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Comparative Evaluation of Efficacy of 0.2% Chlorhexidine and Triphala Mouth Rinse on Prevention of Streptococcus Mutans Biofilm Collected From Excessive Adhesive Flash (EAF)

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Abstract

Objective: To evaluate and compare the efficacy of 0.2% Chlorhexidine and Triphala mouth rinse on prevention of streptoccus mutans biofilm collected from Excessive Adhesive Flash (EAF).

Materials and Method: 20 patients were selected and segregated on the basis of simple random sampling into 2 groups of 10 each: Group 1: 0.2% Chlorhexidine; Group 2: Triphala. Plaque was collected from the labial surface of maxillary lateral incisors using a sterile curette at four intervals: T0-before bonding, T1-1 month after bonding, T2-2months after bonding, T3-3months after bonding. S. Mutans was quantified by colony count procedures.

Results: After analysing the readings by Student's paired and unpaired t-tests, no significant difference was found between the colony counts of both groups at T0, T1, T2, T3.

Conclusion: The preventive efficacy of Triphala was comparable to that of Chlorhexidine.

Keywords: EAF, Triphala

Introduction

Multibracket orthodontic appliances are more prone for dental plaque retention, thereby making maintenance of oral hygiene a cumbersome task for such patients(1). Patients receiving fixed orthodontic appliance treatment are at a considerable risk of enamel demineralization, because of inaccessibility for cleaning the minute areas of

these appliances(2). Orthodontic treatment may bring about oral ecological variations, causing an increase in streptococcus mutans in saliva, and plaque accumulation(3). Dental plaque formation is the initial step in dental caries formation, and streptococcus mutans is considered as one of the main colonizers in the multi-species dental biofilm(4). During fixed orthodontic appliance treatment, up to 5-fold increase in the number of streptococcus mutans has been observed(4). Both these considerations imply the need for prophylactic measures against colonization of streptococcus mutans(4). Also, orthodontic attachments and bonding materials may retain plaque and promote initiation of "white spot lesions" (WSL) and dental caries(5). The most commonly involved teeth include the maxillary lateral incisors, maxillary canines and mandibular premolars(6).

While doing bracket bonding procedures, an unspecified quantity of adhesive is left on the surface of the tooth, invariably around the periphery between the appliance and enamel interface. This is termed as "Excessive Adhesive Flash" (EAF)(2). If this excessive flash is not promptly removed during the process of bonding, it may frequently contribute as a mechanically noxious stimuli causing irritation of the gingiva, specifically on teeth, such as premolars, where the vertical length from bracket pad to marginal gingiva is less(6). Secondarily, bacteria will readily inhabit the rough surface and accelerate the incidence of white spot lesion and dental caries(2). Thereby, EAF acts as a harbour area for anchoring various pathogenic bacteria, especially streptococcus mutans(2).

In orthodontic patients undergoing fixed appliance therapy, optimum mechanical plaque control is impaired due to presence of multiple brackets and bands on the tooth surface, ligatures and wires. Caries-preventive operations, optimum dental hygiene maintenance, non cariogenic diet, and systematic fluoride supplementation are generally inadequate in preventing demineralization and initiation of new carious lesions in fixed orthodontic appliance therapy subjects with increased caries susceptibility(3). Preventive measures in these susceptible populations have been focused on direct inhibition of the cariogenic oral microflora by chemotherapeutic agents, in addition to enhanced oral hygiene. The usage of extraneous anti-caries agents has been demonstrated to be effective for controlling demineralization and caries during orthodontic treatment(2).

Owing to emerging trends in phytodentistry, different ayurvedic formulations for plaque control are being researched upon(7). "Triphala" is an established powdered preparation in Ayurveda, containing equal portions of "Emblica officinalis", "Terminalia Chebula" and "Terminalia belerica"(7). Currently, triphala is being expansively investigated for its numerous healing benefits including its anti-microbial, antioxidant, anti-caries, and anti-collagenase activities(7).

Chlorhexidine, a very broad-spectrum antimicrobial cationic bisbiguanide is considered as gold standard and is widely used alone as well as an adjunct to mechanical oral hygiene procedures(8). The major advantage of chlorhexidine lies in its substantivity by virtue of which it binds to soft and hard tissues in the oral cavity, thereby enabling it to act over a prolonged duration after application of a formulation(8). Chlorhexidine mouthwash is routinely prescribed in the orthodontic patients of our department. But, as chlorhexidine usage is associated with several documented adverse effects, such as tooth discoloration, altered taste sensation, greater supragingival calculus formation and less frequently, desquamation of the oral mucosa which restrict its prolonged use, it was felt that a mouthwash along with its other advantages like easy availability and cost efficiency, could serve as an

effective alternative in orthodontic patients. Even though there are a wide range of herbal mouth rinses available, the target of this research was to compare the anti-cariogenic efficacy of triphala mouth rinse formulated at Mahatma Gandhi Ayurveda College, Hospital and Research Centre, Datta Meghe Institute of Medical Sciences (DMIMS), with the already proven effective chemotherapeutic chlorhexidine.

The goal of this research was to comparatively evaluate the preventive efficacy of herbal triphala mouth rinse over conventional chlorhexidine mouth rinse on streptococcus mutans biofilm formation, over the excessive adhesive flash produced from two different types of orthodontic adhesive resins, in patients undergoing fixed appliance therapy.

Materials and Methods

For this research, a prospective interventional study design was planned and followed. The research was planned in the Department of Orthodontics and Dentofacial Orthopaedics, Sharad Pawar Dental College and Hospital in alliance with the Department of Microbiology, Jawaharlal Nehru Medical College; Department of Oral Pathology and Microbiology, Sharad Pawar Dental College and Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurvedic College and Hospital.

Inclusion criteria

- Patients between 15-25 years of age undertaking fixed orthodontic appliance therapy in the department, immaterial of the type of malocclusion.
- Patients being treated with pre-adjusted edgewise brackets.
- Patients with maxillary lateral incisors devoid of caries, hypoplasia or any other
- developmental anomalies(9).
- Patients with good general health.
- Patients with good periodontal health and without severe gingivitis or periodontitis, with adequate alveolar bone support.
- Patients devoid of any systemic diseases, or on antibiotic therapy having oral manifestations.
- Patients not using any other mouth rinses in the past 3 months.
- Patients with no history of hypersensitivity to the materials being used.

20 patients were selected on the basis of satisfaction of the above inclusion criteria. The patients were then segregated on the basis of simple random sampling into 2 groups:

- Group 1: 0.2% Chlorhexidine mouth rinse (Standard Medical Care-SMC) [Mouthwash 1: CHX]
- Group 2: Triphala mouth rinse [Mouthwash 2: Triphala]

Written consent was acquired from the patients, and the procedure was explained at length. Before the treatment, and during every follow-up visit, patients were explained oral hygiene instructions. Verbal directions and physical demonstration were provided on how to perform effective oral hygiene maintenance, particularly, close to the brackets and ligatures. Patients were asked to refrain from consuming food or beverages or performing any oral hygiene procedures, for two hours prior to collection of plaque samples.

Preparation of Triphala mouth rinse(10):

Initial drug preparation

- Raw materials of plant origin were obtained from Dattatraya Ayurveda Rasashala Sawangi, Wardha.
- These raw materials were then identified and authenticated by a taxonomist.
- Pharmaceutical preparation of Triphala mouth rinse was conducted at Dattatraya Ayurved Rasashala,

Mahatma Gandhi Ayurveda College, Hospital and Research centre, Salod (H) Wardha, Maharashtra.

- The preparation was tested for organoleptic characters, physicochemical analysis, and microbial contamination in analytical lab as per API standards.
- Ingredients of Triphala mouth rinse are depicted as follows:

➢ "Haritaki" ("Terminalia Chebula Retz"): Fruit pulp-1 part.
➢ "Bibhitaki" ("Terminalia Belerica Roxb"): Fruit pulp-1 part
➢ "Amalaki" ("Emblica officinalis Gaertn"): Fruit pulp-1 part
➢ Water for decoction: 16 parts
➢ Alcohol (Ethanol): 5%
➢ Menthol (Peppermint): 0.042%
➢ Thymol (Thymus vulgaris): 0.064%

Preparation of mouth rinse

- The procedure of Kwatha kalpana was followed for the preparation of Triphala mouth rinse.
- All the above-mentioned raw ingredients were coarsely powdered, weighed individually, and then amalgamated together meticulously.
- This amalgamation was diluted 16 times by addition of distilled water, and boiled in a steel vessel at 90 - 100˚ C temperature till the solution had reduced to 1/4th of its original volume.
- Throughout the procedure, the solution was continuously stirred.
- The decoction was then sieved through a piece of cloth.
- 5% alcohol, 0.042% Menthol and 0.064% Thymol were added and mixed with a stirrer to get a homogenous liquid.
- Finally, it was filled in 200 ml amber coloured plastic bottles and packed with air tight lid.

Methodology

- Designated portion of the anterior dentition for plaque collection was isolated with cotton rolls, promptly dried, disclosing agent (Plaque-D by MAARC) was administered for visualisation of biofilm, and plaque was collected from the labial surface of maxillary right lateral incisor using a sterile curette, prior to bonding, to determine the carriage of Streptococcus Mutans by the patient, which was termed as T0.
- The same procedure was repeated for the maxillary left lateral incisor.
- The appliance bonding procedures were then carried out using Transbond XT while maintaining proper isolation.
- The placement of appliances was done by a single operator for all the patients to avoid inter-operator bias.
- After 1 month, plaque samples were obtained from the excessive adhesive flash on the lateral incisor from both the sides, and this reading was termed as T1.
- Such an interval had been chosen in accordance with the assumption that there will be sufficient colonization of bacteria around the surface of the orthodontic adhesive.
- These readings were compared with T0 to understand the difference in colonization of streptococcus mutans, before and after the placement of fixed appliances.
- After taking samples for T1, patients were advised to use mouth rinse for maintenance of oral hygiene, and they were randomly segregated into two groups.
- The two groups were based on the different mouth rinse being used by the patient.

Group 1: received 0.2% Chlorhexidine which is a gold standard

Group 2: received Triphala mouth rinse

- Patients were given instructions about how to use the mouth rinse and a checklist, in the form of an instruction chart, for the purpose of making them adhere to the guidelines of the study and to maintain a level of stringency while following oral hygiene protocol.
- Patients were then recalled after 1 month from the date of prescription of mouth rinse.
- Samples were collected again and the readings were termed as T2.
- Similarly, patients were recalled at the termination of third month of treatment, and T3 readings were recorded for the patient.
- T0, T1, T2 and T3 readings were compared with each other, by appropriate

statistical methods and the difference obtained in the colonization of S. Mutans at each interval was noted to arrive at the conclusion assessing the preventive efficacy of each of the above mouth rinses.

Procedure for sample collection and testing

The amount of S. Mutans was quantified by colony count procedures. The plaque samples were collected while maintaining a dry field from the excessive adhesive flash, using a sterile curette. The samples were then added to a test tube containing 2ml of transport fluid (brain-heart infusion broth), and taken to the microbiology laboratory within 2 hours for processing. Samples were then plated on Mitis Salivarius agar for identification of S. Mutans. A single agar plate was divided into two sections, where one half was used to streak the sample collected from the left side, and the other half was used for the right side. Streaking was done using sterile nichrome loops of 4mm diameter. Agar plates were kept in an incubator set at 37˚C in a candle jar or in an anaerobic jar with gas pack for 48 hours. Total colony count for quantification of S. Mutans was obtained from the Mitis Salivarius agar plate. The number of colonies were counted manually for each sample, and documented.

Statistical Analysis: The software employed for statistical analysis of the data were SPSS 22.0 version and Graph Pad Prism 5.0 version, and $p<0.05$ was considered as level of significance. The statistical tests implemented for the scrutiny of the Results Were

1. Students paired t test
2. Students unpaired t test

Observations and Results

Table 1: Comparison of colony of streptococcus mutans in adh 2 Transbond XT in

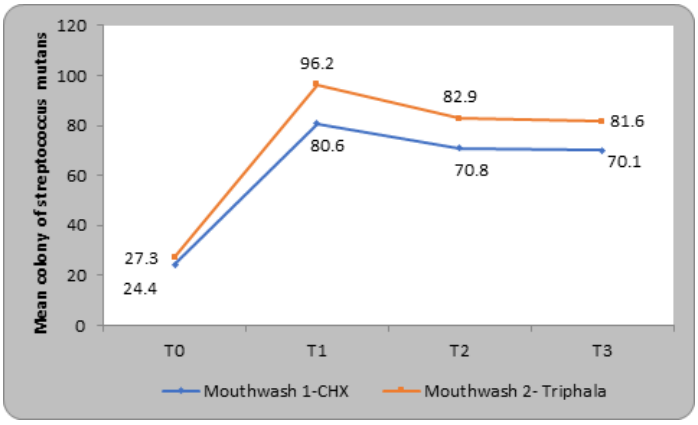
Group-1: mouthwash 1- CHX and

Group-2: mouthwash 2-triphala

Student's unpaired t test

Time	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
T0	Mouthwash 1-CHX	10	24.40	12.13	3.83	0.65	0.52,NS
	Mouthwash 2- Triphala	10	27.30	7.16	2.26		
T1	Mouthwash 1-CHX	10	80.60	23.07	7.29	1.81	0.086,NS
	Mouthwash 2- Triphala	10	96.20	14.28	4.51		
T2	Mouthwash 1-CHX	10	70.80	21.28	6.73	1.61	0.123,NS
	Mouthwash 2- Triphala	10	82.90	10.31	3.26		
T3	Mouthwash 1-CHX	10	70.10	20.62	6.52	1.58	0.130,NS
	Mouthwash 2- Triphala	10	81.60	9.93	3.14		

Graph 1: Comparison of colony of streptococcus mutans in adh 2 Transbond XT in Group-1: mouthwash 1- CHX and Group-2: mouthwash 2-triphala



- Mean colony formation for mouthwash 1: CHX was 24.40±12.13 and for mouthwash 2: Triphala it was 27.30±7.16 at time T0. Statistically no significant difference was observed in mean colony formation among the patients of two groups (t=0.65, p=0.52).
- Mean colony formation for mouthwash 1: CHX was 80.60±23.07 and for mouthwash 2: Triphala it was 96.20±14.28 at time T1. Statistically no significant difference was observed in mean colony formation among the patients of two groups (t=1.81, p=0.086).
- Mean colony formation of mouthwash 1: CHX was 70.80±21.28 and for mouthwash 2: Triphala it was 82.90±10.31 at time T2. Statistically no significant difference was observed in mean colony formation among the patients of two groups (t=1.61, p=0.123).
- Mean colony formation for mouthwash 1: CHX was 70.10±20.62 and for mouthwash 2: Triphala it was 81.60±9.93 at time T3. Statistically no significant difference was observed in mean colony formation among the patients of two groups (t=1.58, p=0.130).

Discussion

For decades now, enamel demineralization has been a commonly associated phenomenon with orthodontic treatment using fixed appliances(12). Early prevention of demineralization during the course of comprehensive orthodontic treatment is a herculean expostulation encountered by practicing clinical orthodontists despite emergence of newer modalities in caries prevention(12). The progression of white spot lesions (WSLs) during orthodontic treatment is credited to prolonged accumulation of dental plaque and its retention around the brackets(12). The biologically programmed self cleansing actions of the oral musculature and saliva are deterred by the roughened surfaces of attachments such as orthodontic bands, brackets, and wires(13). Fixed orthodontic appliances hinder good routine oral hygiene practice; thereby increasing the number of sites for plaque retention on those surfaces of the teeth that are usually less inclined to caries development(12). These lesions are highly unpleasant in terms of aesthetics and health, and cause reversible enamel damage(14). Hence, early recognition of WSLs during orthodontic treatment is of utmost importance, as it would let practitioners to implement preventive measures against demineralization process before lesions progress any further(12).

Chlorhexidine is a proven- effective antimicrobial agent against streptococci and dental caries that has been extensively documented in literature(15). Chlorhexidine has several side effects, such as tooth staining, gustatory alteration, increased supragingival calculus deposition and occasionally, desquamation of the oral soft tissues which limit its long term use(8).

Ayurvedic formulations have been used for eons as oral preparations which are used for periodontal therapeutic purposes. “Triphala” is among the most popular Traditional Ayurvedic Medicinal Formulations in use(16). An amalgamation of the fruits obtained from three trees, “Bibhitaki”, “Amalaki”, and “Haritaki”, Triphala is highly valued for its quality to regulate the process of digestion and elimination(16). Many studies have compared the inhibitory effect on Streptococcus Mutans, as well as periodontal effects of Triphala mouth rinse with Chlorhexidine mouth rinse(8,16).

However, the formulation of Triphala mouth rinse varies from institute to institute, which alters its antibiotic properties. Also, the direct effect of chlorhexidine and Triphala mouth rinses on orthodontic adhesives had so far not been assessed. The above findings indicate that both the mouth rinse groups show a gradual progressive decrease in the number of colony-forming units at T2 and T3 as compared to T1. Patients of the Triphala group show higher mean colony formation as compared to

patients of the chlorhexidine group, but the difference is not statistically significant at both intervals. Similarly, our findings were supported by the study conducted by Tandon(17) et al (2010) and Bajaj(16) et al (2011). However, both the above two studies were conducted on children within the age group of 8-12 years, whereas our study evaluated patients between 15-25 years of age. Also, the subjects of the above two studies were not undergoing orthodontic treatment.

Likewise, Niranjane(8) et al (2016), planned a research comparing the therapeutic changes observed with Triphala and chlorhexidine mouthwashes in 150 patients undergoing fixed orthodontic appliance therapy. The study established a reduction in the plaque score of patients using Triphala and chlorhexidine mouthwashes, stating that the anti-plaque effects of Triphala were similar to that of chlorhexidine.

However, in our study, a reduction in the number of colonies after mouth wash prescription may have also been obtained due to Hawthorne effect, which is the alteration of behaviour by the subjects of a study due to their awareness of being under observation. Even though we assessed the growth in the number of S. Mutans, the specific strain of S. Mutans responsible for WSLs was not isolated. The difference in between the amount of colonization between male and female subjects was not assessed. Variations on the basis of malocclusion were not taken into account. Further studies may be planned with a larger sample size and over a longer time period to assess the prolonged use of Triphala mouth rinse.

Conclusion

The colony counts of Streptococcus Mutans increased in the oral cavity within one month after bonding procedures. Both Triphala and Chlorhexidine mouth rinses, effectively decreased the colony counts of Streptococcus Mutans on the excessive adhesive flash, formed from both adhesives. The preventive efficacy of Triphala was comparable to that of Chlorhexidine. Hence, Triphala mouth rinse can be prescribed as an economic alternative in rural areas, where the availability of chlorhexidine is questionable. It is necessary to educate the patients regarding maintenance of optimum oral hygiene, and constantly motivate them to do so, throughout the orthodontic treatment period. Also, the use of adjunctive oral hygiene measures should be advocated in these patients to ensure better quality of treatment outcomes.

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