component	Definition	Skold-Larsson et al., 2004 [16]	Ogaard et al., 2001[17]	Jenatschke et al., 2001[18]	Madléna and Vitalyos, 2000[19]	Ogaard et al., 1997[20]	Twetman et al., 1995[21]
1. Study design	Description of study design	NE	E	NE	NE	NE	NE
2. Participants	Eligibility criteria for participants	E	E	E	E	E	E
	Entry criteria and exclusion	E	E	E	E	E	E
3. Interventions	Sufficient details	E	E	E	E	E	P
	Description of modifier effects	E	E	E	E	E	E
4. Outcomes	Completely defined	E	E	E	E	E	E
5. Sample size	How sample size was determined	NE	NE	NE	NE	NE	NE
6. Randomization	Method used	P	P	NE	NE	E	NE
7. Blinding	Who was blinded and how	NE	NE	NE	NE	NE	NE
8. Control group acceptable	Definition of control	E	E	E	E	E	E
9. Statistical methods	Statistical methods used	E	E	E	E	E	E
	Methods for additional analyses	E	E	E	E	E	E
10. Participant flow	For each group, losses and exclusions	E	E	E	E	E	E
11. Baseline data	Baseline clinical of each group	E	E	E	NE	E	NE
12. Numbers analyzed	For each group	E	E	E	E	E	E
13. Interpretation	Consistent with results	E	E	E	E	E	E

NE: not explained; E: explained; P= partially.

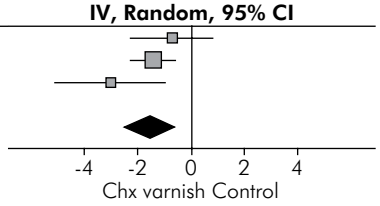
included in both analyses[16]. Two articles[17,20] were excluded from meta-analysis because in addition to the CHX, they applied fluoride varnish, hence constituting a bias.

The Forest plot of the mean difference between the CHX group and control group among the articles is presented in Figure 2. The pooled meta-analysis with continuous data demonstrated that CHX varnish was more effective on caries reduction than the placebo was (p = 0.003), with a mean difference and confidence interval of −1.49 [−2.47, −0.51]. Figure 3 presents the frequency difference among the articles that presented the data as a dichotomous variable. The dichotomous analysis did not demonstrate an association between CHX varnish and caries incidence reduction (OR = 0.52; confidence interval = 0.17, 1.59; p = 0.25). The overall heterogeneity (I^2) among the articles was low.

Table 3. Effect size of the included articles.

Author, year	Exposure	d	r	Type of effect
Skold-Larsson et al., 2004[16]	CHX	0.159	0.079	Low increase
	Control	0.769	0.359	Medium increase
Ogaard et al., 2001[17]	CHX	0.221	0.110	Medium increase
	Control	0.320	0.158	Medium increase
Jenatschke et al., 2001[18]	CHX	0.557	0.268	Medium increase
	Control	0.610	0.292	Medium increase
Madléna and Vitalyos, 2000[19]	CHX	1.140	0.487	High increase
	Control	1.467	0.590	High increase
Ogaard et al., 1997[20]	CHX	0	0	Low increase
	Control	0	0	Low increase

CHX: chlorhexidine.

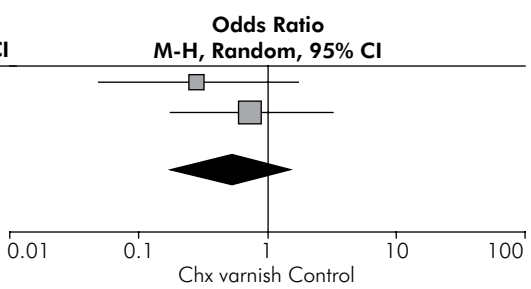


Study of subgroup	Chx varnish Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, Random. 95% CI
Jenatschke et al., 2001	4.12	2.12	18	4.83	2.29	15	28.2%	-0.71 [-2.23, 0.81]
Madiéna et al., 2000	0.65	0.75	24	2.05	1.93	24	53.8%	-1.40 [-2.23, -0.57]
Sköld-Larsson et al., 2004	0.5	1.8	24	3.5	4.5	21	18.0%	-3.00 [-5.05, -0.95]
Total (95% CI)			**66**			**60**	**100.0%**	**-1.49 [-2.47, -0.51]**

Heterogeneity. Tau^a = 0.28; Chi^a = 3.11, df = 2 (P = 0.21); I^a = 36%

Test for overall effect Z = 2.99 (P = 0.003)

Figure 2. Forest plots of the included studies in the continuous data analysis.



Study of subgroup	Chx varnish Events	Total	Control Events	Total	Weight	Odds ratio M-H, Random. 95% CI
Sköld-Larsson et al., 2004	2	24	5	21	39.5%	0.29 [0.05, 1.69]
Twetman et al., 1995	5	18	6	18	60.5%	0.77 [0.19, 3,19]
Total (95% CI)		**42**		**39**	**100.0%**	**0.52 [0.17, 1.59]**
Total events	7		11			

Heterogeneity. Tau^a = 0.00; Chi^a = 0.71, df = 2 (P = 0.40); I^a = 0%

Test for overall effect Z = 1.14 (P = 0.25)

Figure 3. Forest plots of the included studies in the dichotomous data analysis.

Discussion

During orthodontic treatment, proper oral hygiene is more difficult, resulting in biofilm retention and consequently, caries establishment and development. Previous studies attempted to review the evidence regarding CHX varnish in caries prevention. A systematic review performed by James et al.[22] reported that existing evidence does not support the use of CHX varnish for preventing caries in children and adolescents. On the other hand, a systematic review performed by Slot et al.[23] demonstrated that CHX varnish might have a place for high-risk patients, such as the elderly. Although orthodontic patients with fixed appliances are considered high risk, the studies are contradictory regarding the effectiveness of CHX varnish in caries prevention during orthodontic treatment. Therefore, we conducted a systematic review followed by meta-analysis to elucidate this query.

CHX is an oral antiseptic used to prevent gingivitis, periodontitis, and caries[9]. Different concentrations and formulations of CHX exist for oral health. CHX has bacteriostatic action at low concentration, and bactericidal action at high concentration[9]. A sizeable number of publications evaluating CHX in orthodontic patients were performed. A recent systematic review concluded that, indeed, the majority of studies found CHX varnish to be an effective antimicrobial against *mutans streptococci* [24]. However, a significant number of these articles did not evaluate caries as an outcome of the treatment[25,26]. Instead, they attempted to analyze *mutans streptococci* reduction as an outcome. *Mutans streptococci* are associated with caries, primarily initial caries lesions[8,27]. Regardless, we excluded the articles that evaluate only *mutans streptococci* reduction as an outcome because of the fact that presence of these microorganisms does not necessarily reflect the clinical presentation of the disease.

The long-term use of CHX mouth rinses can cause staining of the teeth and tongue[28]. However, this side effect can be eliminated with the use of local CHX varnish application[29]. In the selected articles, different concentrations of CHX varnishes were used. Jenatschke et al.[18] used 40% CHX varnish every 8 weeks; they relayed that the application of the viscous varnish was very difficult. Despite the high concentration of CHX used by the authors, no statistically significant difference was demonstrated. Simply a tendency toward improvement was shown.

CHX binds surrounding tissues and can be released again slowly over extended periods of time, a phenomenon known as substantivity[30]. Although CHX substantively is well reported, half of the selected articles used the split-mouth technique[16,19,21]. The spilt-mouth design could allow a bias for these studies, because of the potential for a carry-over effect from the test side to the control side[22].

The articles included have some methodological differences, although they exhibited low heterogeneity, demonstrating them to be comparable in our meta-analysis results. Although our forest plot of the dichotomous data did not demonstrate statistical difference between groups, this finding must be carefully interpreted. Our meta-analysis of the continuous data demonstrated an association between CHX varnish treatment and caries incidence reduction. The majority of articles included used the DMFS index for caries assessment. Sköld-Larsson et al.[16] used DMFS index and also laser-fluorescence for WSL detection. Although the studies presented slight differences in caries diagnosis, they maintained the same criteria for assessment of the control and experimental groups. We suggested that this is responsible for the low heterogeneity found in our results.

It is important to emphasize that although only the meta-analysis with continuous data presented a statistical difference between the groups, both meta-analyses demonstrated a protective effect for CHX varnish against caries during orthodontic treatment. We attributed this difference to the greater accuracy in values of continuous data. A clear example is the article of Sköld-Larsson et al.[16] included in the meta-analysis providing continuous and dichotomous data. The continuous data resulted in a statistical difference favoring CHX varnish. The dichotomous data favors CHX varnish but resulted in no statistically significant differences.

Results clearly demonstrate the need for additional, high quality clinical trials to examine CHX varnish effectiveness on caries prevention during orthodontic

treatment. However, on the basis of the pooled meta-analysis of the continuous data, our findings demonstrate the effectiveness of the CHX varnish treatment during fixed orthodontic treatment.

Conclusion

Our results suggest that CHX varnish is an effective preventive measurement to decrease caries incidence during fixed orthodontic treatment.

References

1. Küchler EC, Feng P, Deeley K, Fitzgerald CA, Meyer C, Gorbunov A et al. Fine mapping of locus Xq25.1-27-2 for a low caries experience phenotype. Arch Oral Biol. 2014;59(5):479-8. doi:10.1016/j.archoralbio.2014.02.009
2. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ. 2005;83(9):661-9. doi:10.1111/odi.12428
3. Young DA, Nový BB, Zeller GG, Hale R, Hart TC, Truelove EL. The American Dental Association Caries Classification System for clinical practice: a report of the American Dental Association Council on Scientific Affairs. J Am Dent Assoc. 2015;146(2):79-86. doi:10.1016/j.adaj.2014.11.018
4. Heymann GC, Grauer D. A contemporary review of white spot lesions in orthodontics. J Esthet Restor Dent. 2013;25(2):85-95. doi:10.1111/jerd.12013
5. Srivastava K, Tikku T, Khanna R, Sachan K. Risk factors and management of white spot lesions in orthodontics. J Orthod Sci. 2013;2(2):43-9. doi:10.4103/2278-0203.115081
6. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod. 1982;81(2):93-8. doi:10.1016/0002-9416(82)90032-X
7. Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development.. Am J Orthod Dentofacial Orthop. 1988;94(1):68-73. doi:10.1016/0889-5406(88)90453-2
8. Struzycka I. The oral microbiome in dental caries. Pol J Microbiol. 2014;63(2):127-35.
9. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. Minerva Stomatol. 2012;61(9):399-419.
10. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009;62(10):1006-12. doi:10.1016/j.jclinepi.2009.06.005
11. Fowkes FG, Fulton PM. Critical appraisal of published research: introductory guidelines. BMJ. 1991;302(6785):1136-40. doi:10.1136/bmj.302.6785.1136
12. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Method. 2005;5(1):13. doi:10.1186/1471-2288-5-13
13. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. New Jersey: Lawrence Erlbaum; 1988.
14. Colditz GA, Burdick E, Mosteller F. Heterogeneity in meta-analysis of data from epidemiologic studies: a commentary. Am J Epidemiol. 1995;142(4):371-82.
15. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials.1986;7(3):177-88. doi:10.1016/0197-2456(86)90046-2
16. Sköld-Larsson K, Fornell AC, Lussi A, Twetman S. Effect of topical applications of a chlorhexidine/thymol-containing varnish on fissure caries assessed by laser fluorescence. Acta Odontol Scand. 2004;62(6):339-42. doi:10.1080/00016350410010072
17. Øgaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. Am J Orthod Dentofacial Orthop. 2001;120(1):28-35. doi:10.1067/mod.2001.114644
18. Jenatschke F, Elsenberger E, Welte HD, Schlagenhauf U. Influence of repeated chlorhexidine varnish applications on mutans streptococci counts and caries increment in patients treated with fixed orthodontic appliances. J Orofac Orthop. 2001;62(1):36-45. doi:10.1007/PL00001917
19. Madléna M, Vitalyos G, Márton S, Nagy G. Effect of chlorhexidine varnish on bacterial levels in plaque and saliva during orthodontic treatment. J Clin Dent. 2000;11(2):42-6.
20. Ogaard B, Larsson E, Glans R, Henriksson T, Birkhed D. Antimicrobial effect of a chlorhexidine-thymol varnish (Cervitec) in orthodontic patients: a prospective, randomized clinical trial. J Orofac Orthop. 1997;58(4):206-13.
21. Twetman S, Hallgren A, Petersson LG. Effect of an antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. Caries Res. 1995;29(3):188-91. doi:10.1159/000262067
22. James P, Parnell C, Whelton H. The caries-preventive effect of chlorhexidine varnish in children and adolescents: a systematic review. Caries Res. 2010;44(4):333-40. doi:10.1159/000315346
23. Slot DE, Vaandrager NC, Van Loveren C, Van Palenstein Helderman WH, Van der Weijden GA. The effect of chlorhexidine varnish on root caries: a systematic review. Caries Res. 2011;45(2):162-73. doi:10.1159/000327374

24. Tang X, Sensat ML, Stoltenberg JL. The antimicrobial effect of chlorhexidine varnish on mutans streptococci in patients with fixed orthodontic appliances: a systematic review of clinical efficacy. Int J Dent Hyg. 2016;14(1):53-61. doi:10.1111/idh.12163
25. Attin R, Yetkiner E, Aykut-Yetkiner A, Knösel M, Attin T. Effect of chlorhexidine varnish application on streptococcus mutans colonisation in adolescents with fixed orthodontic appliances. Aust Orthod J. 2013;29(1):52-7.
26. Baygin O, Tuzuner T, Ozel MB, Bostanoglu O. Comparison of combined application treatment with one-visit varnish treatments in an orthodontic population. Med Oral Patol Oral Cir Bucal. 2013;18(2):e362-70. doi:10.4317/medoral.18261
27. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008;46(4):1407-17. doi:10.1128/JCM.01410-07
28. Flötra L, Gjermo P, Rölla G, Waerhaug J. Side effects of chlorhexidine mouth washes. Scand J Dent Res. 1971;79(2):119-25. doi:10.1111/j.1600-0722.1971.tb02001.x
29. Matthijs S, Adriaens PA. Chlorhexidine varnishes: a review. J Clin Periodontol. 2002;29(1):1-8. doi:10.1034/j.1600-051x.2002.290101.x
30. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. Iran Endod J. 2008;2(4):113-25.