The effect of active oxygen-containing toothpaste on *Streptococcus mutans* and white spot lesions: An in-vivo randomized controlled trial

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Introduction: Fixed appliances limit patients' ability to clean their teeth, thus making orthodontic patients highly susceptible to developing white spot lesions (WSLs). Using patient compliance would be one of the simplest methods to prevent WSLs. **Methods:** A parallel trial design with 34 patients that met the eligibility criteria were randomized to experimental (received Blue M oxygen for health toothpaste) and control (received Colgate Total fluoridated toothpaste) groups. The eligibility criteria were that the patients should be aged 13-30 years, on fixed mechanotherapy, had undergone therapeutic extraction of all first premolars, had completed leveling and aligning, and had satisfactory oral hygiene (assessed using pretreatment and current Simplified Oral Hygiene Index scores). The objectives were to assess the effects of active oxygen-containing toothpaste on *Streptococcus mutans* (SM) and WSLs in patients undergoing fixed mechanotherapy. The random allocation sequence was generated using the RAND function in Microsoft Excel. The sequence generated was sealed in opaque white envelopes. The investigators and the participants were blinded until allocation. Blinding of the first author (J.A.G.) who dispensed the toothpaste or the participants was not possible. The outcome assessment was blinded by coding the plaque specimens sent for real-time polymerase chain reaction (RT-PCR) to ensure that the final data assessment was blinded. The study setting was the Department of Orthodontics and Dentofacial Orthopedics, Sri Ramachandra Institute of Higher Education and Research (SRIHER) University. The primary outcome was SM counts, which were analyzed using RT-PCR. The secondary outcome was WSL assessment, performed using DIAGNOdent and International Caries Detection and Assessment System (ICDAS) II criteria at baseline and after 4 weeks. The data were subjected to statistical analysis. **Results:** Thirty-four patients were randomized to 17 per group and analyzed. RT-PCR showed lower SM in the experimental group (Ct = 32.25 ± 10.6) than in the control group (Ct = 30.9 ± 10.49). However, this was not statistically significant ($P = 0.70$). ICDAS scores remained the same from baseline and after 4 weeks for both groups. The DIAGNOdent values were in tandem with the ICDAS scores and showed minimal change for the 2 groups ($P > 0.05$). A strong positive correlation was observed for DIAGNOdent and ICDAS scores ($\tau b > 0.30$). No harms or adverse effects were observed or reported by the patients during the experimental period. **Conclusions:** The active oxygen-containing toothpaste showed a greater inhibitory effect on SM than the fluoridated toothpaste. However, this effect was minimal. Both kinds of toothpaste showed negligible effects on WSLs. Thus, active oxygen-containing toothpaste is as effective as fluoridated toothpaste. **Registration:** The Trial was registered in the Controlled Trials Registry India (CTRI/2021/10/037458). **Protocol:** The full protocol can be obtained from any of the authors via e-mail. **Funding:** The project was self-funded. (Am J Orthod Dentofacial Orthop 2022;162:594-600)

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White spot lesions (WSLs) are major adverse sequelae of fixed orthodontic appliance therapy. Fixed appliances increase the number of plaque retention sites and limit patients' ability to clean their teeth, leading to higher levels of acidogenic bacteria, most notably *Streptococcus mutans* (SM).[1] Visible WSLs may develop as early as 4 weeks (ie, within the period of 1 orthodontic appointment to the next) and appear most commonly on the buccal aspects of teeth

around the brackets, especially the labiogingival area of lateral incisors.[2]

Evidence from literature directs attention to the effect of active oxygen on retarding the growth rate of SM and its ability to form biofilms.[3-6] Sudden increases in oxygen concentration in SM cultures decrease the bacterial growth rate and its ability to form biofilms.[4] Assessment of the antiplaque and antigingivitis efficacies of 2 commercial toothpaste products demonstrated that active oxygen might have a role in biofilm reduction.[7] However, these studies were not conducted on orthodontic patients with different microflora.

From 49% to 97% of orthodontic patients exhibit at least 1 WSL. Using patient compliance would be one of the simplest methods to prevent WSLs.[8,9] Any modifications made to constituents of a toothpaste that would reduce the counts of SM would be a simple, relatively inexpensive, and efficient approach to prevent WSLs, as all patients undergoing orthodontic treatment use an orthodontic toothbrush and toothpaste every day. This would also offer the advantage of eliminating additional expenses for the patient, reducing chairside time for the clinician, and improving oral health. Thus, this study aimed to assess the effects of an active oxygen-containing toothpaste on SM and WSLs for 4 weeks in patients undergoing fixed mechanotherapy.

MATERIAL AND METHODS

This was a single-center, parallel-group, 2-arm randomized clinical trial with a 1:1 allocation ratio. The clinical trial was performed in accordance with Consolidated Standards of Reporting Trials 2010 guidelines.[10] No changes were made to the trial design after commencement.

Ethical approval

Approval was obtained from the University Institutional Ethics Committee (Number: IEC/21/AUG/164/55), and the trial was registered in the Controlled Trial Registry India (CTRI/2021/10/037458).

Participants, eligibility criteria, and settings

Patients aged 13-30 years on fixed mechanotherapy who had undergone therapeutic extraction of all first premolars and had completed leveling and aligning were included in the study. All patients were treated with the MBT preadjusted edgewise appliance (Ormco Mini 2000, 0.022-in slot, Ormco, Glendora, Calif). Brackets were bonded using the Enlight bonding system (Ormco) with an etch time of 30 seconds. The screening was done to identify patients with satisfactory oral hygiene and dietary practices. Oral hygiene was assessed using pretreatment and current Simplified Oral Hygiene Index (OHI-S) scores. A consistent OHI-S score <3 at both periods was required to be eligible for the study. Patients with conditions that alter the salivary flow rate, excessive drinking, poor oral hygiene, poor dietary practices, active carious lesions, repeated bracket breakages, and abnormal morphology of the lateral incisors were excluded. Eligible patients were enrolled in the study after obtaining informed consent/assent. The study setting was the Department of Orthodontics and Dentofacial Orthopedics, Sri Ramachandra Institute of Higher Education and Research (SRIHER) University.

Intervention

At baseline (T0), elastomeric modules were carefully removed, and the archwires were disengaged, after which each patient underwent scaling. Visual examination to detect WSLs was performed at T0 and after 4 weeks (T1) using The International Caries Detection and Assessment System II (ICDAS II)[11] and DIAGNOdent pen (KaVo Dental, Biberach Germany)[12] on the maxillary right and left lateral incisors. For recording ICDAS II scores, the labial surfaces were examined after drying the tooth surfaces for 5 seconds with compressed air. The assessment was performed according to the ICDAS II index criteria with a mouth mirror and a blunt probe under clinical lighting.[11] For DIAGNOdent evaluation, the pen was calibrated against the ceramic standard supplied by the manufacturer and then calibrated for each patient by measuring a sound area of the labial surface. Each surface was divided into 4 areas: mesial, gingival, incisal, and distal. Each site was scanned 3 times with the pen, and the highest value from the 3 readings was registered. Finally, the archwires were engaged, new elastomeric modules were placed, and the patients were asked to report after 4 weeks for assessment.

After 4 weeks, plaque specimens were collected using the 4-pass technique as suggested by Pellegrini et al.[13] The samples were placed into coded Eppendorf tubes and transported for storage and real-time polymerase chain reaction (RT-PCR). Assessment using RT-PCR shows the relative quantification of SM present in the samples by evaluating the cycle threshold (Ct) values. The value is inversely proportional to the amount of bacterial genome present.

Bacterial genomic isolation was done using the Purefast Bacterial DNA mini spin prep kit (HELINI Biomolecules, Chennai, India). The *Streptococcus mutans* Real-time PCR kit (HELINI Biomolecules) was used to detect and quantify SM-specific DNA. The target sequence was the gene *htrA*. An RT-PCR reaction mixture of 25 μl was prepared. The polymerase chain

reaction values were obtained using a graph interpreted using Rotor-Gene Q Software (Qiagen, Valencia, Calif).

Outcomes

SM counts were analyzed using RT-PCR at T1 and considered the primary outcome. The secondary outcome was WSL assessment, performed using DIAGNOdent and ICDAS II criteria at T0 and T1. No changes were made to the trial outcomes after commencement.

Sample size calculation

Sample size calculation was based on the data by Santamaria et al,[14] with a significance level of 0.05, 80% power, and 16 patients were required for each group. To account for loss to follow-up, 34 participants with 17 patients per group were recruited. No interim analyses or stopping guidelines were applicable.

Randomization

The random allocation sequence was generated using the RAND function in Microsoft Excel (Microsoft Corp, Redmond, Tex) to generate an experimental group (received Blue M oxygen for health toothpaste [Blue M International, Wapenveld, The Netherlands]) and a control group (received Colgate Total fluoridated toothpaste [Colgate-Palmolive Company, New York, NY]). The sequence generated was sealed in opaque white envelopes. The first author (J.A.G) generated the random allocation sequence, the second author (B.S) enrolled the participants and the first author (J.A.G) assigned the participants to the intervention.

Blinding

The investigators and the participants were blinded until allocation. Blinding of the first author (J.A.G) who dispensed the toothpaste or the participants was not possible. Plaque specimens were coded and sent for RT-PCR to ensure that the final data assessment was blinded.

Statistical analysis

Data were analyzed with SPSS (version 23; IBM Corp, Armonk, NY). Descriptive statistics, mean, and standard deviation were used to describe the data. Intergroup comparisons were made using the Mann-Whitney U test. Intragroup comparisons were made using paired *t* test. The Kendall tau-b correlation was used to determine the correlation between ICDAS II and DIAGNOdent. For all the statistical tests, $P < 0.05$ was considered statistically significant.

RESULTS

Participant flow and baseline data

The Consolidated Standards of Reporting Trials[10] flowchart demonstrates participant flow for each group, the numbers of participants who were randomly assigned and received intended treatment, and were analyzed for the primary outcome with reasons described in Figure 1. There were no exclusions after randomization. The recruitment period was from March 1 to August 31, 2021, and the follow-up lasted 1 month. The trial ended when the study duration was complete. The baseline characteristics of the 2 groups have been described in Table I.

Numbers analyzed for each outcome and subgroup analyses

The analysis was done by original assigned groups.

Real-time polymerase chain reaction

The mean Ct value in the experimental group (32.25 ± 10.60) was greater, indicating lower bacterial genomic incidence when compared with the control toothpaste group (30.90 ± 10.49). However, this was not statistically significant ($P = 0.699$) (Table II).

Assessment of WSLs

The ICDAS II scores from T0 to T1 remained the same and therefore showed no statistical significance ($P > 0.05$) (Table III).

The mean DIAGNOdent T0 to T1 scores exhibited a slight decrease in mean values for maxillary left and right lateral incisors in the experimental group and the maxillary left incisor in the control group. The DIAGNOdent value increased at T1 for the maxillary right incisor in the control group ($P > 0.05$) (Table IV). Intragroup comparisons showed no statistically significant differences (Table V).

The correlations between DIAGNOdent and ICDAS scores showed a strong positive correlation ($\tau b > 0.30$) for maxillary right and left lateral incisors, which was statistically significant (Table VI).

Harms

No harms or adverse effects were observed or reported by the patients during the experimental period.

DISCUSSION

Fixed appliances increase the plaque-retentive sites and predispose the patient to increased cariogenic risk.[15] SM have greater adhesion to orthodontic

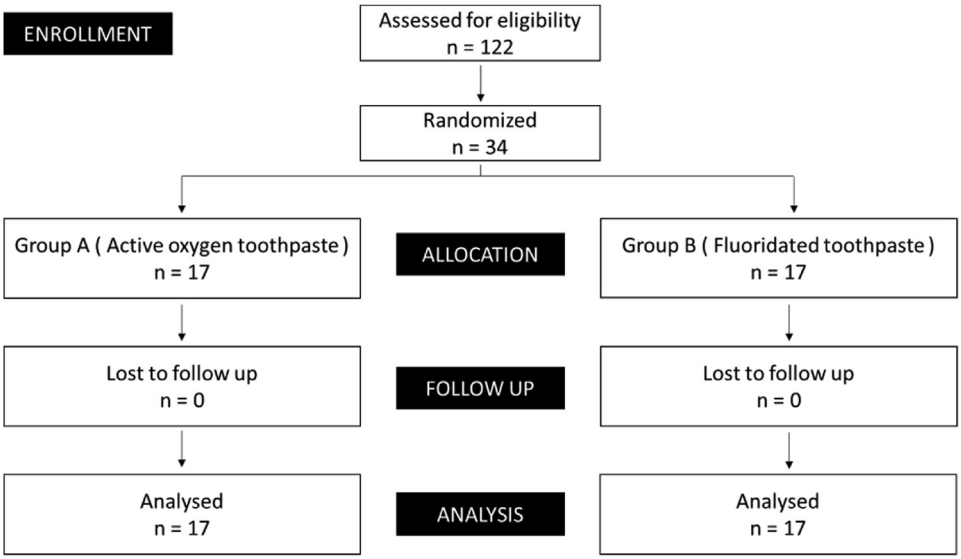

Fig. Consolidated Standards of Reporting Trials flow diagram of the study selection process.

Table I. Baseline characteristics of patients

Characteristics	*Experimental (n = 17)*	*Control (n = 17)*
Male	8	9
Female	9	8
Mean age, y	17.71 ± 2.82	19.00 ± 4.91

Table II. Comparison of Ct values (T1)

Group	*Mean ± standard deviation*	P *value*
Experimental	32.25 ±10.60	0.70
Control	30.90 ± 10.49	

Table III. ICDAS scores at T0 and T1

Group	*Mean ± standard deviation*	P *value*
Right lateral		
Experimental	0.18 ± 0.39	0.29
Control	0.06 ± 0.24	
Left lateral		
Experimental	0.35 ± 0.79	0.14
Control	0.06 ± 0.24	

Table IV. Change in mean DIAGNOdent readings from T0 − T1

Group	*Mean ± standard deviation*	P *value*
Right lateral		
Experimental	0.06 ± 0.24	0.50
Control	−0.41 ± 1.28	
Left lateral		
Experimental	0.35 ± 0.49	0.54
Control	0.29 ± 0.59	

Note. The negative sign indicates that the DIAGNOdent reading increased at T1, indicating demineralization.

Table V. Intragroup comparison of DIAGNOdent values

Group	*Mean ± standard deviation*	P *value*
Experimental		
Right lateral T0	26.82 ± 8.29	0.33
Right lateral T1	26.76 ± 8.38	
Left lateral T0	28.71 ± 12.60	0.43
Left lateral T1	28.59 ± 12.79	
Control		
Right lateral T0	24.24 ± 8.74	0.20
Right lateral T1	24.65 ± 9.10	
Left lateral T0	24.41 ± 8.26	0.27
Left lateral T1	24.24 ± 8.36	

materials and may pose a cariogenic/WSL challenge.[1] The prevalence of WSLs in 50%-97% of patients undergoing fixed mechanotherapy makes it one of the greatest challenges faced by clinicians.[8,9] Thus, methods to manage WSLs are evolving, and newer techniques are being devised.

One of the simplest methods to prevent WSLs is by utilizing patient compliance. All patients undergoing orthodontic treatment are encouraged to adhere to good oral hygiene practices, including brushing with fluoridated toothpaste.[15] Incorporation of material

Table VI. Correlation between DIAGNOdent and ICDAS scores using Kendall's tau-b

Variables	*Correlation value (τb)*	P *value*
Right lateral T0	0.381**	0.009
Left lateral T0	0.366*	0.011
Right lateral T1	0.369*	0.012
Left lateral T1	0.365*	0.012

*Statistically significant at $P < 0.05$; **Statistically significant at $P < 0.01$.

into the toothpaste, which would enhance its anticariogenic properties, would be a simple, more practical, relatively inexpensive, and efficient method to prevent WSLs. Exposure of SM to oxygen has been shown to retard its growth by decreasing the glycolytic rate and causing an extended lag phase. Studies have also reported that aeration significantly increases the susceptibility of SM to acid stress and impairs the capacity of SM to form biofilms.[3-6]

Thus, the aim was to evaluate the anticariogenic potential of active oxygen-containing toothpaste. Blue M oxygen for health toothpaste (Blue M International, Wapenveld, Netherlands) Assessment was performed by quantification of SM and recording WSLs.

All attempts to standardize the samples included streamlining the sample to patients who underwent extraction of all first premolars and were at the end of leveling and aligning and had a passive 0.017 × 0.025-in stainless steel archwire. The sequence of archwire progression and the time taken to reach the working archwire was similar for all patients, thus ensuring uniformity of the sample. Patients' oral hygiene at baseline and various time points was assessed and standardized using OHI-S. An OHI-S score <3 was required to be enrolled in the study. All patients had been treated for at least 6 months. This was done to eliminate the possible confounding effect of dental crowding and variation in archwire materials on bacterial accumulation. At T1, the experimental group showed a greater Ct value than the control group, indicative of a lower SM count, but this was not statistically significant ($P = 0.699$) (Table II). The observed results could be attributed to multiple factors which could not be controlled, such as the sucrose content of the diet, the fluoride content of the tooth and plaque, possible immune mechanisms in the saliva, genetic factors, and inherent characteristics of SM.[16]

The ICDAS II[11] is an evidence-based system for detecting and diagnosing caries that was developed in response to the need for introducing standardized systems to avoid differences in diagnosis, prognosis, and clinical management of caries lesions among different practitioners. The advantages of using the ICDAS II are that it is easy to use, inexpensive, and less time-consuming, but more subjective and less informative for the patient.

Plaque samples were used for the quantification of SM. Salivary samples were not considered for quantification as the SM count in saliva also includes the bacteria on the tongue and soft tissues. The plaque accumulated around an orthodontic bracket is a more specific region and would therefore be more reliable. Further, differences between the salivary SM counts and SM counts in plaque have been demonstrated.[16,17]

The polymerase chain reaction is a simple, extremely sensitive, and specific tool to determine the expression level of target genes. In an RT-PCR assay, a positive reaction is detected by accumulating a fluorescent signal. The Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

Demineralization of teeth can occur without clinical manifestation, and DIAGNOdent is a quantitative and reliable method to assess WSLs and demineralization. The DIAGNOdent pen is more informative for the patient, and it is also easy to use and carry in the clinic, but the disadvantages are that it is expensive and more time-consuming.[18] WSLs were assessed using the ICDAS II criteria and DIAGNOdent pen as a combination of these methods has been suggested to be best for detecting carious lesions.[19,20] Baeshen et al[18] showed that the clinical index correlated well with the DIAGNOdent values and stated that progression and regression of WSLs could be registered using both methods, which is similar to the results obtained in our study (Table VI).

After 4 weeks, DIAGNOdent readings decreased for both lateral incisors in the experimental group, indicating potential remineralization. However, a variable effect was observed for the control group scores in that remineralization was observed for the maxillary left incisor, whereas demineralization was observed for the maxillary right incisor (Table V). The decrease in mean DIAGNOdent scores was observed to be greater for the maxillary left incisor in both groups. This could be due to right-handed brushing being more effective on the left side.[21] All patients included in our study were right-handed. However, these changes were not statistically significant (Table VI). The ICDAS II scores were in tandem with the DIAGNOdent readings indicating a negligible change in the progression or regression of WSLs.

The planned observation period for the study was 4 weeks. This was because visible WSLs have been observed to develop as early as 4 weeks. Furthermore, research has shown that the effect of toothpaste on

SM can be observed in as early as 1 week.[22] As the results did not show statistically significant differences at 4 weeks, we decided not to extend the observation period as we felt it would be unethical to pursue the effectiveness of a toothpaste approximately 10 times more expensive than regular toothpaste and without knowledge of the medium to long term side effects of the oxygen for health toothpaste.

The formation of WSLs is composite; and can be attributed to the complex interplay of factors such as diet, fluoride use, the extent of plaque, caries susceptibility, bacterial and salivary activity, and social and behavioral factors. With current research focusing on preventing WSLs, optimum use of a dentifrice is one of the methods to manage WSLs. This study showed that both types of toothpaste demonstrated comparable effects against SM and WSLs.

The results can be generalized to the orthodontic population and depict the importance of routine oral hygiene measures like tooth brushing in the prevention and treatment of WSLs.

Limitations

All patients were encouraged to follow good oral hygiene and dietary practices. As dental caries is a multifactorial disease, factors such as bacterial and salivary activity, caries susceptibility, and lack of adherence to dietary and oral hygiene practices could have confounded the results, which is a limitation of this study.

CONCLUSIONS

1. At the end of 4 weeks, there was a decrease in SM counts in the active oxygen-containing toothpaste group than in the fluoridated toothpaste group, but this was not statistically significant.
2. The ICDAS and DIAGNOdent scores showed no remineralizing activity or incidence of new WSLs in the patients using the active oxygen-containing toothpaste.
3. The ICDAS and DIAGNOdent scores showed no remineralizing activity or incidence of new WSLs in the patients using fluoridated toothpaste.
4. There were no changes in WSLs between the active oxygen-containing and the control toothpaste groups.
5. Active oxygen-containing toothpaste is as effective as fluoridated toothpaste against SM.

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