ORIGINAL RESEARCH

Efficacy of Fluoride Varnish with Casein Phosphopeptide and Amorphous Calcium Phosphate vs Fluoride Varnish in Prevention of White Spots Lesion in fixed Orthodontic Patients: *In Vivo* Study

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Abstract

Aim: The aim of the study is to compare the *in vivo* efficiency of Michigan (MI) varnish containing casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP) and Fluoritop containing sodium fluoride (5% NaF) in the prevention and remineralization of white spot lesions (WSLs) around orthodontic brackets at days 28 and 56 after bonding.

Materials and methods: A total of 30 patients were selected and divided into two groups I (MI varnish) II (Fluoritop varnish) of 15 patients in each group. All the patients were bonded and then varnish was applied around the brackets. Right-side upper and lower first premolar teeth were taken as the control group and left-side upper and lower first premolar teeth as the experimental group. Also, 14, 24 teeth were extracted on day 28 after bonding and 34, 44 teeth after day 56 of bonding. Samples were collected and sent to laboratory for evaluation of surface microhardness (SMH).

Results: Based on the statistics results, there was a significant decrease in demineralization and an increase in remineralization of WSLs after the application of varnish. No statistical significance was found between the effectiveness of MI varnish and Fluoritop except in the cervical region.

Conclusion: Through our study, we concluded that no statistical significance was found between the effectiveness of MI varnish and Fluoritop except in the cervical region where MI varnish was found to be more effective than Fluoritop in preventing WSLs.

Clinical significance: The results from the above study concluded that CPP–ACP varnish can be an effective method in preventing WSLs in patients undergoing fixed orthodontic treatment.

Keywords: Fluoritop, MI Varnish, Remineralization, White spot lesion.

The Journal of Contemporary Dental Practice (2022): 10.5005/jp-journals-10024-3404

Introduction

White spot lesions are opaque white areas caused by the loss of minerals below the outermost enamel layer. White spot lesions are common and an unfavorable sequelae of the orthodontic treatment which in turn compromise esthetics and can be extremely difficult to reverse.[1] Fixed orthodontic appliances attributes to the development of WSLs by increasing the number of plaque retention sites on the less caries-susceptible surfaces of the teeth and by prolonging plaque accumulation around the brackets.[2–6] Also, a considerable amount of enamel dissolution occurs in patients with fixed orthodontic appliance.[7,8]

White spot lesions can become noticeable around the fixed appliances within 1 month of bracket placement, usually, it takes at least 6 months duration for the formation of regular caries.[9] The main mechanism of WSLs formation around orthodontic appliances is a rapid increase in the acidogenic bacterial flora of plaque mainly *Streptococcus mutans* and *Lactobacilli*. Acidogenic bacterial flora decreases the pH of the plaque in orthodontic patients to a greater extent than in non-orthodontic patients. Therefore, the progression of caries is faster in patients with full orthodontic appliances.[9,10]

Over a period of years, several types of research have been conducted to prevent demineralization and induce remineralization with the help of fluoride application.

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How to cite this article: Rangarajan S, Vikram NR, Dhayananth XL, *et al*. Efficacy of Fluoride Varnish with Casein Phosphopeptide and Amorphous Calcium Phosphate vs Fluoride Varnish in Prevention of White Spots Lesion in fixed Orthodontic Patients: *In Vivo* Study. J Contemp Dent Pract 2022;23(11):1100–1105.

Source of support: Nil

Conflict of interest: None

Fluoride plays a vital role in the prevention of enamel demineralization and also it increases the initial rate of remineralization.[6] Various methods of delivering fluoride to the teeth in patients undergoing orthodontic treatment include topical fluorides (e.g., mouth rinse, gel, varnish, and toothpaste) and fluoride-releasing materials (e.g., bonding materials and elastics).[11]

However, contradictory opinions were also prevalent against the use of high concentrations of fluoride (~12,000 ppm) that it will cause remineralization mainly in the superficial part of the WSLs.[12,13] This superficial layer might prevent calcium and phosphate from penetrating the deeper layers of the enamel, thus inhibiting deeper remineralization and limiting the cosmetic improvement of the WSLs. Thus, the most ideal concentrations and delivery vehicles for fluoride remain controversial.

The active agent, CPP–ACP is thought to stabilize and localize calcium, fluoride, and phosphate at the tooth surface in a slow-release amorphous form, thus enhancing deeper layer remineralization of WSLs.[14]

Few *in vivo* studies investigated the effectiveness of remineralization products to address the problem of WSLs formation during and after orthodontic treatment. However, these studies had used fluoride and CPP–ACP either as a paste or as a solution which does not deliver localized application over teeth region.[13,15]

With that in mind, this study aimed to compare the *in vivo* efficiency of Fluoride varnish with CPP and ACP and Fluoride varnish containing sodium fluoride (5% NaF) in prevention and remineralization of WSLs around orthodontic brackets at days 28 and 56 after bonding.

Materials and Methods

Subject Selection Criteria

The study was conducted in the Department of Orthodontics, Adhiparasakthi Dental College & Hospital, Melmaruvathur, Tamil Nadu, India.

Ethical Clearance

The study design and protocol were approved by the ethical committee of Adhiparasakthi Dental College & Hospital, Melmaruvathur, Tamil Nadu, India. The subjects were explained about the purpose of the study and an informed consent was obtained from them. In the event of the subjects being less than 18 years of age, informed consent was also obtained from the respective parents. Subjects were clinically examined before the study.

Inclusion Criteria

Male and female patients, with age range of 15–25 years with full complement of permanent dentition excluding third molars who are having Angle's class I malocclusion with bimaxillary protrusion [Angle (ANB) of 0–2°], who require extraction of both the maxillary and mandibular first premolars were included in the study.

Exclusion Criteria

Patients with dental abnormalities, history of smoking, nickel allergy, allergic to milk protein, any systemic illness, growth abnormality, bleeding disorders, and history of any trauma or injury to the face were excluded from the study.

Methodology

Patients who reported to the Department of Orthodontics, Adhiparasakthi Dental College & Hospital, Melmaruvathur, Tamil Nadu, India with Angle's class I malocclusion with bimaxillary protrusion who satisfied the inclusion criteria were selected for the study. A total of 30 patients were divided into 2 groups (groups I and II) of 15 patients in each group. Group I consisted of 15 patients who were bonded with the fixed appliance [Mclaughlin Bennett Trevisi (MBT) standard 0. 022 slot bracket]. Tooth 14 (IA) and 44 (IB) were taken as control group and tooth 24 (IC) and 34 (ID) were taken as experimental group. Also, MI varnish (GC America, USA) was coated by applicator tip around 24 and 34 tooth brackets on the day of bonding itself (Flowchart 1).

Group II, consisted of 15 patients who were bonded with the fixed appliance (MBT standard 0.022 slot bracket). Tooth 14 (IIA) and 44 (IIB) were taken as the control group and tooth 24 (IIC) and 34 (IID) were taken as the experimental group. Fluoritop varnish (ICPA Health Products Ltd, India) was coated by applicator tip around 24 and 34 tooth brackets on the day of bonding itself (Flowchart 1). Initial 0.016″ Ni-Ti archwire was secured in both the upper and lower arches with elastomeric modules in all the subjects (Flowchart 1).

Patients were advised to follow the oral hygiene instructions as recommended for the study. Patients were advised not to brush or floss teeth for at least 4 hours after varnish application and also should avoid hard, hot, and sticky food and products which contained alcohol and fluoride (oral rinses and beverages).

All the patients were recalled on day 28 after bonding. On day 28, teeth 14 (IA), 24 (IC); 14 (IIA), 24 (IIC) from groups I and II, respectively, were extracted, cleaned with thymol, and sent to laboratory in transfer media in sterile plastic containers for evaluation of enamel SMH. Transfer media used was normal saline.

All the patients were given next appointment after day 28 of the present appointment, that is, day 56 after bonding. On day 56, teeth 34 (ID), 44(IB); 34 (IID), 44 (IIB) were extracted from groups I and group, respectively, cleaned with thymol, and sent to laboratory in normal saline for evaluation of enamel SMH.

The SMH of the 120 samples were measured using a Vickers micro hardness Tester (HDNS Kelly Instruments and MVD 402, Shanghai) with a load of 200 gm for 10 seconds. All readings were performed by the same examiner using the same calibrated machine. Long axis of the tooth connecting Zenith of gingiva and buccal occlusal tip was marked with the microtip pencil (0.05 mm) for all the sample. For each sample, two indentations were marked 2 mm away from bracket surface, one occlusally and another one cervically along the long axis of the tooth. SMH was measured at these two indentation marks (Fig. 1) with the tester in Vickers hardness number (VHN) units.

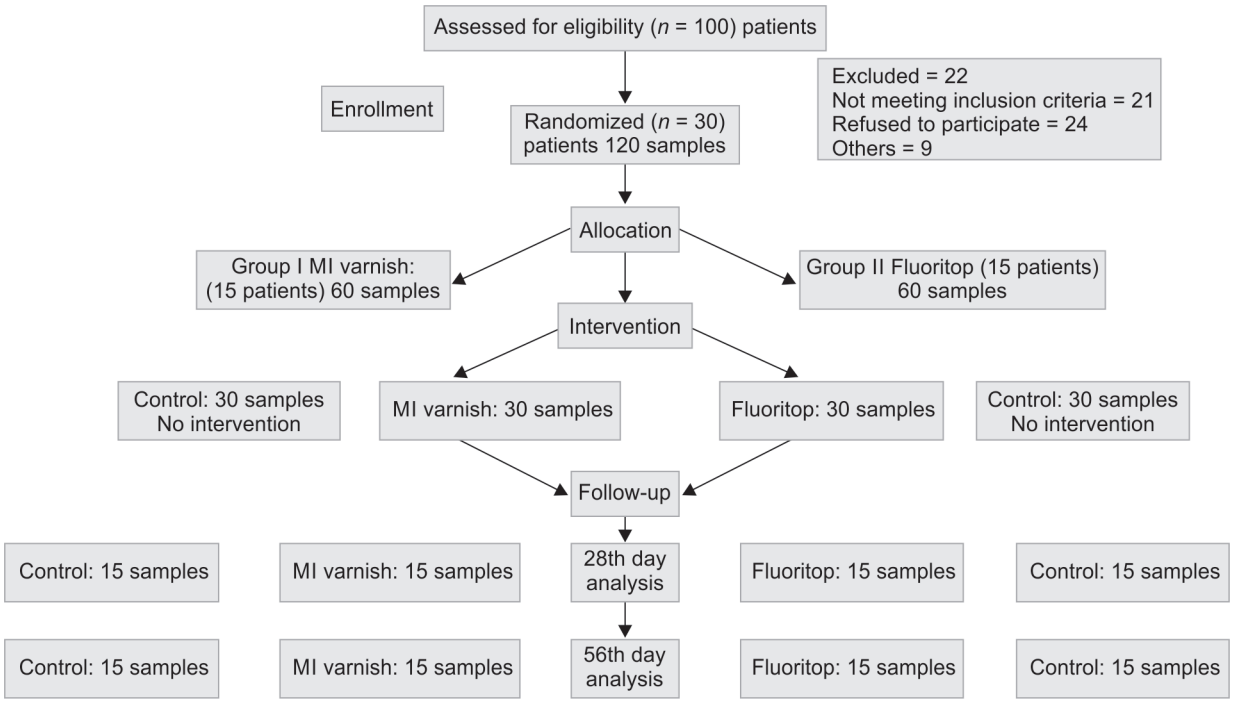
Statistical Analysis

Statistical analysis was performed for all the samples using IBM® SPSS software, version 21. The normality of the data was confirmed using Shapiro–Wilk test. Paired sample *t*-test was performed for intragroup comparison whereas for intergroup comparison unpaired *t*-test was performed; $p < 05$ was considered statistically significant.

Results

The mean distribution of the SMH of group I (MI varnish) on day 28 in sample A at the occlusal region (IA–o) was 274.30 ± 27.25 VHN

Flowchart 1: Consort flowchart



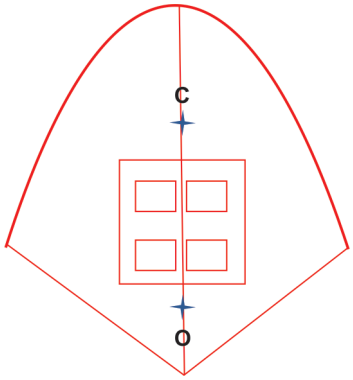


Fig. 1: Indentation marks for the assessment. C, cervical; O, occlusal

Table 1: Descriptive statistics for group I (MI varnish)

Site	*N*	*Minimum (VHN)*	*Maximum (VHN)*	*Mean (VHN)*	*Standard deviation (SD) (VHN)*
IA–o	15	222.30	311.30	274.3067	27.25966
IA–c	15	241.90	355.50	293.0333	27.75998
IC–o	15	230.80	354.70	299.7600	32.50580
IC–c	15	245.80	371.30	307.9200	40.49244
IB–o	15	218.90	279.20	255.3600	17.19704
IB–c	15	229.40	294.70	273.6533	20.17420
ID–o	15	279.10	343.80	304.4267	18.87581
ID–c	15	302.70	382.70	347.2000	27.01457

whereas at the cervical region (IA–c) was 293.03 ± 27.75 VHN and that in sample C at the occlusal region (IC–o) was 299.76 ± 32.50 VHN whereas at the cervical region (IC–c) was 307.92 ± 40.49 VHN (Table 1).

The mean distribution of the SMH on day 56 in sample B at the occlusal region (IB–o) was 255.36 ± 17.19 VHN whereas at the cervical region (IB–c) was 273.65 ± 20.17 VHN and that in sample D at the occlusal region (ID–o) was 304. 42 ± 18.87 VHN whereas at the cervical region (ID–c) was 347.20 ± 27.01 VHN (Table 1).

The results obtained after comparing samples A and C of group I, the SMH at the occlusal region (IA–o and IC–o) was found to be 274.30 VHN and 299.76 VHN, respectively, which was found statistically significant ($p = 0.008$) whereas at the cervical region (IA–c and IC–c) it was 293.03 VHN and 307.92 VHN, respectively which was not statistically significant ($p = 0.21$) (Table 2).

The results obtained after comparing samples B and D of group I, SMH at the occlusal region (IB–o and ID–o) was found to be 255.36 VHN and 304.42 VHN, respectively, which was statistically significant ($p < 0.001$) whereas at the cervical region (IB–c and ID–c)it was 273.65 VHN and 347.20 VHN, respectively, which was statistically significant ($p < 0.001$) (Table 2). The results obtained after comparing samples C and D of group I, the SMH at the occlusal region (IC–o and ID–o) was found to be 299.76 VHN and 304.42 VHN, respectively, which was not statistically significant ($p = 0.59$) whereas at the cervical region (IC–c and ID–c) it was 307.92 VHN and 347.20 VHN, respectively, which was statistically significant ($p < 0.001$) (Table 2).

The mean distribution of the SMH of group II (Fluoritop) on day 28 in sample A at the occlusal region (IIA–o) was 268.44 ± 27.05 VHN whereas at the cervical region (IIA–c) was 276.70 ± 20.64 VHN and that in sample C at the occlusal region (IIC–o) was 285.02 ± 24.34 VHN whereas at the cervical region (IIC–c) was 296.98 ± 20. 89 VHN (Table 3).

The mean distribution of SMH on day 56 in sample B at the occlusal region (IIB–o) was 254.16 ± 16.71 VHN whereas at the cervical region (IIB–c) was 269. 45 ± 21.64 VHN and that on day

Table 2: Comparison between MI varnish group and control group

Site		N	Mean (VHN)	SD (VHN)	p-value
Pair 1	IA–o	15	274.3067	27.25966	0.008
	IC–o	15	299.7600	32.50580	
Pair 2	IA–c	15	293.0333	27.75998	0.21
	IC–c	15	307.9200	40.49244	
Pair 3	IB–o	15	255.3600	17.19704	<0.001
	ID–o	15	304.4267	18.87581	
Pair 4	IB–c	15	273.6533	20.17420	<0.001
	ID–c	15	347.2000	27.01457	
Pair 5	IC–o	15	299.7600	32.50580	0.590
	ID–o	15	304.4267	18.87581	
Pair 6	IC–c	15	307.9200	40.49244	0.001
	ID–c	15	347.2000	27.01457	

Table 3: Descriptive statistics for group II (Fluoritop)

	N	Minimum (VHN)	Maximum (VHN)	Mean (VHN)	SD (VHN)
IIA–o	15	215.30	305.30	268.4400	27.05359
IIA–c	15	235.90	311.80	276.7000	20.64202
IIC–o	15	233.80	314.70	285.0267	24.34723
IIC–c	15	247.80	327.10	296.9867	20.89022
IIB–o	15	211.90	275.20	254.1600	16.71718
IIB–c	15	221.40	295.30	269.4533	21.64172
IID–o	15	269.60	321.30	296.4933	15.95581
IID–c	15	291.30	359.60	317.6667	22.52705

56 in sample D at the occlusal region (IID–o) was 296.49 ± 15.95 VHN whereas at the cervical region (IID–c) was 317.66 ± 22.52 VHN (Table 3).

The results obtained after comparing samples A and C of group II, the SMH at the occlusal region (II A–o and IIC–o) was found to be 268.44 VHN and 285.02 VHN, respectively, which was statistically significant ($p < 0.001$) whereas at the cervical region (IIA–c and IIC–c), it was 276.70 VHN and 296.49 VHN, respectively, which was statistically significant ($p < 0.001$) (Table 4).

The results obtained after comparing samples B and D of group II, the SMH at the occlusal region (IIB–o and IID–o) was found to be 254.16 VHN and 296.49 VHN, respectively, which was statistically significant ($p < 0.001$) whereas at the cervical region (IIB–c and IID–c), 269.45 VHN and 317. 66 VHN, respectively, which was statistically significant ($p < 0.001$) (Table 4).

The results obtained after comparing samples C and D of group II, the SMH at the occlusal region (IIC–o and IID–o) was found to be 285.02 VHN and 296.49 VHN, respectively, which was not statistically significant ($p = 0.07$) whereas at the cervical region (IIC–c and IID–c), it was 296.98 VHN and 317.66 VHN, respectively, which was statistically significant ($p < 0.001$) (Table 4).

The results obtained after comparing groups I and II, SMH at the occlusal region (IC–o and IIC–o) on day 28 day was found to be 299.76 VHN and 285.02 VHN, respectively, which has no statistically significance ($p = 0.17$) and at the cervical region (IC–c and IIC–c),

Table 4: Comparison between Fluoritop group and control group

Site		Mean (VHN)	N	SD (VHN)	p-value
Pair 1	IIA–o	268.4400	15	27.05359	0.001
Pair 2	IIC–o	285.0267	15	24.34723	
	IIA–c	276.7000	15	20.64202	0.001
Pair 3	IIC–c	296.9867	15	20.89022	
	IIB–o	254.1600	15	16.71718	0.001
Pair 4	IID–o	296.4933	15	15.95581	
	IIB–c	269.4533	15	21.64172	0.001
Pair 5	IID–c	317.6667	15	22.52705	
	IIC–o	285.0267	15	24.34723	0.070
	IID–o	296.4933	15	15.95581	
Pair 6	IIC–c	296.9867	15	20.89022	0.001
	IID–c	317.6667	15	22.52705	

it was 307.92 VHN and 296.98 VHN, respectively, which was not statistically significant ($p = 0.36$) (Table 5).

The results obtained after comparing groups I and II, SMH at the occlusal region (ID–o and IID–o) at day 56 was found to be 304.42 VHN and 296.49 VHN, respectively, which was not statistically significant ($p = 0.22$) and at the cervical region (ID–c and IID–c), it was 347.20 VHN and 317.66 VHN, respectively, which was statistically significant ($p < 0.003$) (Table 5).

Discussion

The tooth enamel structure is a unique one that has no residual cellular components to repair when it gets damaged by a cariogenic episode. Demineralization and remineralization (repair or healing) of enamel are continuous and constant processes occurring on the availability of acidogenic bacterial flora on the plaque biofilm and refined carbohydrates. The plaque biofilm facilitates the attachment and spread of acidogenic bacteria resulting in WSLs formation. The plaque biofilm encloses numerous microenvironments that can be disrupted through chemomechanical systems such as applications of topical fluoride, CPP–ACP, and tooth brushing. Reduced enamel subsurface demineralization was found when enamel plaque is exposed to solutions of tryptic peptides of casein. Thus, incorporating casein peptides into enamel plaque increases the plaque content of calcium and phosphate.[16] One topical application of fluoride varnish in high concentration can decrease enamel lesion depth adjacent to bonded brackets up to 40% for 3 months.[17]

Casein phosphopeptide–amorphous calcium phosphate acts as a reservoir of calcium and phosphate. The active CPP is capable of binding to calcium and phosphates of enamel and also stabilizes ACP simultaneously. Reduced pH in the plaque results in calcium and phosphate ions release from CPP resulting in supersaturation which reduces demineralization and promotes the remineralization of enamel by binding to enamel calcium and phosphates.[18,19] With this in mind, this study was conducted to compare the effectiveness of CPP–ACP varnish (MI varnish) and sodium fluoride varnish (Fluoritop varnish) in preventing the formation of WSLs and promoting remineralization of the lesion. In our study, an increased demineralization was found at the occlusal region compared to the cervical region of the tooth in general. This inference was held

Table 5: Intergroup comparison between group I (MI varnish) and group II (Fluoritop)

Groups		*N*	*Mean (VHN)*	*SD (VHN)*	*Mean difference (VHN)*	*p-value*
C occlusal	IC–o	15	299.7600	32.50580	14.73333	0.171
	IIC–o	15	285.0267	24.34723		
C cervical	IC–c	15	307.9200	40.49244	10.93333	0.361
	IIC–c	15	296.9867	20.89022		
D occlusal	ID–o	15	304.4267	18.87581	7.93333	0.224
	IID–o	15	296.4933	15.95581		
D cervical	ID–c	15	347.2000	27.01457	29.53333	0.003
	IID–c	15	317.6667	22.52705		

true for both the control and experimental groups as shown in Tables 2 and 4.

Our results were in contrast to the study by Pascotto et al. where they observed reduced enamel hardness in the cervical region of the bracket compared with that in the occlusal area. *In vivo*, the explanation for this observation was greater dental plaque accumulation and difficulty in cleaning the area. The explanation for this variation of results in our study could be the variation found in the enamel SMH at the occlusal and cervical region of the tooth.[20]

Tables 2 and 4 show that the loss of enamel substance was found to be reduced by the topical application of both MI varnish and Fluoritop in the experimental group when compared to control group at the end of day 28 after bonding. This reduced demineralization was found to be statistically significant for both the MI group and the Fluoritop group except the occlusal region of the MI varnish group. There was no statistical significance found when the effectiveness of both MI varnish and Fluoritop in reducing demineralization was compared at the end of day 28 day of bonding.

At the end of day 56 after bonding also, the demineralization was reduced in both groups which was found to be statistically significant as shown in Tables 2 and 4. By comparing the results obtained at days 28 and 56, it was also noticed that there was not only decrease in demineralization but also there was remineralization of the WSLs, especially in the cervical region which was statistically significant in both MI varnish and Fluoritop groups as shown by pair 6 of Tables 1 and 4, respectively. With this, a time-dependent remineralization could also be concluded. Finally, no statistically significant difference was found between MI varnish and Fluoritop in preventing WSLs. However, in the cervical region, MI varnish was found to be statistically significant compared to Fluoritop.

The results of this study agree with several other clinical trials. A study by Kumar et al.[21] indicated that CPP–ACP containing Tooth Mousse remineralized initial enamel lesions and showed a higher remineralizing potential when applied as a topical coating after the use of fluoridated toothpaste. A study by Giulio et al. stated that topical applications of Tooth Mousse could be effective in promoting enamel remineralization after interdental stripping.[22] A study by Pithon et al.[23] found that MI varnish used without any other oral hygiene procedure would be capable of significantly reducing the depth of carious lesions whereas Duraphat varnish (5 % sodium fluoride varnish) was effective only when it was supported with brushing and fluoridated mouth wash. In another study by Mayne,[15] MI Paste Plus not only prevents WSLs development during orthodontic treatment, but also reduces the number of white spots already present, with greater impact on the gingival surfaces.

In contrast to our results, several randomized clinical trials were reported by Huang et al. where they compared the effectiveness of MI Paste Plus and PreviDent fluoride varnish (22,600 ppm of fluoride) with a standard oral hygiene regimen with toothpaste containing 1,100 ppm of fluoride in and concluded that MI Paste Plus and PreviDent fluoride varnish were not more effective when compared to normal home care for improving the appearance of WSLs over a period of 8 weeks.[24] Another contradictory study by Beerens et al. showed that there were no significant differences between CPP–ACP paste (MI Paste Plus) and the fluoride-free control paste on the remineralization of enamel WSLs and plaque composition during a 3-month follow-up. In both groups, limited changes in fluorescence loss were found 12 weeks after debonding, but a decrease in the percentage of aciduric bacteria and *S. mutans* in the plaque was detected over time.[25] Another randomized trial compared WSLs treated with a low-fluoride mouth rinse (50 ppm) to those treated with a non-fluoride mouth rinse. An explanation for all the above contrary studies could be the retention period or duration of their product used around the brackets. In the above studies, they used products in paste or rinse form which would not have longer retention around the bracket. On the contrary to this, in our study, we used products in varnish form which showed minimum of 3 months to 6 months retention period around the brackets (Farhadian et al.[17]). In our study, the lesions decreased by 26–58%, but no significant differences were found between the CPP and ACP and the Fluoritop groups. The effectiveness of varnish in preventing WSL can be attributed to the retentivity property of varnish.

Limitations

The split mouth technique will have carry-across effect due to fluoride or calcium and phosphate release by the agents on enamel around the brackets. Patient compliance was not monitored and factored into the process. The most effective means of studying decalcification is to carry out a histosection of the enamel of the teeth which was not used for our study.

In the future, patient compliance should also be taken into consideration. Microhardness of both cervical and occlusal regions of tooth should be first evaluated separately for all upper and lower first premolar teeth before the start of the study for standardization. In the future, orthodontic bonding adhesives containing CPP–ACP could become popular. At present, the American Dental Association

Foundation is developing biologically remineralizing composites, or "smart composites," that contain amorphous calcium phosphate. Even sports drinks are being developed that contain CPP–ACP. The possibilities for its applications are endless.

Conclusion

Our study concluded that generalized demineralization occurs around the bracket surfaces of teeth in patients undergoing orthodontic treatment. There was significant decrease in demineralization and increase in remineralization found by topical application of MI varnish as well as Fluoritop at the intervals between weeks 4 and 8 around the bonded tooth. However, there is no statistical significance found in relation to *in vivo* effectiveness comparison of MI varnish and Fluoritop except in cervical region where MI varnish was found to be more effective than Fluoritop in preventing WSLs.

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