To Ascertain If Bracket Material Difference (Ceramic And Stainless Steel) Has An Effect On Streptococcus Mutans Count In Orthodontic Patients When Used With Herbal Dentifrices.

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DOI:10.47750/pnr.2022.13.S08.594

Abstract

Objectives: The present study investigated the role of bracket material difference (Ceramic and Stainless Steel) on Streptococcus mutans count in orthodontic patients when used with Herbal Dentifrices.

Material and methods: This prospective study consisted of 30 patients who were undergoing fixed orthodontic treatment. A total of 60 teeth were included in the study – one tooth from first and third quadrant of the mouth of all the patients. Plaque samples were collected at various time interval. The bacteriological study was conducted by Dilution Plating Method. The data collected was tabulated and subjected to statistical test by SPSS software. One-way ANOVA was used to compare each individual groups. Multiple comparison tests (Tukey HSD) were used to compare different brackets with herbal dentifrices individually.

Results: Among 30 orthodontic patients using herbal tooth pasteit was seen that Streptococcus mutans count was slightlylow with Stainless steel brackets at mean difference 2.5333 & high with Ceramic brackets at mean difference 2.6667.But there was no statistically significant difference between them.

Conclusion: Both statinless steel and ceramic brackets performed well with herbal tooth paste andclinically efficient in reducing Streptococcus mutans colony counts around them .

Keywords: Streptococcus Mutans¸ Herbal toothpaste¸ Stainless steel brackets¸ Ceramic brackets.

INTRODUCTION

Conventionally orthodontic patients use some form of cleansing equipment like a toothbrush aided with dentifrices of their choice. The market is flooded with Ayurvedic products. Most often during orthodontic therapy the patient is left to his personal choice in selecting the toothpaste with most orthodontist prescribing specifically only orthodontic toothbrush/mouthwash. A number of controlled clinical trials have demonstrated that tooth brushing with herbal dentifrices reduces supragingival plaque and gingivitis[1]. MeswakSalvadora persica (Meswak) is a medicinal herbal plant which has been used for centuries as oral hygiene tools. Chemical analysis of S. persica demonstrated the presence of many components exhibiting antimicrobial effect. Meswak (*Salvidora persica*) contains salvadorine and trimethylamine, which are shown to exhibit anti-bacterial effects on cariogenic[2] bacteria such as *Streptococcus mutans.* It has been shown that these active principles support periodontal health, reduces the accumulation of biofilm-like dental plaque formation and exhibits fungistatic activity against *Candida albicans*[2]. The World Health Organization (WHO) endorsed the usage of the meswak for its oral cleanliness features and scientific research therefore validating its anti-bacterial and plaque preventing qualities. Meswak is an eco-friendly, natural and cost effective way to maintain oral hygiene. Due to free availability and exclusive chemical structure the usage of meswak stick and other natural herbs are growing at a rapid speed in both developing and developed nations. Research has confirmed that meswak is as useful as, or may be even better than the current day common dental hygiene aids. The World Health Organization (WHO) has endorsed and suggested the usage of meswak as a good tool for dental hygiene.[3]

Neem (Azadirachtaindica) is a tropical evergreen tree native to India and is also found in other southeast countries. In India, neem is known as "the village pharmacy" because of its healing versatility, and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. Neem is also called 'arista' in Sanskrit- a word that means 'perfect, complete and imperishable'. The seeds bark and leaves contain compounds with proven antibiotic, antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses[3]. It was found that neem could reduce the ability of Streptococcal bacteria to colonize on the surface of teeth, thus providing an explanation for neem's long-standing reputation as a cavity fighter.[3]

Pomegranate is currently finding important applications in the field of dental health. Clinical studies[4] have shown that this popular antioxidant attacks the causes of tooth decay at the biochemical level, with remarkable vigour. The fruit of the pomegranate tree has been used extensively in the folk medicine of many cultures. The healing property of pomegranates was discussed in one of the oldest medical texts, the Ebers Papyrus from ancient Egypt (1500 BC).[4]Babool (Acacia nilotica) is a medium size thorny tree found in the drier parts of India[5]. Babool contains tannin and gallic acid and the leaves, bark and the gum of the tree all have medicinal qualities of antibacterial, antihistaminic, anti-inflammatory, astringent and hemostatic properties and is helpful in cases of gingivitis and periodontitis.[6]

An additional important factor for microbial colonization is the presence of orthodontic appliances. The adhesion of oral microorganisms to bracket surfaces is influenced to a large extent by interactions between salivary components in the pellicle and properties of the different microorganisms, in addition to the adherent patterns of bacteria on different types of orthodontic brackets- dictated by design and material used. Metallic orthodontic brackets have been found to induce specific changes in the oral environment[6] such as reduced levels of pH, increased plaque accumulation, and elevated S. mutans colonization. Nevertheless recent studies on possible differences in the initial affinity and adherence of bacteria on metal, ceramic brackets over time were inconclusive.

Hence, this research project was designed as a microbiological assay of Streptococcus mutans with an objective to study the performance and measure the efficacy of Neem, Meswak, Babool and Pomegranate based herbal toothpaste with conventional design Stainless Steel metal and Ceramic orthodontic brackets.

MATERIAL AND METHOD

Nature of Study

Randomized, prospective, cross sectional single blinded microbiological assay study with each patient acting his /her own control in this study.

Area of Study

Department of Orthodontics and Dentofacial Orthopedics, Divya Jyoti College of Dental Sciences and Research and Microbiological Assay was conducted in Divya Jyoti Hospital.

Ethical Clearance

This study was approved by Institutional Committee (IEC No DJD/IEC/2014/A-001). A written consent was taken from each participating subject.

Sample Size Estimation- N-Master Software (copyright @ Department of Biostatics, CMC Vellore)

$\mathbf{N = (}Z_\alpha + Z_\beta)^2 \times S^2 \times 2 / d^2$

$\mathbf{N= (}1.96+1.282)^2 \times (0.5)^2 \times 2 / (0.3)^2$

N= 10.51 Ẋ 2 Ẋ 0.25/0.09

N=58

S= Average Standard Deviation

d= Mean Difference

Sample

30 patients (15 males and 15 females) with a mean age of 15 -25 yrs. who were undergoing orthodontic treatment in department were selected. A total of 60 teeth were included in the study – one tooth from first and third quadrant of the mouth of each patient was admitted into the study (Table no.1).

Inclusion criteria

- Patient with similar socioeconomic strata & common food habits.
- Patients free of oral/parental antibiotics for one month.
- No periodontal & systemic disorders
- Patients with no crowding and who have had alignment and leveling completed.

Bonding Technique

For study¸stainless steel and ceramic brackets were used (fig-1). Labial and buccal surfaces of the teeth were acid etched with 37% phosphoric acid gel (3M™ ESPE™) for 30 seconds followed by rinsing for 30 seconds and then dried with

oil free compressed air for 20 seconds. Ortho Solo (Ormco) primer was applied on the teeth with a applicator tip and Transbond XT (3M Unitek, Monrovia adhesive were placed on the bracket base. The bracket was then placed on tooth with bracket positioning tweezer applying adequate pressure on bracket to expel flash from bracket. The flash was removed with explorer and care was taken to ensure complete removal of the flash. A single operator bonded the bracket included in the study for uniformity of pressure applied to expel the flash. Curing was done with Light Cure (Blue Luxcer^{TM}) cure the teeth for 20 sec.

Oral Hygiene Instructions

- The subjects were given oral hygiene instructions & requested to refrain from using any other oral hygiene products like mouthwash etc.
- The subjects were instructed to follow standard oral hygiene regime which included brushing twice a day with toothpaste as prescribed in the study regime.
- The patients were advised to rinse thoroughly after every meal.
- The patients were instructed to use herbal toothpaste from 3rd to 8th day (Table-2)

Plaque Collection Method

- Sample collection was done at time intervals T1(prior the use of prescribed dentrifice)¸ T2 (at day 3 after starting the use of prescribed dentrifice) and T3(at day 8 after using prescribed dentrifice).(Table-3).
- Patients were requested to refrain from eating or drinking 1 hour prior to sample collection.
- Plaque sample was collected by Four Pass Technique (Fig. No.2) at midmorning (11 a.m.).
- In this technique the explorer tip is moved around the circumference of the bracket at the bracket tooth interface.
- Four passes, along the tooth at the bracket interface at the gingival, mesial, distal, and occlusal aspects are done to avoid overloading the instrument tip.
- This is considered an effective method of obtaining the total plaque. Plaque samples were placed in sterilized vials having distilled water in it.
- Plaque sample placed in 5ml sterilized vials with1ml distill water.
- Sterilized vials were transported in icebox to the lab (fig-3).
- The bacteriological study was conducted by Dilution Plating Method.
- The growth media used was Mutans-Sanguis Agar.

LAB PROCEDURES

Various laboratory equipments required for studies includes¸ Autoclave¸ Hotplate¸ Petridish¸ Micropipette¸ Laminar flow Cabinet¸ Conical flask¸ Cotton Plug¸ Sterilized Wire loop Incubator¸ Disposable gloves¸ U shape flask¸ and Disposable Mouth mask (Table-4).

a) Protocol under Autoclave (fig-5)

- 100 gm. of Mitis Sanguis Agar (Himedia) was mixed in 1 L of Distilled water and sterilized at 121 ˚C for 20 minutes in an Autoclave. (Table-5).
- Liquid agar was placed in conical flask with cotton plug (Absorbent) and Home foil on top.

b) Protocol under Laminar Air Flow (fig-7)

- Cotton plug (Absorbent) and Home foil was removed from the top of conical flask and melted agar was poured in petridish for solidification in laminar air flow for 10-15 minutes at 37^{0}C.
- A sterilized wire loop was used to spread the plaque sample over the petridish.(fig-6)

c) Protocol Under Incubator(Fig-8)

- The petridish with agar containing sample prepared in the previous step were sealed with Parafilm “M” and incubated in incubator for 48 hours at 37˚C .

d) Data Collection

- The growth was mixed with 1 ml of distill water andincubated again for 24 hours.
- With the help of a micropipette 10 micro liter sample was spread over a slide and then covered with a cover slip (fig-9).
- Colony count was done under the microscope with the help of 40 X (High power lens)
- The data was collected and tabulated for all the groups. Colony counting was done by the researcher to obtain the number of Streptococcus mutans in the sample tested. Random recounting of samples was done at different time interval in order to check intra observer error. No significant errors were found.

Statistical test-

The data collected was tabulated and subjected to statistical test by SPSS software.

- One-way analysis of variance (one-way ANOVA was used to compare each individual groups (SS and Ceramic) in each time frame in the study (T_1,T_2&T_3).

- T-test was used to compare efficacy of the brackets dentifrice combination as checked against colony count of Streptococcus mutans.
- Two-way analysis of variance (ANOVA) test was used to compare two different groups of bracket with herbal dentrifices (SS, Ceramic) at single time frame.
- Multiple comparison tests (Tukey HSD) were used to compare different brackets with herbal dentifrices individually.
- One-way ANOVA was used to measure the significance of the mean count of Streptococcus mutans around two brackets tested.(Table-6).

RESULTS

On Comparison of Streptococcus mutans count around 2 brackets on day 1, 3&8with herbal dentifrices shows:

- **Day 1(Baseline):** The Streptococcus mutans count was lowin C bracket at 3.4333 and highin SS at 3.5555 There was no statistically significant difference between them.
- **Day 3 Herbal:** Both the values were almost similar to that of baseline at Day 1 no statistical significance.
- **Day 8Herbal:** low with SS at 2.5333& high with C at 2.6667.(Table-7).

Multiple Comparisons of Means of Streptococcus mutans Count in Two Brackets on Day 3 by Tukey HSD Test indicates:

Day 3: Herbal based toothpasteshowed no statistically significant difference in inter group comparison of the brackets.(Table-8)

Multiple Comparisons of Means of Streptococcus mutans Count around Brackets on Day 8 by Tukey HSD Test suggests:

Day 8: Streptococcus mutans count around brackets compared after usage of herbal dentifrices. (Table-9).

Between Day 3 & Day 8: Streptococcus mutans count around brackets compared after usage of herbal dentifrices.

- Inter group comparison by Tukey's HSD Test shows no statistically significant difference between the brackets tested,

Comparison of Means of Streptococcus mutans Count at Different Time Intervals around Stainless Steel Bracket by Paired T – Test signifies:

- Paired T test compared the means of Streptococcus mutans count around SS bracket at different time intervals. (Table-10).
- Difference between Day 3 and Day 8 with herbal dentifrice was highly significant statistically
- Difference between Day 1 & Day 8 was also highly statistically significant.

Comparison of Means of Streptococcus mutans Count at Different Time Interval in Ceramic Bracket by Paired T – Test suggests:

- Paired T test compared the means of Streptococcus mutans count around C bracket at different time intervals. (Table-11).
- Difference between Day 3 and Day 8 with herbal based dentifrice was statistically highly significant
- Difference between Day 1 & Day 8 was also highly statistically significant.

Cronbach's alpha reliability test:

- The statistical test retest validity was examined by Cronbach's Alpha reliability test to assess the reduction of streptococcus mutans colony count. The value was found to be 0.771 this indicate the reliability of the data to be acceptable.(Table-12).

DISCUSSION

Plaque accumulating around orthodontic brackets often results in enamel white spot lesions (WSL) adjacent to brackets. Plaque is composed of various microorganisms of which *S. mutans* is the most cariogenic. ***Loesche et al***[7]showed significant association between plaque levels of *S. mutans* and caries. Although WSL occurs in caries development irrespective of orthodontic treatment, it is during orthodontic treatment that they are extremely common and of prime concern for the clinician. Microorganisms play a major role in causation of WSL and dental caries. Entire removal of microorganism from the oral cavity is difficult but their count can be reduced with the help of various preventive measures so that it becomes less cariogenic. The market is flooded with numerous bracket types of different biomaterials. Literature evidences that adherence of plaque to the fixed appliance is largely contributed by the bracket material [8] as it could play a role in the degree of bacterial adhesion and plaque accumulation as well as in the risk of development of WSL. The initial affinity of bacteria on solid surfaces is due mostly to electrostatic and hydrophobic interactions. Surfaces with high surface free energy more easily attract bacteria such as S.mutans.[9]

The clinician is looking to an advantageous combination of dentifrices and /or bracket material and /or design to reduce enamel demineralization and WSL. This research was planned with an objective to ascertain if bracket material had any role in reducing bacterial oral microflora. Hence, stainless steel, ceramic bracket material was tested. These brackets were

of the conventional design. The dentifrices tested were Herbal commercially available product containing Babool, Neem, Meswak and Pomegranate. The participating subjects were given the specific oral hygiene regime and requested to use the dentifrices provided during the specified time periods. The subjects were blinded and this single blinded prospective microbiological assay was conducted to ascertain if any combination of bracket material/design and dentifrice could give clinically viable results of lowering the microbial count of Streptococcus mutans in the mouth during the test period. Both brackets to arch wire ligations were done with ligature wire as it is well known that elastomeric rings accumulate more microbes. The teeth which were chosen for the present study were 12 and 35 as in the study done by***Khalaf***[10] found the highest incidence of WSLs on the maxillary canines and lateral incisors on the maxillary and mandibular premolars and first molars as they showed more plaque accumulation. A standard method of plaque collection by Four Pass technique was done and laboratory Diluting and Palating method for assessing microbial flora.[11]Laboratory culture of Streptococcusmutans have been done on varied culture media.[12] The problem with accuracy in culturing and colony counting lies in actual isolation of the microbe. Strep. sanguinis is cocci which is very similar to Strep. mutans in size and may have a competition and/or coexist in the same niche of oral environment. While Strep. mutans is a pathogen incriminated in WSL and dental caries, Strep. Sanguinis is a commensal of the oral cavity and may play a beneficial role by inhibiting Strep. mutans proliferation. Hence, any research with lab culture must choose an appropriate culture media which will give accurate research findings. In the current research we used Mitis Salivaris Agar [13]which is a differential culture media and differentially allows the growth of Strep. mutans and inhibits Strep. sanguinis. It also helps to differentiate Enterococcus and salivary Mitis. ***Fadia et al*** [14]used Mitis Salivarius agar for bacterial culturing Streptococcus mutans around SLB systems. ***Syed &Loesche***[15]tested different culture media sucrose blood agar, N2C agar, Schaedler agar, and Mitis Salivarius agar and found the Mitis Salivaris Agar is a suitable media for differential culture. ***Emilson&Bratthall***[16]tested total cultivable flora and the selective media of Mitis Salivaris Agar and found it acceptable as a culture media for Smutans. Some researchers like ***Wadeet al***[17]incorporated Bacitracin for superior results. The current study was unique as each individual acted as his/ her own control and herbal *dentifrice* was used at the same time on both brackets being tested simultaneously. Colony counting by in vivo may be very cumbersome and long drawn if many variables are being tested and in vivo testing may not always be ethical practice if the drugs/products being tested are not cleared for human use. ***Nitin et al*** [18]checkedantimicrobial activity of herbal extracts against dental pathogens by using disc diffusion method in which antibiotic-impregnated wafers are used to test whether bacteria are affected by antibiotics. Diluting and Plating method was done in the current study as it is in accordance to the study done by ***Pujari*** [19]and found that diluting and plating method is the reliable method for growth of Streptococcus mutans.

The results of the current research assessing the performance of orthodontic brackets of Stainless Steel and Ceramic (polycrystalline) with herbal dentifrices showed highly significant reduction of Streptococcusmutans around stainless steel bracket with herbal toothpaste. These results of current study were in consonance to the study done by ***Jurelaet al***[20] that the number of colony-forming units of S.mutans and S.sobrinus in stimulated saliva samples does not seem to be significantly different between patients with stainless steel brackets and patients with plastic brackets.

The results of the current research study showed highly significant reduction around Ceramic bracket when herbal toothpaste is used**.** These results were in consonance to the study done by ***Anhouryet al***[21]and found no significant differences between metallic and ceramic brackets with respect to caries inducing *Streptococcusmutans*.

Hence, it may be useful to conduct further research on the effect of different concentrations of the herbal products and essential oils on different bracket material. These research findings should translate to the orthodontic brackets production companies and pharma companies to produce products with optimum concentrations of bracket material and herbal products for best clinical results. The current research validates the use of metal and ceramic material brackets with herbal based dentifrices as a viable modality of maintaining oral hygiene in orthodontic patients.

SUMMARY & CONCLUSION

This research tested if bracket biomaterials of stainless steel and ceramic had any clinical advantage of lowered Strep mutans count with herbal dentifrices. 30 indivividuals with strict selection criteria were enrolled in the study . Teeth Nos.12 &35 in each person were bonded with a specific bracket and 60 teeth were included in the study with 30 teeth/bracket in each study group.This research design added accuracy as the tests on the herbal dentifrices &2 variables of brackets were done at the same time interval in all the participantsThe findings may be summarised as –

- There was no statistically significant difference in the Streptococcus mutans count around the different brackets at baseline for herbal toothpaste .
- On Day 3 (i.e. 24 hrs after the use of herbal toothpaste) statistically significant difference was seen in the bacterial colony counts of Strep mutans around the Ceramic bracket.This could be because the ceramic bracket used was monocrystalline and may have superior efficiency in reducing adhesion of biofilm due to its lattice structure and surface characteristics.
- By Day 8 both brackets performed equally well with the herbal based tooth paste and there was statistically significant reduction of Strep mutan colony counts as compared to baseline values of Day 1 around both the brackets . Inter group comparision tests indicated no significant difference between the brackets.
- Herbal tooth paste have reduced colony forming unit of S.mutansaround both brackets.

Hence, it may be concluded that both statinless steel and ceramic brackets performed well with herbal tooth paste andclinically efficient in reducing Strep mutans colony counts around them .Both brackets performed equally well with slight more reduction of S mutans on statinless steel but nosignificant difference was seen between the brackets.

The herbal products containing neem, babool,pomogranate and meswak may be advised with steel and ceramic bracket during orthodontic treatment.The interesting fact is that patient counselling about regular discipline in oral hygiene is critical as the study showed that even though statistically significant reduction in microbial counts were found 24 hrs after start of the dentifrice. Further research with different concentrations of the active ingredients needs to be tested on larger and diverse sample groups to give insights in developing products which will have a sustained release and antimicrobial effect of reducing the counts lasting for a prolonged period . This would be a useful innovation specially in patients who are periodontally compromised and those who have difficulty in maintaining oral hygiene during orthodontic treatment.

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