ORIGINAL RESEARCH

Effect of Enamel Deproteinization Prior to Etching on the Clinical Performance of Resin-based Pit and Fissure Sealants: A Split-mouth Double-blinded Pilot Randomized Controlled Trial

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Abstract

Background: Enamel deproteinization using sodium hypochlorite entails the oxidation of organic components, resulting in the dissolution of proteinaceous components and subsequent cleansing of the enamel substrate. This oxidative treatment augments surface energy and promotes favorable interfacial interactions, thereby enhancing the adhesion and performance of dental materials, ultimately leading to better clinical outcomes.

Aim: To evaluate the effectiveness of enamel deproteinization prior to etching on the retention of pit and fissure sealants (PFS) at different time intervals among 6–14-year-old children.

Objective: The objective of this study was to evaluate the effect of 5.25% sodium hypochlorite on the clinical performance and retention of pit and fissure sealants in permanent molars.

Materials and methods: A double-blind randomized controlled trial using a split-mouth design was conducted for a period of 1 year. The clinical trial registry was done CTRI/2023/06/053542. About 20 children aged 6–14 years, who required bilateral PFS for permanent molars, were included and divided into 20 units for each group. The contralateral side served as the control for the same patient. Randomization was performed using computer-generated numbers (Randomizer.com). Treatment was divided into two groups: group I: pit and fissure sealant with enamel deproteinization prior to etching, group II: pit and fissure sealant without enamel deproteinization. A blinded evaluator assessed the clinical outcome using modified Simonsen's criteria.

Results: At the end of 12 months of follow-up, the results in the current study show that there is a statistical significance between groups I and II. In group I, only 10 teeth have experienced the event, while in group II, it is maximum, accounting for 33 teeth. It was found that the retention rate in group I is 88.1%, while in group II, it is 60.0%. Thus, we can conclude that the retention of pit and fissure sealant in group I is significantly better than in group II.

Conclusion: The present trial yielded notable enhancements in the retention outcomes of PFS following the incorporation of an adjunctive procedure involving enamel deproteinization utilizing 5.25% sodium hypochlorite, combined with intermediate bonding, over a 12-month observation period. This approach holds promise as a strategic intervention for augmenting adhesion and optimizing the efficacy of PFS as a preventive measure.

Keywords: Children, Dental sealants, Enamel deproteinization, Pit and fissure sealants, Preventive dentistry, Sodium hypochlorite.

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Introduction

Dental caries is a widely prevalent but preventable multifactorial chronic oral disease. Deep and retentive pits and fissures exhibit heightened susceptibility to dental caries development, contributing to approximately 44% in primary teeth among children and 90% in posterior permanent teeth.[1]

An intricate morphology of occlusal fissures makes it conducive for retention of food particles and bacteria, making it challenging to maintain proper oral hygiene. The prevalence of dental caries is intricately associated with multiple factors such as limited access to dental care resources, insufficient awareness and motivation, socioeconomic disadvantage, substandard oral hygiene practices, and reduced levels of parental education. The necessary care for children in this age bracket typically involves restorative procedures and occasionally pulp therapy.

From a pediatric dentistry perspective, the utilization of pit and fissure sealants (PFS) demonstrates economic viability, emphasizing the principle that preventive measures are superior to curative interventions in enhancing oral health among children.

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Extensive research has conclusively established the impact of PFS in preventing demineralization in these specific areas. According to the systematic review conducted by Ahovuo-Saloranta et al.,[2] resin-based sealants (RBPFS) reduced the incidence of caries by a range of 11–51% on a 2-year follow-up, as compared to teeth where sealant was not applied.

The main concern regarding PFS is their loss of retention owing to improper marginal sealing. Factors affecting pit and fissure sealant retention include tooth morphology, patient-related factors, sealant material properties, and operator technique. Anatomical variations in tooth morphology, such as depth and contour of pits and fissures, influence the effectiveness of sealant placement.[3] Patient-related factors like oral hygiene practices and saliva flow rate can also impact sealant retention.[2] Furthermore, the adhesive system used, sealant material properties (e.g., viscosity, filler content), and operator technique play crucial roles in sealant retention.[4] Studies have reported that about 60.4% of sealants retained on sound teeth after 4 years of follow-up and 55.6% when placed on teeth with limited lesions.[5]

In treatments aimed at improving the adhesion and durability of PFS, researchers endorsed the utilization of diverse surfaces such as air abrasion,[5] sodium bicarbonate,[5] rubber cups,[5] and the use of intermediate layers,[6] maleic acid,[7] pumice slurry,[7] and chlorhexidine.[7]

One of the methods is enamel deproteinization, which is a noninvasive method for surface conditioning for removal of the organic component of the enamel-etching pattern and increases type I and type II etching patterns, which improves adhesion along with improved bonding and retention of resin-based materials.[8]

The success of enamel deproteinization of PFS leading to an increase in shear bond strength has been proven in various *in vitro* studies by Garrocho-Rangel et al.,[9] Bayrak et al.,[10] Mohammadi et al.,[11] and in an *in vivo* study by Rishika et al.,[12] where enamel deproteinization with 3% sodium hypochlorite with intermediate bonding improved the retention of PFS.

Since there is a scarcity of literature in incorporating an additional step of using 5.25% sodium hypochlorite as a deproteinizing agent, along with adding an intermediate bonding layer, which could potentially serve as a strategy to improve adhesion and enhance the effectiveness of PFS as a preventive measure, this study was performed to analyze the influence of enamel deproteinization utilizing a 5.25% sodium hypochlorite solution on the retention of PFS.

Materials and Methods

A 1-year double-blind randomized controlled trial employing a split-mouth design was conducted. Ethical approval was obtained from the Institute's Ethical Review Committee (DPU/710/44/2022) before the commencement of the trial, and the clinical trial registry was done (CTRI/2023/06/053542). Parents who consented to their children's participation in the study provided written authorization using a standardized form. This clinical trial adhered to the CONSORT guidelines and included 20 children aged 6–14 years who required bilateral PFS for permanent molars. The children were divided into 20 units for each group, with the contralateral side serving as the control for the same patient. Randomization was performed using computer-generated numbers (Randomizer.com). Participants included in the trial had previously untreated, fully erupted first or second molars with intact contralateral molars meeting the inclusion criteria. These teeth exhibited at least two deep occlusal fissures prone to food lodgment and had no history of prior treatment.

Participants exhibiting parafunctional habits, systemic diseases, allergies to restorative materials, or who were uncooperative or differently abled were excluded. A sample size was estimated using Open Epi Software with a 95% confidence interval and 80% power. Based on the provided data, the minimum required sample size was determined to be 10 in each group. However, after applying the eligibility criteria and accounting for potential observer or instrumentation errors, the sample size was increased to 20 participants.

The study participants were randomly allocated to two groups: group I: pit and fissure sealant with enamel deproteinization prior to etching; group II: pit and fissure sealant without enamel deproteinization. A blinded evaluator assessed the clinical outcome using modified Simonsen's criteria. A single operator applied the sealant for both groups. The data analysis was conducted by two independent evaluators, both of whom were standardized in assessing PFS according to Simonsen's criteria prior to the commencement of the study.

In the present trial, both the intervention group (pit and fissure sealant with enamel deproteinization using 5.25% sodium hypochlorite) and the control group (pit and fissure sealant without enamel deproteinization) underwent a similar procedure for pit and fissure sealant application.

Phase 1 involved organizing oral health check-up camps in selected schools for children aged 6–14 years. Comprehensive screenings identified candidates for dental interventions, and those meeting the inclusion/exclusion criteria were provisionally enrolled. Information on PFS was distributed, and parental understanding was augmented through informational brochures. Upon parental consent, children were formally included in the study. Phase 2 commenced after subject enrollment, with a predetermined schedule for sealant placement visits. A single operator facilitated sealant placement for groups of 5 students, resulting in 10 applications per session. Randomization into two groups determined whether sealants were applied with or without enamel deproteinization. Group A received pit and fissure sealant application with enamel deproteinization, whereas group B underwent pit and fissure sealant application without enamel deproteinization. Standardized protocols were adhered to for sealant application, with postoperative instructions provided.

Procedure for Placement of Pit and Fissure Sealant

In group A (pit and fissure sealant with enamel deproteinization), after obtaining proper isolation with cotton rolls and high vacuum suction, the occlusal surface of the tooth was subjected to the application of a layer of 5.25% sodium hypochlorite with an application tip for 1 minute (Fig. 1) and then washed and air-dried, followed by etching for 15 seconds with 37% phosphoric acid etchant (Fig. 2) and rinsing with water and air-drying. This was followed by the application of ESPE Single Bond Universal adhesive bonding agent for 20 seconds (Fig. 3). 3M™ Clinpro™ pit and fissure sealant was applied with an applicator tip and light-cured for 20 seconds (Figs 4 and 5).

In group B, pit and fissure sealant without enamel deproteinization, except the additional enamel deproteinization with 5.25% sodium hypochlorite step, a similar procedure for pit and fissure sealant application was followed.

The primary objective was to evaluate and compare the retention of PFS between the intervention and control groups after a 1-year follow-up. Participants were contacted to attend follow-up visits.

Fig. 1: Enamel deproteinization—application of a layer of 5.25% sodium hypochlorite (prime) with an applicator tip

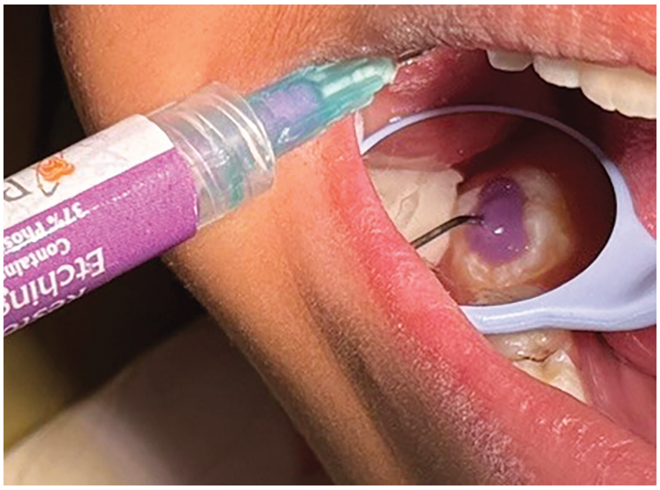

Fig. 2: Application of 37% phosphoric acid etching gel (prime)

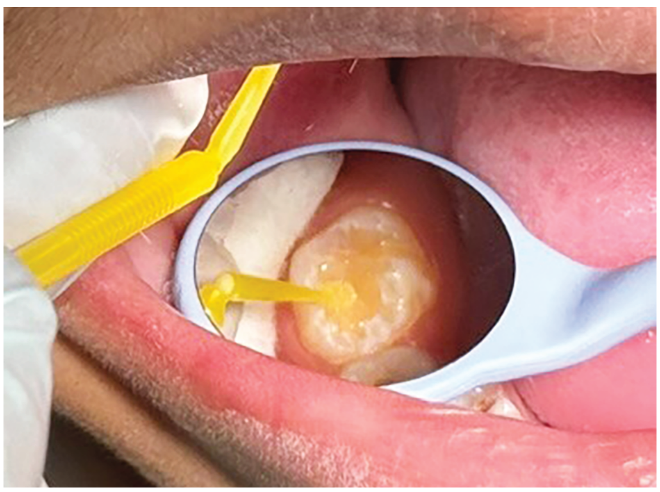

Fig. 3: Application of Single Bond Universal adhesive bonding agent (ESPE) with an applicator tip. Application followed by light curing for 20 seconds

Children/students were evaluated by follow-ups at 3, 6, 9, and 12 months by a second examiner who was blinded, based on modified Simonsen's criteria[13] for sealant retention as follows:

Fig. 4: 3M™ Clinpro™ pit and fissure sealant application followed by light curing for 20 seconds

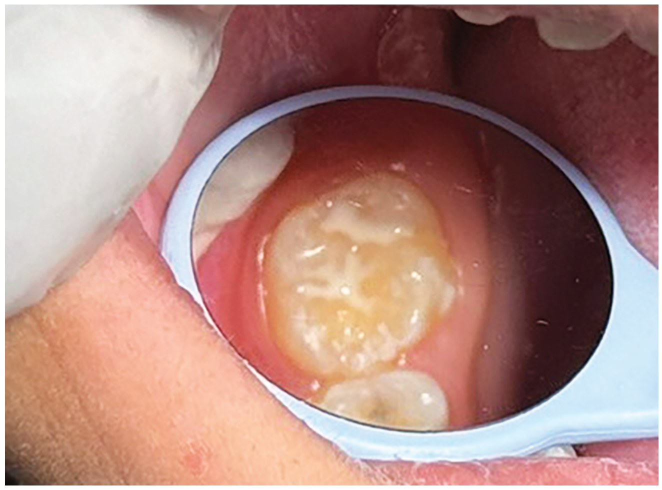

Fig. 5: Postoperative photograph of 16—pit and fissure sealant with enamel deproteinization

Score 0: No loss of sealant and no evidence of caries.
Score 1: Partial loss of sealant and no evidence of caries.
Score 2: Partial loss of sealant and evidence of caries.
Score 3: Complete loss of sealant and no evidence of caries.
Score 4: Complete loss of sealant with caries evidence.

The retention was evaluated visually using a probe and mouth mirror. Data were tabulated in an Excel sheet and transferred to IBM SPSS Statistics version 27.00 for analysis. Normality was assessed with the Kolmogorov–Smirnov test, indicating a nonnormal distribution. Consequently, nonparametric tests, including Friedman's two-way analysis of variance by Ranks, *post hoc* tests with Bonferroni correction, independent-samples Mann–Whitney *U* test, Kaplan–Meier estimator, and Mantel–Cox Log-rank tests, were employed. Analysis was conducted using SPSS version 27.00 (IBM; Chicago, IL).

Results

The primary objective evaluated was to assess the effect of enamel deproteinization on the clinical performance of PFS over various time intervals among children aged 6–14 years old. The sample size was estimated to be 40, with an equal allocation ratio of 1:1 in two groups. All patients were clinically evaluated for retention after 3, 6, 9, and 12 months of application, and retention was assessed based on Simonsen's criteria, which are mentioned in Tables 1 to 3.

Intergroup Comparison

Distribution of sealant retention based on modified Simonsen's criteria and within-group comparison of sealant retention at four intervals in two groups using Friedman's two-way analysis of variance by ranks are seen in Table 1 and Fig. 6. At the 3-month follow-up, group I did not exhibit sealant loss, contrasting with 71.4% in score 0 and 28.6% in score 1 in group II, although the disparity did not reach statistical significance. At the 6-month interval, group I displayed an increase in sealant loss to 4.8% in score 1, while group II experienced an increase of 14.3% in score 1, 4.8% in score 2, and 9.5% in score 3, with a significant difference observed between groups. Subsequently, at the 9-month follow-up, group I maintained a lower loss rate of retention with 85.7% in score 0 and 14.3% in score 1, compared to a retention rate of only 52.4% in score 0, 9.5% in score 1, 28.6% in score 2, and 9.5% in score 3 in group II, a statistically significant contrast. By the end of 12 months, in group I, only 10 teeth had experienced the event, while in group II, it was maximum, accounting for 33 teeth. The results show that the retention rate in group II is 88.1%, while in group II it is 60.0%. By the end of 12 months, in both groups, none of the teeth showed complete loss of sealant with caries evidence.

In summary, at the conclusion of the 12-month observation period, group I exhibited superior retention of sealants compared to group II.

Between group (groups I and II) comparison at 3 follow-up intervals using the independent-samples Mann–Whitney *U* test is shown in Table 2. The results show that there is a statistical significance between groups I and II at 3 months ($p = 0.009$), 6 months ($p = 0.036$), 9 months ($p = 0.006$), and at 12 months ($p = 0.019$). It can be noticed that the retention of the pit and fissure sealant is better in group I in comparison with group II.

The analysis comparing groups I and II across four follow-up intervals reveals statistically significant differences ($p < 0.05$) in sealant retention. At 3 months, group I exhibited a mean rank of 18.50, contrasting with 24.50 in group II. Similarly, at 6 months, group I had a mean rank of 18.93 compared to 24.07 in group II. The disparity persisted at 9 months, with group I recording a mean rank of 17.29 versus 25.71 in group II. By the 12-month mark, group I maintained a mean rank of 17.57, while group II registered 25.43. These findings highlight a consistent pattern of superior sealant retention in group I compared to group II across all follow-up intervals.

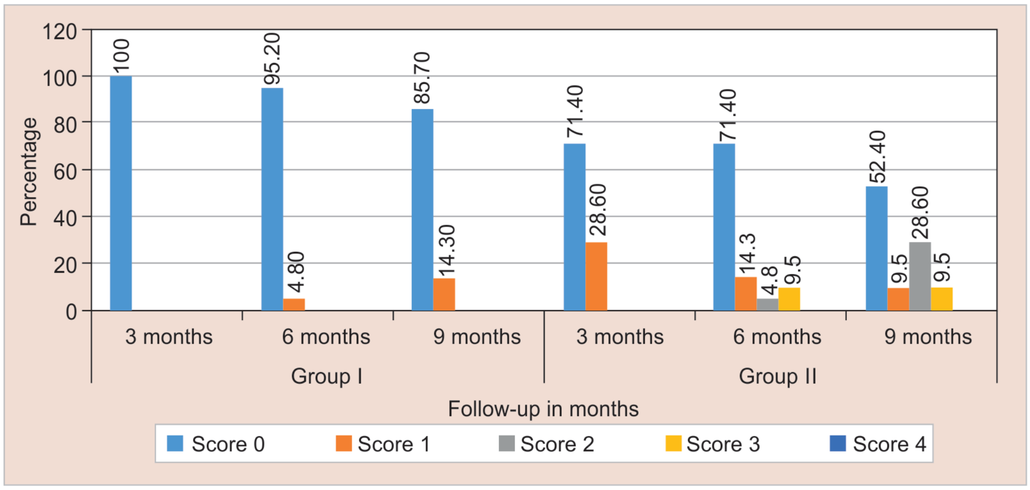

Fig. 6: Percentage distribution of sealant retention based on modified Simonsen's criteria in groups I and II

Table 1: Intergroup and intragroup comparison of sealant retention based on modified Simonsen's criteria using Friedman's two-way analysis of variance by ranks

		Group I				*Group II*			
Scores		*3M*	*6M*	*9M*	*12M*	*3M*	*6M*	*9M*	*12M*
Score 0		21 (100%)	20 (95.2%)	18 (85.7%)	15 (71.4%)	15 (71.4%)	15 (71.4%)	11 (52.4%)	10 (47.6%)
Score 1		0 (0%)	1 (4.8%)	3 (14.3%)	6 (28.6%)	6 (28.6%)	3 (14.3%)	2(9.5%)	1 (4.8%)
Score 2		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.8%)	6 (28.6%)	2 (9.5%)
Score 3		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9.5%)	2 (9.5%)	6 (28.6%)
Score 4		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9.5%)
Total		21 (100.0%)	21 (100.0%)	21 (100.0%)	21 (100%)	21 (100.0%)	21 (100.0%)	21 (100.0%)	21 (100.0%)
Friedman's test	Mean rank	2.26	2.26	2.55	2.83	2.02	2.17	2.86	2.17
	Test statistics	12.60				17.55			
	p-value	0.006 (NS)				<0.001 (S)			

Table 2: Intergroup comparison of groups I and II at three follow-up intervals using the independent-samples Mann–Whitney *U* test

Follow-up		*Mean rank*	*Mann–Whitney U test statistic*	*SE*	*p-value*
3 months	Group I	18.50	283.500	24.10	0.009 (S)
	Group II	24.50			
6 months	Group I	18.93	274.50	25.7	0.036 (S)
	Group II	24.07			
9 months	Group I	17.29	309.00	32.48	0.006 (S)
	Group II	25.71			
12 months	Group I	17.57	303.00	35.14	0.019 (S)
	Group II	25.43			

*SE, standard error; S, significant at p-value < 0.05

Table 3: Survival probability (retention rate) of the pit and fissure sealants using Kaplan–Meier survival estimator and Mantel–Cox log-rank tests

Group	*Total N**	*N* of events*	*Censored N* (%)*	*Mean (SE)*	*Median (SE)*	*Log-rank (Mantel–Cox)*
I	84	10	74 (88.1%)	11.69 (0.15)	–	χ^2: 15.57
II	84	33	51 (60.0%)	10.22 (0.33)	12.00 (0.68)	df: 1
Total	168	43	125 (74.4%)	10.93 (0.19)	–	p-value: <0.001 (S)

N* = total number of teeth per group at three follow-up intervals; SE, standard error; χ^2, Chi-square value; df, degree of freedom; S, significant at p-value < 0.001. Note: modified Simonsen's criteria above 0 is considered as an event

Survival probability (retention rate) of the PFS using the Kaplan–Meier survival estimator and Mantel–Cox log-rank tests is shown in Table 3.

At the end of 12 months, in group I, only 10 teeth had experienced the event, while in group II, it was maximum, accounting for 33 teeth. It was found that the retention rate in group I is 88.1%, while in group II it is 60.0%. The Mantel–Cox log-rank test was conducted to evaluate differences in the survival distribution (retention rate), if any. The survival distributions for the two interventions were statistically significantly different, $\chi^2 = 15.57$, $p < 0.001$.

The Kaplan–Meier survival estimator analysis for pit and fissure sealant retention shows that group I had a higher retention rate compared to group II. In group I, 88.1% of teeth retained the sealants, while in group II, only 60.0% retained them. The Mantel–Cox log-rank test confirms a significant difference between the two groups ($p < 0.001$). Overall, the total retention rate across both groups was 74.4%.

It was found that the retention of the pit and fissure sealant is significantly better in group I—PFS with enamel deproteinization—in comparison with group II—PFS without enamel deproteinization.

Therefore, we can also conclude that PFS with enamel deproteinization have shown better retention rates than PFS without enamel deproteinization.

Discussion

Dental caries is a significant oral health concern affecting children worldwide. Despite advancements in dental care and preventive measures, dental caries remains a prevalent issue among pediatric populations. Untreated dental caries in permanent teeth, as per the World Health Organization, is identified as the foremost prevalent health concern, according to the assessment of the Global Burden of Disease 2019.

Unaddressed, untreated dental conditions, and dental caries can result in pain and challenges related to eating and sleeping. This pain often necessitates emergency dental visits, hospitalization, and the requirement for invasive treatments, consequently giving rise to systemic health issues and a decline in overall quality of life.

The occurrence of dental caries within the Indian population in mixed dentition was reported to be 58%, whereas in primary dentition, it was noted at 54%.[14] A study examining the geographical distribution of caries prevalence in India revealed that the regional prevalence in the western part of India has notably elevated, reaching 72%.[15]

Various preventive methods, including topical fluoride therapy, mechanical barriers such as PFS, community water fluoridation, dietary sugar control, and plaque management, are widely acknowledged as primary contributors to the overall reduction in caries prevalence. These interventions have led to a significant decrease in the occurrence of carious lesions on smooth surfaces.[5,6]

The intricate morphology of occlusal fissures makes it conducive to the retention of food and bacterial particles, making it challenging to maintain proper oral hygiene. Additionally, the limited access of saliva into these fissures diminishes the effectiveness of fluoride in this area. The utilization of PFS is undeniably vital in averting decay in both primary and permanent teeth.

The extended eruption phase of the first permanent molar contributes to its heightened vulnerability to caries, as the enamel is still immature during this developmental stage. Clinical evidence indicates that teeth are particularly susceptible to caries during the initial 2–4 years after eruption.[16] Many researchers illustrate that amounts of biofilm accumulated are higher on the occlusal surface of partially erupted teeth as compared to completely erupted first permanent molars.[14,16] Applying PFS during this vulnerable period proves highly effective in preventing occlusal caries.

Evidence-based recent guidelines from the American Dental Association (ADA) and the Academy of Pediatric Dentistry (AAPD) recommend pit-and-fissure sealing over no sealant or fluoride varnish to decrease caries prevalence. About 71% of occlusal decay can be prevented with a single sealant application.[17]

The emergence of contemporary methods and implementation of PFS has transformed the dental treatment landscape, transitioning from the conventional "drill and fill" approach to the proactive "seal and heal" methodology. The integration of PFS into the National Oral Health Program (NOHP) in India is indicative of their endorsement and their role in its success in curbing dental decay in primary and permanent teeth.

Enamel deproteinization is a noninvasive method for surface conditioning to remove the organic component of the enamel-etching pattern and increases type I and type II etching patterns, which improves the adhesion of resin-based materials.[8] This procedure notably enhanced the mechanical bonding of adhesives to enamel, as noted by Ayman et al.[18] They highlighted that despite lower etching ability, sodium hypochlorite deproteinized and increased the surface, allowing the etching material to penetrate more deeply and create a type II etching pattern, consequently improving bond strength. Espinosa et al.[19,20] similarly documented a types I and II etching pattern characterized by an enlarged total etched area. They also suggested that etching enamel with 37% phosphoric acid after deproteinization resulted in longer adhesive tags, enhancing retention.

In our study, at the end of 12 months of follow-up, the results show that there is statistical significance between groups I and II. In group I, only 10 teeth experienced the event, while in group II, the maximum accounted for 33 teeth. It was found that the retention rate in group I is 88.1%, while in group II, it is 60.0%. Thus, we can conclude that the retention of pit and fissure sealant in group I is significantly better than in group II.

In line with the current study, Ekambaram et al.,[21] Hasija et al.,[22] and Aras et al.[23] found that deproteinization postetching significantly enhanced bond strength compared to conventional etching. The findings of the current study also align with those of Rishika et al.,[12] who demonstrated that the application of 3% sodium hypochlorite and an intermediate layer significantly improves the retention of PFS.

On the contrary, studies by Ahuja et al.[24] and Ramakrishna et al.[25] showed no significant improvement in enamel retention with deproteinization after etching. Roopa et al.,[26] in their *in vivo* study, stated that deproteinization does not offer any significant benefit over the standard acid-etching method for enhancing pit and fissure sealant retention. Deproteinization after etching was found to be better compared to deproteinization before.

The split-mouth design employed in this study was selected for its recognized advantages in minimizing intersubject variability, thereby enhancing statistical power and efficiency in comparative analyses. However, a notable limitation is the sample size. This limitation is acknowledged within the context of the present investigation, which constitutes a pilot randomized controlled trial and intends to address it through the implementation of a subsequent, more extensive study with a longer follow-up period, informed by the outcomes derived from this preliminary study.

Conclusion

The present trial yielded notable enhancements in the retention outcomes of PFS following the incorporation of an adjunctive procedure involving enamel deproteinization utilizing 5.25% sodium hypochlorite, combined with intermediate bonding, over a 12-month observation period. This approach holds promise as a strategic intervention for augmenting adhesion and optimizing the efficacy of PFS as a preventive measure.

Clinical Significance

Incorporating this technique into routine dental practice and school-based dental camps can be a highly effective long-term preventive strategy for the prevention, control, and management of dental caries in primary and permanent teeth.

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