

Study Protocol

Evaluation of Antimicrobial efficacy of *Triphala* Oral Spray in patients undergoing Orthodontic treatment- A randomized clinical trial.

Abstract:

Background: Malocclusion is one of the most susceptible cause of development of periodontal diseases and dental caries in young individuals undergoing fixed orthodontic treatment. It provides a surface area for the accumulation of food particles and which further leads to plaque formation. Various mechanical and chemical aids are been used regularly to decrease the microbial load and accumulation of plaque. Chemical adjunct such as chlorhexidine mouthwash is widely used as antimicrobial agent which has significant efficacy against oral Streptococci and Actinomyces species. Conventional medications have been replaced by herbal agents due to development of antibiotic resistance. Among herbal medicaments *Triphala* is the well-known drug formulation which can be used as an adjuvant for commercially available chemical aid. Chlorhexidine mouth wash requires an assistance of the caregivers. Therefore, there is a need of a newer modality to maintain oral hygiene in patients undertaking orthodontic corrections. **Objectives:** **1.** To evaluate the antimicrobial efficacy of *Triphala* oral spray on Streptococcus mutans levels in children undergoing orthodontic treatment. **2.** To evaluate the efficacy of *Triphala* oral spray in reduction of dental plaque in children undergoing orthodontic treatment. **3.** To evaluate the efficacy of *Triphala* oral spray on in reduction of gingivitis in children undergoing orthodontic treatment.

Methodology: A randomised control trial will be done among – participants with the age group of 10 to 15 years of age. A total of 25 children are included in the study. The participants will be told to use the spray two times a day for one weeks. After a period of 7 days, plaque index and gingival index will be re-recorded and plaque sample will be collected for post-intervention microbial analysis.

Results: *Triphala* based oral spray will be effective in reduction of plaque score, reduction in gingival inflammation and will show inhibitory effects on microbial count.

Conclusion: *Triphala* oral spray can be used as an adjuvant by the orthodontic patients for reduction of the microbial load. It also provides better acceptability by the children as it does not require any assistance of the caregivers.

Keywords: *Triphala* based Oral spray, Dental Plaque, Gingivitis.

Introduction:

Malocclusion is one of the most susceptible cause of developing the periodontal disease and dental caries in children.¹ Microorganisms reside passively in the oral cavity and further accumulation of these microorganisms may lead to biofilm formation. These biofilms as plaque, enhance synergistic associations among virulent pathogenic microorganisms. It is responsible for

dental caries, periodontal as well as other oral diseases.² The circumstances of increasing dental caries & periodontal disease is reduced in patients who are using removable appliances.³ Nevertheless, fixed orthodontic appliances provides a surface area for the accumulation of food particles and which further leads to plaque formation.

Patients undertaking orthodontic treatment experiences enamel demineralization prompting initiation of dental caries, chiefly in the locations adjacent to the brackets. Mostly chemical and mechanical measures are being used regularly to decrease the microbial load and accumulation of plaque. Frequently used aids for mechanical elimination of plaque in individuals undergoing orthodontic treatment are soft bristles toothbrush and interdental brushes. Antimicrobial mouth rinses are also used as an adjunct aid.

Chemical adjunct such as chlorhexidine mouthwash is widely used as antimicrobial agent which has significant efficacy against oral Streptococci and Actinomyces species. In current studies, It has been coated that sodium fluoride rinses effective in treating halitosis, plaque, gingivitis and aids in decrease the in appearance of white spots in children who are undergoing orthodontic corrections. Acidulated phosphate fluoride-based rinses is promoted commercially to decrease the enamel decalcification and microbial colonization all around the orthodontic brackets. The major purpose for utilization of any antimicrobial mouth rinse as it involves all the tooth surfaces which are not self-cleansable by mechanical measures. Oral sprays have also been observed as a possible substitute for chemical plaque control as it is well accepted by the patients along with their parents due to its lesser ill effects particularly in patients who are unable to follow the instructions for mouth rinsing.⁴

Lately, the conventional medicines are being exchanged by herbal agents due to increasing problem of antibiotic resistance. Amongst the herbal medications, *Triphala* is a well-known drug formulation used in traditional ayurvedic medicine. It comprises of *Emblica officinalis* (Amlaki), *Terminalia chebula* (Haritaki) and *Terminalia bellerica* (Bibhitaki).⁵ *Triphala* preparation have effectively prevented plaque accumulation on the surface of the tooth, as it inhibits the sucrose induced attachment and prevents glucan induced accumulation. The antimicrobial, antifungal, anti-inflammatory and antioxidant properties of *Triphala* are proven in in-vitro studies and numerous randomized controlled clinical trials using *Triphala* mouthwash.⁵

Rationale:

Orthodontic treatment leads to poor oral hygiene maintenance. Mechanical aids such as tooth brushing and flossing are not sufficient to maintain oral hygiene. Though, mouth rinses are prescribed as an adjunctive aid, their use is limited due to the presence of certain disadvantages such as the decrease in the bond strength of the orthodontic brackets, development of white spot lesions, and demineralization of enamel.⁶ Moreover, it is advised to use mouth rinse under the supervision of parents or caregivers. Therefore, there is a need of a newer modality to maintain oral hygiene in patients undertaking orthodontic corrections.

Aim:

To evaluate the antimicrobial efficacy of *Triphala* oral spray in patients undergoing Orthodontic treatment- A randomized clinical trial.

Objectives:

1. To assess the antimicrobial efficiency of *Triphala* oral spray on Streptococcus mutans levels in children undergoing orthodontic treatment.
2. To assess the efficiency of *Triphala* oral spray in reduction of dental plaque in children undergoing orthodontic treatment.
3. To assess the efficiency of *Triphala* oral spray on in reduction of gingivitis in children undergoing orthodontic treatment.

Material and Methods:

Statistical Analysis:

The sample size was calculated using:

$$n = (2\alpha + 2\beta)^2 [\delta_1^2 + \delta_2^2 / K] \div \Delta^2$$

Where;

2α is the level of significance at 5% i.e; 95%

Confidence interval = 1.96

$Z\beta$ is the power of the test = 80% = 0.84

Δ_1 = SD of GI in control group = 0.63

Δ_2 = SD of PI in CHX group = 0.35

$\Delta = 0.08 - 0.50 = - 0.42$

$K = 1$

$N = (1.96 + 0.84)^2 (0.63^2 + 0.35^2/1) \div (0.42)^2$

= 23.08

25 patients.

Sample Collection:

- Diagnostic instruments
- Gloves
- Mouth mask and head cap
- *Triphala* oral spray
- Sterile paper points
- (HiMedia) Disposable Eppendorf Tubes with stand & box
- Phosphate buffered saline
- Periodontal probe

For Microbial Analysis:

- Centrifugating machine
- Agar to be used- Mitis salivarius agar
- A standard loop
- Specimen collecting loops
- Anaerobic jar

Method

Study Design

This randomized clinical trial will be conducted at the Department of Pediatric and Preventive Dentistry, Sharad Pawar Dental College in collaboration with Department of Orthodontics, Sharad Pawar Dental College, Department of Dravyaguna, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod, Wardha and Institution of Pharmaceutical Education and Research, Borgaon, Meghe, Wardha.

The study protocol will be explained to the participating children and their parents. Further, a printed prior permission will be taken from the care givers.

Inclusion Criteria:

- Healthy children with the age range of 10 to 15 yrs.
- Children undertaking fixed orthodontic corrections.

Exclusion Criteria:

- Special health care needs
- Children who were on medications that could influence gingival tissue eg. Phenytoin, Cyclosporine, etc.
- Children who have taken antibiotics or anti-inflammatory drugs in the past 3 months
- Children suffering from systemic diseases.

Data Collection:

The data collected was calculated on the basis of the previous literature using the sample size formula. A total of 25 children will be included for the study. Simple random sampling method will be adopted for selection of the participants. They will be instructed to follow routine oral hygiene practices prior to intervention. Clinical parameters namely, plaque index and gingival index will be recorded and plaque sample will be collected for at baseline for microbial analysis. Subsequently, *Triphala* oral spray will be provided to the parents. The participants will be told to use the spray two times a day for one week. They will be instructed to apply the spray on the both the side of the mouth and on the both buccal/labial and lingual/palatal aspects of the teeth. Parents/guardians will be asked to apply the spray and supervise children during oral hygiene procedures. After a period of 7 days, plaque index and gingival index will be re-recorded and plaque sample will be collected for post-intervention microbial analysis.

Sample Collection:

Plaque samples will be collected from each individual at baseline and after 7 days. Patient will be instructed to perform pre procedural mouth rinsing with saline so as to eliminate all the food debris prior to the collection of plaque specimens. Collection of the plaque specimens will be performed under sterile environment with sterile paper points from a particular area (palatal groove of maxillary first molar) to a particular depth, so as the homogenous portion of the paper point (colored area) is exposed to the tooth for 5 secs. The samples will then be incorporated in 1 ml of phosphate buffered saline (PBS).

Microbial Assays:

The plaque samples will be swirled for 10 secs and instantly will be sub cultured on Mitis salivarius agar for streptococcus species taking 5 ml of plaque in PBS. A semi quantitative that is four quadrant streaking method will be adopted. With the help of standard loop, the saliva will be streaked on the Mitis salivarius agar.

The plates will be incubated anaerobically at 37°C for the period of 18 – 48 hrs. for successive listing of *S. mutans* colonies with the help of dissecting microscope. The colony-forming units for each millilitre will be estimated. The gained figures will be arranged and exposed to statistical evaluation.

Statistical Analysis:

Analytical test like Paired t-tests will be carried out using a P-value <0.05 to determine if remarkable differences exist between the time interval. All the statistical analysis will be performed using SPSS software, version 20. Data obtained will be tabulated and compared between two groups.

Discussion:

Rathore K et al.⁽¹⁾ assessed the antimicrobial Effect of Mouthwashes in Patients Undergoing Orthodontic Treatment. The study was conducted on 30 patients undergoing fixed orthodontic treatment between the ages of 15-25years. Patients were divided into 3groups - Group 1 control group, Group 2 using freshclor for 30 secs twice daily and group 3 using HiOra mouth wash for 30sec twice daily. Samples from tooth surfaces were collected at 1st day, 30th day and 90th day interval and were incubated for 48 hours. Colonies were counted using digital colony counter. Freshclor and HiOra mouthwashes showed the maximum potential for the control of pathogenic organisms, and prevention of gingivitis and bacterial plaque inhibition than patients those were not using mouthwash.

Fard B et al.⁽³⁾ evaluated Effectiveness of Mouth Washes on Streptococci in Plaque around Orthodontic Appliances. A double-blind randomized cross-over clinical trial on 25 orthodontic patients, classified into 6 groups was done to assess MS in plaque and saliva with the side specific modified Strip-Mutans technique and the plaque (PI) was measured before and after rinsing using 3 types of commercial mouth-rinses. Listerine was not effective on accumulation of MS in plaque or saliva and Ortho-kin was more effective than Listerine and Oral-B in decreasing PI.

Olympio K et al.⁽⁷⁾ evaluated Effectiveness of a chlorhexidine dentifrice in orthodontic patients. . Volunteers in fixed orthodontic therapy used the following dentifrices: 1100 ppmF, NaF (group A, n 5 27); experimental, 1100 ppmF, NaF and chlorhexidine 0.95% (group B, n 5 28); and experimental, chlorhexidine 0.95% (group C, n 5 28). At baseline, after 6, 12 and 24 weeks, clinical examinations were carried out. The results suggested that the use of dentifrices containing chlorhexidine seems to be effective for the treatment of gingivitis in orthodontic patients.

Saxena S et al.⁽⁸⁾ conducted a study to evaluate the efficacy of *Triphala*, *T. chebula*, *T. bellirica* and *E. officinalis* aqueous extract rinses separately on *Streptococcus mutans* count at various time intervals. The study concluded that *triphala* in 10% concentration used once in a day as a mouth rinse brought down the oral streptococci count by the end of 60 minutes significantly. When individual ingredients of *triphala* were used separately as mouth rinses they were not as

effective. *E. officinalis* was found to be least effective. *Triphala* extract may be recommended as an antibacterial mouthwash in prevention of dental caries. Few of the related studies were reviewed⁽⁹⁻¹¹⁾.

Scope:

The study will encourage the use of preventive measures in children undergoing orthodontic treatment and prevent the further incidence of caries.

Implications:

The present study will help in maintenance of the oral hygiene in children who are treated with orthodontic treatment.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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