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Effect of Chlorhexidine on Immediate and Delayed Bond Strength between Resin and Dentin of Primary Teeth: A Systematic Review and Meta-Analysis

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A B S T R A C T

Objectives: The purpose of this study was to systematically review the literature and use meta-analysis to investigate whether chlorhexidine (CHX) application after acid etching as an adjunct treatment has any influence on the immediate and delayed bond strength to primary dentin.

Materials and Methods: In this review, PubMed, ISI (all data bases), Scopus and Cochrane were searched according to the selected keywords up to April 30, 2018. The full texts of all published articles that met our primary inclusion criteria were obtained. The studies were analyzed in two parts: in vitro studies that evaluated the effect of CHX application during the bonding procedures (application after acid etching) on immediate and delay dentin bond strength of resin-dentin interface.

Results: The initial search yielded 214 publications, of which 8 were selected after thorough methodological assessment. None of the clinical studies fulfilled the eligibility criteria. Our results indicated that in comparison to the control group, CHX significantly reduced immediate resin-dentin bond strength (P=0.043). These values were increased after aging (P<0.001).

Conclusion: Based on this invitro Meta-analysis CHX application, improve resin-dentin bond strength durability in primary teeth.

Keywords: Dental Stress Analysis; Chlorhexidine; Tooth, Deciduous

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INTRODUCTION

Considerable evidence based on past research, noted that bonds created in dentin by resin-based adhesives may not be durable [1,2] and bond degradation occurs in etch and rinse and etch and dry adhesives with different mechanisms. Many studies noted that bond degradation in etch and rinse adhesives mainly transpires because of degradation of naked collagen fibrils in the demineralized dentin zone under the hybrid layer by host-derived matrix metalloproteinases (MMPs) in the dentin matrix [3-5]. MMPs are capable of degrading organic dentin matrix after demineralization [6].

MMPs are a group of zinc/calcium-dependent endopeptidases that are responsible for degrading collagen fibrils, in their natural [7] or denatured [8] form. These proteins are expressed by odontoblasts and other pulp

cells during extracellular matrix synthesis [9] and MMP-8 is the dominant abundant MMP in dentin [10]. MMPs are activated in dentin due to the local acidic pH as observed during the carious process or acid-etching step of adhesive protocols [11].

In etch and rinse adhesives, when the depth of the dentin demineralization exceeds the capacity of resin infiltration, a resin-free demineralized dentin zone with exposed collagen fibrils is created at the base of the hybrid layer [4,12,13], which is susceptible to hydrolytic and enzymatic degradation, especially by dentin MMPs that are activated during acid etching [11]. Thus, MMPs are mainly effective in the destruction of the bond in etch and rinse adhesive. Hebling et al. [5] demonstrated that in exfoliating primary molars, clinical degradation of hybrid layers by host-derived MMPs, occurred at a rate 3-5 years faster than accelerated aging required for such conditions to be identified in vitro.

The use of synthetic substances that inactivate MMPs enzymes like biologic MMP inhibitors, may increase the stability of resin–dentin bonds.

It has been noted that chlorhexidine (CHX) solutions, beside their antimicrobial activity, are able to inhibit MMPs and therefore reduce the collagen fibril solubility in an aqueous medium [3,5,14].

Many studies have demonstrated that the application of CHX to permanent dentin between the acid-etch and adhesive systems was able to prevent, or at least decelerate, the degradation of exposed collagen fibrils at the bottom of the hybrid layer after aging without influencing immediate bond [15,16].

As primary teeth are less mineralized and present a higher organic material content in comparison with their permanent counterparts [17], the hybrid layers created on primary dentin would be more susceptible to degradation over time [18]. It was therefore postulated that the response of primary dentin to CHX can be different from that of permanent dentin because of the differences inherent in their structures.

The purpose of the current investigation was to perform a systematic review and meta-analysis to examine whether or not application of CHX after acid-etching affects short or long term bonding strength as measured by resin-dentin bond strength to primary dentin.

MATERIALS AND METHODS

Eligibility criteria and search strategy

This study was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [19].

Population, intervention, comparison, outcome, and study design (PICOS) respectively included primary tooth dentin restored with composite resin, use of chlorohexidine after etching, no application of chlorohexidine after etching, bond strength (MPa) measured over time, and clinical or in vitro studies that measured bond strength immediately and six months or more afterwards.

A literature search was performed in four databases PubMed, Web of Science™ (consists of Core Collection, Biosis Citation Index, Biosis Previews, Current Contents Connect, Data Citation Index, Derwent Innovations Index, KCI Korean Journal Database, MEDLINE, Russian Science Citation Index, SciELO Citation Index, Zoological Record), Scopus and Cochrane, without any language or time limitation with the earliest citation in 2003 and the last search was performed on April 30, 2018.

The PubMed search strategy was as follows:

(("Tooth"[Mesh] OR teeth OR tooth OR "Mouth"[Mesh] OR mouth OR oral OR dent*) AND ("Chlorhexidine"[Mesh] OR Chlorhexidine OR (Chlorhexidine Hydrochloride) OR (Hydrochloride, Chlorhexidine) OR Tubulicid OR Novalsan OR (Sebidin A)) AND ("Dentin"[Mesh] OR Dentin OR Dentine OR Dentins OR Dentines) AND ("Tooth, Deciduous"[Mesh] OR (Tooth, Deciduous) OR (Milk Teeth) OR (Primary Teeth) OR (Baby Tooth) OR (Baby Teeth)))

In order to find out additional studies not included in the initial literature search, the cited reference lists of all the included studies were manually screened.

Table 1: Detailed summary of studies included in the meta-analysis

Study	Storage time	Bond strength test	Adhesive system	Without CHX group		With CHX group		CHX %/time (s)
				Sample size sticks	Bond strength (MPa) Mean±SD	Sample size sticks	Bond strength (MPa) Mean±SD	
Ersin (2009) [22]	24h water	MTBS	Prime&Bond NT/surefilrage	10-15	23.2±6.2	10-15	22.6±6.9	2/60
Kapdan (2015) [23]	24h water/MTBS	MTBS	Prime&Bond NT/Compomer	26	19.82±6.16	26	15.07±6.58	2/30s
Leitune (2011) [15]	24h water	MsBS	Scotch Bond Multi Purpose/Z350	10	22.37±3.69	10	22.30±3.66	2/30
	6 months			10	19.93±2.05	10	24.48±2.24	2/30
Lenzi (2014) [24]	24h water	MTBS	Adper Single Bond/Filtek Z250	38	70.1±3.8	34	32.8±3.8	2/60
	6 months			36	24.2±3.6	36	31.3±2.6	2/60
Manfro (2012) [25]	24h water	MTBS	Single bond/Filtek Z250	35	50.8±12.8	35	49.3±2.6	0.5/30
							32.3±7.9	
	12 months			35	20.4±3.7	35	44.0±8.7	2/30
							34.6±5.1	
Oznurhan (2015) [26]	24h water	MTBS	Prime and bond NT /Tetric N-Ceram	10	6.38±2.47	10	7.58±3.18	2/20
Ricci (2010) [14]	24h water	MTBS	Adper Single Bond	23	41.4±11.9	31	47.4±9.5	2/60
			Prime Bond NT	26	40.8±13.4	40	48.0±9.8	
			Excite	29	43.4±12.0	21	45.2±9.2	
Vieira Rde (2003) [27]	24h water	SBS	Single bond/Filtek Z 250	10	19.88±1.04	10	17.99±1.15	2/40

MTBS: micro tensile bond strength; SBS: shear bond strength; CHX: chlorhexidine; MSBS: micro shear bond strength

Screening and selection

As we found no relevant clinical studies in our selected articles, the inclusion criteria were in vitro studies that evaluated the effect of CHX application on immediate dentin bond strength of resin-dentin during the adhesive step (application after acid etching) and after aging of the adhesive interface (at least 6 months using any type of aging). Only studies that evaluated the bond strength on primary dentin and had a control group (without application of CHX as comparison) were included. The included studies must have complete report of statistical data such as the sample size, mean, and standard deviation. Papers that did not provide such data after at least twice e-mail request to authors, were excluded. Moreover, studies in which CHX was applied before etching or with the storage time shorter than 6 months were excluded.

At first, two independent reviewers evaluated the titles of studies identified in the searches. If the title indicated possible inclusion, the abstracts were reviewed and selected per the reviewer's agreement. Then the full texts of eligible articles were read to determine whether each met the criteria. Some data were requested of corresponding authors whenever necessary.

A summary of the included studies is presented in Table 1.

Data extraction

One author collected the data from each study then another author checked the collected data for accuracy by using a standardized outline. The data included storage time, type of bond strength test, adhesive system, sample size, bond strength in MPa and standard deviation, CHX application time and CHX concentration.

In the selected studies, only the data of interest were extracted to be analyzed in the meta-analysis. As this study was based on in-vitro studies, to assess individual risk of bias in each eligible study, 6 methodological items [20] were analyzed as follows: teeth randomization, use of caries or restoration free teeth, use of materials according to the manufacturer's instructions, application of restoration by same operator, description of sample size calculation, blinding of the operator of the testing machine. If the authors presented the criteria, the paper had a Y (yes) on that specific criteria; if the information was not reported, the paper received an N (no).

Papers that reported 1 or 2 items were classified as high risk of bias, 3 or 4 as medium risk, and 5 to 6 as low risk.

Statistical analysis

Mean and standard deviation of bond strength data including shear bond strength (SBS) or micro tensile bond strength (MTBS) data, and the sample size of each study were used to compare the included articles. The Cochrane Q test was used to assess heterogeneity of the treatment effect among the eligible studies, in which the significance level was set at P=0.05. Furthermore, we used the I^2 index to quantify the degree of heterogeneity, in which values greater than 50% were considered indicative of high heterogeneity [21].

The first global analysis was carried out using random effect model and two subgroup analyses were carried out to find the heterogeneity between the studies: 1) resin-dentin bond strength after the CHX application against control at baseline; 2) resin-dentin bond strength after the CHX application against control after at least 6 months of aging. Comprehensive Meta-Analysis Version 2 (Biostat Inc., Englewood, NJ, U.S.A.) was used for the statistical analyses. The influence of the time of the CHX application on studies on resin- dentin bond strength was analyzed using descriptive statistics.

RESULTS

Risk of bias

Of the 8 studies included, 12.5% showed low risk of bias, while the majority (87.5%) were medium-risk and none of them demonstrated high risk of bias (Table 2).

Study search and meta-analysis

The PRISMA flowchart of the included studies is presented in Fig. 1. An initial assessment of the articles led to 214 studies. However, after removing duplicates and excluding non-relevant records, 8 articles that met our inclusion criteria were analyzed for scientific and methodological accuracy.

Table 2: Risk of Bias Considering Aspects Reported in the Materials and Methods Section

First Author (year)	Teeth randomization	Teeth free of caries or restoration	Materials used according to manufacturers' instructions	Adhesive procedures performed by the same operator	Sample size calculation	Blinding of the operator using the testing machine	Risk of bias
Ersin (2009)	Yes	Yes	Yes	Yes	Yes	No	Low
Kapdan (2015)	Yes	Yes	Yes	No	Yes	No	Medium
Leitune (2011)	Yes	Yes	Yes	No	Yes	No	Medium
Lenzi (2014)	Yes	Yes	Yes	No	Yes	No	Medium
Manfro (2012)	Yes	Yes	Yes	Yes	No	No	Medium
Oznurhan (2015)	Yes	Yes	Yes	No	No	No	Medium
Ricci (2010)	Yes	Yes	Yes	Yes	No	No	Medium
Vieira Rde(2003)	Yes	Yes	Yes	No	Yes	No	Medium

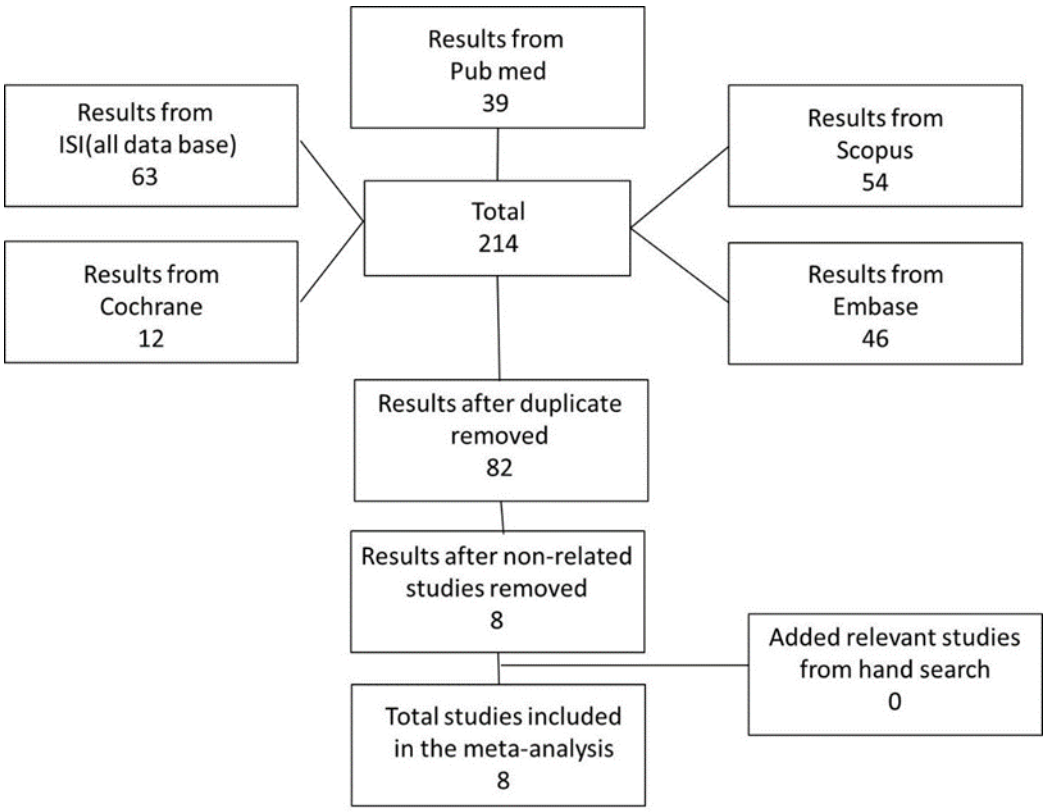


Fig. 1. PRISMA flowchart of the included studies and the used search strateg.

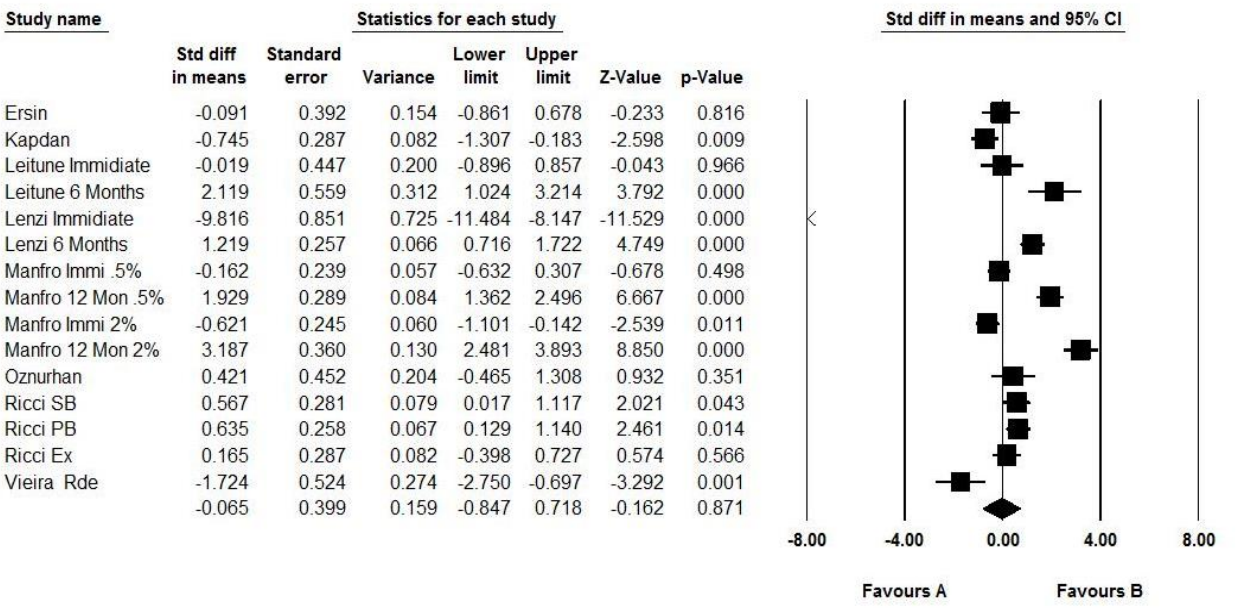


Fig. 2. Forest plot comparison of the resin- dentin bond strength after CHX application (Global analysis)

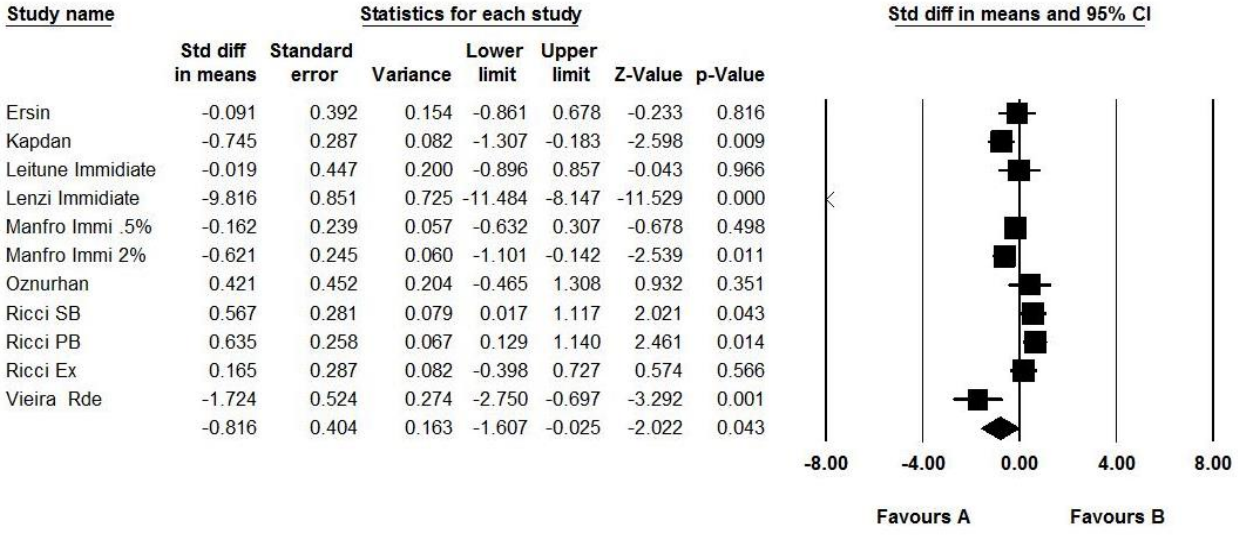


Fig. 3. Forest plot comparison of the resin- dentin bond strength after CHX application vs. control at baseline.

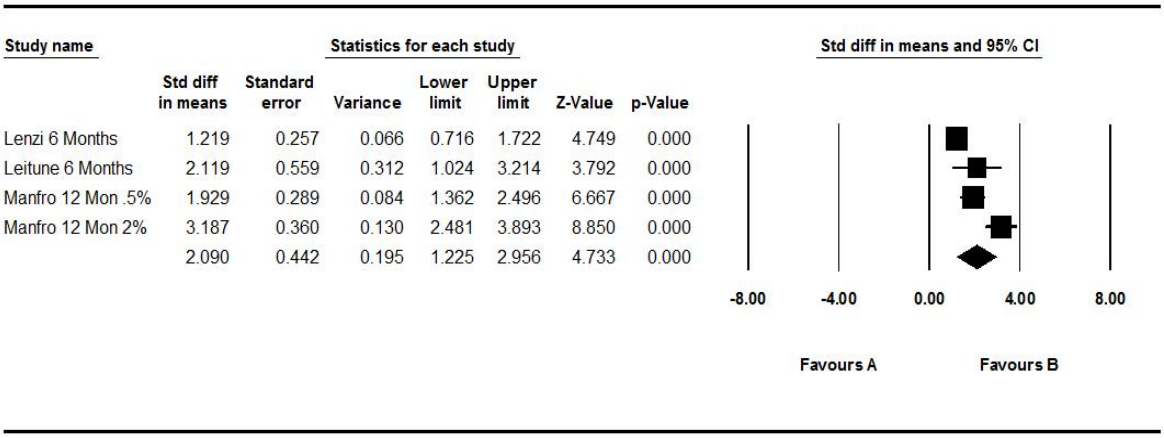
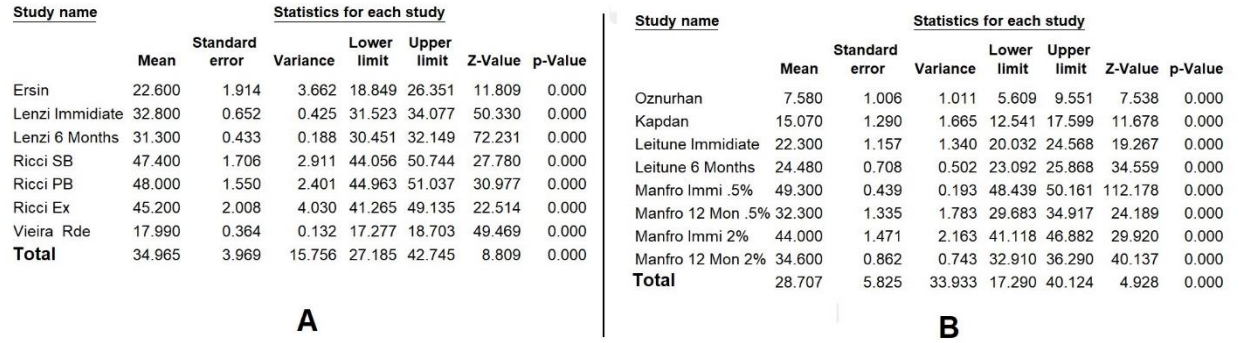


Fig. 4. Forest plot comparison of the resin-dentin bond strength after CHX application vs. control after aging.



Study name | Statistics for each study

	Mean	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Ersin	22.600	1.914	3.662	18.849	26.351	11.809	0.000
Lenzi Immidiate	32.800	0.652	0.425	31.523	34.077	50.330	0.000
Lenzi 6 Months	31.300	0.433	0.188	30.451	32.149	72.231	0.000
Ricci SB	47.400	1.706	2.911	44.056	50.744	27.780	0.000
Ricci PB	48.000	1.550	2.401	44.963	51.037	30.977	0.000
Ricci Ex	45.200	2.008	4.030	41.265	49.135	22.514	0.000
Vieira Rde	17.990	0.364	0.132	17.277	18.703	49.469	0.000
Total	34.965	3.969	15.756	27.185	42.745	8.809	0.000

A

Study name | Statistics for each study

	Mean	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Oznurhan	7.580	1.006	1.011	5.609	9.551	7.538	0.000
Kapdan	15.070	1.290	1.665	12.541	17.599	11.678	0.000
Leitune Immidiate	22.300	1.157	1.340	20.032	24.568	19.267	0.000
Leitune 6 Months	24.480	0.708	0.502	23.092	25.868	34.559	0.000
Manfro Immi .5%	49.300	0.439	0.193	48.439	50.161	112.178	0.000
Manfro 12 Mon .5%	32.300	1.335	1.783	29.683	34.917	24.189	0.000
Manfro Immi 2%	44.000	1.471	2.163	41.118	46.882	29.920	0.000
Manfro 12 Mon 2%	34.600	0.862	0.743	32.910	36.290	40.137	0.000
Total	28.707	5.825	33.933	17.290	40.124	4.928	0.000

B

Fig. 5. Effect of 60 seconds (A) and 30 seconds (B) usage of chlorohexidine on bond strength to the primary dentin

The characteristics of the included studies are presented in Table 1. Eight studies were included in the global analysis, which was performed using random effect model (Fig. 2). Accordingly, CHX application showed no significant effect on the resin-dentin bond strength regardless of aging (P=0.87). The values of the Cochran's Q and I^2 tests were 310.7 and 95.5, respectively. In subgroup analysis of the resin-dentin bond strength after CHX application in comparison to control at baseline (no aging group), CHX application significantly reduced the resin-dentin bond strength (P=0.043). The values of the Cochran's Q and I^2 tests were 164.3 and 93.9, respectively. In subgroup analysis of the resin-dentin bond strength following CHX application compared with control after at least 6 months of aging, CHX application showed significantly higher values of resin-dentin bond strength (P<0.001, Q= 19.97, I^2= 84.98). Figures 3 and 4 demonstrate forest-plot comparisons of the subgroups. As shown in Fig. 5, descriptive analysis indicated that both 60 seconds and 30 seconds application of CHX had significantly positive effects (P<0.001).

DISCUSSION

This meta-analysis demonstrated that although CHX application after acid-etching had a significant detrimental effect on the immediate bond strengths of etch and rinse systems to primary dentin, it significantly arrested bond deterioration over time.

Many studies showed that in permanent dentin, collagen fibril degradation at the base and also within the hybrid layer,

occurred after one year of storage. This was due to the function of collagenolytic host-derived enzymes such as matrix MMPs that are trapped within the mineralized dentin matrix during tooth development [28]. These MMPs regulate the physiological and pathological mechanism of collagen-based tissues [29]. These enzymes are converted to the activated form when the dentin is solubilized by caries [28] or the dentin bonding process [11].

It was assumed that phosphoric acid could have activated MMPs of the dentin [30], resulting in collagenolytic activities within the hybridized dentin. On the other hand, Mazzoni et al. [31] demonstrated that 37% phosphoric acid, being very acidic (pH=-0.7), caused partial denaturation of the MMPs that are exposed during etching. Moreover, simplified etch-and-rinse adhesives can reactivate the endogenous enzymes present in dentin, which were previously inactivated by phosphoric acid-etching. In mild self-etch adhesive (pH≥ 2.3) dentin collagenolytic activities triggered without denaturing these enzymes, while the more acidic versions of these adhesives may activate, but partially denature these enzymes [32].

The action of MMPs was inhibited with the use of inhibitors such as cysteine and serine protease. CHX also showed a desirable MMP inhibitor activity for MMPs by altering their three-dimensional structure and depleting metal ions (Ca^{2+}, Zn^{2+}), which were necessary for their function [33-35].

Primary tooth dentin presents a higher organic and lower mineral content than permanent dentin. These differences may reduce resin-dentin bond strength in this substrate and also increase the chance of degradation over time [17,18]. Therefore, the effect of MMP inhibitors on bond strength preservation could be more evident in primary teeth than in permanent teeth.

While many studies have evaluated the influence of CHX pretreatment on dentin bond strength, controversy still exists regarding whether CHX decreases immediate bond strength [3,25,36-38]. In the present meta-analysis, CHX treatment caused lower immediate dentin bond strength to primary dentin. In line with the present results, Gunaydin et al. [39] and Kapdan and Öztaş [23] observed that 2% CHX caused a decrease in immediate dentin bond strength values of etch and rinse adhesives in permanent and primary dentin. However, Campos et al. [40] showed that CHX application had no influence on immediate bond strength values of etch-and-rinse adhesives, but reduced the bond strength of self-etch adhesives. Hiraishi et al. [41] reported that CHX is more likely to bind to loose apatite remnants within the smear layer when it is applied to smear-covered dentin surfaces than when it is applied to acid-etched dentin surfaces where phosphorate groups are depleted due to etching and rinsing. Bonding of CHX to these loose, superficial apatites could interfere with functions of E&D primer (Kuraray, Japan) monomers. Thus, the authors of the present study concluded that the use of CHX with composite resin restorations appeared to be material specific regarding the interactions with various dentin bonding systems and the ability to seal dentin.

Lenzi et al. [24] and Manfro et al. [25] found that the application of CHX on dentin of primary teeth resulted in no bond strength degradation after at least six months of water storage, which supports the results of our study. Many in vivo studies also demonstrated that the use of CHX prior to the application of an etch-and-rinse adhesive system arrested the clinical sign of degradation of hybrid layers produced in primary and permanent teeth after six months [3,5,42,43].

In vitro, CHX is able to inactivate all MMPs existing in dentin at a concentration of only 0.02% [16,33]. Furthermore, CHX holds the advantage of a high substantivity. This term describes the chemical property of CHX to remain in situ due to its positive charge-related non-specific binding, and thus exerts its influence beyond the mere duration of application. For this reason, it may be assumed that the concentration of CHX is of minor importance [33,44,45]. On the other

hand, although Carrilho et al. [45] noted that after CHX application, rinsing with water should be avoided, because water can remove CHX from dentin, the incorporation of CHX into phosphoric acid is also effective in the preservation of bond strength [37].

This study in the descriptive analysis showed that the 60 second application of CHX was more effective than the 30 second application; however, there were no studies to compare the time of application of CHX in bond strength of primary dentin. As CHX is likely to bind to collagen fibrils at a very fast rate, thus even short periods of time such as 30 seconds appear to be sufficient to guarantee such binding. Accordingly, recommendations of usage range from 2% CHX for 60 seconds to 0.002% CHX for 15 seconds [33,46]. However, there is scientific unanimity with respect to the form of application: CHX should always be used as a pure aqueous solution, rather than in the form of conventional mouthwash solutions, which potentially contains preservatives that may negatively affect the adhesive bond. Pure aqueous CHX solutions can be purchased or prepared by pharmacies.

The results of the present review should be interpreted with caution because laboratory studies have intrinsic limitations when attempting to simulate in vivo conditions. Well-designed randomized controlled trials with long follow-up periods are needed to provide the ultimate answer as to whether CHX application prior to bonding results in improved clinical success rates compared with conventional bonding procedures.

CONCLUSION

This meta-analysis showed that CHX application significantly reduced immediate resin-dentin bond strength, but increased aged bond strength values.

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CONFLICT OF INTEREST STATEMENT

None declared.

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